Review Article

A Review on Poly(amidoamine) Dendrimers: Properties, Synthesis, and Characterization Prospects

Piyushkumar Sadhu[1*], Mamta Kumari[1], Falguni Rathod[2], Niyati Shah[1], Shivkant Patel[3]

[1]Department of Pharmaceutics, Department of Pharmacy, Sumandeep Vidyapeeth Deemed to be University, Piparia, Vadodara, Gujarat, India. [2]Medical Surgical Nursing, Faculty of Nursing, Noble University, Junagadh, Gujarat, India. [3]Department of Pharmaceutical Chemistry, Department of Pharmacy, Sumandeep Vidyapeeth Deemed to be University, Piparia, Vadodara, Gujarat, India.

Abstract

Scientists have recently paid a lot of attention to the use of dendrimers in biomedicine. The properties of dendrimers, such as their branching, well-defined globular structures, excellent structural regularity, multivalency, diverse chemical composition, and great biological compatibility, make them appealing for biomedical applications. Several biologically active substances can be incorporated into the three-dimensional structure of dendrimers to create biologically active conjugates. First, a brief overview of dendrimers is given in this state-of-art review, with an emphasis on Poly(amidoamine) (PAMAM) dendrimers and optical sensors. Dendrimers are a newer type of monodisperse polymer with tree-like spherical structures and well-defined sizes and forms. Their peculiar structure significantly affects both their chemical and physical characteristics. PAMAM dendrimer-based optical sensors, employed for the detection of pH, cations, and other analytes, have recently seen advancements, according to reports. Due to its robust synthesis, availability, dendritic structure, and peptide/protein mimic properties, poly(amidoamine) (PAMAM) dendrimers have received the most research attention of all the dendrimers that have been described. The current review is thorough and addresses a different generation of PAMAM dendrimer and related aspects, including i) properties, ii) synthesis, and iii) characterization. The focus is on their uses as well as the state of ongoing medication targeting research at the moment.

Keywords: Poly(amidoamine), PAMAM, Targeted drug delivery, Drug carrier, Dendrimer

Introduction

Dendrimers are nanoscale molecules having a monodisperse, homogeneous, and well-defined structure made entirely of tree-like arms or branches. *Fritz Vogtle* 1978, *Donald Tomalia* in the 1980s, and *George R. Newkome* in the same time in 1980s discovered these hyperbranched molecules independently [1]. The term dendrimers come from the Greek word 'dendrite' which means 'tree' and 'mero's' which means 'units'. They are also known as cascade molecules, arborols, or starburst polymers, and their distinctive structures and features make them attractive building blocks [2]. Dendrimers are a molecule that is highly branched, globular, multivalent, and monodispersed with a wide range of applications [3]. The interaction between dendrimers and solid or liquid surfaces is being studied and provides much important information regarding their prospective functions due to the structure specificity. Since the structure of dendrimers is so distinct from other conventional polymers, scientists used specific synthetic methods rather than polymerization to create them [4]. Dendrimers are prepared by either divergent or convergent methods [1]. On market, there are a few dendrimer products available, such as vivagel, which is made of G_4 Polylysine dendrimers and is used to treat bacterial vaginosis and protect against HIV [5]. Surface modification of dendrimers can be made with many ligands such as polyethylene glycol (PEG), folic acid, amino acids, peptides, proteins, carbohydrates, vitamins, surfactants, antibodies, p-hydroxybenzoic acid, and hyaluronic acid which facilitate the formulation being targeted to the specific site of action [6]. Some examples of dendrimers a listed in **Table 1**.

Table 1. Name of dendrimers and their inventors

Sr. No.	Type of Dendrimer	Inventor(s)	Year
1.	Poly(propyleneimine) (PPI) dendrimer	Vogtle *et al.*	1978
2.	Poly(amidoamine) (PAMAM) dendrimer	Tomalia *et al.*	1983
3.	Arbosols	Newkome *et al.*	1985
4.	Polylysine dendrimer	Denkewalter *et al.*	1981
5.	Poly(aryl ether) dendrimer	Frechet and hawker	1990
6.	Polyether dendrimer	Frechet and Grayson	2001

Address for correspondence: Piyushkumar Sadhu, Department of Pharmaceutics, Department of Pharmacy, Sumandeep Vidyapeeth Deemed to be University, Piparia, Vadodara, Gujarat, India. piyush.sadhu@yahoo.in

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Poly(amidoamine) (PAMAM) Dendrimer

The Poly(amidoamine) (PAMAM) dendrimer is a polymeric molecule with several branching monomers originating from a central core with numerous active amine groups and with many reactive groups on the surface. PAMAM dendrimer can be engineered with various functional groups for specific targeting ability [7, 8]. They are highly reactive because of enormous functional groups, high density of surface-active groups, structural uniformity, and adjustable size. Specifically, amine-terminated PAMAM dendrimers have found wide acceptance in metal ion adsorption [9, 10]. Drugs can be enclosed within the PAMAM's vast interior cavity, entrapped on the surface, and/or distributed throughout the dendritic structure, preventing them from being degraded physiologically. It is also appropriate for passive drug targeting, decreasing the side effects of loaded drugs and get accumulated within the tumor cells via enhanced permeation and retention (EPR) [11, 12]. The PAMAM dendrimer's core can be constructed up of linear chain molecules containing primary amines, which start the stepwise polymerization process, which is the most important structural variations process [13]. Its' molecular weight and number of active surface units increased exponentially with each generation from G0 to G6, but their diameters increase linearly **(Figure 1)** [12, 14].

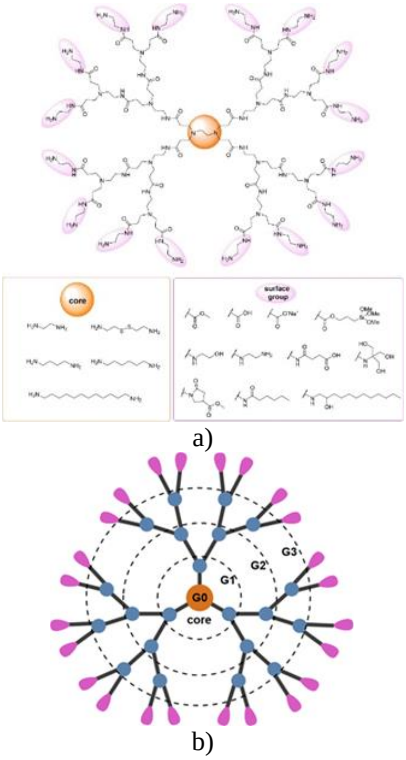


Figure 1. a) Chemical Structure of Poly(amidoamine) dendrimer with core and surface functional group; b) Cartoon illustration of dendrimer structure shows the generations. The central core is generation 0 (G0); generation 1 (G1), generation 2 (G2), and generation 3 (G3) of dendrimer refer to the 1st, 2nd, and 3rd levels of branching, respectively [12].

Physicochemical Properties of PAMAM Dendrimer

Dendrimers are a relatively novel chemical class characterized by a unique molecular geometry and size. Dendrimers have several advantages over other forms of delivery systems, including; i) three-dimensional and globular design, ii) structure and size that can be controlled, iii) reduced molecular volume compared to linear polymers of the same molecular weight, and iv) drug encapsulation. Furthermore, these nanosystems have significant physicochemical features, making them an excellent alternative for medicinal excipients [15, 16].

Size

The topologies, functions, and dimensions of dendrimers are remarkably similar to the biological polymer. They have several features that are similar to proteins. Dendrimers can be recognized as artificial proteins with biomimetic features because they exhibit nanometric dimensions and other protein-like qualities [17]. The extravasation time of PAMAM dendrimers increased exponentially as the size and/or molecular weight of the dendrimers increased. Large cationic PAMAM Platelet aggregation is induced by large size cationic PAMAM dendrimers that disrupt cell membrane integrity [18].

Higher Solubilization Potential

PAMAM dendrimers are biocompatible, non-immunogenic, water-soluble, and have amine functional groups at the end that can be modified to bind various targeted or guest molecules. Dendrimer-mediated solubility can be influenced by parameters like generation type, temperature, pH, core, dendrimer concentration, and terminal functionality. The protonation of nitrogen whether at the periphery or dendrimer interiors is influenced by pH [19]. The core, layers of repeating units (generations), and terminal groups make up a typical PAMAM dendrimer. End groups can have cationic, anionic, or neutral charges and are primarily responsible for the molecules' high solubility, reactivity, and toxicity. Dendrimers are ideal for drug delivery systems because of their unique characteristics. PAMAM dendrimers may have potential applications in enhancing the solubility of low water solubility medications, as the generation of PAMAM increases from G0 to greater. The solubility of furosemide at different pH was studied by B Devearkonda *et al.* [20]. They also noticed that the solubility of the nifedipine drug increased linearly with increasing the concentration of amine-terminated PAMAM dendrimers at pH 7 and 10. The scientists also discovered that the solubility of Nifedipine was maximum at pH 7, decreased at pH 10, and was lowest at pH 4. The protonation of tertiary amines in amine-terminated dendrimers at pH 4 was thought to create an environment with significant polarity inside the dendrimer [19].

High Loading Capacity

Dendrimers' structure can be utilized to load and store a wide range of inorganic and organic compounds via electrostatic interactions on the surface, covalent bonding with the surface

groups, and encapsulation of the medication within the dendrimer's cavities **(Figure 2)** [15]. Guest-guest electrostatic interactions are protonated under physiological conditions and dominated under external complexation. Interior encapsulation occurs within the dendrimer branches' internal chambers and is primarily driven by van der waals and hydrogen bonds [21].

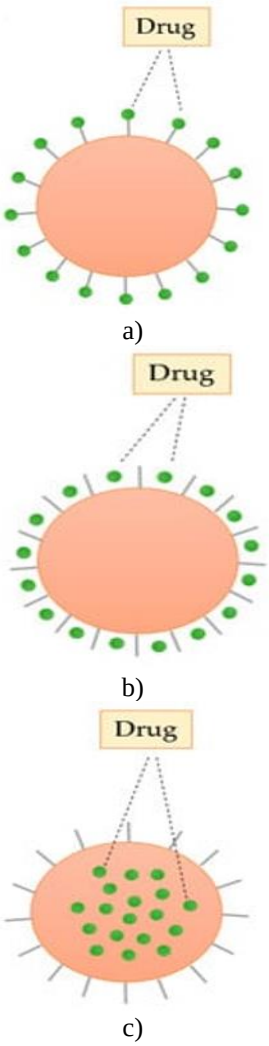


Figure 2. Loading of the drug into a dendrimer structure in a three-way; a) electrostatic interaction, b) covalent bonding and c) encapsulation (15)

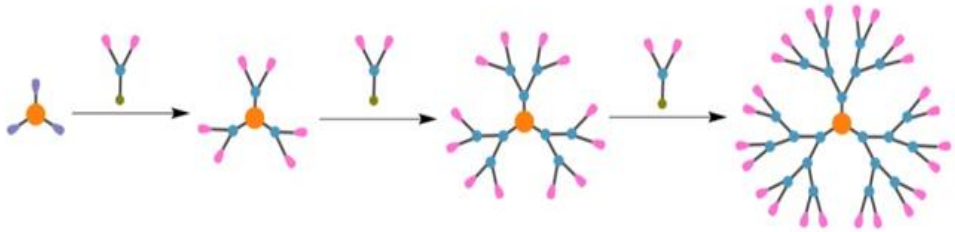
Synthesis of PAMAM Dendrimer

PAMAM may be synthesized by three approaches which are; a) Divergent method, b) Convergent method, and c) Combined approach.

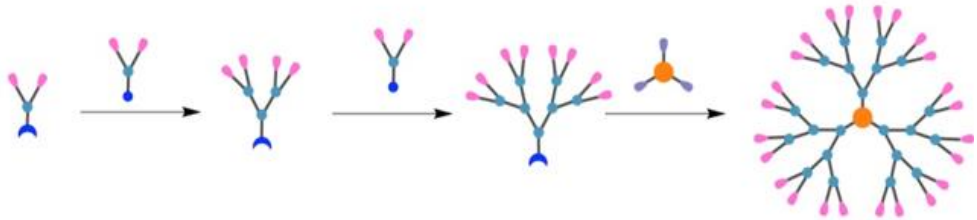
Divergent Method

The "divergent method," as introduced by *Tomalia,* was the first method to synthesize PAMAM dendrimer. Divergent synthesis **(Figure 3a)** involves iterative monomer unit addition to develop the dendrimer structure from the initiator core to the periphery multifunctional core. The first generation of PAMAM dendrimers is formed as a result of such reactions. The new perimeter molecule is then activated for supplementary reactions with other monomers, and the cycle is repeated until the required number of generations has been reached [22]. To create amine-terminated PAMAM dendrimers, *Tomalia et al.* used this strategy and attached N-(2-aminoethyl) acrylamide monomers to an ammonia core. Each branching unit is made in a two-step process that begins with an exhaustive Michael addition of the acrylate ester to the ammonia core and ends with excess ethylenediamine (EDA) amidation. The first step produces a half-generation and adding the diamine in excess amount completes the process. Side reactions that produce incomplete or imperfect dendrimers can make this synthesis difficult [23].

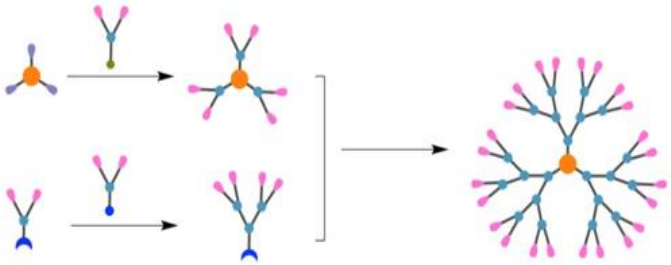
Beginning with Ethylenediamine (EDA) in excess amount, the technique entails a sequence of Michael additions and an ester amidation reaction. Following the addition of a primary amine (EDA in the first step) to methyl acrylate, the resultant multi-ester of EDA was amidated. In the second step, the terminal carbomethoxy group (-COCH3) of methyl acrylate was amidated with EDA, and in such a way, PAMAM dendrimers were synthesized [24]. The PAMAM dendrimer's outer surface has a high density of primary amino groups, which gives the dendrimer a positive charge at physiological pH. Because of the high surface density of the polymer branches, steric hindrance prevents ideal growth. Because the molar mass of the dendrimer is duplicated in each generation-adding step, the divergent method is ideal for the synthesis of large amounts of dendrimers. However, side reactions that result in incomplete or defective dendrimers can put a stop to this method of synthesis. Another disadvantage of this method is the difficulty in purifying the final products, as well as the lengthy multistep reactions [25].



a) Divergent synthesis



b) Convergent synthesis



c) Combined divergent/convergent synthesis

Figure 3. Schematic representation of different methods of synthesis of PAMAM dendrimers; a) divergent synthesis, b) convergent synthesis, and c) combined divergent/convergent synthesis [12].

Convergent Method

Researchers, *Hawker* and *Frechet* first described the convergent method. The purpose of appraising this method was to overcome some difficulties of the divergent method. In this top-down method **(Figure 3b)**, dendrimers are synthesized from the surface towards the center, usually through "one-to-one" monomer coupling. To overcome the increasingly low reactivity experienced in stepwise divergent synthesis of large oligopeptides on solid-phase, segment coupling strategies were first used in peptide synthesis. Peptide synthesis has progressed one step closer to the pure chemical synthesis of high molecular weight polypeptides and proteins, and to making highly monodisperse dendrimer structures. This new tool was quickly adopted by other synthetic chemists working in the dendrimer field as a powerful alternative to the divergent approach [26]. Because of the large "molecular difference" between the reactant molecule and the product, purifying the final desired product in the convergent method is simple [12]. During the purification process, this situation makes it easier to separate the reactants and the product, and this is how around 98 percent of the purified dendrimer is created [22, 25]. This method also has the advantage of fewer nonideal growth events and improved final dendrimer monodispersity. As a result, during the proliferation process, the number of reactive sites is kept to a minimum, resulting in faster reaction rates and yields. However, there are some disadvantages to this method, including low yields in the synthesis of large structures and the failure to form high generations of dendrimer due to steric crowding in the reactions of the dendrons and core [26].

Combined Divergent/Convergent Approach

This method of synthesis of the dendrimer is also called a double-stage exponential approach. Kawaguchi and his team members developed this method to combine the benefits of the divergent and convergent methods. This method, first, involves the synthesis of the branched group by the divergent method and is followed by the development of dendrimers by coupling with the core by the convergent method. This results in the development of the first generation of the dendrimer. Then the parent dendron can be grown by attaching to an activated dendron and thus next generation is developed **(Figure 3c)**. The advantage of this method is to develop a more efficient higher generation dendrimer compared with the divergent or convergent method. Moreover, this method can alter the terminal groups of branches while synthesis, providing the dendrimers structural variety (12,24,26).

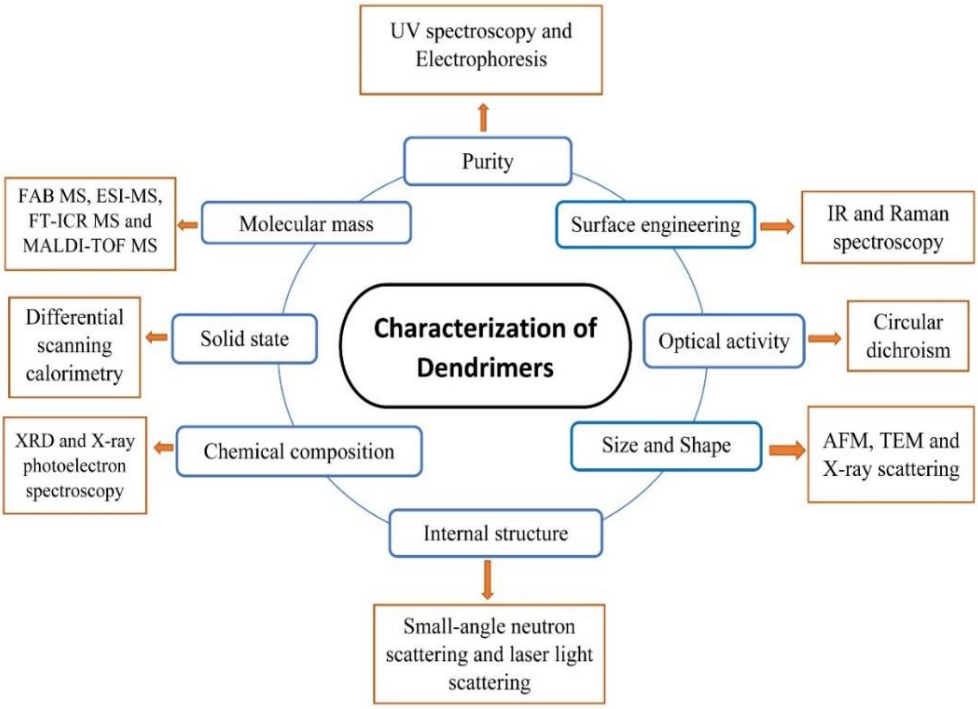


Figure 4. Different characterization techniques of PAMAM dendrimers reproduced from [27].

Various physicochemical characterization techniques of Pamam Dendrimers are illustrated in **Figure 4**.

Conclusion

Dendrimers have unique qualities that make them potential candidates for a variety of applications. Since the first dendrimers were created, the relevance of dendrimer chemistry has grown quickly. Even 20 years after dendrimers were discovered, the multi-step synthesis still takes a lot of work. The most frequently investigated dendrimer class, PAMAM dendrimers, are synthesized using a variety of methods that we have discussed in this brief overview. In addition to their well-defined dendritic topologies and intrinsically produced multivalent cooperativity, PAMAM dendrimers are widely used in a variety of industries due to their ready availability and peptide/protein mimic characteristics. Using the iterative reaction process established by Tomalia, PAMAM dendrimers are easily produced and may be conjugated with a variety of functions. Significantly, several PAMAM dendrimers are inexpensively offered on the market. As a result, research on PAMAM dendrimers has exploded, and they are now widely used in a wide range of applications, including molecular devices, sensors, catalysts, medicines, and drug delivery systems.

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