AN OVERVIEW OF POLYAMIDOAMINE DENDRIMERS:
STRUCTURE, PHYSICAL PROPERTIES, AND BIOMEDICAL APPLICATIONS

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ABSTRACT
Polyamidoamine (PAMAM) dendrimers are the primary complete dendrimer family to be orchestrated, described and marketed. Dendrimers are hyper branched, monodispersed macromolecules with multivalent useful end bunches. Dendrimers have been investigated as nanocarrier for many drugs like anticancer, antiviral, antimalarial, antiprotozoal, anti-tubercular drugs. In spite of the fact that a number of various sorts of dendrimers containing distinctive center atoms, fanning monomers and surface practical gatherings, so they have been planned till date for medication conveyance applications, yet PAMAM dendrimers have been the most investigated dendrimers in such manner. In this review, we have focuses on relative information on PAMAM dendrimers especially significant to their properties, synthesis, lethality, biomedical applications and drug delivery characteristics.

KEYWORDS: PAMAM dendrimers.

INTRODUCTION
The term “dendrimer” is originated from two Greek words “dendron”— which means tree and meros— which means part. The dendritic design is a standout amongst the broadest topologies watched all through natural frameworks.[1] The primary motivation for blending such atomic level tree like structures by D. A. Tomalia, yet maybe initially conceptualized by Flory, the principal effective research facility amalgamation of such dendritic many-sided
quality did not happen until the late 1970s. In the historical backdrop of manufactured polymer science, this was the first occasion when those abiotic macromolecules were orchestrated without the utilization of an organic framework. Therefore, macromolecular engineering, now perceived as dendrimers. Another polymer researcher Dr. George R. Newkome likewise reported the blend of a comparative macromolecule and named it as "arborol" originate from Latin word "arbor" otherwise called “tree”. There are some different terms like "course" atom is likewise utilized yet "dendrimer" is the most widely recognized one.

Dendrimers resemble tree shape simulated macromolecules. They are mono-scattering, three-dimensional and hyper-spread particles, having a property of host–guest entanglement and characterized atomic weights. Dendrimers are set up from fanned monomer units in an orderly way and it is conceivable to control their shape, measurement, atomic size, adaptability, thickness and dissolvability by picking surface useful gatherings and distinctive building-expanding units. In addition, they have polymers and natural atoms as a piece of their structure and consequently, gain uncommon synthetic and physical properties.

Till now, dendrimers have been utilized in numerous fields, for example, electrochemistry, photochemistry, supra-molecular chemistry, host–guest chemistry, synthesis of the nanoparticle, decolonization of dye, contamination administration, curing of epoxy pitches, impetuses, readiness of monomolecular membranes, delivery of the medication and transfection of the quality. In late years, dendrimer's utilization in conveyance frameworks procured more considerations when contrasted with other fields.

Internal cavities are empty, open and often found in low generation dendrimers, as a result they can encapsulate drug molecules which are hydrophobic in nature. As compare to the conventional molecules, they possess a large number of functional groups at their surface. The solubility of drugs increases due to the presence of these functional groups in dendrimers. The presence of a large number of these functional groups on the surface of the outer shell increases its reactivity, thus causing conjugation or modification of dendrimers with a chain of different guest molecules. Encapsulation of both the drugs and guest molecules can occur in the hydrophobic cavities of dendrimers or the conjugation with surface functional groups can occur. These properties are indicative that dendrimers are a suitable agent for delivery of drugs. Research is also being conducted in the biomedical field for the application of dendrimers.
The first three generations dendrimers resemble with ordinary organic molecules. Dendrimers have no consistent or specific three-dimensional structure, instead they are small and floppy shaped in appearance. At generation 4 (G4), they appear to be spherical in shape. After G5 they show consistent and specific three dimensional structures. After G5 they become highly spherical in structure resembles with a tree having large number of branches. The diameter of dendrimers increase with the increase of generation number, similarly the surface group number also increases geometrically with increase in generation number.

**STRUCTURES, SYNTHESIS, AND PROPERTIES OF DENDRIMERS**

**Structures of PAMAM dendrimers**

Generally, dendrimers have two types of shape, globe shape and ellipsoid shape with three kinds of specific component, one is the central core, second is repeated branch and third one are the functional groups on surface. Two functional groups are attached to the central core which are reactive in nature. Branches are arranged in concentric layers and are repeated radically, these layers are known as “generations.” In the surface of dendrimers, the functional groups determine its physical properties in aqueous solution or in solid state. Molecular weight, number of functional groups and dendrimer size varies with the generation of dendrimer and also controlled by dendrimer synthesis.
Synthesis of PAMAM dendrimers

Generally, the dendrimers are produced by two methods, divergent method and convergent method. In the year 1984, Tomalia first introduced the divergent method for the synthesis of PAMAM dendrimers. Later Fewchet and Hawker mentioned about the convergent method in 1994. The two methods are different in one way, convergent method using top-down approach and starting from the margin of the dendrimers while the divergent one starting from the central core and widening in the direction of surface.

Through divergent method the high generation dendrimers are created but it has some on the surface of high generation dendrimers as a result of steric resistance. Cleaning of the product after each series of preparation is necessary. But the convergent method has ease in characterization and purification and have the potential of attracting Dendron’s of different types to one dendrimer.

The synthesis of PAMAM dendrimers starts using the tertiary amine as a focal point or core point which produces the desired generation of dendrimers with poly amide branches. The PAMAM dendrimers are commonly available in two kinds of impurity grades, these are technical grade which is suitable for general commercial application with high purity and another one is Medical grade which is mainly used for biomedical science application.
For the half generation dendrimer, amine-terminated cores and subsequent primary amine-terminated dendrimer generations are reacted with methyl acrylate initially, then these ester terminated, half generation intermediates are then release to react with large excesses of EDA to form higher full generation dendrimer (i.e., G=1.0,2.0,3.0) by repeating these reactions again interactively and sequentially.

For the full generation dendrimer, EDA was dissolved in methanol and then cooled on dry ice at -30°C. on the other side the Multiester was also dissolved in methanol and the mixture was cooled down. In the presence of argon, the EDA was added at -25°C temperature. Then the mixture was kept at room temperature and excess EDA was evaporated as azeotrope.¹⁶

**COMPARISON BETWEEN DENDRIMERS AND LINEAR POLYMERS**

In compare with the linear type polymers, dendrimers are mono dispersed macro molecules. Dendrimers show improved chemical and physical properties of specific molecular architecture in compared with the mostly used linear polymers. Linear polymers are more viscous than the dendritic solution.¹³ The intrinsic viscosity will also increase as the molecular mass increases in dendrimer at generation 4 then starts to decrease. But in the case of linear polymer the molecular mass increase with the intrinsic viscosity continuously.¹⁴

**COMPARISON BETWEEN PAMAM AND PPI DENDRIMERS**

Nowadays PAMAM and PPI dendrimers are playing a very important role in the nanomedicine field.¹⁷,¹⁸ PAMAM dendrimers have polymeric shape which changes as the
generation goes high, in the low generation (0.5-4.0G) it shows the planner and elliptical shape but in the higher generation (5.0-10.0G) it shows densely packed dendritic structure.\cite{19,20} Surface groups, molecular weight and size is the main factor which effect the number of the generations. In lower generation PPI like G 0.0,G 1.0 and G 2.0 it shows an open structure with high polarity range.\cite{21,22} Normally the PPI dendrimers are non-polar in nature but PAMAM is polar in nature with tertiary amine, amido group and alkyl chain in its structure. PAMAM dendrimers are larger in length compared with the PPI, as PAMAM has seven bonds where PPI only have four bonds.\cite{23,24} The structures PPI and PAMAM have large distinct behavior in their ionic structure. PPI dendrimers are more hydrophobic in nature then the PAMAM. In the drug release behavior also it shows a different nature, it totally depends upon the number of generation and groups present on the surface of dendrimers.\cite{25} By the addition of different group like alcohols, amines, carboxylic acids or nitrile groups to a large number in surface functional group will make new properties of generation.\cite{26}

The recent study reveals that amine-terminated PAMAM dendrimers shows low toxicity as compared with the liner polymer because of its globular structure attachment with the cellular surface.\cite{27} Mainly PAMAM dendrimers are more toxic than secondary and tertiary amines. In cytotoxic and hemolytic effect both PAMAM and PPI shows the same results. Cationic PAMAM dendrimers shows less in vivo applications because it has high cytotoxicity on different cell lines and hemolytic activity on the blood which help to clear the blood rapidly. For this kind of problems, the PAMAM dendrimers are checked by different methods like acetylation, glycosylation or PEGylation.\cite{28,29} PAMAM and PPI both shows little toxic effects on red blood cells because of the realizing hemoglobin at low concentration.\cite{30} This toxic effects can be reduced by surface functionalization as this toxic effects have direct relationship with the amino groups and positive charge density. Normally high generation PPI and native PPI dendrimers get accumulated in the liver and causes problem but PEGylated dendrimers do not affect any bad in liver.\cite{31,32}
Figure 4: Schematic chemical structures of poly(L-lysylated) PAMAM dendrimers (a) and PLL dendrimers (b). In the hypothetical structure of poly(L-lysylated) PAMAM dendrimers, the residues of L-lysine (balls) may be linked to surface terminal groups either directly (via peptide bond) or with the use of a linker.

TYPE OF PAMAM DENDRIMERS
The first commercialized and synthesized by divergent is PAMAM or Poly-amidoamine. The structure of the PAMAM starts with an ammonia (NH2) or ethylene diamine (C2H8N2) as a core which binds to the amine (R-NH2) and amide (-CONH2R) groups. As the generation goes higher the size of the dendrimers also get bigger, between 1.1-12.4 nm mainly because of drug-polymer conjugation (5-20 nm), viruses (25-240 nm) and proteins (3-8 nm) conjugation. PAMAM has known as the ideal drug carrier for the drug delivery only because of the presence of different surface groups. To form Poly (amidoamine) organosilicon (PAMAMOS), PAMAM was combined with the silicon and this is the first dendrimer which was commercialize. This are inverted unimolecular micelles with interior hydrophilic and exterior hydrophobic polyamidoamine. Based on polylysine, polyether, poly (disulfide amine) or polyester some biodegradable dendrimers are designed with modified surface structure which shows a promising antibacterial, antiviral, chemotherapeutic and vaccine carrier characteristics. Glycodendrimers consist with carbohydrates and saccharide which make them a great drug carrier. There are many other dendrimers which are used in various other pharmaceutical applications like acid -based dendrimers, hydrophobic dendrimers, peptide dendrimers and asymmetric dendrimers.
There are some FDA approved dendrimer-based products are available in the market like Dade Behring, which are dendrimer linked with monoclonal antibody mainly used for cardiac diagnostic testing. Other dendrimers like SuperFect (qiagen) which are also used with many other cell lines.[35]

**DENDRIMERS AND DRUG MOLECULES INTERACTIOS**

The last group of the dendrimer can be changed for obtaining the novel biological properties of a new molecule like receptor-ligand interaction by which the dendrimers will be able to interact with poorly soluble drugs[36] There are two different methods by which the dendrimers are used as a drug carrier,

1. Lipophilic drugs encapsulated inside hydrophobic dendrimer cavity for increasing water solubility, 2. Covalently attached onto the surface of dendrimers. the drug can also be attached to the exterior of the dendrimer in the case of drug /dendrimer conjugate.[3] Drugs can also be attached to the exterior of the dendrimer in case of drug/dendrimer conjugates.

This kind of conjugates are mainly pro drugs which are inactive in nature and or decreased activity. The covalent conjugation of drugs is mainly used for achieving and targeting the higher drug payload but the noncovalent drug interactions are mainly used to increase the solubility of the insoluble drugs.[37]

![Figure 5: Dendrimer drug-delivery systems, a schematic view. The dark oval shows an active substance: (a) Encavitated guest, (b) Dendrimer-drug networks, (c) Current Opinion in Chemical Biology.](image-url)
1. Drug Encapsulation inside the Dendritic Structure

The drugs with the coulomb attraction and acid-base reaction inside the dendrimer, mainly are the reason which trapes the drugs inside the dendrimer structure and hydrogen bonding keep them together. First time dye was encapsulated inside the dendrimer in 1994 as reported by Jansen and co, it named “dendritic box”.[8] After the successful encapsulation of dye Kojima and co fist encapsulated anticancer drug like doxorubicin and methotrexate[38] using G3 and G4 PAMAM, later some groups were attached with methotrexate and folic acid to target the tumor cells by using this drug-dendrimer conjugates.[39] Dendrimers which consist of a polar shell and an polar core are called as Unimolecular micelles. The structure of the dendrimer does not depend on dendrimer concentration because of this nature to control the drug release from the dendrimer core is really difficult to control. Dendrimers can be modified by PEG and produces a unimolecular micelle by creating hydrophobic shell on the dendritic core.[40] Cored dendrimers are modified with dendritic structure to encapsulate the drugs. The ester bond core was removed by synthesis but the structure remains the same as a result of new ether linkage.[41]

2. Drug Dendrimer conjugation

The loading capacity of the dendrimer is high as the outer surface of the dendrimer have many interaction sites. As the generation goes higher the available surface group also increases and also the drug interaction increases. Drugs are conjugated through amide, ester or any other linkage which depends on dendritic surface which can be hydrolyzed by endosomal or lysosomal enzymes inside the cell.[36] The release of the drugs can be increased by using different kind of linkers, it mainly depends on the length of the linker and the flexibility. There are some kind of linkers which are ph. sensitive and proved to enhance the drug release.[42] The drugs which are covalently attached have more control over drug release then the drugs which are electrostatically attached.

3. Dendritic Gels

Hydrogels are hydrophilic in nature, mainly used for drug delivery because of high water absorbing capacity.[43] In situ forming gels are used in many different formation of drugs including vaginal, nasal, oral, rectal and injectable.[44] Because of their low molecular weight and structure and nature in between traditional gel polymers and the organic compound this can be synthesized and used in self-assembled supramolecular gels.[45] The polymer networks are mainly made of the cross linkers during polymerization.[46]
BIOMEDICAL APPLICATIONS OF DENDRIMER

Dendrimers are used for many different applications. It mainly depends on the different end groups and molecular weight. Some of the applications are described below.

As A Drug Carrier

Dendrimer is a mono dispersed drug carrier and the size of the dendrimer can also be controlled which makes the dendrimer the best drug carrier for many molecules which are encapsulated or make and interaction with the terminal group of the dendrimer. The lipophilic molecules get attached with the dendrimer core via Van der Walls or polar forces. Dendrimers are mainly used to enhancement of solubility, drug protection, controlled release, drug delivery and others.

For Solubility Enhancement

By inserting a gust molecule to the dendritic structure or gating interaction with the terminal groups can increase the solubility. It mainly depends on some factors like generation of the dendrimer, core of the dendrimer, concentration, pH, temperature and terminal groups. Dendrimers use ionic interaction, hydrophobic interaction and hydrogen bonding for control there solubilizing property.\[^{47}\] The solubilizing property of artemether can be increased three to fifteen times by increasing the concentration, generation and type of micelles of MPEG 2000 and 5000.\[^{48}\]

As Vectors for GENE Delivery

PAMAM dendrimer can carry genetic materials.\[^{49}\] The dendrimer with amino acid at the end of the group, can easily attached with the nucleic acid at the phosphate group which results the development of transfection complex. This transfection complex reagents commonly known as Super-Fect TM. Activated dendrimers can carry large quantity of genetic materials in compare with the viruses.\[^{50}\] The DNA complex known as Super Fect have the greater solubility and can easily transfer DNA into the nucleus then liposomes. The transfection efficiency depends on low pk and shape of amines.

Dendrimers as Drugs

Dendrimers bind with the viral component or with the cell surface with the electrostatic force which inhibit of infection at the stage of entry of virus cells and also inhibit the replication.\[^{51}\] Anionic dendrimers have antiviral activity. In recent days’ dendrimers are also used against HSV, RSV and HIV.\[^{52}\] It also shows antimicrobial activity. Mainly the cationic surface
group interact with negatively charged prokaryotic membranes and destabilize them which starts the lysis of bacterial cell.\textsuperscript{[53]}

**Dendrimers in Drug Delivery**

Nanoparticle drug delivery system was mainly used to increase the stability and selectivity of the drug. Drug delivery system with dendrimers is widely used these day. The guest molecules are encapsulated in the cavity of dendrimers, the dendrimer drug network is easy to be visualize. Sometimes the drugs are attached to the dendrimer with covalent or non-covalent bonds.\textsuperscript{[28]} A dendrimer with hydrophobic and hydrophilic core-shell having an PAMAM inside and a long alkane chain outside can bind with 5-flourouracil which is a water soluble anti-tumor drug. Coating with the dendrimer macromolecule phospholipid can increase the oral bioavailability of 5-fluorouracil.\textsuperscript{[54]} liposomal formulations with dendrimers inside can control the drug release. Dendrimer having saccharides in their surface can used directly to target and then deliver covalently attached or complexed drugs.\textsuperscript{[55]}

**As Diagnostic Tools**

Dendrimers are used as a valuable methodological diagnostic tool in these days, and they are also used to construct the contrast agent for imaging, widely used as magnetic resonance imaging, can also be modified to carry a particular ionic agent (Mn\textsuperscript{3+} Gd\textsuperscript{3+},Mn\textsuperscript{2+}) by covalent interaction or by chelation while minimizing toxicity and controlling their bio distribution.\textsuperscript{[56]} Gadomer-17 was the fist commercially used dendrimer based contrast agent which was developed by schering AG and it have a polylysine-DTPA dendrimer along with Gd\textsuperscript{3+}. Dendrimers are also used for increasing sensitivity of the assays in microarray and ELISA bioassays, by enhancing the signal generation because of multivalent binding. Dendrislides TM from Genopole are dendrimer based DNA Chips which are used for oligonucleotide detection and highly amplified labeling.

**Other Applications**

There are many other applications of dendrimers like in the field of biological chemistry. And also in some highly sensitive analytical device like MRI, EPR, the dendrimers are used.\textsuperscript{[57]} Other than that burn treatment, prion research\textsuperscript{[58]}, contrast agents in this areas also the dendrimers are useful.\textsuperscript{[59]}
FUTURE PROSPECTS
Dendrimers mainly used to deliver the drug molecules but it also can be used as drug targeting agents. This are monodispersing macromolecule with a large number of surface groups which varies from generation to generation. The surface agent presents in the dendrimer can easily be modified and controlled to design a carrier system with a desired property which is not possible for other available carrier system. It can also become a useful tool for optimizing the drug delivery system.

SUMMARY AND CONCLUSION
Dendrimers are showing an remarkable changes in different fields mainly in drug delivery but it has a bright future in applications of pharmaceutical industries and also in diagnostic fields because of its exceptional characteristics like high degree of branching, molecular weight, globular architecture. There are many drugs which are facing problems like poor solubility, permeability and bioavailability but dendrimers can improve the therapeutic efficacy of the product and also minimize the side effects by modifying the surface agents.

REFERENCES


