REVIEW ARTICLE

Dendrimer a versatile polymer in drug delivery

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Dendrimers are a unique class of synthetic macromolecules having highly branched, three-dimensional, nanoscale architecture with very low polydispersity and high functionality. Structural advantages allow dendrimers to play an important role in the fields of nanotechnology, pharmaceutical and medicinal chemistry. This review discusses several aspects of dendrimers, including preparation, dendrimer-drug coupling chemistry, structural models of dendrimer-based drug delivery systems, and physicochemical and toxicological properties. Dendrimers have emerged as one of the most interesting themes for researchers as a result of their unique architecture and macromolecular characteristics. Several groups are involved in exploring their potential as versatile carriers in drug delivery. The use of dendrimers in drug delivery has been reviewed extensively. The increasing relevance of the potential of dendrimers in drug delivery emphasizes the need to explore the routes by which they can be administered. The high level of control possible over the architectural design of dendrimers; their size, shape, branching length/density, and their surface functionality clearly distinguish these structures as unique and optimum carriers in those applications. The bioactive agents may be encapsulated into the interior of the dendrimers or chemically attached/physically adsorbed onto the dendrimer surface, with the option of tailoring the carrier to the specific needs of the active material and its therapeutic applications. This review clearly demonstrates the potential of this new fourth major class of polymer architecture and indeed substantiates the high hopes for the future of dendrimers.

Key words: *Biomaterials, dendrimer, drug delivery, nanoscopic drug carriers, polyamidoamine (PAMAM) dendrimer*

INTRODUCTION

Dendrimers, a nanoparticle-based drug-delivery system have numerous applications in pharmaceuticals such as enhancing the solubility of poorly soluble drugs, enhancing the delivery of DNA and oligonucleotides, targeting drug at specific receptor site, and ability to act as carriers for the development of drug delivery systems.

The therapeutic effectiveness of any drug is often diminished by its inability to gain access to the site of action in an appropriate dose. This is often due to the poor solubility of the drug in the body's aqueous environment. Medicinal chemists initially attempted to address this problem by synthesizing a water-soluble derivative of the drug moiety; unfortunately, even small structural changes can often reduce the efficacy of a drug. Another method of aiding solubilization is to encapsulate the drug within the hydrophobic domains of a colloidal or surfactant-based micellar system (i.e., emulsions, liquid crystals or micelles). However, the unstable dynamics of these systems, as well as their sensitivity to other functionality and pH, can lead to uncontrolled and premature release of the bound drug moieties, once again rendering this approach ineffectual. Ideally, a static or covalent micellar system is desirable. Other approaches to increase solubility includes solubilization by surfactant (e.g., using tween, span, etc.), solubilization by complexation (e.g., using cyclodextrin), solubilization by salt formation, solubilization by prodrug formation, solubilization by cosolvent, etc. In 1991 Newkome *et al.* observed that dendrimers have "container" properties in the solution, which makes them analogous to unimolecular micelles with the ability to maintain their structure stable at even higher concentrations of solvents.[1] The study of dendrimer-mediated solubilization has been found to be superior to cyclodextrin-mediated solubilization.[2] Several hydrophobes such as nifedipine,[3] niclosamide,[4,5] methotrexate,[6] 5-fluorouracil,[7,8] indomethacin,[9] propranolol, ibuprofen, flurbiprofen[10] etc. have been successfully solubilized in dendrimers.[11]

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BRIEF HISTORY OF DENDRIMERS

The evolution of construction of macromolecules (polymers) possessing branched architecture has three general eras. The first period was from late 1860s to the early 1940s when branched impurities were thought to be the insoluble byproducts formed in polymerization reactions. However, purification and

separation techniques were too primitive at the time to prove the structures of these impurities.

In the second period of dendrimer history, from early 1940s to the late 1970s, branched polymer structures were considered mostly from the view of polymer physicists, who developed a theory that described that behavior of macromolecules and branched species. The synthesis of these branched structures was attempted using differentiated monomers in a traditional one-pot synthesis used for the preparation of linear polymer chains. Flory noted in the 1950s that the breadth of distribution is coupled with the impossibilities of selectively fractionating, branching, and molecular weight separately makes this approach impractical. Attempt to investigate branching by such means consequently have been notably fruitless. However, he did examine the scaling properties of branched polymers, where he envisioned a branched system synthesized with an AB_2 type monomer resulting in 1®2 branching system [Figure 1].

In the third period from 1970s and early 1980s, the concept of repetitive growth with branching was successfully reported in by Buhleier *et al*. for the construction of low molecular weight amines. This was followed closely by the parallel and independent development of the divergent, macromolecular synthesis of "true dendrimers" in the Tomalia group. The first article that used the term "dendrimer" and described in great detail the preparation of poly (amidoamine) (PAMAM) dendrimers was presented in 1984 at the 1st International Polymer Conference, Society of Polymer Science, Japan, which was then published in 1985.[12] In the same year, a communication reported the synthesis of arborols by Newkome *et al*.

DENDRIMER

Generally, when one considers the term polymer, a simplistic image of a straight chain molecule of one or two repeating units, such as that of poly (ethylene), comes to mind. However, with imagination, one can see that polymers could become considerably complex with branching limits. Traditionally polymers are classified as follows:[13]

- Linear polymers – These are straight chain polymers made up of any number of monomers linked end to end.
- Cross linked polymers – These become denser as the cross-linked bonds increase in number and strength.
- Branched polymers – These are composed of polymers constructed from monomeric units bearing a nonpolymerizing molecular branch; the resulting molecule is an essentially linear polymer with any number of branches.

Extending this idea generates a fourth category capable of additional polymerizing branch as the major class of macromolecular architecture called hyperbranched polymers have evolved; the category of hyperbranched polymers is referred to as dendritic polymers owing to their similarity to branches of tree. They consist of the following four subcategories:

- **Random hyperbranched:** It is the subclass of dendritic macromolecules, prepared by polymerization of AB_x monomers, where x is 2 or more. Polymerization of such monomers gives highly branched polymers, as shown in Figure 2. In these polymers, structure and size control is limited.
- **Dendrigraft:** It is prepared by reactive oligomers or polymers used to protect-deprotect. These are having larger structure than dendrimers.Structure of Dendrigraft shown in Figure 3.
- **Dendrons and dendrimers:**[14] A dendrimer is defined as hyperbranched ordered, monodisperse, high molecular weight polymer possessing a central core with void spaces, radially extending repeat units and a terminal functional group as abundant surface as shown in Figure 4. Dendron is a segment of the dendrimer.[15]

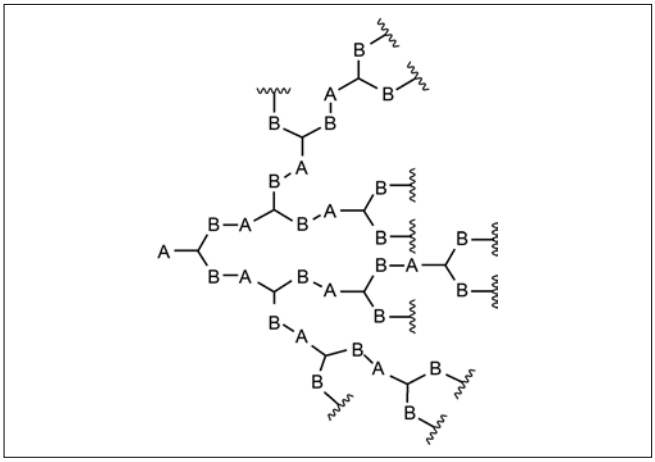

Figure 1: Flory's branched architecture created by reaction of AB_2 type monomer.

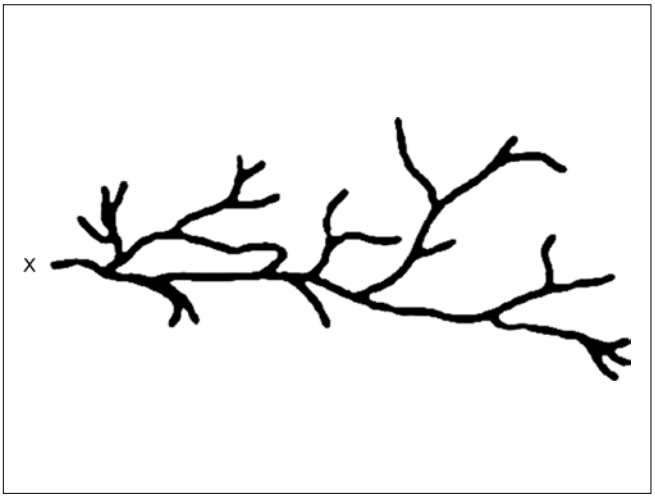

Figure 2: Random hyperbranched polymer

The present dissertation is concerned with the last category, namely that of dendrimers. The word dendrimer is derived from the Greek word dendri (tree-branch-like) and meros (part of) and was coined by Tomalia. Other names for dendrimers are "arborols" from Latin also referring to a tree and "cascade polymers." Although the earlier name "cascade molecule" is more directly connected to nomenclature, the expression "dendrimer" has been generally accepted from about 30 years ago.[16]

A dendrimer is generally described as a macromolecule, which is characterized by its extensively branched 3D structure that provides a high degree of surface functionality and versatility. Its structure is always built around a central multi-functional core molecule, with branches and end-groups. Dendrimers are synthesized in a stepwise manner, i.e., each successive shell, known as a generation, is formed in an individual step. Dendrimers are composed of three distinct regions:

- An initiator core
- A series of inner microdomains or internal cavities made up of repetitive molecular units
- Terminal moieties from which future branching may take place [Figure 5].[17,18]

SCOPE OF DENDRIMER RESEARCH

The scope of dendrimer research depends on its applicability. Based on literature survey, it is found that there are more than 30 areas that have wide scope in dendrimer research, out of which important areas of interest are synthesis, drug delivery, biotechnology, nanotechnology, contrast agent, detection, cosmetic, and catalyst.[19] Patents growths of dendrimer from 1981 to 2008 were shown in Figure 6.

DENDRIMER PATENT TRENDS: AN EXPLOSIVE GROWTH

By searching for and reviewing hundreds of dendrimer patents, we could elucidate several important dendrimer patent trends. Dendrimer patenting is the more exotic; biospace is common now, whether for pharmaceuticals, drug delivery, diagnostics, nanotechnology or biotechnology. Starpharma continues to build its patent portfolio. Based on application, dendrimer patents were weighted. Some patents, for example, may briefly recite dendrimers as a potential embodiment, but do not expressly claim or exemplify dendrimers. Such patents were given relatively little weight compared to patents that

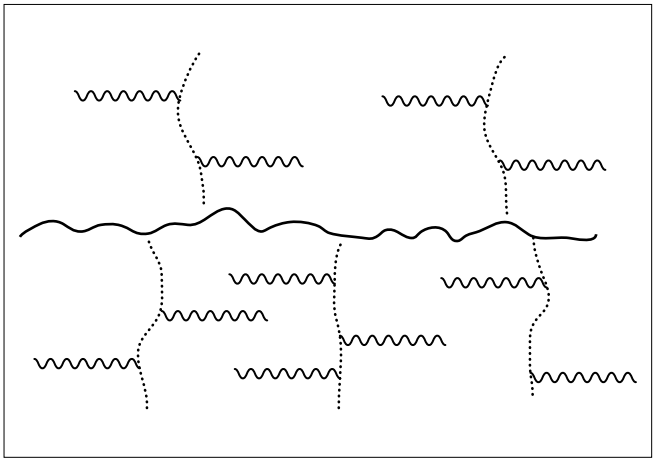

Figure 3: Dendrigraft polymer

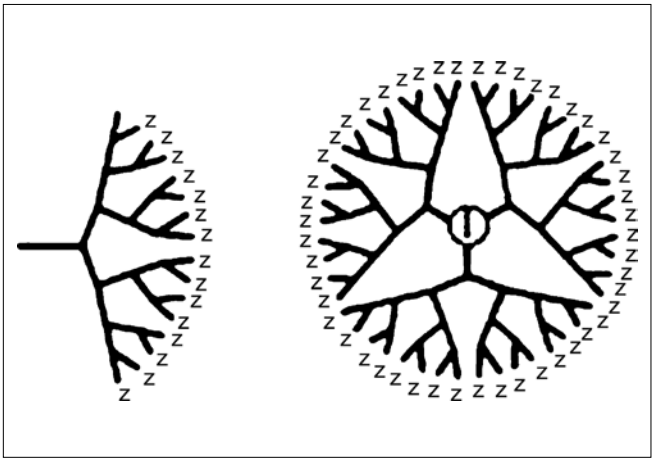

Figure 4: Dendron and dendrimer

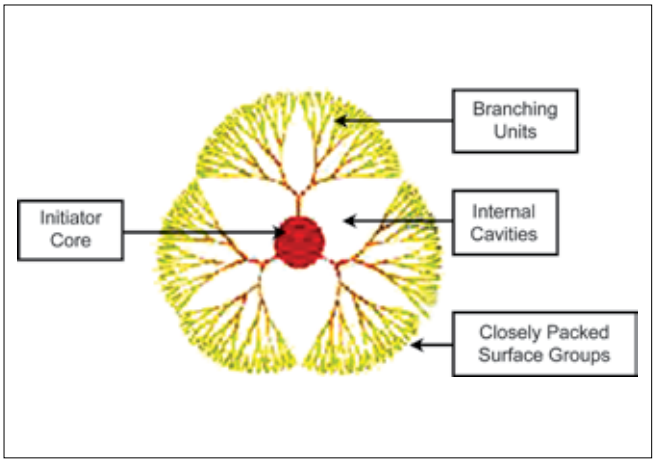

Figure 5: Dendritic structure

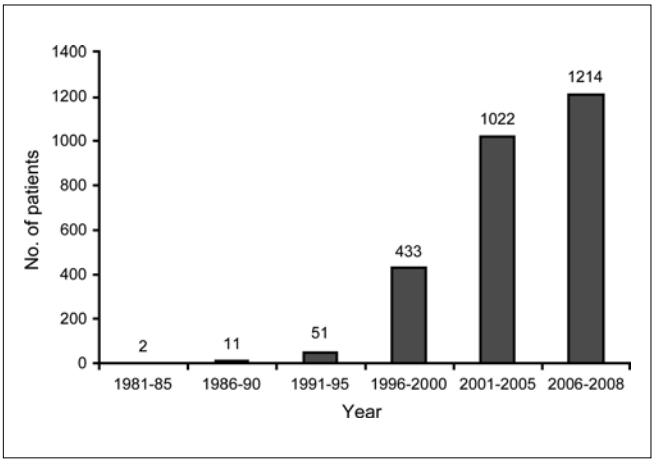

Figure 6: Dendrimer patents - An explosive growth

expressly focused on dendrimers. Based on US patent search, we found following potential areas and patent trends.[20, 21]

Synthesis drug delivery

- Biotechnology
- Nanotechnology
- Cosmetics

Comparison with linear polymer

Dendrimers have the unique properties unlike the traditional polymers. Because of their molecular architecture, dendrimers show some significantly improved physical and chemical properties when compared to traditional linear polymers. Dendrimer solutions have lower viscosity than linear polymers. In solution, linear chains exist as flexible coils; in contrast, dendrimers form a tightly packed ball. This has a great impact on their rheological properties. Dendrimer solutions have significantly lower viscosity than linear polymers.[22] When the molecular mass of dendrimers increases, their intrinsic viscosity goes through a maximum at the fourth generation and then begins to decline.[23] Comparison of dendrimers with linear polymers is shown in Table 1.

General synthetic approach of dendrimers

Synthesis approach of dendrimer were shown in Figure 7. Dendritic polymers or dendrimers are synthesized using a stepwise repetitive reaction sequence that guarantees a very highly monodisperse polymer, with a nearly perfect hyperbranched topology radiating from a central core and grown generation by generation.[24] The synthetic procedures developed for dendrimer preparation permit early complete control over the critical molecular design parameters such as size, shape, surface/interior chemistry, flexibility, and topology.

Divergent synthesis

In the divergent approach, the dendrimer is prepared from the core as the starting point and built up generation by generation as shown in Figure 8. In the divergent way, problems occur from an incomplete reaction of the end groups, since these structure defects accumulate with the buildup of further generation. As the side products possess similar physical properties, chromatographic separation is not always possible. Therefore, the higher generations of divergently constructed dendrimers always contain certain structural defects. To prevent side reactions and to force reactions to completion, a large excess of reagents is required; however, this causes some difficulties in the purification of the final product.

Convergent synthesis

The convergent approach starts from the surface and ends up at the core, where the dendrimer segments (dendrons) are coupled together as shown in Figure 9. In this way, only a small number of reactive sites are functionalized in each step, giving a small number of possible side-reactions per step. Therefore, each synthesized generation of dendrimers can be purified, although purification of high-generation dendrons becomes more cumbersome because of increasing similarity between reactants and formed product. However, with proper purification after each step, dendrimers without defects can be obtained by the convergent approach. On the other side, the convergent approach does not allow the formation of high generations because steric problems occur in the reactions of the dendrons and the core molecule.

General synthetic procedure for PAMAM dendrimer

The construction of an EDA-core PAMAM dendrimer consists of two consecutive steps: Michael addition of primary amine

Table 1: Comparison of dendrimers with linear polymers

Property	Dendrimers	Linear polymers
Structure	Compact, globular	Not compact
Architecture	Regular	Irregular
Size	Certain	Uncertain
Shape	Spherical	Random coil
Aqueous solubility	High	Low
Viscosity	Low	High
Reactivity	High	Low
Polydispersity	Monodisperse	Polydisperse
Architecture	Highly symmetric	Unsymmetric
Monomer units	Only 2	At least 20

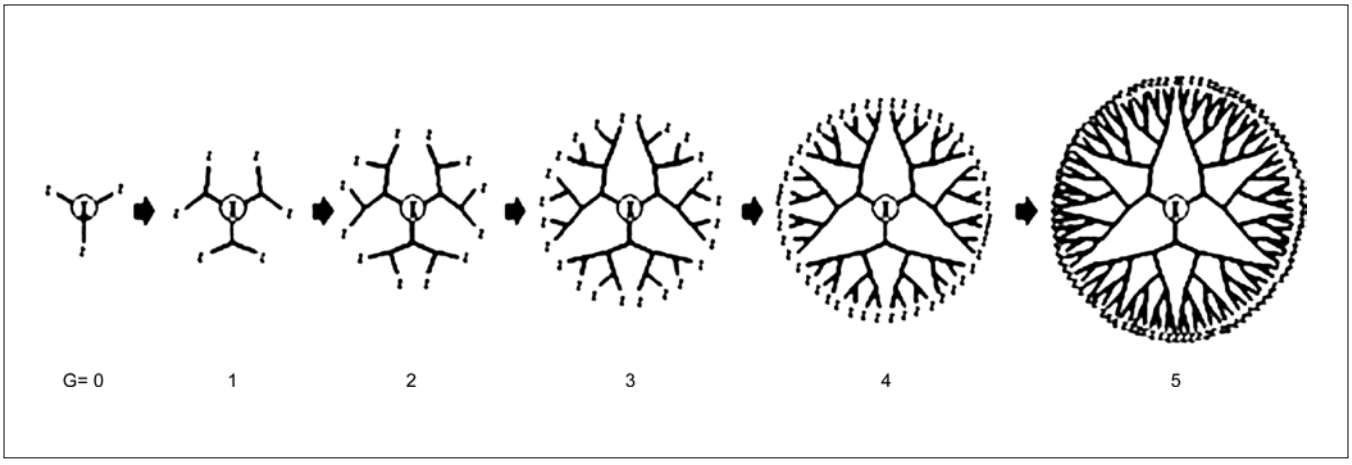

Figure 7: Synthesis of dendrimers

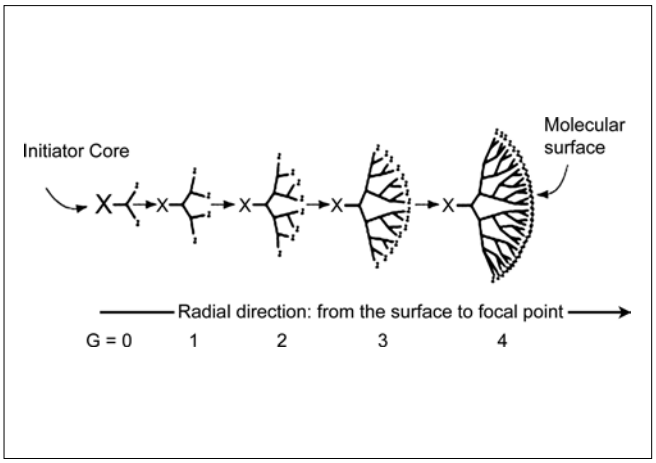

Figure 8: Divergent synthesis of dendrimer

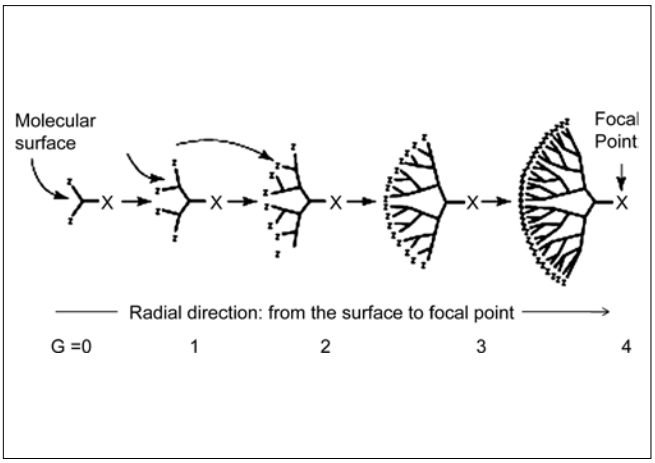

Figure 9: Convergent synthesis of dendrimer

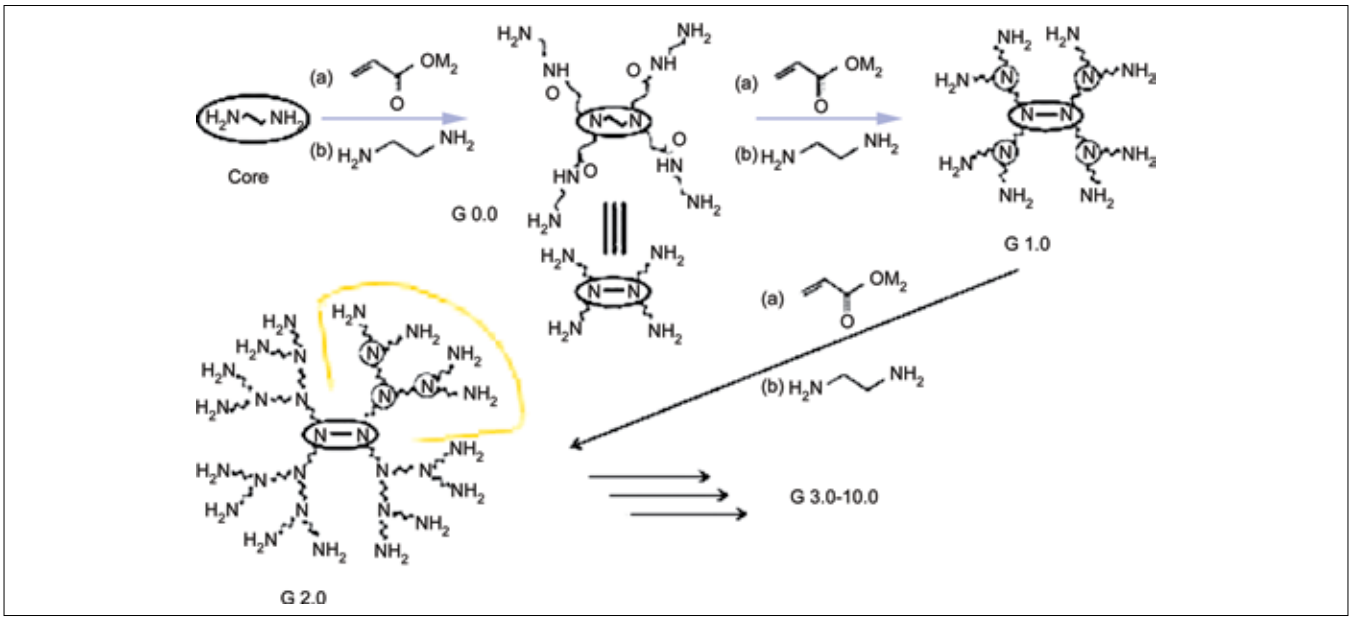

Figure 10: General synthesis of PAMAM dendrimer

(EDA in the very first step) to methyl acrylate followed by the amidation of the formed multiester (tetraester at the very beginning) with EDA[25,26] [Figure 10]. The tetraester is called PAMAM dendrimer of generation −0.5 (G-0.5) and the initial EDA itself may be considered as the generation G-1. The following amidation of the tetraester with EDA yields generation zero (G0) of the dendrimer with four terminal amino groups. The reiteration of this two-step procedure leads to higher dendrimer generations. Such a strategy of constructing dendrimers is known as the divergent synthesis method.

Surface modification of dendrimers

In order to increase water solubility, biocompatibility and to reduce toxicity, it is necessary to change the surface of the dendrimers. Different attempts have been made for the surface modification of dendrimers. Now a day, considerable effort has been dedicated to the preparation of dendrimers that are designed to be highly biocompatible and water soluble. In addition, some dendrimers have been designed to be biodegradable, e.g., polylysine.[27] Dendrimers having carbohydrate moiety at core or periphery have been widely explored and are emerging as promising immunological tools because of their multivalent binding capacity.[28] Grinstaff *et al*.[29] prepared different polyester dendrimers incorporating monomers such as glycerol, succinic acid, phenylalanine, etc. Bhadra *et al*.[30] synthesized PEGylated dendritic nanocarrier for the delivery of fluorouracil. The PEGylated dendrimer has shown to increase drug loading but reduced drug release and hemolytic toxicity of dendrimers.[31] Majoros studied the nature of the acetylation reaction and reported a method for the preparation of an acylated macromolecule, which can serve as a scaffold for complex dendrimeric structures. Acetylated G5 PAMAM dendrimer represents a more compact structure than the nonacetylated G5 PAMAM dendrimer. In

Ref,[32] the enhancement of gene transfer activity mediated by mannosylated dendrimer/alpha cyclodextrin activity conjugate has been studied. Jevprasesphant *et al*. prepared lauryl-dendrimer conjugates to enhance transepithelial transport and reduce cytotoxicity.[33]

Biocompatibility of dendrimers

Dendrimers have to exhibit low toxicity and be nonimmunogenic in order to be widely used in biomedical applications. To date, the cytotoxicity of dendrimers has been primarily studied *in vitro*; however, a few *in vivo* studies have been published. As observed for other cationic macromolecules, including liposomes and micelles, dendrimers with positively charged surface groups are prone to destabilize cell membranes and cause cell lysis. For example, *in vitro* cytotoxicity IC50 measurements (i.e., the concentration where 50% of cell lysis is observed) for amino-terminated PAMAM dendrimers revealed significant cytotoxicity on human intestinal adenocarcinoma Caco-2 cells. Furthermore, the cytotoxicity was found to be generation-dependent, with higher generation dendrimers being the most toxic. Some recent studies have shown that amino-terminated PAMAM dendrimers exhibit lower toxicity than more flexible amino-functionalized linear polymers perhaps due to lower adherence of the rigid globular dendrimers to cellular surfaces.[34] Comparative toxicity studies on anionic (carboxylate-terminated) and cationic (amino-terminated) PAMAM dendrimers using Caco-2 cells have shown a significantly lower cytotoxicity of the anionic compounds. In fact, lower generation PAMAM dendrimers possessing carboxylate surface groups show neither hematotoxicity nor cytotoxicity at concentrations up to 2 mg/ml. One way to reduce the cytotoxicity of cationic dendrimers may reside in partial surface derivatization with chemically inert functionalities such as PEG or fatty acids. A partial derivatization with as few as six lipid chains or four PEG chains on a G4-PAMAM, respectively, was sufficient to lower the cytotoxicity substantially. Only a few systematic studies on the *in vivo* toxicity of dendrimers have been reported so far. Upon injection into mice, doses of 10 mg/kg of PAMAM dendrimers (up to G = 5), displaying either unmodified or modified amino-terminated surfaces, did not appear to be toxic. Hydroxy-or methoxy-terminated dendrimers based on a polyester dendrimer scaffold have been shown to be of low toxicity both *in vitro* and *in vivo*. At very high concentrations (40 mg/ml), these polyester dendrimers induced some inhibition of cell growth *in vitro* but no increase in cell death was observed. Upon injection into mice, no acute or long-term toxicity problems were observed. The nontoxic properties make these new dendritic motifs very promising candidates for drug delivery devices.[35]

General characterization of dendrimers

Brief history of characterization of dendrimers

The earliest work on dendrimer characterization was concerned with aspects of the organic chemistry. The development of mass spectroscopic techniques such as matrix assisted laser desorption (MALDI) and electrospray mass spectrometry has allowed the absolute determination of dendrimer perfection.[36]

Hydrodynamic sizes from intrinsic viscosity (IV), gel permeation chromatography (GPC), and holographic relaxation spectroscopy (HRS) map the changes in size with generation. Light scattering techniques measure the radius of gyration (Rg) of dendrimers, which is an average of the spatial arrangement of spatial distribution of all the units. Transmission electron microscopy has been used to image individual dendritic molecules, usually the larger generations. More recently, atomic force microscopy has also been used to image dendritic molecules.

Spectroscopy and spectrometry

UV-Vis method

UV-Visible spectroscopy can be used to monitor the synthesis of dendrimers. The intensity of the absorption band is essentially proportional to the number of chromophoric units. UV-Vis has been used also to define morphological information.[37]

Infra-red spectroscopy

Infra-red (IR) spectroscopy is mainly used for the routine analysis of the chemical transformations occurring at the surface of dendrimers, such as the disappearance of nitrile groups in the synthesis of PPI dendrimers.[38]

Nuclear magnetic resonance spectroscopy

Routine Nuclear magnetic resonance (NMR) analyses are especially useful during the step-by-step synthesis of dendrimers, even up to high generations, because they afford information about the chemical transformations undergone by the end groups. Special techniques of NMR, e.g.,[13] C NMR etc have also been used to probe their size and morphology.[39]

Fluorescence

The high sensitivity of fluorescence has been used to quantify defects during the synthesis of dendrimers. When a fluorescent unit is attached to the core of a dendrimer or dendron, changes in the fluorescence spectra resulting from changes in size and shape are observed after a certain generation.[40]

Mass spectrometry

Due to their mass limitation, classical mass spectrometry techniques such as chemical ionization or fast atom bombardment (FAB) can be used only for the characterization of small dendrimers, whose mass is below 3000 Da. For higher molecular weights, techniques developed for the characterization of proteins and polymers have to be applied.[41]

Scattering techniques

Small angle X-ray scattering (SAXS) technique is often used for the characterization of polymers can be applied to dendrimers to obtain information about their average radius of gyration (Rg) in solution. The intensity of the scattering as a function of angle also provides information on the arrangement of polymer segments and segment density distribution within the molecule.[42]

Microscopy

Two types of microscopy, different in principles, have been used for imaging dendrimers. In transmission microscopy, electrons or light produce images that amplify the original, with a resolution ultimately limited by the wavelength of the source. In scanning microscopy such as Atomic Force Microscopy (AFM), the images are produced by touch contact Q at a few angstroms of a sensitive cantilever arm with the sample. Visualizing single molecules by optical microscopy has been successfully carried out for dendrimers having a fluorescent core.[43]

Size exclusion chromatography

Size exclusion (or gel permeation) chromatography allows the separation of molecules according to size. A detector such as a differential refractive index or a LLS detector (see above) is connected to the size exclusion chromatography (SEC) apparatus for the determination of the polydispersity, which is generally very close to unity. Most types of dendrimers were characterized by SEC, even self-assembled dendrimers.[44]

Electrical techniques

Electron paramagnetic resonance

Electron paramagnetic resonance was used for the quantitative determination of the substitution efficiency on the surface of PAMAM dendrimers or was able to detect interactions between the end groups of large PPI dendrimers.[45]

Electrochemistry

Electrochemistry may afford mainly three types of information concerning the structure of dendrimers. Exhaustive coulometry has been used to measure the number of electroactive groups, in most cases ferrocenes, linked to the surface of PPI, poly (aryl ether), and also for naphthalene groups linked to PAMAM dendrimers.[46]

Electrophoresis

Gel electrophoresis is widely used in biology for the routine analysis and separation of biopolymers such as proteins and nucleic acids. This technique was used for the assessment of purity and homogeneity of several types of water-soluble dendrimers such as PAMAM dendrimers having NH_3^+ or COO^- end groups. Gel electrophoresis is also used for studying the interaction between positively charged dendrimers and DNA in view of transfection experiments. It was found that complex formation depends both upon the generation (size), and the charge ratio for PAMAM dendrimers.[47]

Rheology, physical properties

Intrinsic viscosity

Rheology and particularly dilute solution viscosimetry studies can be used as analytical probe of the morphological structure of dendrimers.[48] Dendrimers should exhibit a maximum in the dependence of the intrinsic viscosity [g] on generation, because the volume grows faster with generation than the molecular weight for the first generations, whereas the contrary occurs after a certain generation. This behavior is experimentally observed for several series of dendrimers.

Differential scanning calorimetry

The Differential scanning calorimetry (DSC) technique is generally used to detect the glass transition temperature (Tg), which depends on the molecular weight, entanglement and chain-end composition of polymers. The Tg is affected by the end group substitutions, and molecular mass DSC and Temperature modulated calorimetry (TMC) can also used to detect physical aging of dendrimers.

Miscellaneous

Of course, elemental analyses are usually performed with dendrimers, but due to their repetitive structure, this technique is uninformative, especially for high generations. Some techniques have been rarely used for characterizing dendrimers. Among them, one can cite sedimentation for lactosylated PAMAM dendrimers, and PMMH dendrimers, or titrimetry to determine the number of NH_2 end groups of PAMAM dendrimers.

Applications

There are following precise reasons which make the dendrimer an effective carrier for the drug delivery system.

- Forms uniform shell structure.
- Can be precisely designed and manufactured, permits tunable solubility, low toxicity and bioattachment capability.
- Possibilities of adjusting physical and chemical properties by altering chemistry.

Dendrimers in drug delivery

Dendrimer-drug conjugates

Drug molecules can be either chemically conjugated to the dendrimer surface or physically encased inside a dendrimer core. For chemical conjugations, a good coupling efficiency may be achieved if functional groups are activated prior to coupling. Hydroxyl (OH), carboxyl (COOH), primary amine (NH_2), thiol (SH) and guanidino are commonly found functional groups in drug molecules and polymers. Hydroxyl groups can be converted to active intermediates that favor nucleophilic reactions. For example, coupling hydroxyl groups with primary amine groups causes primary amine groups to form secondary amines or stable carbamate bonds. Amides are relatively stable in basic, acidic and enzymatic conditions. In some cases, direct coupling may lead to the deactivation of drugs, so that the conjugated drugs (i.e., prodrugs) will not have any pharmaceutical effect until they are cleaved from the bonding.[49] PAMAM dendrimers form conjugates with 5FU, which are water soluble and releases free 5FU slowly on hydrolysis of the conjugate, thereby reducing the toxicity.[50]

Site specific delivery

Dendrimers can be used as coating agents to protect or deliver drugs to specific sites in the body or as time-release

vehicles for biologically active agents. Well defined and macromolecular structure of dendrimer offers the polyvalent characteristic. Through polyvalent interactions with receptors and binding sites, dendrimers may be designed to achieve higher activity than small molecules. In addition, dendrimers may be constructed and modified to have longer duration of action, reduced side effects and other beneficial effects compared with currently available pharmaceuticals. Branched poly (l-glutamic acid) chains were centered around PAMAM dendrimers to create new biodegradable polymers with improved biodistribution and targeting ability. These constructs were surface-terminated with poly (ethylene glycol) chains to enhance their biocompatibility and folic acid receptors to introduce cell-specific targeting.[51]

Solubility enhancer

PAMAM dendrimers possess empty internal cavities that can encapsulate hydrophobic guest molecules in the macromolecule interior. Drugs or other molecules can either be attached to dendrimers' end groups or encapsulated in the macromolecule interior. These specific properties make dendrimers suitable for drug delivery systems. Dendrimers can be used as the carriers to increase the solubility of drug.[6] The effect of PAMAM dendrimer generation, its size and surface functional groups on the aqueous solubility, and therefore, the bioavailability of nifedipine has been studied.[52] The solubility enhancement of nifedipine was higher in the presence of ester-terminated dendrimers than their amino-terminated analogues possessing the same number of surface groups.

Transdermal drug delivery

PAMAM dendrimers enhanced the bioavailability of indomethacin in transdermal delivery applications.[52] Wang *et al*.[54] reported the utilization of polyhydroxyalkanoate and G 3 PAMAM dendrimers as Transdermal drug delivery (TDDS). Cheng *et al*.[55] investigated TDDS for anti-inflammatory drugs and concluded that the bioavailability of anti-inflammatory drugs was increased; it may be due to the facilitated skin penetration of such drugs.

Ocular dug delivery

Vandamme and Broberck[56] have reported the development of ophthalmic vehicles in ocular drug delivery using PAMAM dendrimers for pilocarpine nitrate. They found that there is more ocular residence time and significantly increased bioavailability by using PAMAM dendrimers.

Starpharma[57] has discovered a large number of dendrimer compounds that have demonstrated efficacy against a range of major diseases. Starpharma's lead vaginal microbicide compound is expected to be the first dendrimer, and perhaps more significantly, the first synthetic nanosized structure, to be used in pharmaceutical products for human use. Starpharma is presently carrying out research on the possible use of dendrimers for the treatment of sexually transmitted diseases, malaria, hepatitis B, HIV, dengue virus type 2 and respiratory infections by respiratory syncytial viruses, Influenza A and B and Adenovirus, etc.

Dendrimers in gene transfection

Dendrimers can act as carriers, called vectors, in gene therapy. Vectors transfer genes through the cell membrane in to the nucleus. Currently, liposomes and genetically engineered viruses have been mainly used for this. PAMAM dendrimers have also been tested as vectors.[58] Dendrimers are very actively under investigation for the delivery of DNA and small organic molecule drugs, especially for cancer therapy. PAMAM and PPI dendrimers have been studied thoroughly as vectors for gene delivery, enhancing the transfection efficiency DNA.[59] It is noteworthy that dendrimers of high structural flexibility and partially degraded high-generation dendrimers (i.e., hyperbranched architectures) appear to be better suited for certain gene delivery operations than intact high-generation symmetrical dendrimers. Perhaps, this is due to their enhanced flexibility, which allows the formation of more compact complexes with DNA. The maximum transfection efficiency is obtained because of net positive charge on the complexes (i.e., an excess of primary amines over DNA phosphates).[60]

Dendrimers as imaging agents

Macromolecular contrast agents have become very important tools of modern diagnostic medicine. An early application of dendrimer to imaging technology was disclosed in the US patent.[61] 'Bone seeking Tc-99M complexes of phosphonate derivatives of "polyamidoamines." The patent discloses the new stable complexing agent for radionucleotide-derivatized phosphonate dendrimers imaging the skeletal system in mammals. Dendrimers provide multiple binding sites on the periphery, allowing many magnetic resonance imaging (MRI) contrasting agent complexes to attach to them. One dendrimer molecule can host up to 24 contrasting agent complexes (depending on generation), thereby attaining a higher signal to noise ratio.[62]

Dendrimers as biomimetic artificial proteins

Based on their dimensional length scaling, narrow size distribution, and other biomimetic properties, dendrimers are often referred to as artificial proteins.[63] Within the PAMAM family, dendrimers closely match the sizes and contours of many important proteins and bioassemblies. For example, insulin (3 nm), cytochrome C (4 nm), and hemoglobin (5.5 nm) are approximately the same size and shape as ammonia-core PAMAM dendrimers generations 3, 4 and 5, respectively. Furthermore, G5 and G6 PAMAM dendrimers have diameters approximately equivalent to the thickness of lipid bilayer membranes (~5.5 nm) of biological cells, while a generation 2 dendrimer matches the width (2.4 nm) of DNA duplexes. These duplexes form stable complexes with histone clusters to condense and store DNA within the nucleosome of cells. Undoubtedly, the close match in size and shape between histone clusters and PAMAM dendrimers of generations 7-10 accounts for the extraordinary stability of DNA-PAMAM complexes, as well as the enhanced

gene expression observed for these histone mimics compared with lower generation (G = 1-5) PAMAM dendrimers.

Miscellaneous

Catalysis and reaction sites

Dendrimers have nanoscopic cavities that act like a microenvironment for molecular reactions. The cavities provide nanoscale reactor sites for catalysis. There are two possible catalytic sites being investigated, one at core and the other at the surface.[64]

Nanocomposites

It was discovered that PAMAM dendrimer forms stable interior nanocomposites with metal cations, zerovalent metals, other electrophilic ligands and semiconductor particles. These materials are actively being investigated in electronics, optoelectronics.[65]

Nanodevices

The characteristic nontoxicity of PAMAM dendrimers to biological systems makes their biocompatibility considerably greater than that of many other materials currently researched for use as controlled, chemotherapeutic drug delivery systems.[66] The multifunctionality and biocompatibility of dendrimer-based nanodevices are crucial for the development of targeted drug delivery technology. Multifunctional cancer therapeutic nanodevices have been designed and synthesized using the PAMAM dendrimer as a carrier. Partial acetylation of amine-terminated PAMAM dendrimer can be used to neutralize a fraction of the primary amino groups, provide enhanced solubility of the dendrimer during the conjugation reaction of fluorescein isothiocyanate (FITC) in dimethyl sulfoxide (DMSO), and prevent nonspecific targeting interactions (*in vitro* and *in vivo*) during delivery.

Cross linking agent

Dendritic macromolecules are ideal candidates as cross-linking agents. Newly emerging dendritic cross-linked hydrogels can be made by using multifunctional dendrimers as cross-linking agents. The number and hence the density of the junctions can be controlled by varying the generation of the dendrimer.

CONCLUSION

The high level of control over the architecture of dendrimers, their size, shape, branching length and density, and their surface functionality, makes these compounds ideal carriers in biomedical application such as drug delivery, gene transfection and imaging. The bioactive agents may either be encapsulated into the interior of the dendrimers or they may be chemically attached or physically adsorbed onto the dendrimer surface with the option to tailor the properties of the carrier to the specific needs of the active material and its therapeutic applications. Furthermore, the high density of surface groups allows attachment of targeting groups as well as groups that modify the solution behavior or toxicity of dendrimers. Surface-modified dendrimers themselves may act as nanodrugs against tumors, bacteria, and viruses. This review of biomedical applications of dendrimers clearly illustrates the potential of this new "fourth architectural class of polymers" and substantiates the high optimism for the future of dendrimers in this important field.

REFERENCES

1. Gupta U, Agashe HB, Jain NK. Polypropylene Imine: Dendrimer Mediated Solubility Enhancement: Effect of pH and Functional Groups of Hydrophobes. J Pharm Pharmaceut Sci 2007;10:358-67.
2. Devarakonda B, Hill RA, Liebenberg W, Brits M, De Villiers MM. Comparison of aqueous solubilization of practically insoluble niclosamide by polyamidoamine (PAMAM) dendrimers and cyclodextrins. Int J Pharm 2005;300:193-209.
3. Devarakonda B, Li N, De Villiers M. Effect of Polyamidoamine (PAMAM) Dendrimers on the *in vitro* release of water-insoluble nifedipine from aqueous gels. AAPS PharmSciTech 2005;6:504-12.
4. Liu M, Kono K, Frechet JM. Water-soluble dendritic unimolecular micelles: Their potential as drug delivery agents. J Control Release 2000;65:121-31.
5. D`Emanuele A, Jevprasesphant JP, Penny J, Attwood D. The use of a dendrimer propranolol prodrug to bypass efflux transporters and enhance oral bioavailability. J Control Release 2004;95:447-53.
6. Milhem OM, Myles C, McKeown NB, D'Emanuele A. Polyamidoamine Starburst dendrimers as solubility enhancers. Int J Pharm 2000;197:239-41.
7. Beezer AE, King AS, Martin IK, Mitchel JC, Twyman LJ, Wain CF. Dendrimers as potential drug carriers;encapsulation of acidic hydrophobes within water soluble PAMAM derivatives. Tetrahedron 2003;59:3873-80.
8. Khopade AJ, Caruso F, Tripathi P, Nagaich S, Jain NK. Effect of dendrimer on entrapment and release of bioactive from liposomes. Int J Pharm 2002;232:157-62.
9. Chauhan AS, Sridevi S, Chalasani KB, Jain AK, Jain SK, Jain NK, *et al.* Dendrimer-mediated transdermal delivery: Enhanced bioavailability of indomethacin. J Cont Rel 2003;90:335-43.
10. Asthana A, Chauhan AS, Diwan PV, Jain NK. Poly (amidoamine) (PAMAM) Dendritic Nanostructures for Controlled Site-specific Delivery of Acidic Anti-Inflammatory Active Ingredient. AAPS PharmSciTech 2005;13:200-8.
11. Bhadra D, Bhadra S, Jain S, Jain NK. A PEGylated dendritic nanoparticulate carrier of fluorouracil. Int J Pharm 2003;257:111-24.
12. Tomalia DA. Dendritic macromolecules: Synthesis of starburst dendrimers. Macromolecules 1986;19:2466-7.
13. Kim C. Advanced pharmaceutics: Physicochemical principles. New York: CRC Press;p. 409-12.
14. Esfand R, Tomalia DA. Poly (amidoamine) (PAMAM) dendrimers: From biomimicry to drug delivery and biomedical applications. Drug Discov Today 2001;6:427-36.
15. Chauhan AS, Jain NK, Diwan PS, Khopade AS. Solubility enhancement of indimethacin with PAMAM dendrimer and targeting to inflammatory regions of arthritic rats. J Drug Targeting 2004;12:575-83.
16. Tomalia DA. Birth of a new macromolecular architecture: Dendrimers as quantized building blocks for nanoscale synthetic polymer chemistry. Prog Polym Sci 2005;30:294-324.
17. Jain NK. Advances in controlled and novel drug delivery: Delhi: CBS Publishers and Distributors;2000. p. 361-70.
18. Available from: http://www.dnanotech.com. [last accessed January 2006].
19. Available from: http://www.pubmed.com. [last accessed March 2000].
20. Available from: http://www.uspto.gov. [last accessed September 2005].
21. Rutt SJ, Maebus SB. 3rd annual BBC conference on nano/bio convergence. Cambridge;Foley and Lardner LLP;2004. p. 1-5.
22. Pushkar S, Philip A, and Pathak K. Dendrimers: Nanotechnology derived

novel polymers in drug delivery. Ind J Pharm Edu Res 2006;40:153-7.
23. Barbara K, Maria B. Dendrimers: Properties and application. Acta Biochemica Polinica 2001;48:199-208.
24. Newkome GR. Dendrimers and dendrons: Concept, Synthesis, Applications. New York: Wiley- VCH;2001. p. 44, 51, 68, 61
25. Tomalia DA, Svenson S. Dendritic macromolecules: Synthesis of starburst dendrimers. Macromolecules 1986;19:2466-7.
26. Peterson J, Ebber A, Veiko A. Synthesis and CZE analysis of PAMAM dendrimers with an ethylenediamine core. Proc Estonian Acad Sci Chem 2001;50:156-66.
27. Denkewalter RG. Macromolecular highly branched diaminocarboxylic acids. Chem Abstr 1984;100:103907.
28. Bezouska K. Design, functional evaluation and biomedical applications of carbohydrate dendrimers (glycodendrimers). J Biotechnol 2002;90:269-90.
29. Grinstaff MW. Biodendrimers: New polymeric biomaterials for tissue engineering. Chemistry 2002;8:2839-46.
30. Bhadra S, Bhadra D, Jain NK. PEGylated Dendritic nanoparticulate carrier of fluorouracil. Int J Pharm 2003;257:111-24.
31. Istvan JM, Scott W, Baker JR. Acetylation of Poly (amidoamine) Dendrimers. Macromolecules 2003;36:5526-9.
32. Uekama K, Chihara, Y, Koki, W, Enhancement of gene transfer activity mediated by mannosylated dendrimer/alpha cyclodextrin activity conjugate. J Control Release 2006;116:64-74.
33. Jevprasesphant R, Penny F, Attwood D, McKeown NB. Engineering of dendrimer surfaces to enhance transepithelial transport and reduce cytotoxicity. Pharma Res 2003;20:1543-50.
34. Svenson S, Tomalia DA. Dendrimer Biomedical applications reflection on field. Adv Drug Del Rev 2005;57:2106-29.
35. Duncan R, Izzo L. Dendrimer biocompatibility and toxicity. Adv Drug Del Rev 2005;57:2215-37.
36. Frechet JM, Tomalia DA. Dendrimers and other dendritic polymers, Wiley series in polymer science. John Wiley and Sons. 2004. p. 256-60.
37. Achar S. Organoplatinum dendrimers formed by oxidative addition. Angew Chem Int Ed Engl 1994;33:847-9.
38. Liu Z, Michael A. FT-IRAS spectroscopic studies of the interaction of avidin with biotynylated dendrimer surfaces. Colloids and surfaces. Biointerfaces 2004;35:197-203.
39. Miller TM, Kwock EW, Neenan TX. Synthesis of four generations of monodisperse aryl ester dendrimers based on 1, 3,5-benzenetricarboxylic acid. Macromolecules 1992;25:3143-8.
40. Wilken R, Adams J. End group dynamics of fluorescently labeled dendrimers. Macromol Rapid Commun 1997;18:659-65.
41. Hummelen JC, Van JL Dongen, Meijer EW. Electrospray mass spectrometry of poly (propylene imine) dendrimers-the issue of dendritic purity or polydispersity. Chem Eur J 1997;3:1489-93.
42. Rietveld IB, Smit AM. Colligative and viscosity properties of poly (propylene imine) dendrimers in methanol. Macromolecules 1999;32:4608-14.
43. Hofkens J, Verheijen W, Shukla R. Detection of a single dendrimer macromolecule with a fluorescent dihydropyrrolopyrroledione (DPP) core embedded in a thin polystyrene polymer film. Macromolecules 1998;31:4493-7.
44. Zeng F. Supramolecular polymer chemistry: Design Synthesis, characterization and kinetics, thermodynamics, and fidelity of formation of self assembeled dendrimers. Tetrahedron 2002;58:825-43.
45. Bossman AW, Janseen Meijer EW. Five generations of nitroxyl-functionalized dendrimers, Macromolecules 1997;30:3606-11.
46. Brothers HM. Slab gel and capillary electrophoresis characterization of poly amidoamine dendrimers. J Chromatogram 1998;A814:233-46.
47. Shi X, Patri AK, Lesniak W, Islam MT, Zhang C, Baker JR Jr, *et al.* Analysis of poly (amidoamine) succinamic acid dendrimers by slab-gel electrophoresis and capillary sone electrophoresis. Electrophoresis 2005;26:2960-7.
48. Caminade AM. Characterization of dendrimers, Adv Drug Deliv Rev 2005;57:2130-46.
49. Yang H, Kao WJ. Dendrimers for pharmaceutical and biomedical applications. J Biomater Sci Polymer Edn 2006;17:3-19.
50. Zhuo RX. *In vitro* release of 5-fluorouracil with cyclic core dendritic polymer. J Control Release 1999;57:249-57.
51. Tansey W, Cao XY, Pasuelo MJ, Wallace S. Synthesis and characterization of branched poly (l-lutamic acid) as a biodegradable drug carrier. J Control Release 2004;94:39-51.
52. Devarakonda B, Hill RA, Villiers MM. The effect of PAMAM dendrimer generation size and surface functional group on the aqueous solubility of nifedipine. Int J Pharm 2004;284:133-40.
53. Chauhan AS, Jain AK, Jain NK. Dendrimer-mediated transdermal delivery: Enhanced bioavailability of indomethacin. J Control Release 2003;90:335-43.
54. Wang ZX, Yoshiaki I, Yoshifumi H. Mechanism of enhancement of effect of dendrimers on transdermal permeation through polyhydroxyalkanoate matrix. J Bioscience Bioeng 2003;96:537-40.
55. Cheng YY, Man N, Xu TW. Transdermal delivery of nonsteroidal anti inflammatory drugs mediated by PAMAM dendrimers. J Pharm Sci 2006;96:595-602.
56. Vandamme TF, Broberck L. PAMAM dendrimers as ophthalmic vehicles for ocular delivery of pilocarpine nitrate and tropicamide. J Control Release 2005;102:23-38.
57. Available from: http://www.starpharma.com.[last accessed December 2007].
58. Davis FJ. Polymer Chemistry A practical approach G.R. Oxford: Oxford University Press;2004. p. 188-98.
59. Bielinska AU, Chen C, Johnson J, Baker JR. DNA complexing with polyamidoamine dendrimers: Implications for transfection. Bioconjug Chem 1999;10:843-50.
60. Choi JC, Nam K, Park JY. Enhanced transfection efficiency of PAMAM dendrimers modification with L-arginine. J Control Release 2004;99:445-56.
61. Fahlvik, Simon. Ultrafine lightly coated superparamagnetic particles for MRI .US patent 4606907.
62. Sheela DK, Michael A, Steven W. Specific targeting of folate-dendrimer MRI contrast agents to the high affinity folate receptor expressed in ovarian tumor xenografts. MAGMA 2001;12:104-13.
63. Svenson S, Tomalia DA. Dendrimer Biomedical applications reflection on field. Adv Drug Del Rev 2005;57:2106-29.
64. Chakib H, Rainer H. Hyperbranched polymers as platforms for catalysts. Top Organomet Chem 2006;20:149-76.
65. Esumi K, Matsumoto T, Seto Y. Preparation of gold-gold/silver-dendrimer nanocomposites in the presence of benzoin in ethanol by UV irradiation. J Colloid Interface Sci 2005;284:199-203.
66. Istvan JM, Thommey PT, Chandan B. Poly (amidoamine) dendrimer-based multifunctional engineered nanodevice for cancer therapy. J Med Chem 2005;48:5892-9.

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