



REVIEW ARTICLE

Starburst pamam dendrimers: Synthetic approaches, surface modifications, and biomedical applications



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PAMAM;
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Synthesis;
Biomedical application;
Nanotherapeutics;
Cytotoxicity

Abstract Dendrimers are having novel three dimensional, synthetic hyperbranched, nano-polymeric structure. Among all of the dendrimers, Poly-amidoamine (PAMAM) dendrimer are used enormously applying materials in supramolecular chemistry. This review described the structure, characteristic, synthesis, toxicity, and surface modification of PAMAM dendrimer. Various strategies in supramolecular chemistry of PAMAM for synthesizing it at commercial and laboratory scales along with their limitations and applications has also discussed. When compared to other nano polymers, the characteristics of supramolecular PAMAM dendrimers in nanopolymer science has shown significant achievement in transporting drugs for molecular targeted therapy, particularly in host–guest reaction. It also finds its applications in gene transfer devices and imaging

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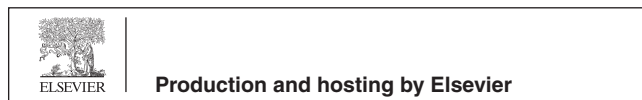
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of biological systems with minimum cytotoxicity. From that viewpoint, this review has elaborated the structural and safety aspect of PAMAM for targeted drug delivery with pharmaceuticals in addition to the biomedical application.

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1. Introduction

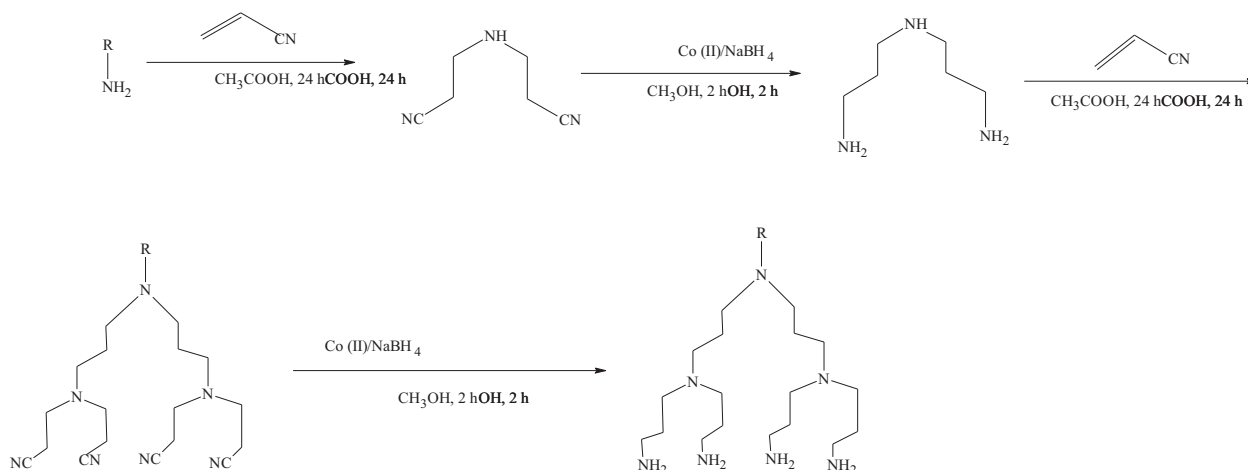
From the current research of polymeric chemistry and technology, significant work had already been completed for developing the therapeutically effective dosage form and transportation of adequate medicament to target site (Al-Jamal et al., 2005; Tupally et al., 2015). Synthetic biodegradable polymeric macromolecules and nano biopolymers have enlightened the field of pharmaceutical and biomedical applications of novel drug delivery systems (Tomalia et al., 1985). In that perspective, dendritic polymers provide an essential scientific breakthrough in the structural designing of different bioactive materials to enhance and exposed their specific functions (Bosman et al., 1999; Tomalia et al., 1985).

In 1978, Vogtle and co-workers reported to synthesize and propose the dendritic structure of polypropylene amine through repetitive monomer addition and activation of branched molecules shown in Scheme 1. The above synthesized dendritic structure initially named “cascade molecule” (Tomalia and Fréchet, 2002). However, this concurrent synthetic investigation Tomalia and associates at Dow chemical’s

near the beginning of 1980, synthesized hyperbranched monodisperse poly(amidoamine) PAMAM molecules (Tomalia et al., 1986). George Newkom’s parallelly synthesized the analogous macromolecules called arborols, which means trees in Latin, but ‘dendrimer’ is the best established one (Newkome et al., 1986).

Dendrimers are well repeating branched unit monodispersed spherical macromolecule characterized with a multifunctional core with high density and precise functional groups attached to a surface (Alper, 1991; Newkome et al., 1986). Table 1 represents different types of dendrimers with its structural specificity and applications. Compared to conventional linear, branched, and hyperbranched polymers of the same molecular weight, dendrimers have attracted wide attention due to unique functional properties confined within a small three-dimensional space (Caminade et al., 2005).

According to Tomalia, dendritic structures categorized into four sub-classes that are random hyperbranched polymer, dendrigraft polymers, dendrons, and dendrimers (Tomalia et al., 1990, 1987). Among all the dendrimers poly(amidoamine) PAMAM dendrimer is used enormously as applying materials



Scheme 1 Synthesis of cascade molecule by Vogtle and co-workers (Tomalia and Fréchet, 2002).

in supramolecular chemistry (Naylor, 1989). PAMAM provides the perfect structure for the development of active drug carriers (Jansen et al., 1994), gene transfer devices, host-guest interactions (Hu et al., 2012), and imaging biological systems with minimum cytotoxicity (Hsu et al., 2017; Roberts et al., 1996). From the above aspects, we have discussed synthesis, structure, cytotoxicity, characteristic, surface modification of PAMAM dendrimers, and their possible biomedical, as well as pharmaceutical applications in the field of nanotechnology.

2. Structural chemistry

In 1984, Tomalia and co-workers developed, characterized, reported, and commercialized the first absolute PAMAM dendrimers family. These molecules start from an ammonia (NH_3) or ethylenediamine ($\text{C}_2\text{H}_8\text{N}_2$) core and bind amine or amide groups on the periphery (Esfand and Tomalia, 2001). From the central core, ‘wedges’ or ‘dendrons’ radiates toward the periphery, resulting in homo-structural layers (branching unit) (Nanjwade et al., 2009; Santos et al., 2019). Each layer of concentric branching units considered as one generation (Menjoge et al., 2010). Commercially PAMAM dendrimers with terminal carboxylic groups are called half generation (0.5, 1.5, 2.5...9.5), and the amino group are called a full generation (1, 2, 3...10). Fig. 1 represents the structure of PAMAM dendrimer with the generation's number and the peripheral end groups (Twyman et al., 2002). Table 2 gives information about the diameter of PAMAM, which linearly increased according to generation and peripheral functional groups.

“Starburst dendrimer” is a trade name for the subclass of PAMAM starlike, three-dimensional, highly ordered oligomeric compounds (Vlasov, 2006), which has been commercially manufactured by Dendritech TM (U.S.A.) since 1990 (Abbasi et al., 2014). PAMAM dendrimer becomes compactly crowded towards the periphery of the higher generation (Sadekar and Ghandehari, 2012). The steric crowding of branches increases after PAMAM G7, which leads in the formation of defective structure (De Gennes dense packing effect). After the tenth generation, the synthetic yield has reduced (Bugno et al., 2015), and dendrimers cannot grow because of insufficient space. This effect is known as ‘Starburst effect’ (Gupta and Nayak, 2015; Maiti and Goddard, 2006).

After this step, the speed of reaction drops suddenly, and further reaction with the end group cannot occur (Peterson et al., 2001; Scott et al., 2005).

The molecular weight of the dendrimer can be predicted mathematically using Eq. (1) (Jackson et al., 1998; Walter and Malkoch, 2012).

$$M = M_C + n_c \left[M_m \cdot \left(\frac{n_m^G}{n_{m-1}} \right) + M_t \cdot n_m^G \right] \quad (1)$$

where

- M_C : The molecular weight of the core
- M_m : Molecular weight of the branched monomer
- M_t : Molecular weight of terminal groups
- n_c : Core multiplicity
- n_m : Branch juncture multiplicity
- G : Generation number.

The number of dendrimer terminal groups increases with geometric progression (Eq. (2)) (Jackson et al., 1998; Walter and Malkoch, 2012).

$$Z = n_c \cdot n_m^G \quad (2)$$

This important contribution of Tomalia in well-defined structure holds great promise in nanotechnological approaches.

3. Properties of pamam dendrimers

Dendrimers are well distinguished from all other traditional linear polymers through considerably enhanced physicochemical properties. Dendrimers also have immense potential in the field of novel and targeted drug delivery (Nishikawa et al., 1996). The physical and chemical properties of PAMAM with immense potential in the field of drug delivery are shown in Fig. 2. Due to the controlled fundamental properties, including monodispersity, size and shape, peripheral charge, flexibility or rigidity, structural design, and fundamental composition, PAMAM proved itself best over the other nanocarriers (Thiagarajan et al., 2013). Researchers provide sufficient information for predicting and elucidating some techniques

Table 1 Different types of dendrimers with its structural specificity and applications.

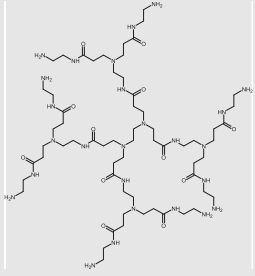
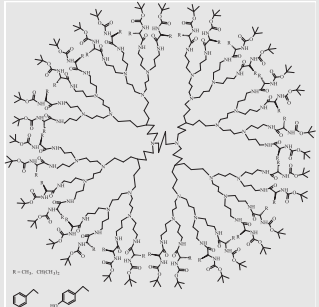
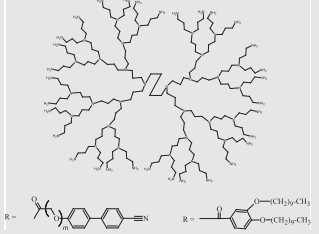
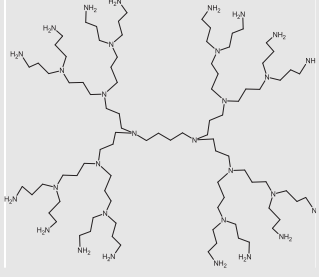
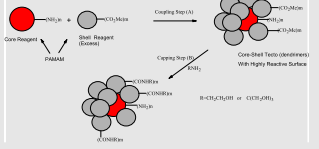

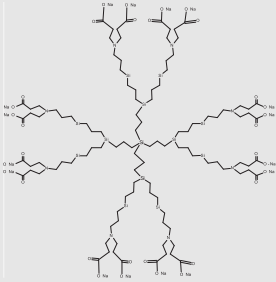
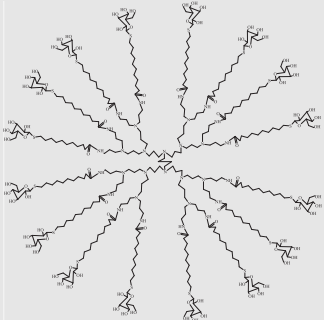
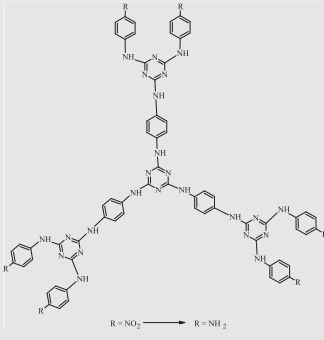
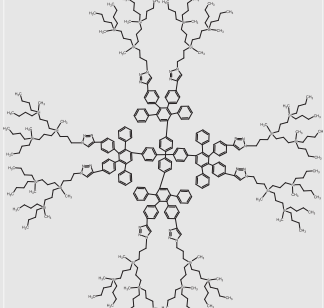
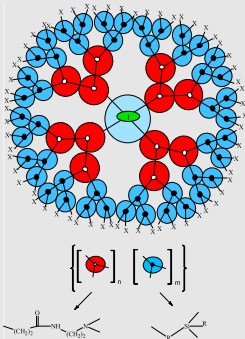
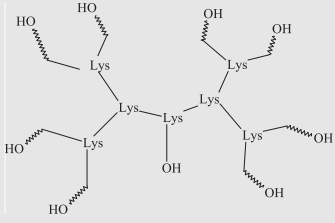
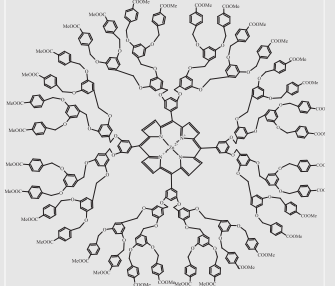
Sr. No.	Types of dendrimers	Synthesis/First reported by	Structural specificity	Applications	Structure	References
1	Polyamidoamine dendrimers (PAMAM)	Divergent method/ Tomalia et al.	Spheroidal or ellipsoidal with the presence of several peripheral functional groups and internal cavities	Diagnostic agent. Targeting drug delivery and gene therapy.		Naylor, 1989; Negar and Rouhollah, 2013; Pagé and Roy, 1997; Tomalia et al., 1985
2	Chiral dendrimers	Convergent method/ Diederich et al.	Peripheral primary amines groups and tertiary propylene amines as a core. Chirality based on the constitutionally different but chemically analogous branches.	Enantioselective catalysis and molecular recognition process.		Ritzen and Frejd, 1999; Smith and Diederich, 1998
3	Liquid crystalline dendrimers	Divergent or convergent method /Virgil percec et al.	Mesogenic liquid crystalline monomers or thermotropic liquid crystalline phases which are rod-like (calamitic) or disk-like (Discotic) molecules.	Use for specific physical properties.		Cheng et al., 2015a, 2015b; Guillon and Deschenaux, 2002; Percec et al., 1992; Smith and Diederich, 1998
4	Polypropylene ether imine dendrimers (PPI)	Divergent method/ Newkome et al.	Amino butane core with primary amines as functional end groups.	Antimicrobial and solubility enhancement agent.		Gillies et al., 2005; Inoue, 2000; Kaur et al., 2016; Newkome et al., 1986
5	Tecto dendrimers	Divergent method/Tam et al.	Fluorescein core (thermotropic agent) and folate as a functionally active peripheral moiety.	Used for Multidrug delivery, gene therapy, and environmental remediation.		Schilreff et al., 2012; Tam and Lu, 1995; Welch and Welch, 2009
6	Peptide dendrimers	Convergent method/Sadler et al.	Amino acid as peripheral and interior unit.	Used for the diagnostic purpose, vaccine delivery, protein replacement, Chemotherapeutic agent and gene therapy.		Ammonium, 1995; Backbone, 1990; Sadler and Tam, 2002

Table 1 (continued)

Sr. No.	Types of dendrimers	Synthesis/First reported by	Structural specificity	Applications	Structure	References
7	Carbosilane dendrimers	Divergent and convergent method/ Bermejo et al.	Two different cores $1,3,5-(\text{HO})_3\text{C}_6\text{H}_3$ and $\text{Si}(\text{C}_3\text{H}_5)_4$ having carboxylate and sulfonate as an end functional groups.	Potential application in catalysis, host-guest chemistry, novel polymer topology.		Bermejo et al., 2007; Kesharwani et al., 2014
8	Glycodendrimers	Divergent and convergent method/ Benjamin Davis et al.	Glycodendrimers include sugar moieties such as mannose, glucose, galactose, and disaccharide.	In biotechnology and material science.		Benjamin, 2002; Roy and Baek, 2002;
9	Triazine dendrimers	Convergent method (cycloaddition reaction or triazine substitution)/ Astruc et al.	The core is containing triazine trichloride and other triazine based compounds.	Used in the synthesis of simazine herbicide also applied in non-viral gene delivery.		Astruc et al., 2012; Steffensen et al., 2006
10	Hybrid dendrimer	A Divergent method (Diels-Alder cycloaddition) by Roovers et al.	These are hybrids of dendritic and linear polymers. Composed of elastic carbosilane core and rigid aromatic shell.	As drug carriers.		Kesharwani et al., 2014; Roovers and Comanita, 1999
11	PAMAMOS (Poly amidoamine Organosilicon Dendrimers)	Divergent method/Peter Dvornic et al.	PAMAM as an interior and hydrophobic organosilicon as a peripheral moiety.	Use for drug conjugate and nanocarrier.		Dvornic, 2006; Tomalia, 1993; Torchilin, 2006

(continued on next page)

Table 1 (continued)

Sr. No.	Types of dendrimers	Synthesis/First reported by	Structural specificity	Applications	Structure	References
12	Multiple antigen peptide dendrimers	Convergent and Divergent method/Tam et al.	Dendron like molecular configuration based on a polylysine framework.	Improved efficiency of antibiotics and antimicrobial molecules.		Joshi et al., 2013; Tam and Lu, 1995
13	Fréchet-type dendrimers	Convergent method/Hawker and Fréchet et al.	Structural framework based polybenzyl ether with Carboxylic acid group act as a functionally active peripheral moiety.	Pharmaceutically active compound, imaging agent, radioligand and targeting molecule.		Hawker and Fréchet, 1990; Ihre et al., 2002

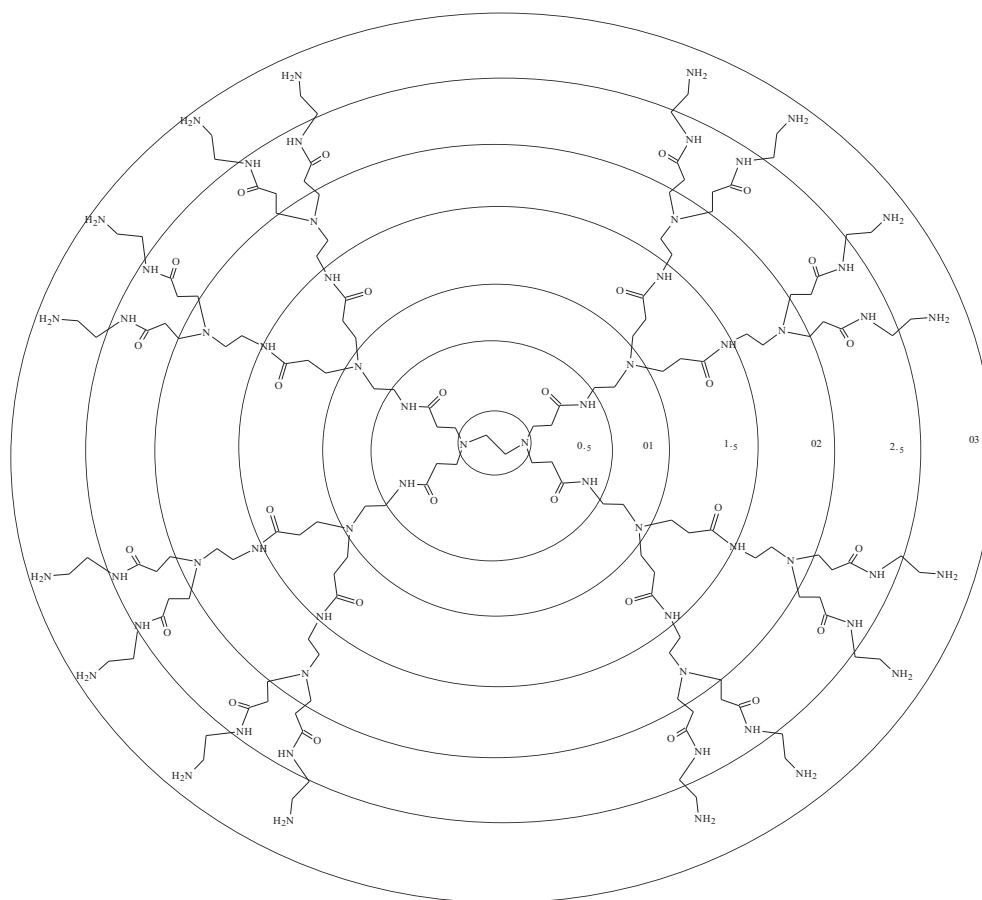
**Fig. 1** Structure of PAMAM dendrimer with the generation's number and the peripheral end groups.

Table 2 Theoretical structural characteristics of PAMAM G 0-7dendrimers (De Araujo et al., 2018; Peterson et al., 2001).

Generation	Molecular formula	Hydro dynamic diameter	Ammonia core		Ethylenediamine (EDA) core	
			Molecular mass	No of end groups	Molecular mass	No of end groups
0	C ₂₄ H ₅₂ N ₁₀ O ₄	1.5 nm	359	3	516	4
1	C ₆₄ H ₁₃₂ N ₂₆ O ₁₂	2.2 nm	1043	6	1428	8
2	C ₁₄₄ H ₂₉₂ N ₅₈ O ₂₈	2.9 nm	2411	12	3252	16
3	C ₃₀₄ H ₆₁₂ N ₁₂₂ O ₆₀	3.6 nm	5147	24	6900	32
4	C ₆₂₄ H ₁₂₅₂ N ₂₅₀ O ₁₂₄	4.5 nm	10,619	48	14,196	64
5	C ₁₂₆₄ H ₂₅₃₂ N ₅₀₆ O ₂₅₂	5.4 nm	21,563	96	28,788	128
6	C ₂₅₄₄ H ₅₀₉₂ N ₁₁₁₀ O ₅₀₈	6.7 nm	43,451	192	57,972	256
7	C ₅₁₀₄ H ₁₀₂₁₂ N ₂₀₄₂ O ₁₀₂₀	8.1 nm	87,227	384	116,340	512
8	C ₁₀₂₂₂ H ₂₀₄₄₈ N ₄₀₉₀₀ O ₂₀₄₄	9.8	174,779	768	233,076	1024
9	C ₂₀₄₆₂ H ₄₀₉₂₈ N ₈₁₈₆₀ O ₄₀₉₂	11.4	349,883	1536	466,548	2048
10	C ₄₀₉₄₂ H ₈₁₈₈₈ N ₁₆₃₇₈₀ O ₈₁₈₈	~13.0	700,091	3072	933,492	4096

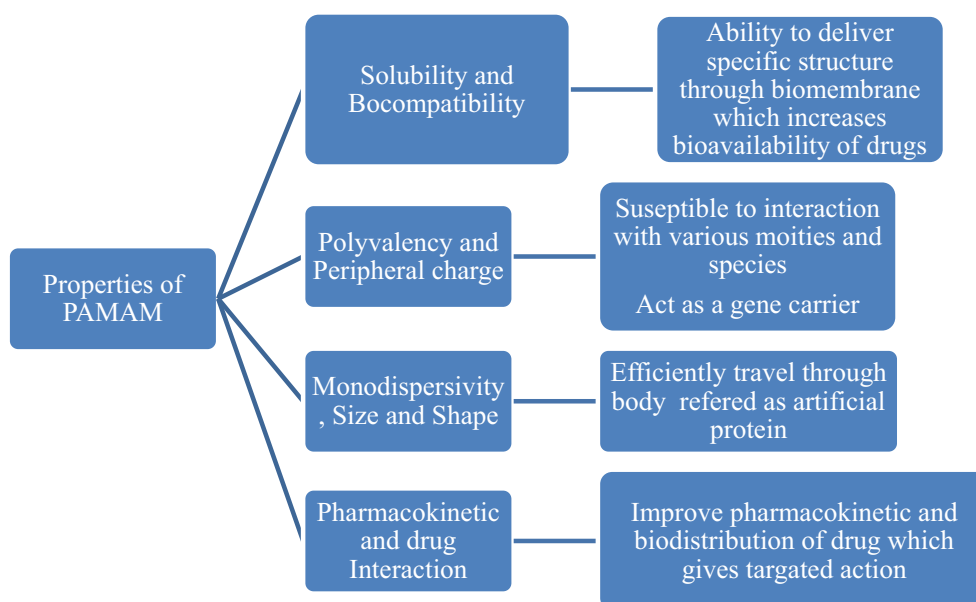


Fig. 2 Physical and chemical properties of PAMAM with immense potential in the field of drug delivery.

for characterization of PAMAM dendrimer molecule which are described below.

3.1. Monodispersity, size, and shape

Monodispersity means a well distinct molecular arrangement without variations, which shows a significant role in predicting the pharmacokinetic behaviour of the molecule (Kaminskas et al., 2011). PAMAM is the class of dendritic polymers having a well distinct molecular arrangement, and reproducible nanoscale size through their controlled synthesis and purification process (Zhong et al., 2011). The Monodispersity of PAMAM confirmed by various analytical techniques includes mass spectroscopy, size exclusion chromatography, gel electrophoresis, and transmission electron microscopy (TEM) (Kaminskas et al., 2011; Zhong et al., 2011). Mass

spectroscopic data explicitly defined monodispersity of lower generation PAMAM (G1 - G5) synthesized by the divergent method. Subsequent generation leads to polydispersity because of the partial elimination of ethylenediamine in every forward generation. The polydispersity index is less than 1.08 for G5–G10 PAMAM dendrimer, which shows that even particle size distribution in every step of generation (Lin et al., 2015; Maiti et al., 2005).

The size of PAMAM dendrimers for G0 to G10 ranges from 10 Å to 130 Å. Lower generations possess open, highly planar, elliptical, asymmetric shapes as compared to a higher generation. The spheroidal structure of PAMAM dendrimers contains the ability to incorporate drug molecules (Lin et al., 2005; Malik et al., 2000), due to the presence of peripheral protonated primary amino groups at a physiological pH (Lin et al., 2005; Percec

et al., 1998). G3, G4, and G5 PAMAM dendrimers with ammonia core have structural similarity with insulin (30 Å), cytochrome C (40 Å), and hemoglobin (55 Å), respectively. Because of its biostructural similarity, PAMAM efficiently travels throughout the body and called 'artificial protein' (Lee and Larson, 2008; Liu et al., 2009). Earlier researchers reviewed various scattering techniques together with small-angle X-ray diffraction, small-angle neutron scattering, and laser light scattering for characterization of PAMAM dendrimers, suggesting two types of microscopy, including transmission electrons microscopy (TEM) and atomic force microscopy (AFM) for imaging hydrodynamic radius, the structure of complete dendrimer, and radius of gyration in solution (Caminade et al., 2005). Monodispersity and size concern its pharmacokinetic behaviour in a biological system for pharmaceutical application.

3.2. Polyvalency and peripheral charge

Polyvalency determined the number of reactive sites present on the surface of the dendrimer. PAMAM consists of several peripheral moieties with positive, negative, or neutral charges, which makes them more susceptible to interact with various materials and species (Cakara et al., 2003; Pryor et al., 2014). Caminade et al. have proposed electron paramagnetic resonance technique (EPR) to identify the interaction between end groups and the substitution efficiency of PAMAM. They also suggested the electrophoresis technique and differential scanning calorimetry (DSC) for the assessment of PAMAM surface groups (Caminade et al., 2005; Pryor et al., 2014). PAMAM dendrimers with a positive surface charge can be applied as a gene carrier because it forms complex with negatively charged DNA (Nomani et al., 2010). Maiti et al. also demonstrated the significance of peripheral charge on PAMAM. He explained that how peripheral charge plays a vital role to synthesize PAMAM encapsulated nanoparticles (Maiti and Messina, 2008; Nomani et al., 2010; Pryor et al., 2014).

The peripheral positive charge of polycationic PAMAM dendrimers interacts with the negatively charged biological membrane; showing smooth intracellular delivery. Among these advantages, polycationic PAMAM dendrimers shows some toxicities (Nomani et al., 2010; Pryor et al., 2014). Nomani et al. synthesized self-assembled antisense/PAMAM dendrimer nanoparticles. He observed that cytotoxicity of nanoparticles depends on generation as well as the charge ratio of PAMAM. He also noticed in-vitro cytotoxicity of Polyfect® (G6 PAMAM) and Superfect® (G6 PAMAM), which can be overcome by surface modification (Nomani et al., 2010; Sadekar et al., 2013). In that concern, polyvalency of PAMAM has a potential role in intracellular targeted drug delivery by surface modification.

Sadekar and Ghandehari studied the effect of peripheral charge and PAMAM generation in oral delivery. They observed the effect of PAMAM dendrimer's surface charge on cytotoxicity, transepithelial transport, and cellular uptake. In their study, they used Caco-2 cell lines to transport dendrimers by paracellular and transcellular mediated routes (Ernandez and Pedro, 2014; Sadekar and Ghandehari, 2012). After the study, they concluded that the surface-modified

PAMAM dendrimers give reduced toxicity with enhanced transepithelial transport.

3.3. Solubility and biocompatibility

As compared to linear polymer, PAMAM dendrimer generally showed higher solubility and miscibility due to the presence of many branching ends. The solubility of PAMAM dendrimers is robustly dependent on various components such as peripheral group, number of generations and nature of terminal functional units (Cheng et al., 2007; Kayser et al., 2016). Generally, PAMAM are soluble, and therefore it acts as solubilizers for low solubility drugs by ionic interaction, hydrogen bonding, and hydrophobic interactions.

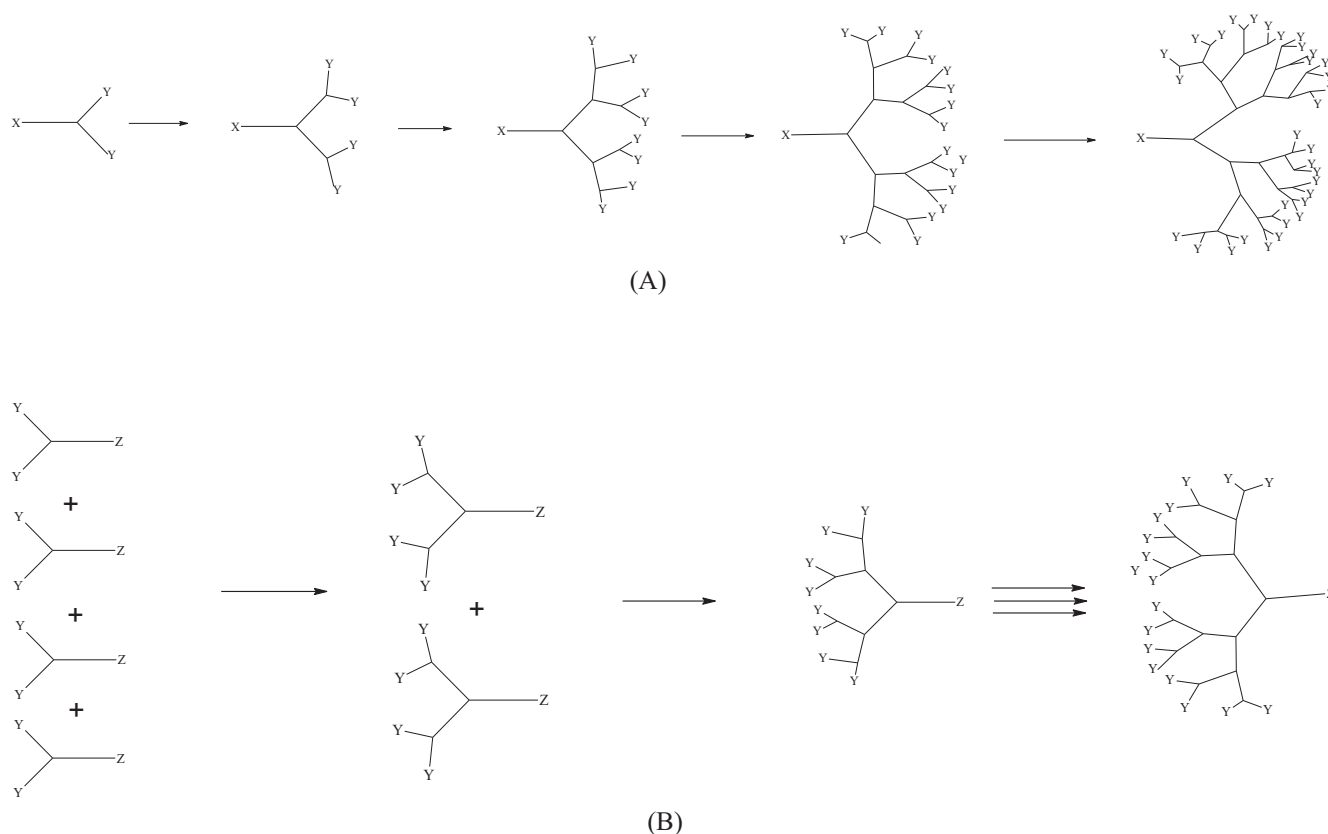
PAMAM dendrimers with hydrophilic surface groups showed solubility in polar solvents, while hydrophobic surface groups showed solubility in nonpolar solvents. High degree hydration of PAMAM dendrimers forms gel-like structures due to repulsive interaction between positive functional groups (Wang et al., 2017). As a result, PAMAM dendrimers with hydrophilic surface groups produced large hydrodynamic radius as compared to that in a dry condition. PAMAM dendrimers can also solubilize water-insoluble drugs. Consequently, these drugs easily transport through the biomembrane and increase its bioavailability (Dib et al., 2019; Negar et al., 2014).

PAMAM dendrimer should accomplish a variety of requirements for potential drug carriers such as non-toxicity, non-immunogenic, ability to cross biological barriers, stay in blood circulation long enough, and ability to deliver specific structure (Agashe et al., 2006; Markowicz-Piasecka et al., 2014).

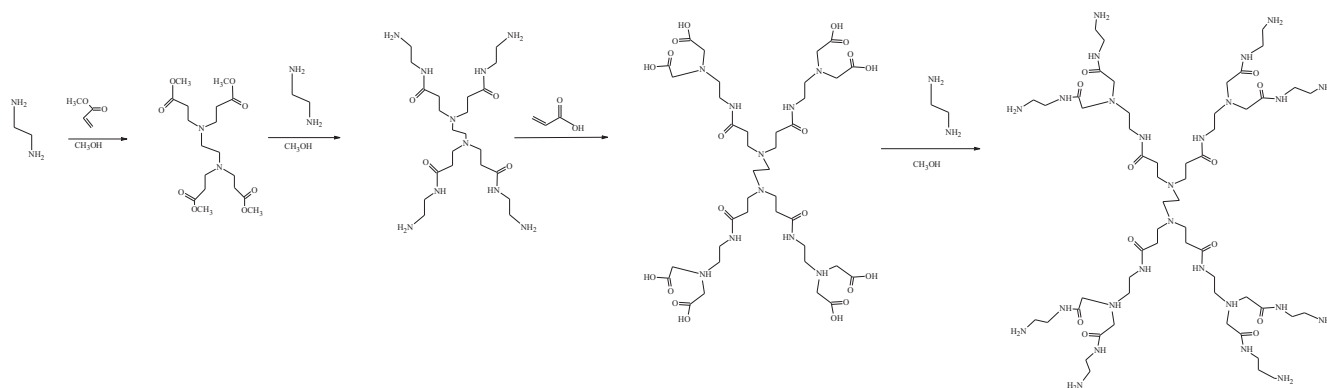
Piasecka et al. examined concentration and generation dependent biocompatibility and adverse effect of PAMAM towards the endothelial monolayers. They also examined the impact of PAMAM on clot formation without disturbing the thrombin activity (Markowicz-Piasecka et al., 2014). On that background, Duncan et al. designated PAMAM dendrimers as 'safe' molecules and the chemistry of PAMAM used in specific applications like biomedical and pharmaceutical applications (Duncan and Izzo, 2005; Fuchs et al., 2004). Therefore, PAMAM dendrimers are capable of enhancing solubility with related biodistribution, biocompatibility and efficacy of several drugs.

3.4. Pharmacokinetic and drug interaction

Pharmacokinetic properties and their interference with biological components of PAMAM depend on its physicochemical properties (Jevprasesphant et al., 2003a, 2003b). The design of PAMAM dendrimers has modified to improve the pharmacokinetics and biodistribution of drugs with the aim of targeted action. Surface modified PAMAM dendrimers reported for giving improved pharmacokinetic profiles than the basic one. Amine terminated G3, and G4 dendrimers showed very rapid initial clearance and the high initial volume of distribution (Jevprasesphant et al., 2003a, 2003b). In that concern, researchers studied the effect of surface charge on the excretion profile of non-biodegradable G5 PAMAM dendrimer. The result showed that neutral charge dendrimer was excreted via



Scheme 2 Diagrammatic illustration of divergent (A) and convergent (B) synthetic approach for dendrimers.



Scheme 3 Divergent approach for synthesizing PAMAM dendrimer (Labiencic-Watala and Watala, 2015).

urine and feces twice over seven days as compared to polycationic dendrimer (Nigavekar et al., 2004). Nigavekar and his coworkers compared PAMAM G5 dendrimer with surface modified acetylated PAMAM dendrimer for biodistribution. They used B16 melanoma cell line and DU145 human prostate cancer cell line. They observed that tissue deposition of polycationic PAMAM is better as compared to the neutral surface charge (Nigavekar et al., 2004). From that observation, they conclude that neutralization of surface charge of PAMAM dendrimers is required to utilize it as a systemic biomedical

delivery device. Drugs interact with the PAMAM dendrimer molecule and encapsulate into the interior cavities by electrostatic interactions or by conjugation with the end groups.

Sadekar et al. compared the effect of the multiple branched PAMAM dendrimers and linear N-(2-hydroxypropyl)methacrylamide (HPMA) copolymers on tumor-bearing mice. They identified the effect of molecular weight and hydrodynamic size of PAMAM and HPMA on biodistribution using compartmental pharmacokinetic analysis. They observed that hydroxyl-terminated PAMAM dendrimers have higher tumor

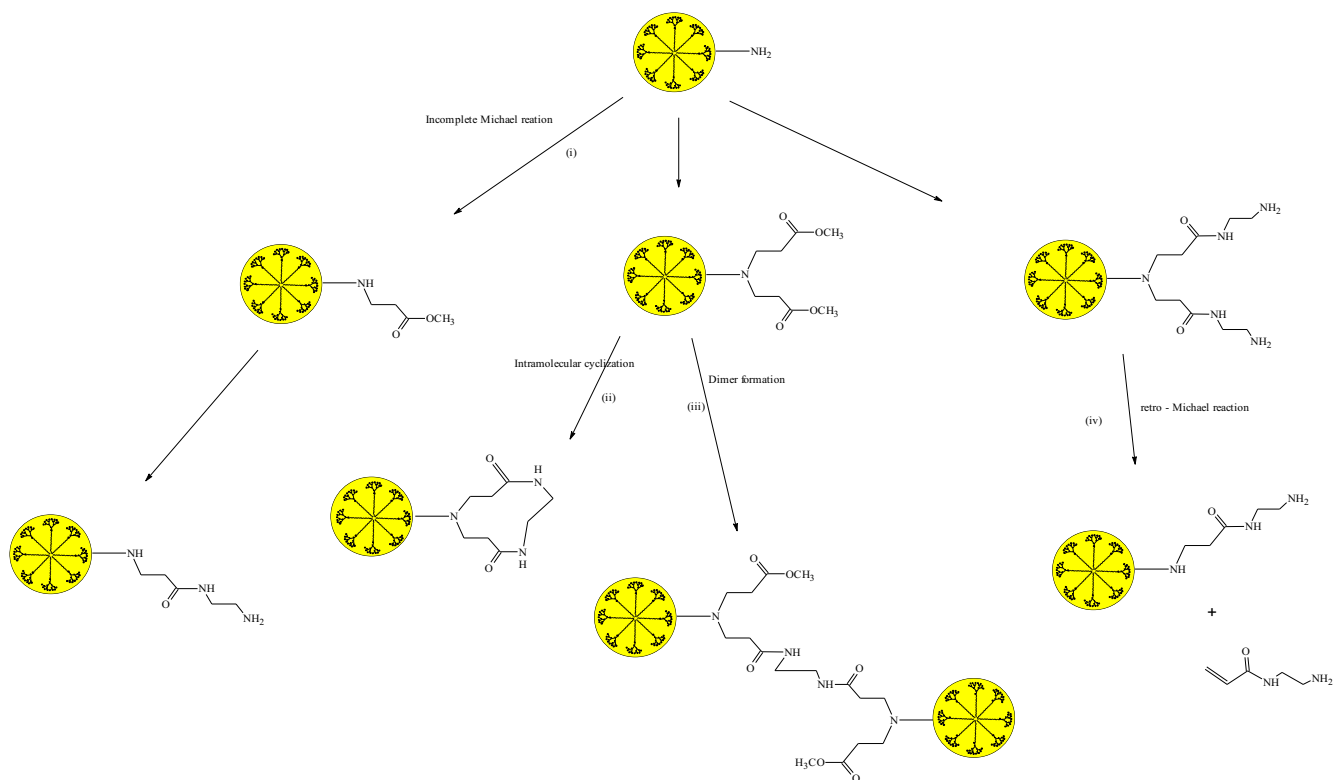


Fig. 3 Structural defects occurred by the different side reaction in the synthesis of PAMAM dendrimer (Grayson and Fréchet, 2001; Miller, 1990).

extravasations rate than linear HPMA copolymers. Therefore they conclude that after circulation, hydroxyl-terminated PAMAM dendrimers have a greater affinity towards the tumor with a smaller amount of clearance than linear HPMA copolymer (Sadekar et al., 2013). From the above discussed prospective, PAMAM dendrimer-drug interaction shows significant importance to design and optimize the PAMAM-based drug delivery system. Molecular PAMAM properties and characteristics are central to releasing their potential.

4. Synthesis of pamam

PAMAM dendrimers are effectively used in various biomedical applications. For that purpose, it must be readily available in the required quantity with consistent quality. Unlike conventional polymers, dendrimers are synthesized via iterative reaction to maintain controlled branched structures and minimum polydispersity (Labieniec-Watala and Watala, 2015; Svenson and Tomalia, 2005). The synthesis of this supramolecular structure is complicated with low yield because the purification processes are difficult. Besides, the design and synthesis of PAMAM dendrimers require the appropriate handling of energy parameters, called “the architecture of the molecule,” to target the supramolecule having different structural and physicochemical properties (Hernández Ramírez et al., 2020).

4.1. Conventional synthesis

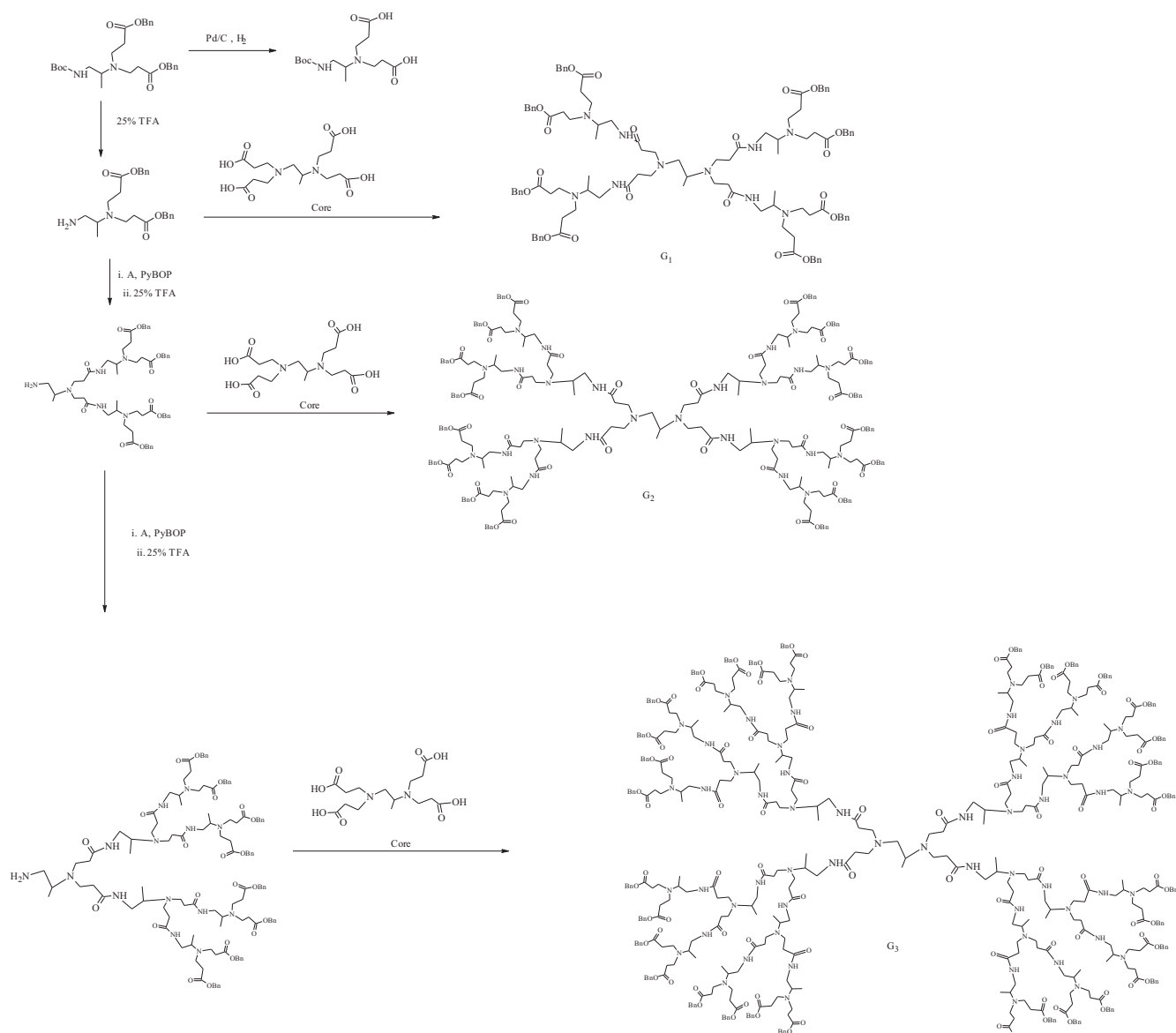
Dendrimers were usually synthesized using a series of iterative and activation steps. Since PAMAM having perfectly

branched structure, the synthesis requires to apply the robust organic reactions that can efficiently proceed even at a macromolecular level (Alper, 1991; Caminade et al., 2005). Scheme 2 provides two main synthetic strategies, divergent and convergent approach to accomplish dendrimers (Zhong et al., 2011).

4.1.1. Solution synthesis

In conventional solution synthesis of PAMAM, generally, two schemes were used that are divergent synthesis and convergent synthesis. However, the divergent scheme is superior for controlled branched PAMAM structure and predominantly employed for solid-phase synthesis of PAMAM dendrimers (Ihre et al., 2003).

4.1.1.1. Divergent approach. PAMAM dendrimers were synthesized by the divergent approach in a stepwise manner, starting from multifunctional core molecule ethylenediamine (EDA) as a precursor and building up the molecule towards periphery using Michael addition and amidation reactions (Tomalia et al., 1987). Half generation of PAMAM dendrimer synthesized by the Michael addition, where as amidation reaction involved two reactions steps (i) coupling reaction for the activation of carboxylic group (ii) amidation step generating amine-terminated full generation PAMAM dendrimers. The above two steps continue for multiple times to get optimized generations of PAMAM (Ihre et al., 2003) given in Scheme 3. After reaching the desired generation, the functionally active end groups are available for further post-functionalization. The divergent growth approach is also called the inside-outside approach (Labieniec-Watala and Watala, 2015).

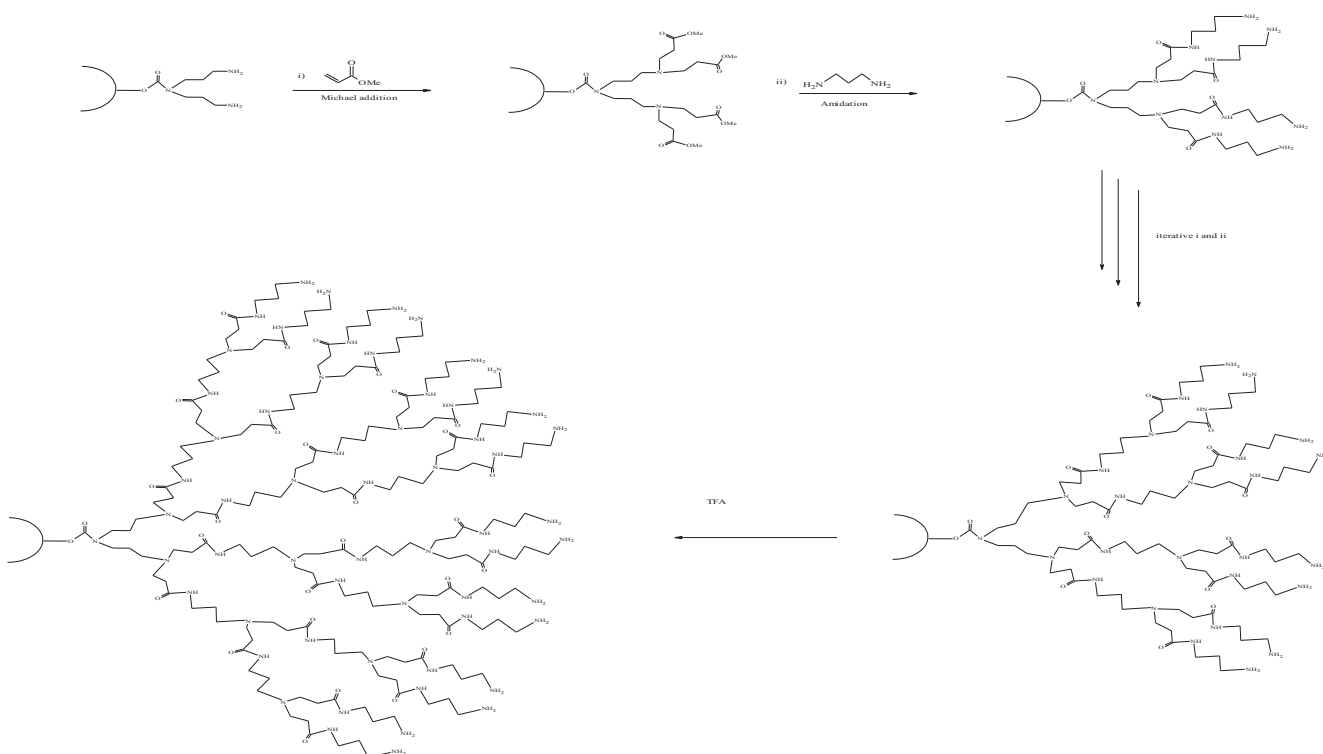


Scheme 4 The synthesis of PAMAM dendrimers via the convergent growth approach using peptide synthesis chemistry (Huang et al., 2013).

Structural imperfection is a significant concern in the growth of PAMAM dendrimer beyond G4 that unavoidably occurs from imperfect Michael addition reactions, retro-Michael reactions, intramolecular cyclization, dimerization during amidation and steric hindrance. It is difficult to separate the defective dendrimers from intact dendrimers because of their similar chemical compositions and physicochemical properties (Miller, 1990; Grayson and Fréchet, 2001). Fig. 3 provides brief information about structural defects occurred in the synthesis of PAMAM dendrimer.

In incomplete Michael addition reaction, nitrogen substitute one of the amine hydrogens of EDA (Michael donor), and another one fails to react. This missing moiety remains as such, and synthesis proceeds using such incomplete form of dendrimer. Sometimes intramolecular cyclization occurs in the amidation step between two branches with the same EDA molecule that stop the further expansion of PAMAM

dendrimer (Gupta and Nayak, 2015). This type of reaction can only occur in the synthesis of full generation dendrimer. There is a chance of ester hydrolysis, which also generates defects in dendrimer. Beginning from the first generation, sometimes there are chances of retro Michael reactions in which arm degrades back to free -NH_2 group. An excess of EDA avoids the side reactions in the amidation step. Peterson et al. suggested the most uncomplicated and easy Capillary zone electrophoresis (CZE) technique for the evaluation of different generations and analysis of homogeneity of a single generation. They also gives valuable information about “structural errors” in generations of dendrimer by electropherogram, which allows assessing the presence of side products in synthesized dendrimers (Peterson et al., 2001). Therefore it can be observed that the synthesis of PAMAM by divergent approach is at present accomplished with the well-known reaction in a controlled manner, where efficient



Scheme 5 Solid-phase synthetic approach of PAMAM dendrimers using peptide synthesis chemistry via iterative Michael addition and amidation (Swali et al., 1997; Huck et al., 1998).

protection or deprotection and coupling reactions are of primary importance.

4.1.1.2. Convergent synthesis. Besides divergent synthesis, Hawker and Fréchet in 1990 introduced a convergent growth scheme for the synthesis of PAMAM dendrimer (Hawker and Fréchet, 1990). In the convergent approach, the synthesis of PAMAM dendrimer forwarded from the surface towards the core by “one to one” coupling of monomers (Gupta and Nayak, 2015). This method relies on the construction of perfectly branched dendrons (dendrimer wedges), coupled to a core moiety after activation of their focal point (Grayson and Fréchet, 2001). They first synthesized dendrons from G1 to G3 via iterative amide linkage and amine-deprotection. The obtained amine-containing dendrons were then attached to the core, as shown in Scheme 4 (Brouwer et al., 2001; Lee et al., 2006).

PAMAM dendrimers synthesis via the convergent growth scheme presented a significant challenge for solution-phase synthesis because the use of an excess of reagent is not feasible. However, this approach overcomes the impurity and structural imperfection issues of divergent synthesis (Pittelkow and Christensen, 2005). By this method more consistent and symmetric dendrimers can be produced, although it has some limitations due to steric hindrance between dendrons which are going to attach the core of high generation dendrimers. Therefore by using the convergent approach, eight or fewer generation dendrimers can be obtained (Pittelkow and Christensen, 2005; Wells et al., 1998).

This approach is more accessible to synthesized heterogeneous or asymmetric dendrimers by incorporating several active sites in one dendrimer molecule. Pittelkow et al. report a convergent approach to synthesize a series of unsymmetrical internally branched PAMAM dendrimers using a highly effective protection group in combination with a very efficient peptide coupling reagent (Pittelkow and Christensen, 2005). The purity of dendrimers were confirmed by using the purity of dendrimers by SEC (scanning electron microscope), NMR (nuclear magnetic resonance), and MALDI-MS (mass spectroscopy) (Huang et al., 2013).

4.1.2. Solid phase synthesis

Other than solution synthesis, in 1988, Tam and his co-workers first reported the solid-phase synthesis approach for the synthesis of PAMAM dendrimer (Tam and Lu, 1995; Tam and Merrifield, 2009). They synthesized polylysine dendrimers by divergent approach through a conventional solid-phase peptide route (Tam and Lu, 1995). Bradley et al. synthesized PAMAM dendrimers conveniently and efficiently on the solid support by using Tomalia iterative growth for solution synthesis, viz., Michael addition and amidation (Egner et al., 1995; Guillier et al., 2000; Wells et al., 1998). Scheme 5 represents the synthetic scheme for PAMAM dendrimers using peptide synthesis chemistry via the iterative growth approach. They achieved the growth of PAMAM dendrimers up to G4 with good homogeneity, characterized by ^1H and ^{13}C NMR, and electrospray mass spectrometry. They also reported that these solid-phase dendrimers employed for the bead loading

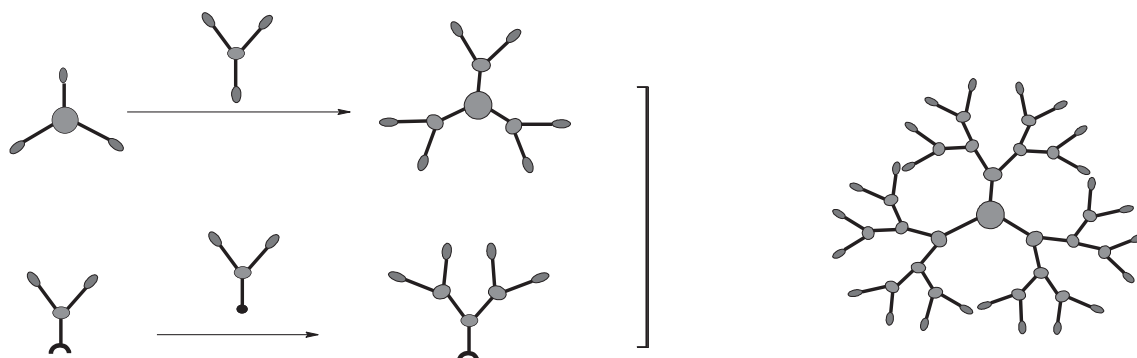


Fig. 4 Combined divergent/convergent synthesis (Pyzowski et al., 2003; Huang et al., 2012).

amplification tool as well as for the effective synthesis of dendrimer conjugates (Egner et al., 1995). By solid-phase synthesis approach, peptides and small drug molecules can be attached to the dendrimer due to their multivalency (Grayson and Fréchet, 2001; Swali et al., 1997).

Huang et al. developed a concise solid-phase synthesis of inverse PAMAM dendrimers by using peptide synthesis chemistry. In this synthetic scheme, the PAMAM dendrimers designed using the peptide synthesis approach with amide linkage in the reverse direction. Therefore dendrimers synthesized by that approach were called inverse PAMAM dendrimers. He prepared dendron by incorporating 1,3-propylene diamine moiety as a core instead of ethylenediamine and carboxylic acid and amine as a peripheral end moiety (Huang et al., 2013). In contrast to the conventional PAMAM synthesis, the synthetic peptide approach appreciably reduces the formation of side products, showing structural defect that begins from Michael addition and amidation. This approach also shortened the reaction steps with greater yield as compared to the conventional synthetic approach (Kawaguchi et al., 1995). Huang et al. ensured that the inverse PAMAM dendrimers were similar in size to the PAMAM dendrimers prepared by the conventional approach with reduction in the costs and time associated with prepared dendrons, particularly higher-generation dendrons (Huang et al., 2013). Despite all these valuable advantages, solid-phase dendrimer synthesis has not commonly used because the production of PAMAM in the large quantity is still a challenging task (Huck et al., 1998; Kawaguchi et al., 1995).

4.2. Other approaches

4.2.1. Hybrid convergent-divergent synthesis

In 1994, Kawaguchi et al. reported a hybrid convergent-divergent synthesis method also called “double exponential growth” synthesis by combining the advantages of divergent and convergent synthesis with the additional advantage that, the hyper core grows along with the peripheral monodendrons (Pyzowski et al., 2003). Double exponential dendrimer growth is the fastest convergent approach for the formation of monodendrons via bi-directional synthesis as exposed in Fig. 4. This approach was more efficient to prepare higher PAMAM generation by reducing the synthesis and refining step. This approach has the facility to alter the dendrons and synthesize the dendrimers with structural diversity (Lyu et al., 2019; Pyzowski et al., 2003). Besides all these advantages, a double

exponential dendrimer growth approach has some disadvantages, which are requirement of a pair of orthogonal protecting groups (rather than one) and formation of the restricted generation before steric hindrance (Grayson and Fréchet, 2001; Pyzowski et al., 2003).

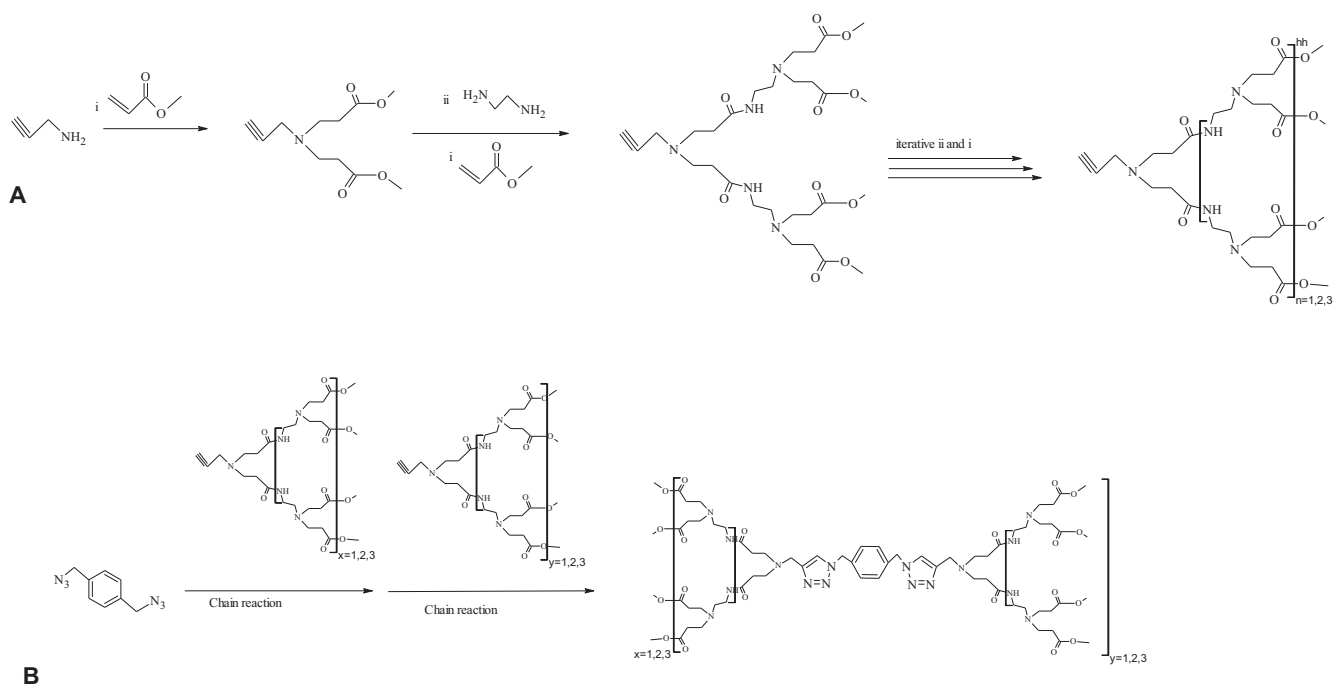
4.2.2. Lego chemistry

Several approaches have implemented to reduce the time as well as the cost for simplified PAMAM synthesis. Pyzowski et al. described the lego chemistry approach for the synthesis of PAMAM dendrimers (Pyzowski et al., 2003). This approach was new, simple, and straightforward employed for the synthesis of phosphorus dendrimers by using two-branched monomers and multi functionalized cores. By using this approach, each generation obtained in a single quantitative step with 48 to 250 surface end groups along with nitrogen and water as by-products (Lyu et al., 2019; Menjoge et al., 2010). In this G4 PAMAM dendrimer is obtained only in four steps with alternative phosphines, and hydrazines end groups. This method has the advantage of utilizing the least volume of solvent and simplified purification procedure. However, two reactions should be simultaneously run for synthesizing G1 is the only disadvantage of this synthetic approach (Huang et al., 2012).

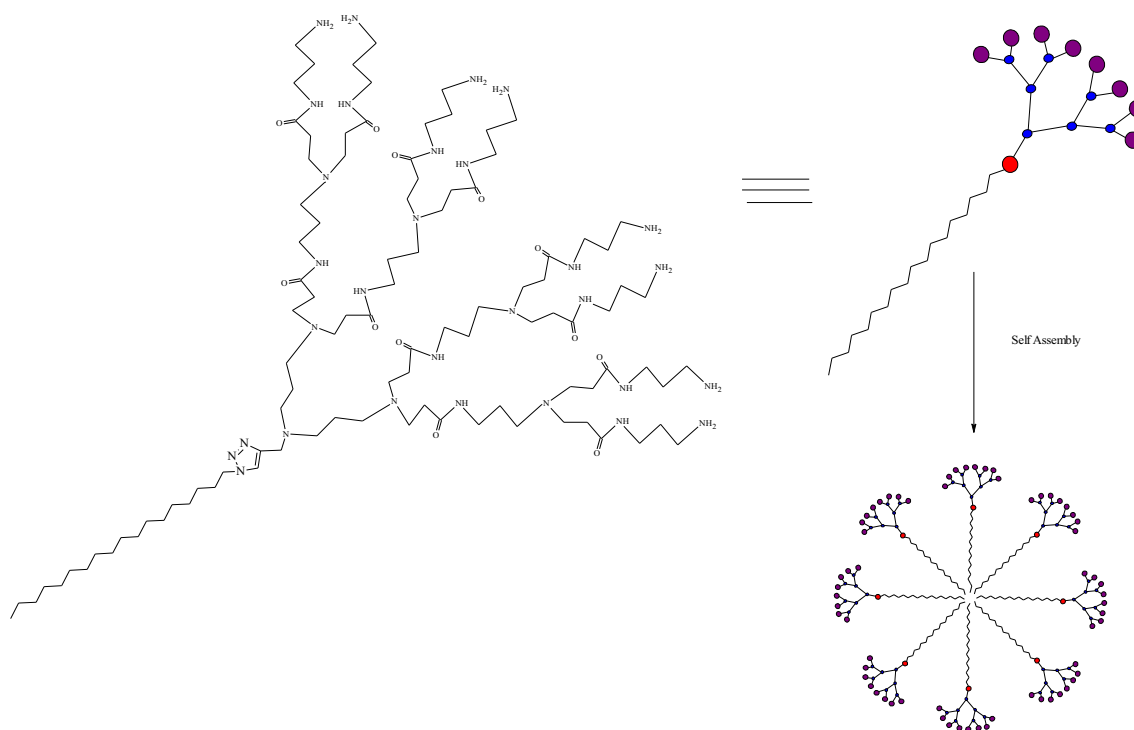
4.2.3. Click synthesis approach

With the asset of good production yield, high efficiency, and minimum reaction time with mild reaction conditions, ‘Click chemistry’ has come forward as an acceptable approach for synthesizing different dendrimers. In click chemistry, Sun et al. successfully implemented divergent, convergent, and double exponential growth approaches (Sun et al., 2015). Dendrimers synthesized by click chemistry approach give a link between dendritic architectures and nanomaterials. Among the various click reaction, formation of 1,2,3-triazole ring by copper (Cu)-catalyzed cycloaddition reaction of alkyne and azide moiety is of great importance (Geraldo et al., 2011; Sun et al., 2015).

Huang et al. synthesized G5 PAMAM dendrimer by peripheral conjugation of three azido moiety, viz., methotrexate (MTX), folic acid, and fluorescein having cyclo-octene ligands through copper-free click chemistry. They prepared mono-, di-, trifunctional PAMAM dendrimer conjugates via mixing of various azido modified functionally active moiety and therapeutic drug MTX. They concluded that copper-free



Scheme 6 (A) Divergent synthesis of PAMAM dendrons bearing the alkyne functionality. (B) Convergent synthesis of symmetrical and asymmetrical PAMAM dendrimers using click chemistry (Pittelkow and Christensen, 2005; Sun et al., 2015).



Scheme 7 Supramolecular dendrimers constructed via self-assembly of long hydrophobic alkyl chains and small hydrophilic PAMAM dendrons (Sun et al., 2015; Zeng and Zimmerman, 1997).

click reactions give excellent yield and in vitro cytotoxicity study of folic acid facile conjugated G5 PAMAM dendrimer with MTX gives efficient binding to KB cells (Huang et al., 2012).

Lee and co-workers synthesized azide moiety containing PAMAM dendrons and azidopropylamine as a core by the divergent method. They suggested these dendrons are useful for the synthesis of uniform and controlled structure of

PAMAM containing 1, 2, 3- triazole rings as linkage. Attachment of these triazole rings with different alkynes is based on Cu-catalyzed cycloaddition reaction between an alkyne and an azide. [Scheme 6](#) gives a synthetic pathway for the synthesis of symmetrical as well as asymmetrical PAMAM dendrimers using click chemistry ([Lee and Larson, 2008](#)).

4.2.4. Supramolecular synthesis

In a supramolecular synthetic approach, dendrimers synthesized by the use of three strategies. The first strategy includes synthesizing dendrons with a core unit having the capability to recognize itself. This step helps for the spontaneous formation of a dendrimer ([Lyu et al., 2019](#); [Sun et al., 2015](#)). In the second strategy, add generation non-covalently to the peripheral groups. Finally, the higher generation of PAMAM dendrimer formed by using the self-assembly of monofunctionalised dendrons. Step by step reaction of supramolecular synthesis are as given in [Scheme 7](#). Large non-covalent supramolecular dendrimer synthesized by this method gives a guarantee for structural accuracy. The above approach reduces the steps from the conventional multistep approach ([Chen et al., 2004](#); [Geraldo et al., 2011](#)). Assembly of monofunctionalized dendrons into large supramolecular dendrimers is the collective effect of multiple non-covalent interactions ([Al-Jamal et al., 2005](#)). Zimmerman et al. reported the synthesis of a fourth-generation disk-shape supramolecular dendritic structure from small dendritic building blocks by a hydrogen bond ([Zeng and Zimmerman, 1997](#); [Zimmerman, 1997](#)). Percec and his colleagues reported the synthesis of monodendrons, which are self-assembled in rod-like, cylindrical, and spherical supramolecular dendrimers molecules. Thermotropic hexagonal columnar lattice and thermotropic cubic liquid crystal lattice self-organized and formed cylindrical and spherical supramolecular dendrimers, respectively ([Percec et al., 1998](#)). Geraldo et al formulated and evaluated a gel containing supramolecular complexes of CdSe by the supramolecular chemistry of PAMAM. Geraldo first synthesized a new quantum dots (QDs)/PAMAM dendrimers containing water-soluble 3-mercaptopropionic acid. Finally, this supramolecular PAMAM was conjugated with folic acid and produced a new QDs/PAMAM-folate derivative. This QDs/PAMAM-folate complex showed an efficient and selective marker for gastric cancer cells ([Geraldo et al., 2011](#)).

Lyu et al. recently prepared supramolecular dendrimers by using small amphiphilic dendrimers, which contain long hydrophobic alkyl chains and small hydrophilic PAMAM dendrons via click chemistry. These amphiphilic dendrimers form stable, controlled, uniformly spherical nanoscale supramolecular dendrimers with different length of hydrophobic chain. The amphiphilic dendrimers functionalized with several copies of a biologically active molecule or imaging agent ([Lyu et al., 2019](#)). These self-assembling supramolecular dendrimers applied for drug delivery and bioimaging, respectively. In polycationic supramolecular PAMAM dendrimers, peripheral amine groups can interact with negatively charged nucleic acids and form nanoscale dendriplexes in non-viral gene therapy ([Lyu et al., 2019](#); [Mignani et al., 2014](#)). Sun and Yang et al. reviewed the future outcomes of siRNA therapeutics in the field of non-viral gene therapy. They analyzed supramolecular dendrimer models for siRNA targeted delivery ([Sun et al., 2015](#)).

5. Purification of PAMAM dendrimers

The purification of PAMAM dendrimers is an essential step in laboratory synthesis because each generation batch has several trailing generation defects, which can affect the PAMAM properties. However, by removing many of these defects, the purification process improves the structural uniformity of PAMAM ([Mullen et al., 2012](#)).

Through purification, PAMAM has been prepared for non-viral gene delivery agents, host-guest interaction, and modification with functional moieties such as therapeutic molecules or imaging agents. The pharmacodynamic and biodistribution of PAMAM is size-dependent property. The structural defect possess different water solubilizing capability which likely to influence the biodistribution. So that it is essential to reduce the variability in the relative structural defect, which will improve the pharmacodynamic and biodistribution properties. After purification, it is essential to perform a characterization of PAMAM ([Mullen et al., 2012](#); [Rundel et al., 2007](#)).

Tomalia et al. already recommended a purification of PAMAM by azeotropic distillation with a 9:1 mixture of toluene and methanol. In the beginning, the PAMAM dendrimer was dissolved in the above mixture, removing all volatile byproducts on a rotary evaporator. This procedure repeated until the evaporation of EDA. After that, the toluene residues from the PAMAM was removed by similar azeotropic distillation with pure methanol. During this step, the water bath temperature of the rotary evaporator is not more than 30 °C to avoid retro Michael reaction ([Esfand and Tomalia, 2002](#)). Mullen et al. used a membrane dialysis procedure for purification of generation 5 PAMAM dendrimer. They demonstrated its effectiveness and limitations using a lot of biomedical grade dendrimers. For purification study, he used 10,000 molecular weight cut off dialysis tubing and washed with nanopure water before use ([Mullen et al., 2012](#)).

6. Cytotoxicity and surface modification

Cytotoxicity of PAMAM dendrimer mainly attributed due to the interaction of cationic peripheral groups with negatively charged biomembrane. PAMAM causes hemolytic toxicity and cytotoxicity by damaging cellular membrane ([Jones et al., 2012](#); [Kesharwani et al., 2011](#)) mentioned in [Table 3](#). Cationic G7 PAMAM dendrimer interacts with lipid bilayers of host cells and open with 15–40 nm diameter, which disturbs the flow of electrolyte and causes cell death ([Cancino et al., 2013](#); [El-Sayed et al., 2003](#)). [Fig. 5](#) represents the mechanism of interaction of polycationic PAMAM dendrimer with the biological membrane. Anionic PAMAM dendrimers were 10-folds more tolerated and less toxic than cationic ones. Hemotoxicity is an additional undesired type of cytotoxicity of cationic charge PAMAM ([Otto and De Villiers, 2018](#)).

Pryor et al. evaluated the cytotoxicity of various full and half generation PAMAM with surface modification by cationic, anionic, and neutral terminal groups. For the above study, they used zebrafish models ([Pryor et al., 2014](#)), and observed neutral or anionic dendrimers which are not showing significant lethal effect, related cardiac impact, and pericardial edema. The authors also observed that the cytotoxicity of PAMAM was dependent on the number of terminal groups, generation, and size of molecules. However, in cationic

Table 3 Level of cytotoxicity of PAMAM dendrimer.

Sr. No.	Dendrimer	Cell line	Level of cytotoxicity IC ₅₀	Reference
1	PAMAM G5 core-PAMAM G2.5 shell Tecto dendrimer	HaCaT((Human epidermal Keratinocytes) CaCo ₂ (Colon adenocarcinoma) SK-Mel-28	Up to 50 µM Nontoxic Up to 50 µM Nontoxic IC ₅₀ = 7.5 µM	Schilrreff et al., 2012
2	PAMAM generation 1.5–3.5 PAMAM generation 1–2 PAMAM generation 3–4	B16F10(Murine malenoma cells)	IC ₅₀ > 155 µM IC ₅₀ > 614 µM 35 µM	Kesharwani et al., 2011; Lin et al., 2005
3	PAMAM dendrimers G4 PEG2 G4PEG4	CaCo ₂ (Colon adenocarcinoma)	PEG2/PEG4 IC ₅₀ = 120/790 µM	Jevprasesphant et al., 2003a, 2003b; Nigavekar et al., 2004
4	PAMAM dendrimers Generation4(G4) Generation 5(G5) Generation 6(G6)	HaCaT (Human epidermal Keratinocytes) SW 480(Primary adenocarcinoma of colon)	G4/G5/G6 IC ₅₀ = 3.21/1.07/1.02 µM IC ₅₀ = 16.35/1.89/1.30 µM	Fischer et al., 2003
5	PAMAM- Pyrrolidone dendrimer	N2a(Mouse Neuroblastoma)	IC ₅₀ > 200 µM Nontoxic	King Heiden et al., 2007
6	Acylation of PAMAM dendrimer G1–G4	Caco-2 cell monolayer	Non toxic	Kolhatkar et al., 2007; Sweet et al., 2009
7	Lauroyl and PEGylated PAMAM dendrimers	Caco-2 cell	Seven fold reduction in cytotoxicity as compared to unmodified PAMAM	Jevprasesphant et al., 2003a, 2003b
8	PAMAM dendrimers Generation4(G4) Generation 5(G5) Generation 6(G6)	PLHC1 (Fish hepatoma cell line) RTG-2(rainbow trout gonad tissue)	G4/G5/G6 IC ₅₀ = 2.08/12.93/0.68 µM IC ₅₀ = 0.56/6.07/0.27 µM	Naha and Byrne, 2013
9	PAMAM- Pyrrolidone dendrimer	B14(Chinese Hamster fibroblast) mHippoE18(mouse embryonic hippocampal cell) BRL-3A(Rat liver derived cells)	Non Toxic	Janaszewska et al., 2017, 2013
10	Carboxymethyl chitosan/Poly (amidoamine) (CMChT/PAMAM) Dendrimer	U ₈₇ MG (Glioblastoma) GBM HTERT/E6/E7(Human immortalized astrocytes)	Non-Toxic	Pojo et al., 2013
11	Amino terminated PAMAM dendrimer G3 and G4	H4IIE(Rat hepatoma)	G3/G4(µg/mL) IC ₅₀ = 12.96/38.3	Hernando et al., 2012

dendrimers, they observed a contradictory effect that lower generations of polycationic PAMAM dendrimers were more toxic than a higher generation (Jain et al., 2010a, 2010b; Pryor et al., 2014). However, Cancino et al. used Caco-2 model and reported exactly the opposite statement that decreases in cell viability with a higher generation of PAMAM (Cancino et al., 2013; Woller and Cloninger, 2001).

Cancino et al. focused on the effects of single-walled carbon nanotube (SWCNT), PAMAM, and SWCNT-PAMAM nanomaterials on C2C12 murine cell line. They observed SWCNT-PAMAM causes DNA damage in C2C12 murine cell. Finally, the authors concluded that the toxicity of nanomaterial was firmly due to peripheral charge (Mukherjee and Byrne, 2013). Thiagarajan et al. evaluated PAMAM for oral drug delivery. They studied the effect of surface modification and

the size of PAMAM on the epithelial barrier of the gut in CD-1 mice. They concluded that polycationic PAMAM caused more toxicity, whereas anionic surface-modified PAMAM was ten times more tolerable in oral doses. G7 cationic amine or hydroxyl-terminated dendrimers showed severe signs of toxicity include hemobilia and splenomegaly with dose 30 mg/kg. Whereas anionic G 6.5 carboxyl-, amine-, or hydroxy-terminated dendrimers tolerated dose up to 500 mg/kg with no sign of toxicity (Thiagarajan et al., 2013). From the above perception, researchers pointed out that peripheral positive charge of PAMAM is responsible for its cytotoxicity, which limits their biomedical application (Cancino et al., 2013; Woller and Cloninger, 2001). Therefore modification of cationic surface groups is one of the ways which reduces the toxicity, improve biocompatibility and circulation time of PAMAM

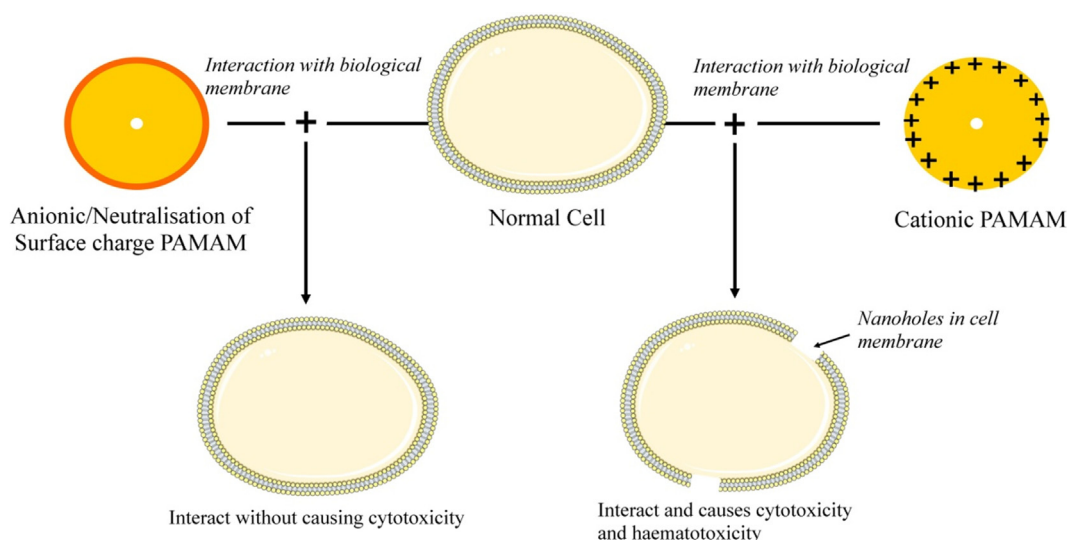


Fig. 5 Mechanism of interaction of polycationic PAMAM dendrimers with the cellular membrane.

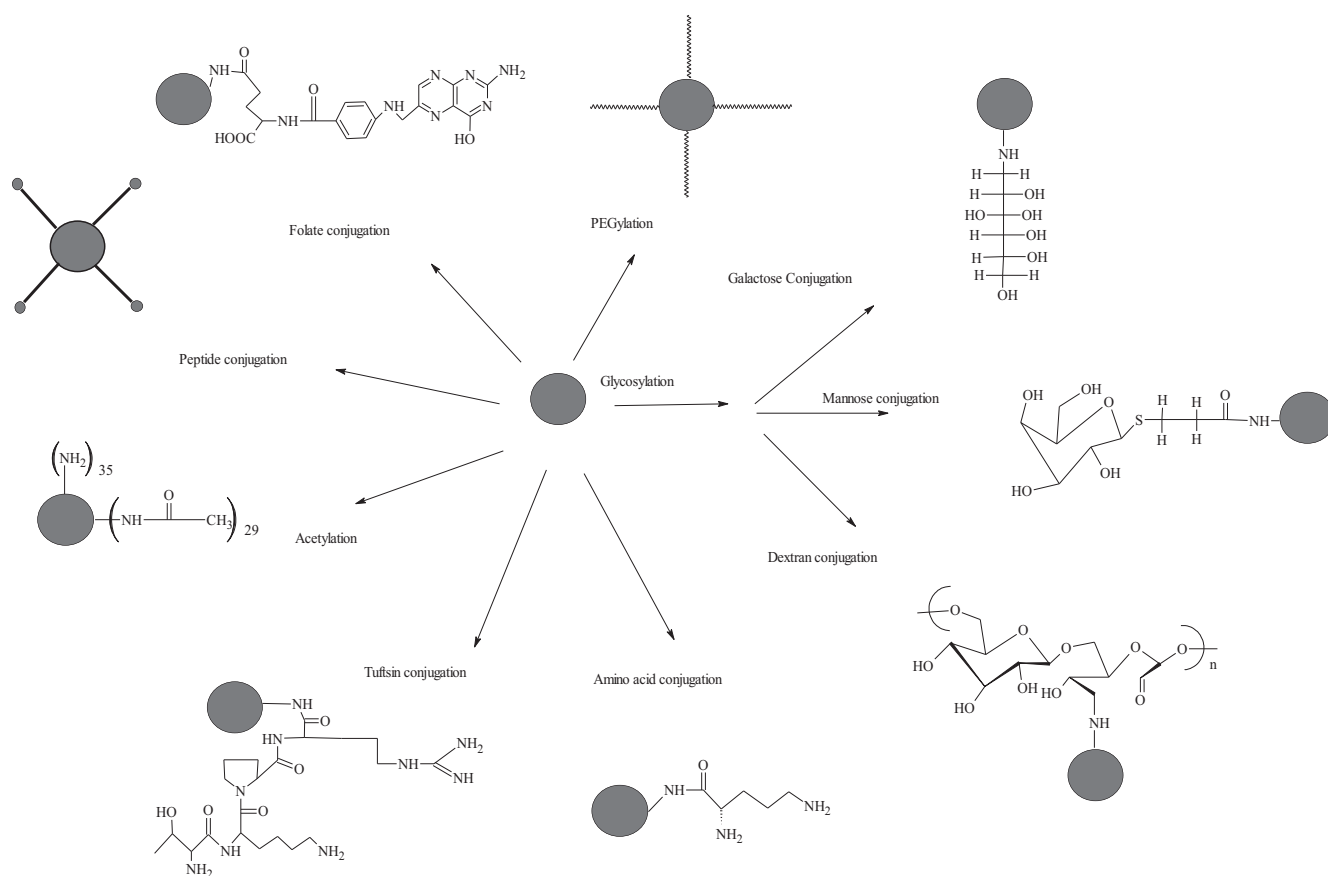


Fig. 6 Modification of surface groups of PAMAM to improve biocompatibility and circulation time of PAMAM dendrimers in the body via neutralization of charge.

dendrimers in the body (Fischer et al., 2003; Janaszewska et al., 2013). However, this surface modification has done by neutralizing charge through PEGylation (polyethylene glycol conjugation), acetylation, folate, and peptide conjugation or by neutralizing the negative charge (King Heiden et al.,

2007; Kolhatkar et al., 2007; Sweet et al., 2009). Fig. 6 gives a diagrammatic representation of attachment of other molecules to dendrimer surface groups, which are the most outstanding solution to reduce cytotoxicity. Fig. 7 shows various strategies for modification of the PAMAM dendrimers

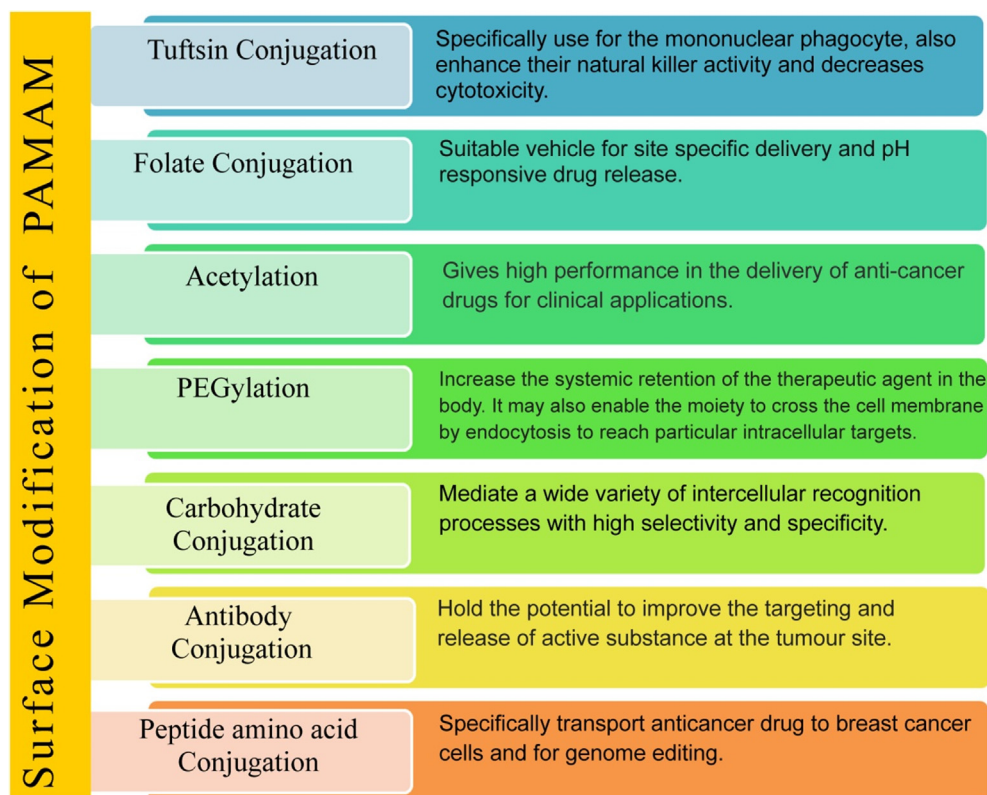


Fig. 7 Various strategies for modification of the PAMAM dendrimers to improve its physicochemical properties for targeted drug release.

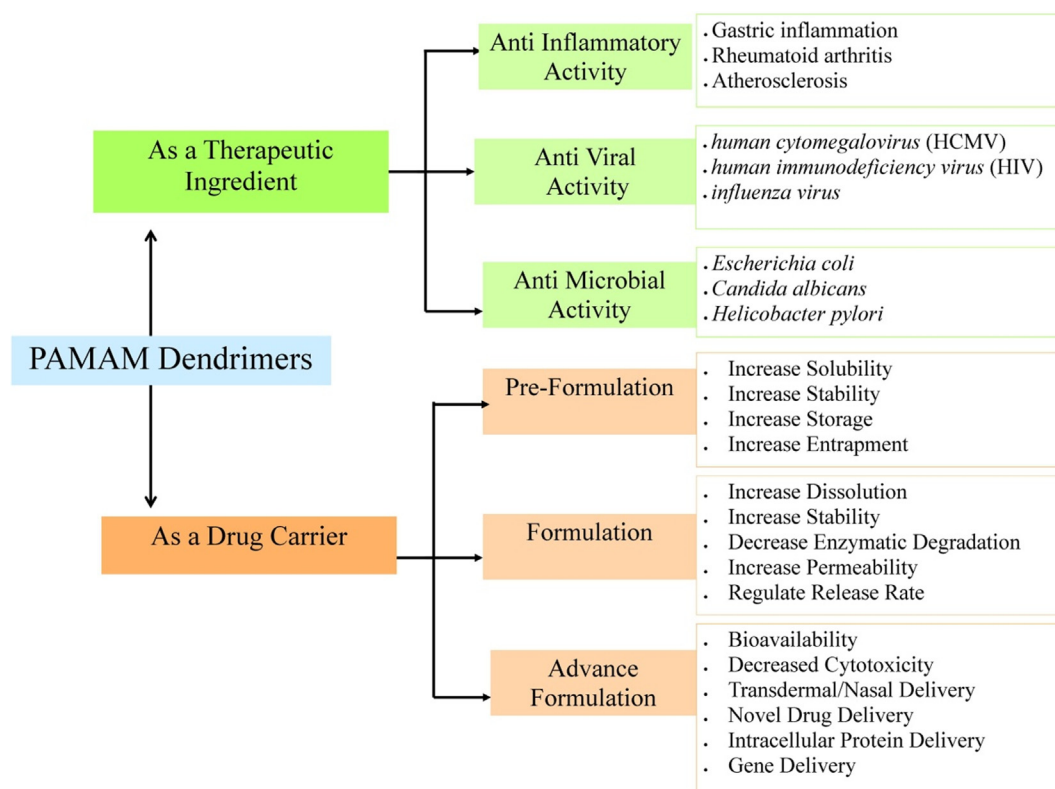


Fig. 8 Schematic representation of the potent application of PAMAM dendrimer as a therapeutic agent and as a drug carrier.

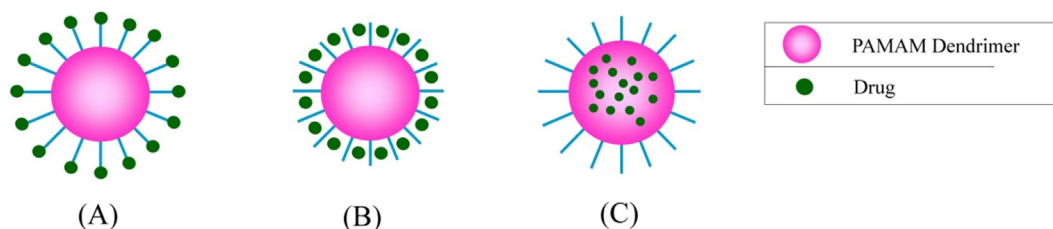
Table 4 Different biomedical and diagnostic applications of PAMAM dendrimers.

Applications	Dendrimers	Drugs	Outcome	References
Enhance solubility	PAMAM G1-G3	Clotrimazole and Ketoconazole	Improve antifungal activity by increasing drug solubility in water	Matsubara et al., 2010
	Folic acid (FA)-PAMAM conjugate	Flavonoid 3,4-difluorobenzylidene diferuloylmethane	Targeted FA-PAMAM-CDF showed high anticancer activities on HeLa and SKOV3 cell line with increasing solubility	Longmire et al., 2008 ; Zong et al., 2012
	Hybrid PAMAM dendrimer G4	Paclitaxel	Lipid-PAMAM hybrid nanosystem proved to be a promising approach for delivering hydrophobic drugs	Parsian et al., 2016 ; Tajarobi et al., 2001
	G0 and G3 PAMAM functionalized with lauryl chain	Naproxen	The lauroyl chain conjugated to lower PAMAM generation terminal groups (G0) rather than high PAMAM generations terminal groups for solubility of Naproxen, which gives optimal drug delivery.	De Araújo et al., 2018 ; Najlah et al., 2017 ; Sadekar et al., 2013
Targeted action	Half generations magnetic PAMAM dendrimers	Gemcitabin	An effective system for targeted gemcitabine delivery with great therapeutic potential at the tumor site and to minimize the side effects in normal tissue	Parsian et al., 2016
	Folic acid (FA)-modified generation 5 (G5) PAMAM dendrimers through a pH-sensitive cis-aconityl linkage Trastuzumab (TZ) grafted PAMAM dendrimer	doxorubicin (DOX) Docetaxel	The developed G5 FA-DOX conjugates used as a promising nanodevice for targeted cancer chemotherapy The developed TZ-G4 PAMAM dendrimer use to improve the delivery of Docetaxel to HER2-positive breast cancer cells.	Cheng et al., 2015a, 2015b, 2011 Kulhari et al., 2016
Transdermal delivery	4.0 G PAMAM dendrimers with amino and hydroxyl terminal and 4.5 G PAMAM dendrimers	Indomethacin	In vivo, pharmacokinetic and pharmacodynamic studies in Wistar rats show significant indomethacin concentration in blood.	Chauhan, 2018
	5.0 G PAMAM dendrimers	Ketoprofen	3–4 times permeation rate increase due to complex as compared to plain ketoprofen	Chauhan, 2018 ; Chauhan et al., 2003
	Lower generation PAMAM	5- Fluorouracil	PAMAM dendrimer enhanced the rate of penetration of 5-fluorouracil by increasing partitioning in the skin.	Venuganti and Perumal, 2008
Sustain drug delivery	PAMAM with carboxyl or hydroxyl active peripheral group	Pilocarpine	Increase the retention time of pilocarpine within the eyes.	Vandamme and Brobeck, 2005
Enhance Bioavailability	G2, G2.5, and G3 PAMAM dendrimers	Heparin and Enoxaparin	Enoxaparin –PAMAM complex is effective in preventing deep vein thrombosis with increasing bioavailability up to 40% without any adverse effect on mucociliary transport rate.	Bai and Thomas, 2007
Enhanced Cellular Uptake	Carboxyl-terminated PAMAM G3.5 PAMAM	Carboplatin	Prepared for nano-based drug delivery system with good cytocompatibility	Cheng et al., 2015a, 2015b
		Sialic acid	They are potentially inhibiting the infection caused by H3N2 (influenza virus) by increasing the delivery of sialic acid and reduced toxicity.	Schilrreff et al., 2012
MRI contrast agents (contrast or imaging agent)	PAMAM G4	(1B4M-Gd)64	MRI agent for a kidney in Nude mice	Guo et al., 2011
	PAMAM-Cystamine G 6.0 PAMAM-Cystamine G 8.0	(1B4M-Gd) 256 (1B4M-Gd)1024	MRI agent for breast cancer in mice MRI agent for sentinel (mammary) lymph nodes in mice	Wang et al., 2012 Kojima et al., 2011
Immunotherapy (antibody conjugate)	PAMAM dendrimers (5.0 G)	60bca and J591	Carrier	Longmire et al., 2008 ; Oddone et al., 2016

(continued on next page)

Table 4 (continued)

Applications	Dendrimers	Drugs	Outcome	References
Gene transfection	PAMAM dendrimers and PAMAM-Arginine dendrimers	DNA	Lysine functionalized PAMAM dendrimers as a novel vaccine delivery vector for a schistosomiasis japonica DNA vaccine	Bahadoran et al., 2017
	4.0 G PAMAM	siRNA	Effective intracellular accumulation of siRNA with co-delivery of the drug was obtained with a developed system	Bahadoran et al., 2017; Dutta et al., 2010; Heegaard et al., 2010
Therapeutic Activity	PAMAM G0 and G1	Conjugates with carbon nanodots and Tetracyclin	Conjugated PAMAM G0 and G1 dendrimers with carbon nanodots with drug observed synergic antibacterial action.	Khandare et al., 2012; Mukherjee et al., 2010a, 2010b
	PAMAM	BRI-2923 and BRI-6195	Inhibit the replication of HIV by interfering virus adsorption.	Winnicka et al., 2011
	PAMAM G3-G5		Covalently bound to DC-SIGN receptor and interfering with virus adsorption.	Nishikawa et al., 1996
	PAMAM G7		Potentially able to inhibit the growth gram-negative and positive bacteria	Landers et al., 2002; Matsubara et al., 2010

**Fig. 9** Schematic representation of entrapment of drug in PAMAM dendrimer. (A) Covalent binding (B) Electrostatic interaction (C) Encapsulation.

to improve its physicochemical properties for targeted drug release.

Kolhatkar et al. pointed out acetylation for decreasing cytotoxicity of PAMAM. The author observed that cytotoxicity of PAMAM could be reduced 10-fold with retaining the permeability of the cell by surface modification with acetyl moiety (Kolhatkar et al., 2007). They also observed that acetylation also increases the water solubility of PAMAM, which is essential for biomedical use. In order to improve the biocompatibility of PAMAM researchers modified G5 PAMAM dendrimers by conjugating them with polyethylene glycol (PEG) of two different molecular weights (Bertero et al., 2014; Ladd et al., 2017). They observed dendrimers conjugated with PEG of higher molecular weight were less cytotoxic as compared to conjugation with lower molecular weight PEG. This study provided a novel idea to decrease the cytotoxicity of dendrimer by PEGylation. Cytotoxicity reduction of dendrimer depends on molecular weight and chain length of PEG (Ladd et al., 2017). Kolhe et al. reported enhanced Ibuprofen loading in G4 and G3 PAMAM by facile conjugation with PEG. PEGylated G4 and G3 PAMAM dendrimers enhanced drug loading, drug release, and drug solubility with improved biodistribution and biocompatibility (Kolhe et al., 2003).

PAMAM dendrimers have a great interest in non-viral gene delivery because of the presence of primary amine groups on their periphery, which can bind to DNA and promote the

cellular uptake of genes (Eichman et al., 2000; Qi et al., 2009). However, cationic PAMAM dendrimers are associated with their cytotoxicity, hemolysis, and liver toxicity. Cationic PAMAM dendrimers interact with the negatively charged cell surfaces, which limit their in vitro and in vivo applications (Faraji and Wipf, 2009). Qi et al. conjugated G5 and G6 PAMAM dendrimer to PEG at three different molar ratios of 4%, 8%, and 15% to improve its characteristics as gene delivery carriers. They observed 8% PEG conjugated PAMAM dendrimers are the most efficient muscular gene expression with decreased in-vivo and in-vitro cytotoxicity (Maciejewski, 1982; Qi et al., 2009).

Bhadra et al. developed and explored the PEGylated and non-PEGylated G4 PAMAM dendrimers for delivery of the anti-cancer drug, 5-fluorouracil. The results showed that PEGylated PAMAM dendrimers increased drug loading capacity, reduce drug release rate, and hemolytic toxicity. Hence, they conclude that, PEGylated dendrimers can be used as a nanoparticulate depot system for drug administration (Maciejewski, 1982; Bhadra et al., 2005). From the above data it can be concluded that, PAMAM dendrimers are promising structure for drug and gene delivery but, its cytotoxicity and hematotoxicity is a basic problem. However, diminishing the cationic charge on surface reduces the therapeutic activity i.e., ability to create complex with nucleic acid. Therefore, partial modification with perfect molecule is applied to get balance between reduced toxicity and retained activity.

7. Application of PAMAM

PAMAM dendrimer has several applications in pharmaceutical as well as the biomedical field. PAMAM dendrimer can improve biological properties such as bioavailability, solubility, and specific target selectivity by linking or entrapping bioactive compounds. Fig. 8 shows the potential applications of PAMAM dendrimers. The summary of the applications of PAMAM dendrimers in both biomedical and diagnostic purpose is tabulated in Table 4.

7.1. Drug delivery agent

Dendrimers have excellent host-guest properties (Faraji and Wipf, 2009). By these unique characteristics, PAMAM has applied in the targeted delivery of drugs by physical interaction or covalently bonding. Drug molecules can be encapsulated in the interior space of dendrimers or attached to the peripheral group, which is shown in Fig. 9 (Maciejewski, 1982; Peng et al., 2008).

The core of dendrimers is hydrophobic, while the peripheral part is hydrophilic, which is analogous to micelles like structure and able to bind ionic surfactant molecules via hydrophobic and ionic interaction (Hu et al., 2012). This structural property makes it a potential carrier in targeted drug delivery (Choudhary et al., 2017; Hsu et al., 2017). Hydrophobic drug molecules formed inclusion complexes with internal tertiary amine and peripheral amine groups of PAMAM (Choudhary et al., 2017; Jain et al., 2010a, 2010b). The peripheral hydrophilic groups help for drug solubilization. The unique properties of PAMAM dendrimers allow them to administer by different routes such as oral, injectable, transdermal, ocular, nasal, colon, rectal, and pulmonary delivery systems (Mehta et al., 2019; Mintzer and Grinstaff, 2011). Nanjwade et al. reported PAMAM are excellent carriers for drug delivery due to multiple branching structures which terminated with cationic amine ($-\text{NH}_2$), neutral hydroxyl ($-\text{OH}$), or anionic carboxylic acid ($-\text{COOH}$) surface groups which conjugated with biological agents and to control the release rate of the drug in the body (Nanjwade et al., 2009; Peng et al., 2008).

Jansen and co-workers reported the concept of “dendritic box” for trapping small molecules of rose Bengal and p-nitrobenzoic acid. They observed that the solubilization of drugs depends on various factors, including generation, size, concentration, pH, focal point, and terminal active groups (Jansen et al., 1994). The drug entrapment efficiency of PAMAM increases with generation as well as the concentration of PAMAM (Jansen et al., 1994; Malik et al., 2000; Padilla De Jesús et al., 2002). Chauhan et al. reported the encapsulation efficiency of indomethacin in G4 and G4.5 PAMAM dendrimers through electrostatic interaction. They also observed the encapsulation of ibuprofen in G4 PAMAM dendrimers and conclude that solubility of Ibuprofen molecules was increased by encapsulation in PAMAM dendrimer (Chauhan et al., 2003). PAMAM also transports the bioactive compounds by a covalent bond (dendrimer prodrug), ionic interaction, or adsorption in the internal space of PAMAM (Dias et al., 2020).

Twyman and Astruc et al. reported the drug release from arborol type dendrimers by calorimetric experiments. They compared four antibacterial and antifungal compounds for

study (Astruc and Chardac, 2001; Twyman et al., 2002). Najlah et al. explored the potential of PAMAM in oral drug delivery. They concluded that the oral drug delivery potential of PAMAM increases with branching and surface amine groups, which immobilize drugs, enzymes, antibody, or other bioactive agents. In this study, the authors studied trans-epithelial permeability of Naproxen-PAMAM conjugate. They observed PAMAM-Naproxen conjugate enhance the oral bioavailability and serve as the most potent candidate for controlled release (Najlah et al., 2017).

Mignani et al. introduced the concept of “dendrimer space” to provide medicinal chemists with a new approach by identifying original dendrimer based drug delivery systems. According to their observations, dendrimers space shapes the boundaries of a new drug cluster that included in a considerable volume of chemical space. This concept fully integrated into the drug discovery process (Mignani et al., 2014).

Wiwattanapatapee et al. reported in vitro cell uptake of polycationic and polyanionic PAMAM in an account of its size, charge, and concentration (Wiwattanapatapee et al., 2000). The result of Wiwattanapatapee experiment showed transport of I-labelled dendrimers across the intestinal membrane was charge-dependent, and tissue uptake of cationic PAMAM was higher than anionic (Malik et al., 2000; Wiwattanapatapee et al., 2000). Dendrimers have also investigated for oral delivery of the anticancer drug. Zong et al. reported that facile conjugated folic acid G5 PAMAM improved the target release of MXT by 2500–170,000 fold due to specific binding with folate receptors (Cheng et al., 2011; Zong et al., 2012).

The highly impermeable and lipophilic stratum corneum layer limited the transdermal drug delivery (Chauhan, 2018). To this end, dendrimers found to be the effective and efficient carrier of drugs to improve drug penetration across the skin by increasing drug solubility and drug partitioning. Among various surface modifications in PAMAM, PAMAM with amino groups shows a higher perfusion rate than neutral and carboxyl terminated PAMAM dendrimers (Chauhan, 2018; Chauhan et al., 2003). Jain et al. investigated G4 PAMAM for transdermal delivery of Indomethacin. They performed in vivo pharmacokinetic and pharmacodynamic studies with Wistar rats. They observed a significant increase in the concentration of Indomethacin in blood with PAMAM dendrimers as compared to pure drug suspension (Chauhan et al., 2004). Venuganti et al. found that cationic lower generation PAMAM dendrimers increased penetration of 5-fluorouracil by increasing partitioning into the skin (Venuganti and Perumal, 2008).

Intra-ocular delivery of bioactive suffers from poor bioavailability problems due to the elimination of the drug by nasolacrimal duct mediated drainage of fluid (Wang et al., 2009). Dendrimers have explored to overcome the above limitations by increasing the retention time of the drug in eyes. Vandamme and Brobeck reported the PAMAM dendrimers with carboxyl or hydroxyl active peripheral groups increases the retention time of Pilocarpine within the eyes (Vandamme and Brobeck, 2005). Dendrimers have also evaluated for pulmonary delivery of heparin and enoxaparin by Bai et al. In this study, they assessed G2, G2.5, and G3 PAMAM dendrimer for pulmonary absorption of Enoxaparin and observed that the resulting drug dendrimer complex is effective in preventing deep vein thrombosis. They also observed that cationic

charged G2 and G3 PAMAM dendrimer increases the bioavailability of Enoxaparin about 40% without any adverse effect on mucociliary transport rate (Bai et al., 2007).

Dendrimers also used as a passive as well as active targeting delivery of medicament due to the presence of active surface groups (Kolhe et al., 2003). In cancer therapy, drug targeting is essential to maximize the therapeutic potential at the tumor site with minimizing side effects in normal tissues, which can be achieved by entrapment of drugs into the PAMAM or facile conjugation of drugs with the terminal active group of PAMAM (Cheng et al., 2015a, 2015b; Parsian et al., 2016). Patri et al. compared the efficacy of covalently attached MTX G5 PAMAM dendrimer complex with noncovalent methotrexate G5 PAMAM dendrimer. They concluded that covalently attached methotrexate conjugated dendrimer was more stable compared to noncovalent MTX dendrimer complex (Patri et al., 2005).

Cytosolic protein delivery shows a significant important role in protein therapies. Cheng et al. reported that cationic polymer like PAMAM encompasses great characteristic features in gene delivery. However, cytosolic protein delivery was limited because of cationic polymers can not prepare stable complexes with proteins (Cheng et al., 2020). Liu et al. reported that Boronic acid rich dendrimers can strengthen the binding affinity of cationic polymer with protein with high efficiency for cytosolic delivery of native proteins (Liu et al., 2019b, 2019a). They also demonstrated that supramolecular chemistry used to describe the reversible connection of the cationic PAMAM. He also ensured complex stability and minimal toxicity by shielding the cationic charge with anionic polymer or lipid PEG (Lv et al., 2019; Cheng et al., 2020). Araujo reviewed that PAMAM dendrimers activate the immune response and, therefore, positively charged dendrimers employed as vaccine carriers, due to their ability to increase cytokine production. He reported that PAMAM was not immunogenic by itself. However, when conjugation with protein carrier, (albumin, Interleukin-3) is induced the formation of antibody and specified antigenic effect (De Araújo et al., 2018).

7.2. Diagnostic agent

As compared to low molecular weight substances, high molecular weight substances mainly used as contrast agents. These agent obtained an X-ray image with high resolution and easily detect disease lesions or organs (Roberts et al., 1990). From that perception, unique and distinct properties of PAMAM dendrimers have become an exciting class of polymer to develop it as a diagnostic agent (Roberts et al., 1996, 1990). Biocompatible PAMAM dendrimer has efficiently used in radiotherapy, as imaging agent or tracer, X-ray or magnetic resonance imaging (MRI) contrast agent, and radionucleotide to detect cancerous cells. Dade International Inc. (U.S.A.) introduced a new method for cardiac test by the use of blood proteins. These blood proteins binds to immunoglobulins with the help of PAMAM dendrimers (Roberts et al., 1990; Tajarobi et al., 2001). Oddone et al. reported the cellular uptake by using the fluorescein isothiocyanate (FITC) tagged PAMAM dendrimers (Oddone et al., 2016).

Guo et al. demonstrated a novel concept for enhancement of X-ray attenuation property in potential computed

tomography (CT) and angiography. They combined gold and iodine within single dendrimer based nanodevice (Guo et al., 2011). G5 PAMAM dendrimers was used as a template for gold particles entrapment (AuNPs), after that it was conjugated with diatrizoic acid via 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) coupling reaction (Guo et al., 2011). He observed that the above nano-complex encompasses high efficacy as a contrast agent in dynamic computerized tomography (CT) imaging and angiography.

Wang et al. also synthesized gold (Au) entrapped PAMAM nanoparticles with distinct sizes. They evaluated the effect of size on CT imaging of passively targeted tissues (Wang et al., 2012). They observed that, contrast agent with small sizes was a natural aspect for CT imaging of tumor by using liver endocytosis. These nanoparticles were also useful in tumor imaging because of the enhanced permeability and retention time (Guo et al., 2011; Wang et al., 2012).

In addition to the imaging agent, PAMAM-gadolinium (Gd) chelate used for MRI and as a contrast agent. However, their applications restrict due to their rapid clearance from the blood (Yang and Chuang, 2012). Several complexes of Gd with high molecular weight compounds identified to overcome this problem. Dendrimers based diethylenetriaminepentaacetate (DTPA) complexes potentially improved the limitations of previously used MRI contrast agent and improve the visualization of vascular structure (Tomalia et al., 2007). Kojima et al. evaluated PEGylated PAMAM (G4 and G5)-Gd chelates for pharmacokinetic and paramagnetic properties. They observed that PEGylated Gd-PAMAM dendrimers showed negligible liver and kidney cell uptake with less plasma clearance and prolonged retention time (Kojima et al., 2011).

The generation and size of PAMAM also play a significant role in altering the pharmacokinetics of contrast agents by altering blood retention time, tissue perfusion rate, and excretion rate. The circulation half-life of the contrast agent was increased by 2–10 times with increasing generation. G7, G8, and G2 PAMAM identified as to be the best diagnostic agent for imaging intratumoral, lymphangiography, and renal imaging, respectively (Barrett et al., 2017). However, compared to hydrophilic PAMAM, hydrophobic PAMAM shows a better contrast effect in liver imaging. Surface conjugating PAMAM with antibodies and ligands such as folic acid used for the targeted delivery of contrast agents (Longmire et al., 2008).

In molecular biology, PAMAM efficiently works as a molecular probe by immobilizing the sensor unit on the branching ends. Surface modified PAMAM intended to apply as a molecular probe for avidin, DNA, amino alcohols, and pH (Barrett et al., 2017; Longmire et al., 2008).

7.3. Gene therapy

Gene therapy is a new technology for DNA transfection by expressing target protein or small interfering RNA to silent the target gene, which is represented in Fig. 10. It provides a new platform to eradicate genetic disease (Kesharwani et al., 2015). Viruses were an attractive gene delivery vectors for gene therapy. However, due to the lack of specificity, a low transfection rate, immunogenicity, toxicity, poor cell uptake, oncogenicity, and susceptibility to nuclease degradation limits the use of viruses for gene therapy. Therefore the non-viral vectors like cationic polymers and lipids are safer and recommend

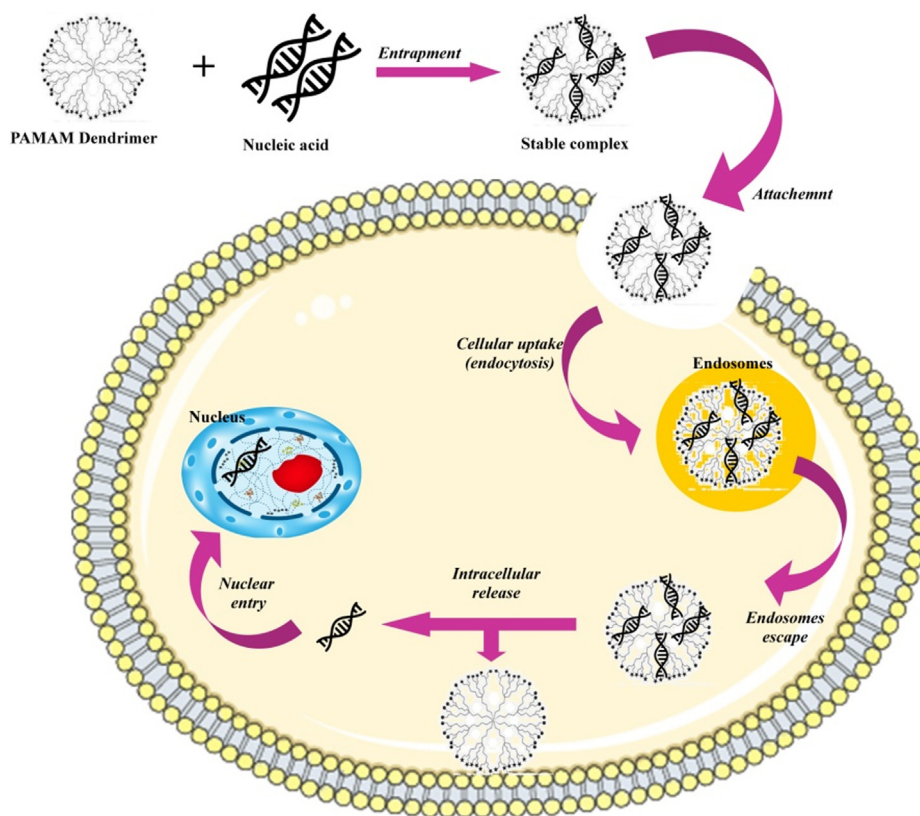


Fig. 10 Mechanism of efficient non-viral mediated gene delivery with emergence use of PAMAM dendrimers (Kesharwani et al., 2015).

more flexibility for gene transfection agents. In this context, dendrimers are acting as efficient non-viral-mediated vectors for gene delivery system (Li et al., 2018). Peripheral amino functional groups of PAMAM dendrimer can effectively bind to the phosphate group of nucleic acids in physiological pH. Terminal amino groups of PAMAM leads to continuous formation of transfection complexes with nucleic acid (Heegaard et al., 2010; Wu et al., 2005).

A transfection reagent “Superfect TM” is a trademark name for activated dendrimers which carry a high quantity of genetic material than viruses. The transfection efficiency of PAMAM may not only due to the well-defined structure but depend on low pKa of amines (3.9 and 6.9). The low pKa value of the PAMAM dendrimer produces it to buffer for pH change in the endosomal compartment (Abedi-Gaballu et al., 2018; Shcharbin et al., 2009). Haensler and Szoka published the report of dendrimer mediated transfection using luciferase and beta-galactosidase containing the plasmid. This plasmid dendrimer complex showed high efficiency towards the DNA transfection into different mammalian cell lines. They also found that the transfection efficiency of PAMAM dendrimer depended on both dendrimers–DNA ratio and diameter of the dendrimers. They also observed that the transfection rate has reached a stable level in G8 PAMAM dendrimers. They used different strategies to improve the properties of PAMAM for non-viral mediated gene delivery agents (Haensler and Francisco, 1993).

Nussbaumer et al. use group II chaperonin thermosome (THS) nanoparticle hollow protein for entrapment of

macromolecules. They conjugated PAMAM to THS for entrapment of siRNA and observed THS-PAMAM protects siRNA from degradation by RNase A and traffics Kinesin family member-11 (KIF 11). This molecule showed a good efficiency for gene delivery, carrying KIF11 and glyceraldehydes-3-phosphate dehydrogenase (GAPDH)-siRNA to cancer cells with significantly inhibit the proliferation of that cell (Nussbaumer et al., 2016). Wang et al. developed a lysine (lys) modified PAMAM dendrimers as a novel vaccine delivery vector for a *Schistosomiasis japonica* DNA vaccine. They investigated its ability to enhance a protective effect against *Schistosoma japonicum* infection. It was observed that when PAMAM-lys combined with DNA vaccine, it exhibited a higher efficacy as compared to the free DNA with reduced infection by 45–50% (Wang et al., 2014).

Dutta et al. explored the potential of dendrimers based siRNA against E7 and E6 cervical cancer. They optimized dendrimer generation by nitrogen to phosphate ratio using dendrimer- fluorescein isothiocyanate oligo complexes (Dutta et al., 2010). The in-vitro study showed that these formulations were able to inhibit target genes against E6 and E7 cervical cancer with the decrease side effect of drugs, overcome drug resistance and increase the survival rate of individual infected by cervical cancer (Dutta et al., 2010; Wu et al., 2013). Bahadoran developed a new method for delivering the H5-DNA vaccine against avian influenza. He designed a complex system with an H5-green fluorescent protein gene cloned into the expression vector pBud (pBud-H5-GFP), then conjugated to transactivator transcription (TAT) and PAMAM

(PAMAM-TAT) (Bahadoran et al., 2017). He observed that the above system demonstrated low toxicity with good skin permeation. The cellular uptake of the above complex system was also better than free dendrimer (Bahadoran et al., 2017). Recently Cheng et al. also observed the boronic acid-rich dendrimer intracellular delivery of Cas9 protein into various cell lines with high efficiency in CRISPR/Cas9 genome editing (Liu et al., 2019b). In the current study Pishavar et al. used modified PAMAM (G4 and G5) dendrimers for effective gene therapy in colon adenocarcinoma. They used alkyl-carboxylate, PEG and cholesteryl chloroformate for enhancement of cellular transfection efficiency while reducing cytotoxicity. Gene delivery efficiency of the above novel vectors was evaluated by a green fluorescent protein reporter gene in the colon cancer cell, in-vitro and in-vivo. After a complete evaluation, they represented that cholesterol-grafted PAMAM dendrimer with alkyl-PEG surface modifier was the best gene delivery vector in-vitro as well as in-vivo (Pishavar et al., 2018). Lee et al. also concluded that PAMAM with histidine and KRTR surface modification could be used as a potential gene vector with prolonged gene therapy with the enhancement of gene expression (Lee et al., 2019).

7.4. Therapeutic activity

In recent times, dendrimer has significant attention in the invention of drugs by their therapeutic value against prion and infectious diseases like viral, parasitic infection, Alzheimer's disease, HIV, Herpes simplex virus (Tyssen et al., 2010) shown in Fig. 8. Dendrimers act as a therapeutic agent in viral infection by preventing the formation of amyloid fibrils and disaggregate previously formed fibrils and prevent viral adhesion and replication (Mhlwatika and Aderibigbe, 2018). Polycationic PAMAM dendrimers (generation greater than 3) react with bacterial membranes and disturb their integrity, while the lower generation did not show antimicrobial activity (Khandare et al., 2012). PAMAM also improves the cell uptake of antibiotics into the bacterium and, as a result showing synergistic effects (Khandare et al., 2012; Mukherjee et al., 2010b). Modified PAMAM dendrimers have shown to be efficacious in tissue repair also have the potential for neutralizing toxins and removing drugs and metal overdose from the body. Ngu-Schwemlein et al. conjugated PAMAM G0 and PAMAM G1 dendrimers with carbon nanodots to increase their amine cationic concentration and evaluated their antimicrobial activity. The author additionally assessed the above conjugate with tetracycline and cilistin, observed synergic antibacterial action (Ngu-Schwemlein et al., 2016).

Dendrimers with certain functional groups on their periphery show anti-retroviral activity by breaking key contact of the virus to target cell. It includes three potential binding sites, i.e., CD4 receptor, glycosphingolipids (GSLs), and dendritic cell-specific ICAM-3-grabbing on integrin (DC-SIGN). Hence dendrimers act at several stages of viral infection for prevention of viral genome replication in the host cells and act as an antiretroviral. The phenyl dicarboxylic acid BRI-2923 and naphthyl disulfonic acid BRI-6195 are fourth-generation PAMAM dendrimer built from ammonia and ethylenediamine core respectively. BRI-2923 is fully capped with 24 naphthyl disulfonic acids group while BRI-6195 capped with 32 phenyl dicarboxylic acids group (Tyssen et al., 2010). BRI-2923 and

BRI-6195 dendrimers inhibit the replication of HIV by interfering with virus adsorption. A gp120 binding assay and virus adsorption assay indicated that the PAMAM has an effect on docking of HIV to the host cells also inhibit binding of TAT protein to trans-acting responsive element (TAR) RNA of HIV (Mhlwatika and Aderibigbe, 2018; Mukherjee et al., 2010b). Araujo and Santos et al. measured the binding affinity of PAMAM to the DC-SIGN receptor and observed that PAMAM covalently bound to it. DC-SIGN act as a receptor with a high affinity for mannose glycans moieties, which are present on the surface of the virus (De Araújo et al., 2018). HIV possesses a high density of Mannose- α 1 and 2-mannose motif on gp 120. Thus PAMAM encoded with carbohydrate focused on mannose and its derivatives suggested that an assembly of mannose with aryl group at the peripheral region is beneficial for DC-SIGN binding. This modified PAMAM intensively block the interaction between gp120 and DCs at 10 μ M, whereas a natural polymer mannose-mannan only partially inhibited the interaction (Mukherjee et al., 2010b; Wang et al., 2009, 2014). Hong Zhao studied the binding of PAMAM dendrimer to TAT peptide using microgravimetric quartz crystal microbalance (QCM) analysis (Zhao et al., 2004). They observed 3–5 generation PAMAM dendrimers easily prevented the binding of TAT protein to TAR RNA. They showed that PAMAM dendrimers could form complexes with TAR RNA and disrupt the interaction of TAT protein with TAR RNA (Mukherjee et al., 2010a; Zhao et al., 2004).

PAMAM –G7 potentially able to inhibit the growth gram-negative and positive bacteria, and their cytotoxicity was dependent on the exposure time and the concentration (Rastegar et al., 2017). Rastegar et al synthesized PAMAM G7 and evaluated for antibacterial properties against gram-positive and gram-negative bacteria. The dendrimer exhibited a good response against both bacteria. According to authors, the antibacterial property of dendrimer is due to disruption of the bacterial membrane by terminal amine groups, which interact with the cytoplasmic membrane, resulting in its disruption and disintegration (Winnicka et al., 2011; Rastegar et al., 2017).

Winnicka et al. reported the PAMAM dendrimer to improve the antifungal activity of clotrimazole by increasing drug solubility in water. Besides, authors also formulated hydrogel of polyacrylic acid containing clotrimazole and ketoconazole, PAMAM–G2, and PAMAM–G3, which was demonstrated 16 times more potent against *Candida* with induced bioadhesive feature as compared to free drug. PAMAM dendrimer G1-G3 also improve amphotericin B water solubility and sustained release action (in-vitro) with higher generation (Winnicka et al., 2011).

Influenza commonly treated with antiviral drugs such as Oseltamivir, Amantadine, and Rimantadine, which have developed resistance due to losing its ability to bind and inhibit the function of the virus nucleic acid proteins (Landers et al., 2002; Naha and Byrne, 2013). Landers et al. conjugated sialic acid-based PAMAM dendrimers for the inhibition of hemagglutinin adhesion of influenza subtype A (H3N2 and H2N2). Results showed that the dendrimers were potentially inhibiting the infection caused by H3N2 by increasing the delivery of sialic acid and reduced toxicity (Landers et al., 2002). PAMAM-G4 conjugated to glutathione was assessed for Parkinson's disease. Glutathione (GSH) is a tripeptide that is an important antioxidant and antiapoptotic in the brain. PAMAM–GSH

was able to lower intracellular reactive oxygen species and to reduce the level of cleaved caspase-3 at low concentration (Pojo et al., 2013). Recently Gao et al. reported the use of G4 PAMAM to remineralize human teeth to treat dentine hypersensitivity. They demonstrated that PAMAM created precipitates on the surface and within the dentinal tubules with a strong ability to resist acid and showed great potential in the treatment of dentine hypersensitivity (Gao et al., 2017).

8. Conclusion

PAMAM dendrimers are hyperbranched organic molecules with distinguished characteristics from all other traditional linear polymers like size, shape, monodispersity, surface charge and peripheral functional group. For achieving these unique characteristics optimally, it must be synthesized with consistent quality and purity. Various approaches for synthesizing PAMAM dendrimers have been available, including divergent, convergent along with click chemistry, lego synthesis and hybrid convergent-divergent synthesis. However, preparation of high generation and defect-free PAMAM dendrimers on a large scale remains challenging because the purification processes are complicated and require the appropriate handling of energy parameters.

Due to its physicochemical properties and bio structural similarities, it determined its immense potential in the field pharmaceuticals as well as the bio-medicals. PAMAM dendrimer can improve biological properties such as bioavailability, solubility and specific target selectivity by linking or entrapping bioactive compounds. The peripheral positive charge of PAMAM is responsible for its cytotoxicity which can be overcome by neutralizing charge through PEGylation, acetylation, folate, and peptide conjugation. The structure of PAMAM dendrimers with a wide scope of applications will provides several opportunities for commercialization in the future.

Declaration of Competing Interest

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