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# DENDRIMERS FOR CANCER THERAPY: A REVIEW

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**Abstract:** There are a variety of nanoparticles systems currently being explored for cancer therapeutics. The properties of each nano particle system have been developed to enhance the delivery to the tumour cells. The types of nanoparticles currently used in research for cancer therapeutic applications include dendrimers, liposome, polymeric nano particles, micelles, Protein nanoparticles, ceramic nanoparticles, viral nanoparticles, metallic nanoparticles, carbon nanotubes.

**Keywords:** Dendrimers, Nanoparticles, Controlled drug delivery, Polymer

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## INTRODUCTION

### Nanotechnology and targeted drug delivery

Nanoscale devices are 100 to 10000 times smaller than human cells but are similar in size to large bio molecules such as enzymes and receptors. Nanoscale devices smaller than 50nm can easily enter most of the cells and those smaller than 20nm can move out of the blood vessels and circulate throughout the body. Thus Nanodevices are suitable to serve as targeted drug delivery vehicles to carry large doses of chemotherapeutic agents or therapeutic genes into malignant cells sparing healthy cells. The effectiveness of a cancer therapeutic device is measured by its ability to reduce and eliminate tumours without damaging the healthy tissue. Therefore, a distinct capacity to target tumours is essential in the success of a therapeutic device. An increased site specificity and internalisation can improve the efficacy of the treatment and decrease the possibility of side effects that cancer patients often experience. The ultimate goal of the cancer therapeutics is to increase the survival time and the quality of life of the patient. Thus nanoparticle systems offer major improvement in therapeutics through site specificity and their ability to escape from multi-drug resistance, and the efficient delivery of an agent.

### Current nanoparticle systems for cancer therapies

There are a variety of nanoparticles systems currently being explored for cancer therapeutics. The properties of each nano particle system have been developed to enhance the delivery to the tumour cells. The types of nanoparticles currently used in research for cancer therapeutic applications include dendrimers, liposome, polymeric nano particles, micelles, Protein nanoparticles, ceramic nanoparticles, viral nanoparticles, metallic nanoparticles, carbon nanotubes.

### Polymers in controlled drug delivery system

### Polymer

The term “polymer” comes from the Greek word meaning “many parts”. Polymers are macromolecules composed of repeated structural units (monomers) connected by covalent chemical bonds. This specific molecular structure (chain like) is responsible for its mechanical properties. They have better physical, chemical and biological properties for their efficient drug delivery.

### Classification of polymers

**1. Based on chemical structure:**

- Linear polymer : consists of long chain monomers.
- Branched polymer : consists of branches covalently attached to the main chain.

- Cross linked polymers : consists of monomers cross linked to form a giant macromolecule. Two types of cross linked polymers.

1. Elastomers: Loosely cross linked networks.

2. Thermosets: densely cross linked networks.

**2. Based on chemical type of monomers:**

- Homopolymers : consists of monomers of same type.
- Copolymers : consists of different repeating units of monomers

**3. Based on arrangement of monomers:**

- Random copolymers : Two or more different repeating units of monomers distributed randomly.
- Alternating copolymers : made of alternating sequence of different monomers.
- Block copolymers : Long sequences of monomers.
- Graft copolymers : consists of chain made of one type of monomer with branches of another.

**4. Polymers based on back bone:**

- Polymers with carbon chain back bone: Polyethylene, Polypropylene, Polystyrene Poly vinyl chloride, Polyacrylamide.
- Polymers with hetero chain back bone: Poly ethylene oxide,Poly propylene oxide, pectinic acid.

**5. Natural or synthetic polymers:**

- **Natural polymers:**

✓ Protein based : Albumin, Collagen, Gelatin Etc.

✓ Poly saccaharides : Agarose, Alginate, Chitosan, Dextran, Cyclo dextrin's etc.

- **Synthetic polymers:**

✓ **Bio degradable:**

➢ Polyesters : Polylacticacid, Polyglycolicacid, etc

➢ Polyandrides : Polyadipic acid, Polysebacic acid etc.

➢ Polyamides : Polyiminocarbonates, Polyamido acids etc.

➢ Phosphorus based: Polyphosphates, Polyphosphonates etc.

➢ Others : Polycyanoacrylates, Polyurethanes, Polyortho esters

✓ **Non-biodegradable:**

➢ Cellulose derivatives: Carboxymethyl cellulose, Ethylcellulose etc.

➢ Silicones : Polydimethylsiloxane, colloidal silica etc.

Sixty million people patients benefit from advanced drug delivery systems today, receiving safer and more effective doses of the medicine, to fight with a variety of human ailments including cancer.

Controlled drug delivery occurs when a polymer, whether natural or synthetic, is judiciously combined with a drug or other active agent in such a way that the active agent is released from the material in a predesigned manner. The release of the active agent may be constant over a long period, it may be cyclic over a long period, or it may be triggered by the environment or other external events. These act by controlling the diffusion of drugs by (1) controlling the movement of molecules through the polymer matrix (2) controlling the movement of dissolved drug molecules through channels or pores in a matrix permeated by the dissolution medium

A large number of both natural and synthetic polymers are used for application in controlled drug delivery. The advantage of synthetic polymers is their advantageous properties and wide choice of availability. Two synthetic poly vinyl pyrrrolidine, polyethylene glycol acrylate based hydrogels are developed for many biomedical applications because of their ability to form copolymers with macromolecules and biocompatibility. On the other hand natural polymers have the advantage of high compatibility and immunogenicity. Collagen and gelatin composites are widely used as carriers in controlled drug delivery.

In addition to their application in controlled drug delivery, polymers are used in many other areas in medical research particularly in the development of surgical sutures, medical prosthetics etc.

**Dendrimers**

Dendrimers are hyper branched macromolecules with definite molecular weight, shape, size and with a high degree of surface functionality and versatility and less than five nanometres in length. The word "***dendrimer***" originated from two words, the Greek word '***dendron***', meaning tree, and '***meros***' means part. Dendrimers are also called as cascade molecules. Dendrimer chemistry was first introduced in 1978 by Fritz vogtle and co-workers [(1)]. He synthesized the first "cascade molecules". In 1985, Donald.A.Tomalia, first synthesized the first generation of dendrimers. They are often been referred to as the "Polymers of the 21$^{st}$ century".

**Production of dendrimers**

Dendrimers are formed using a nano-scale, multistep fabrication process. Each step results in a new "generation" that has twice the complexity of the previous generation - a first generation dendrimer is the simplest, a tenth generation dendrimer is the most complex and can take months to engineer. Donald Tomalia, a researcher working for chemical giant Dow, first synthesized and named dendrimers in 1979. Commercial

development of dendrimers has been slow because of the difficulty of scaling-up production and because their cost is prohibitively high. Diagnostic-grade, tenth generation dendrimers go for US$1,650/100mg. A new, copper-catalyzed process for dendrimer synthesis announced in 2004 has reportedly increased yields. Dendritic Nanotechnologies has reportedly filed for patents on a new, one-step process to synthesize dendrimers, which could potentially drive down the cost of production.

**Properties of dendrimers**

Because of their precise architecture and construction, dendrimers possess inherently valuable physical, chemical and biological properties. These properties include:

- Efficient membrane transport - Dendrimers have demonstrated rapid transport capabilities across biological membranes.
- High loading capacity - Dendrimer structures can be used to carry and store a wide range of metals, organic or inorganic molecules by encapsulation and absorption.
- High uniformity and purity - The synthetic process used produces dendrimers with uniform sizes, precisely defined surface functionality, and very low impurity levels.
- Low toxicity - Most dendrimer systems display very low cytotoxicity levels.
- Low immunogenicity- Dendrimers commonly manifest a very low or negligible immunogenic response when injected or used topically.

**Benefits of dendrimers**

- They are synthesised as a single molecular entity having high structural and chemical homogeneity.
- They offer precisely controlled macromolecular surface, with a far lower cost base than proteins.
- New properties emerge when compounds are multiply presented on a dendrimer compared to a single presentation (“polyvalency”).
- They have broad applicability to interfere with protein-protein interactions.
- They can be used to precisely control pharmacokinetics of drugs.
- They provide a scaffold for attachment of multiple functional elements in precise ratios and positions.

**Opportunities for dendrimers**

Dendrimers are “stealth molecules” that have many potential applications, including diagnostic and therapeutic applications. By customizing and controlling dendrimer “architecture,” nanotechnologists are developing dendrimers for drug delivery,

diagnostic imaging and as carriers of genetic material. Dendrimers can easily move across biological membranes and they can store a wide range of metals, organic or inorganic molecules among their branches. Companies developing these synthetic molecules claim that most dendrimers don't trigger the immune system when injected or used topically, and have low cytotoxicity (that is, toxicity to cells). However, some forms of dendrimers can induce clotting in the bloodstream - a potential concern for *in vivo* applications[2].

Dendrimers could also be used in coatings and materials, electronics and photonics. A look at the patent assignees for dendrimer technology reveals the wide range of potential applications - patents are assigned to chemical, petroleum, tire, cosmetics and pharmaceutical companies, among others.

Dendrimer-based products (and those in the pipeline) include, for example:

- A dendrimer-based tool for detecting cardiac damage is being developed by Dade Behring, one of the world's largest medical diagnostic firms.
- The world's first drug based on dendrimers, developed by Australian-based Starpharma, is a topical gel for use as a "liquid condom" to reduce the risk of HIV infection in women. StarPharma's "VivaGel" microbicide has gone through initial animal testing and phase-one safety trials in humans.
- The US Army Research Laboratory is developing a dendrimer-based anthrax detection agent, dubbed "Alert Ticket";

ExxonMobil owns patent 5,906,970 on a "flow improver" based on dendrimer technology - an additive that will increase the flow of oil in cold temperatures.

**This review aims majorly on five areas:**

**A) Architecture**

**B) Synthesis**

**C) Properties**

**D) Characterisation**

**E) Applications in cancer therapy**

**A) ARCHITECTURE**

Dendrimers are built from a starting atom, such as nitrogen, to which carbon and other elements are added by repeating series of chemical reactions that produce a spherical branching structure. As this process repeats, successive layers are added, and the sphere can be expanded to the size required. The result is a spherical macromolecular structure whose size is similar to albumin and haemoglobin, but smaller than multimers as the gigantic IgM antibody complex. Many dendrimers have been shown to be flexible, whereas a few of the largest seem to be fairly rigid. These macromolecules acquire significant rigidity only at high generation. Small dendrimers, and especially those that involve long and flexible connectors between branch points,

are generally quite malleable and may even collapse into high-aspect ratio ovoids or flattened pancake-like shapes once solvent is evaporated after spreading on a surface.

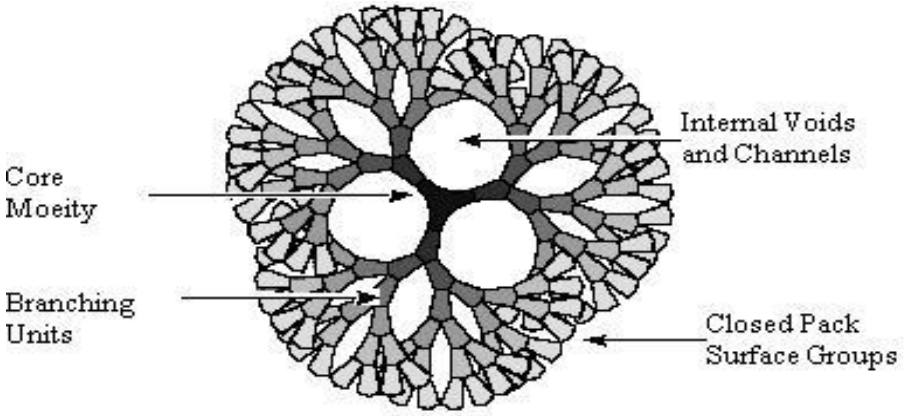
Dendrimers possess three distinguished architectural components [3], namely

1. An initiator core.

2. Interior layers (generations) composed of repeating units, radially attached to the initiator core.

3. Exterior (terminal functionality) attached to the outermost interior generations.

**Figure: 1 Structure of a dendrimer**




**Components of a dendrimers structure**

**Generation:**

It is the hyper branching when going from the centre of the dendrimers towards the periphery, resulting in homostructural layers between the focal points (branching points).The number of focal points when going from the core towards the dendrimer surface is the generation number. The core part of the dendrimer is denoted as generation "zero" or as "G0".A dendrimer having five focal points when going from the centre to the periphery is denoted as $5^{th}$ generation dendrimer or "G5"dendrimer.

**Shell:**

The dendrimer shell is the homo-structural spatial segment between the focal points, the "generation space". The "outer shell" is the space between the last outer branching point and the surface. The "inner shells" are generally referred to as the dendrimer interior.

**Pincer:**

In dendrimers, the outer shell consists of a varying number of pincers created by the last focal point before reaching the dendrimer surface. In PPI and PAMAM dendrimers the number of pincers is half the number of surface groups (because in these dendrimers the chain divides into two chains in each focal point).

**End-group:**

It is also generally referred to as the "terminal group" or the "surface group" of the dendrimer. Dendrimers having amino end groups are termed as "amino-terminated dendrimers".

**Figure: 2 Components of a dendrimer**

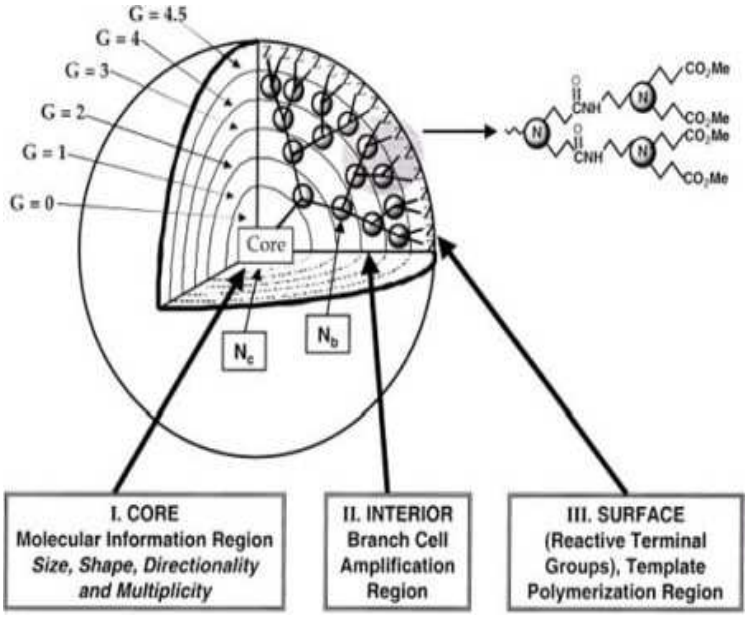
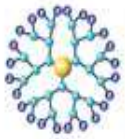
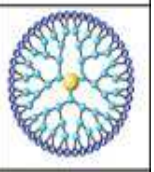



**Table: 1 Generations of a dendrimer**

| Generation | G0 | G1 | G2 | G3 | G4 |
|---|---|---|---|---|---|
| # of Surface Groups | 3 | 6 | 12 | 24 | 48 |
| Diameter (nm) | 1.4 | 1.9 | 2.6 | 3.6 | 4.4 |
| 2D Graphical Representation |  |  |  |  |  |
| 3D Chemical Structure View |  |  |  |  |  |

**Types of dendrimers**

**PAMAM dendrimers**

Poly amido amine dendrimers (PAMAM) are synthesized by the divergent method starting from ammonia or ethylenediamine initiator core reagents[4].

**PAMAMOS dendrimers**

Radially layered poly amidoamine-organosilicon dendrimers (PAMAMOS) are inverted uni- molecular micelles that consist of hydrophilic, nucleophilic PAMAM interiors and hydrophobic organo-silicon (OS) exteriors.

**PPI dendrimers**

Poly propylene imine dendrimers are poly-alkyl amines having primary amines as the end groups, the dendrimer interior consists of numerous tertiary tris-propylene amines.

**PEI dendrimers**

Sub class of PPI dendrimers with poly ethylene imine dendritic branches and diamino ethane or diamino propane as core moiety.

**Hybrid Dendrimers linear polymers**

These are the hybrids (block or graft polymers) of dendritic and linear polymers.

**Amphiphilic Dendrimers**

They are built with two segregated sites of side chain end, one half is electron donating and the other half is electron with drawing.

**Micellar Dendrimers**

These are unimolecular micelles of water soluble hyper branched poly phenylenes.

**MAP dendrimers (Multiple antigen peptides)**

Dendron like molecular construct based up on a polylysine skeleton with alkyl amino side chain as a good monomer.

**Tecto Dendrimers**

These are composed of core dendrimer, surrounded by dendrimers of several steps to perform a function necessary for a therapeutic nanodevice.

**Multilingual Dendrimers**

In these dendrimers, the surface contains multiple copies of particular functional group.

**Chiral Dendrimers**

The chirality in these dendrimers are based upon the construction of a constitutionally different but chemically similar branches to chiral core.

**Frechet-Type Dendrimers**

These dendrimers usually have carboxylic acid groups as surface groups and has good surface functionalisation.

**B) SYNTHESIS**

The synthesis used for dendrimer preparation permit almost entire control over the critical molecular design parameters such as size, shape, surface chemistry, flexibility and topology. Many dendrimer syntheses rely upon traditional reactions, such as the Michael reaction or Williamson ether synthesis whilst others involve the use of modern techniques and chemistry, such as solid-phase synthesis, organo-metal chemistry, organo-silicon chemistry, organo-phosphorous chemistry, or other contemporary methodologies[5]. There three main methods of dendrimer synthesis. They are

1. Divergent dendrimer growth

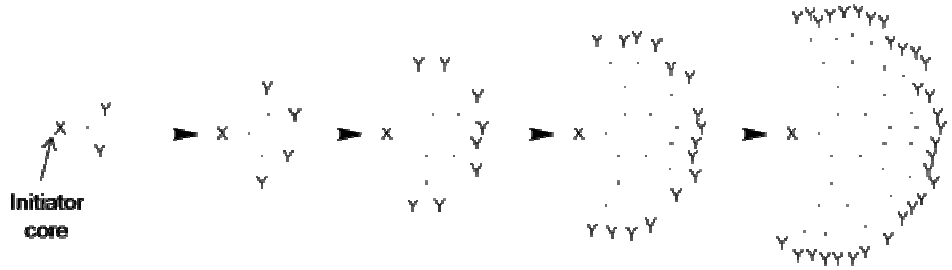
2. Convergent dendrimer growth

3. Double exponential and mixed growth

1. **Divergent dendrimer growth:**

The dendrimer is assembled from a multifunctional core, which is extended outward by a series of reactions, commonly a Michael reaction. Each step of the reaction must be driven to full completion to prevent mistakes in the dendrimer, which can cause trailing generations (some branches are shorter than the others). Such impurities can impact the functionality and symmetry of the dendrimer, but are extremely difficult to purify out because the relative size difference between perfect and imperfect dendrimers is very small[6].

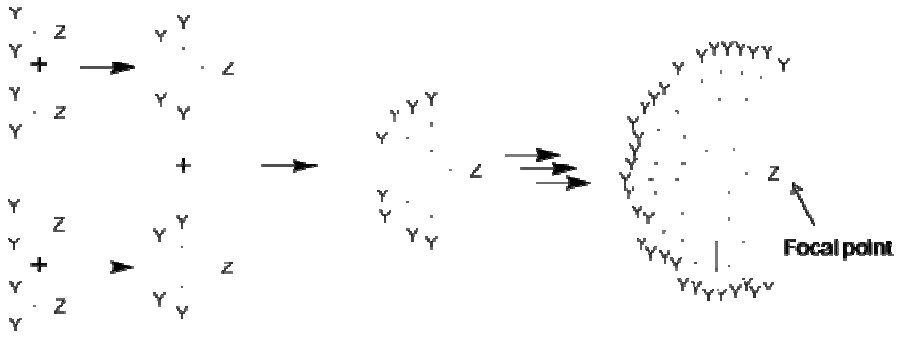
**Figure: 3 Divergent growth synthesis**




**2. Convergent dendrimer growth:**

Dendrimers are built from small molecules that end up at the surface of the sphere, and reactions proceed inward building inward and are eventually attached to a core. This method makes it much easier to remove impurities and shorter branches along the way, so that the final dendrimer is more monodisperse. However dendrimers made this way are not as large as those made by divergent methods because crowding due to steric effects along the core is limiting.

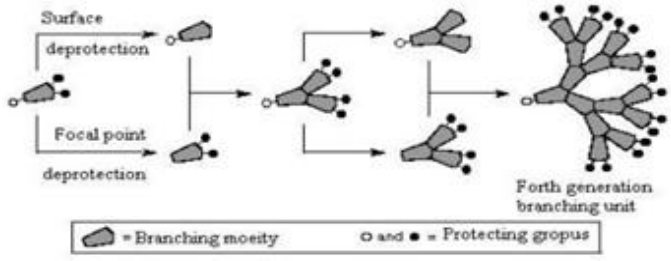
**Figure: 4 Convergent growth synthesis**




**3. Double exponential and mixed growth:**

The most recent fundamental breakthrough in the practice of dendrimer synthesis has come with the concept and implications of 'double exponential' growth. . Double exponential growth, similar to a rapid growth technique for linear polymers, involves an $AB_2$ monomer with orthogonal protecting groups for the A and B functionalities. This approach allows the preparation of monomers for both convergent and divergent growth from a single starting material .These two products are reacted together to give an orthogonally protected trimer, which may be used to repeat the growth process again. The strength of double exponential growth is more subtle than the ability to build large dendrimers in relatively few steps. In fact, double exponential growth is so fast that it can be repeated only two or perhaps three times before further growth becomes impossible. The double exponential methodology provides a means whereby a dendritic fragment can be extended in either the convergent or the divergent direction as required. In this way, the positive aspects of both approaches can be accessed without the necessity to bow to their shortcomings.

**Figure: 5 Double exponential growth synthesis**




**C) Properties**

The classical polymerization process, which results in linear polymers, is usually random in nature and produces molecules of different sizes, whereas size and molecular mass of dendrimers can be specifically

controlled during synthesis. Dendrimers are monodisperse macromolecules, unlike linear polymers. Because of their molecular architecture, dendrimers show some significantly improved physical and chemical properties when compared to traditional 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. Lower generation dendrimers, which are large enough to be spherical but do not form a tightly packed surface, have enormous surface areas in relation to volume. In contrast to linear polymers the intrinsic viscosity of dendrimer solutions does not increase linearly with mass but shows a maximum at a specific generation and then begins to decline. Such behavior is unlike that of linear polymers. This is likely to be because of the way in which dendrimer shape changes with generation, i.e. lower generations adopt a more open planar elliptical shape with transition to a more compact spherical shape for higher generations. The presence of many chain ends is responsible for high solubility and miscibility and for high reactivity. In the structure of dendrimer the molecular density is theoretically highest in the periphery of the dendrimers. It has been suggested that back folding of the terminal branches leads to a more uniform or even reverse density profile[7]. In nature tree-like structures have evolved to maximize the exposed surface area, e.g. to maximize the light exposure/number of leaves of a tree. Dendritic architecture creates molecules where a large proportion of the groups are exposed at the surface and which can have very high molecular surface to volume ratios (up to 1000 $m^2/g$). Dendrimers tend to show high solubility, reactivity, and binding.

**Figure: 6 Intrinsic viscosity vs. Molecular weight (MW)**

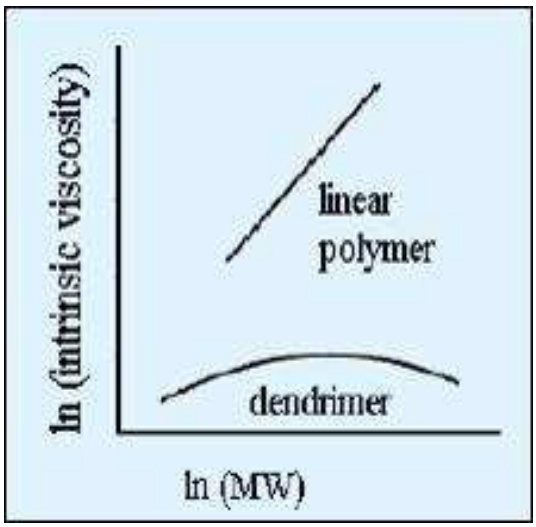


**Figure: 7 Solution structure**

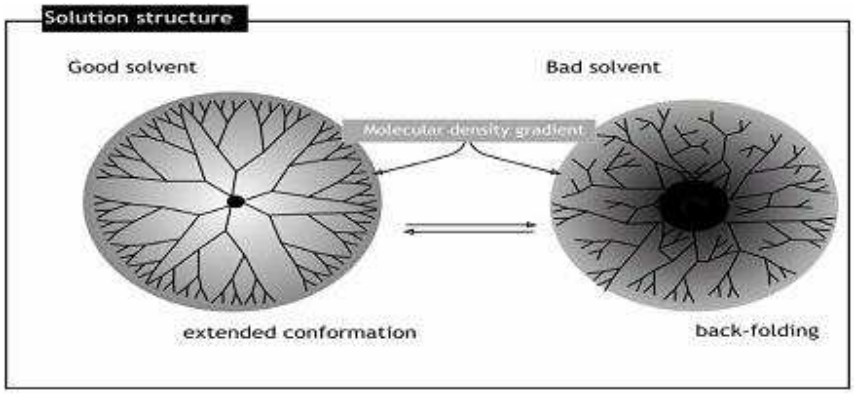



**Table: 2 Properties of dendrimer and linear polymers**

| Sr. No. | Property | Dendrimers | Linear Polymers |
|---|---|---|---|
| 1 | Structure | Compact, Globular | Not compact |
| 2 | Synthesis | Careful & stepwise growth | Single step polycondensation |
| 3 | Structural control | Very high | Low |
| 4 | Architecture | Regular | Irregular |
| 5 | Shape | Spherical | Random coil |
| 6 | Crystallanity | Non-crystalline, amorphous materials<br>-lower glass transition temperatures | Semi crystalline/crystalline materials<br>-Higher glass transition temperatures |
| 7 | Aqueous solubility | High | Low |
| 8 | Nonpolar solubility | High | Low |
| 9 | Viscosity | Non linear relationship with molecular weight | Linear relation with molecular weight |
| 10 | Reactivity | High | Low |
| 11 | Compressibility | Low | High |
| 12 | Polydispersity | Monodisperse | Polydisperse |

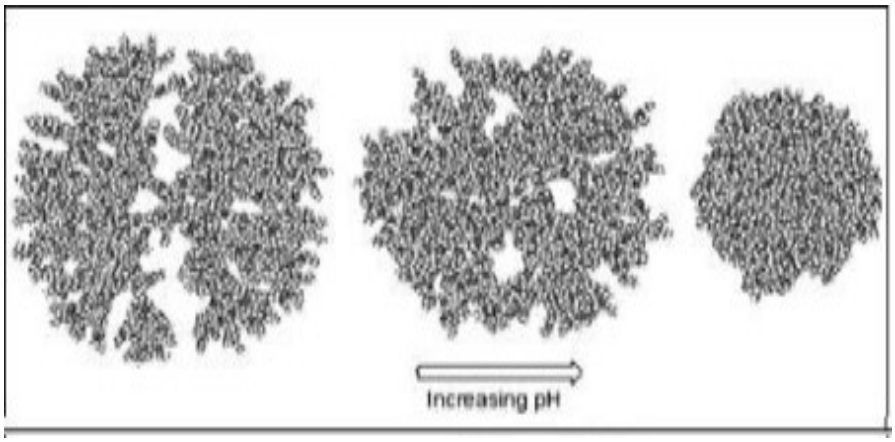
**Effect of Various Factors on the Properties of Dendrimers**

**Effect Of pH**

**Amino-terminated PPI and PAMAM dendrimers** have basic surface groups as well as a basic interior. For these types of dendrimers with interiors containing tertiary amines, the low pH region generally

leads to extended conformations due to electrostatic repulsion between the positively charged ammonium groups. Applying molecular dynamics to predict the structural behavior of PAMAM dendrimers as a function of pH show that the dendrimer has an extended conformation, based on a highly ordered structure at low pH (pH<4). At this pH, the interior is getting increasingly "hollow" as the generation number increases as a result of repulsion between the positively charged amines both at the dendrimer surface and the tertiary amines in the interior. At neutral pH, back-folding occurs which may be a consequence of hydrogen bonding between the uncharged tertiary amines in the interior and the positively charged surface amines. At higher pH (pH>10) the dendrimer contract as the charge of the molecule becomes neutral, acquiring a more spherical (globular) structure, where the repulsive forces between the dendrimer arms and between the surface groups reaches a minimum. At this pH, the conformation has a higher degree of back-folding as a consequence of the weak "inter-dendron" repulsive forces.

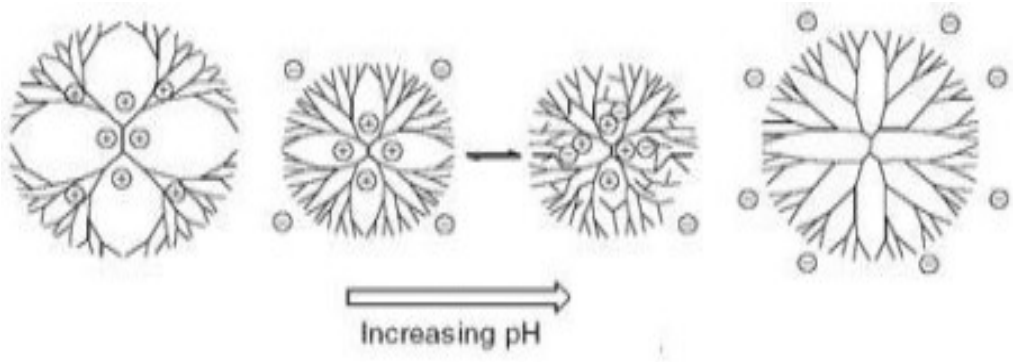
**Figure: 8 Three dimensional structure of a G6-pamam dendrimer under different pH**




pH-dependent conformational changes of **PPI dendrimers having acidic (carboxylic acid) end-groups**, the picture is somewhat different compared to what is observed for their amino-terminated counterparts. Small angle neutron scattering (SANS) and NMR measurements of self-diffusion coefficients at different pH values show that at pH 2 the dendrimer core has the most extended conformation due to the electrostatic repulsion between the positively charged protonated tertiary amines, leading to a large radius of the core, whereas the dendrimer reaches its minimum radius at pH 6, where the amount of positively charged amines equals the amount of negatively charged carboxylic groups (isoelectric point) resulting in a "dense core" conformation more subjective to back-folding. Thus, at pH 6 some degree of back-folding occurs as a result of attractive interactions between the negatively

charged surface carboxy-groups and the positively charged tertiary amines in the inner shells of the dendrimer. At pH 11 the electrostatic repulsion between the negative charged forces the surface groups apart to give a more extended conformation with a highly expanded surface area[8].

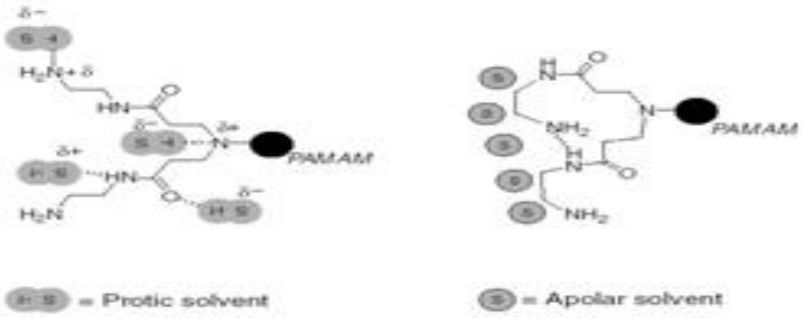
**Figure: 9 Two dimensional depiction of conformational changes up on different ph of carboxy terminated PPI dendrimer.**




**Effect of Solvent**

Dendrimers of all generations generally experience a larger extent of back-folding with decreasing solvent quality, *i.e*. decreasing solvation. However, being more flexible, the low generation dendrimers show the highest tendency towards back-folding as a result of poor solvation compared to the higher generation dendrimers. NMR studies performed on PPI dendrimers conclude that a nonpolar solvent like benzene, poorly solvates the dendrons favoring intramolecular interactions between the dendrimer segments and back-folding. However, a weakly acidic solvent like chloroform can act as a hydrogen donor for the interior amines in a basic dendrimer like PPI, leading to an extended conformation of the dendrimer because of extensive hydrogen bonding between the solvent and the dendrimer amines. Both experimental as well as theoretical studies on amino-terminated PPI and PAMAM dendrimers (polar dendrimers) show the tendency that nonpolar aprotic (poor) solvents induce higher molecular densities in the core region as a result of back-folding, whereas polar (good) solvents solvate the dendrimer arms and induce a higher molecular density on the dendrimer surface. Back-folding of the polar surface groups may expose the more hydrophobic dendrimer parts to the surroundings leading to a decreased surface polarity of the back-folded dendrimer

**Figure:10 Proposed scheme for solvation of a dendrimer under different solvent conditions.(a)Solvation of a polar dendrimer in aprotic solvent ("good") leading to extended conformation exposing a polar surface.(b) Solvation of a polar dendrimer in an apolar solvent("poor") leading to the exposure of an apolar surface consisting of alkyl chains by back folding.**




**Effect of Salt**

High ionic strength (high concentration of salts) has a strong effect on charged PPI dendrimers and favours a contracted conformation of dendrimers, with a high degree of back-folding somewhat similar to what is observed upon increasing pH or poor solvation. At low salt conditions, the repulsive forces between the charged dendrimer segments results in an extended conformation in order to minimize charge repulsion in the structure.

**Figure: 11 Three dimensional conformational change of a PPI dendrimer upon increasing ionic strength.**

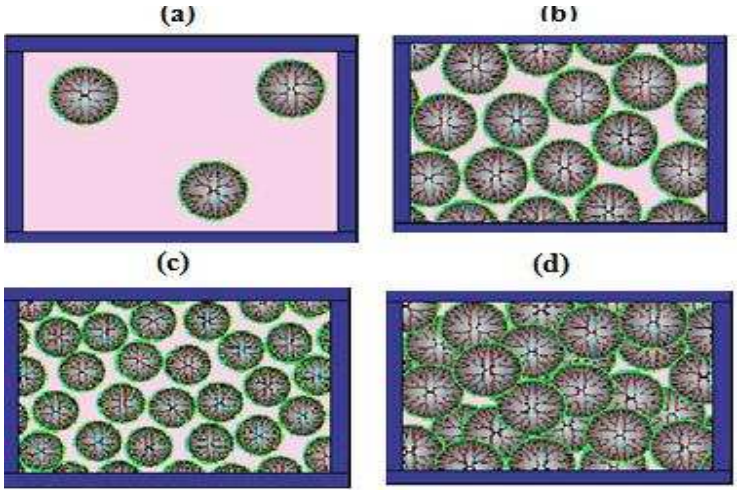

**Effect of Concentration**

In dendrimers with flexible structures the conformation is not only affected by small molecules like solvents, salts or protons, but may also be sensitive to larger objects, such as other dendrimers or surfaces which can have a great affect on the molecular density and conformation of the dendrimer. Small angle X-ray scattering (SAXS) experiments performed on PPI dendrimers (G4, G5) in a polar solvent like methanol show that the molecular conformation of dendrimers upon increasing concentration becomes increasingly contracted. This molecular

contraction may minimize the repulsive forces between the dendrimer molecules and increase the ability of the dendrimers to exhibit a more tight intermolecular packing.

**Figure: 12 Dendrimers at different concentrations (a) Dilute (b) Contact (c) Collapse**

**(d) Interpenetrate**




**D) CHARACTERISATION**

The various techniques used for the characterisation of the chemical composition, the morphology, the shape, and the homogeneity of dendrimers includes NMR, IR, Raman, fluorescence, UV visible, circular dichroism, X- ray diffraction, Mass spectrometry, SAXS, SANS, Laser light scattering, Microscopy, SEC, EPR, Electrochemistry, electrophoresis, Intrinsic viscosity, DSC and Dielectric spectroscopy.

**Spectroscopy and spectrometry**:

**Nuclear magnetic resonance (NMR)**:

Nuclear magnetic resonance is certainly the most widely used in routine analysis for characterizing dendrimers. The special techniques have also been probe their size and morphology.NMR analyses are especially during the step by step synthesis of dendrimers. In high generations, because they afford information about the chemical transformations undergone by the end groups. For organic dendrimers, such as PPI, polyphenylester, and poly (ether ketone) dendrimers, $^{1}H$ and $C^{13}$ NMR are the most used.

**Infra red (IR) and Raman**:

Infra-red 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, the occurrence of hydrogen bonding in PPI glycine functionalized dendrimers , or the disappearance of the aldehydes during the synthesis of PMMH

dendrimers . Detailed IR analyses combined with calculations were also done for these phosphorus dendrimers , Near IR spectroscopy was used to characterize delocalized k–k stacking interactions between end groups of modified PAMAM Raman spectroscopy gave relevant information about the degree of cyclodehydrogenation of polyphenylene dendrimers and the characterization of PPI and phosphorus dendrimers.

**Ultra-violet–visible (UV–vis)**

Dendrimers in which a growth and decay of the metal to ligand charge transfer band is observed. The intensity of the absorption band is essentially proportional to the number of chromophoric units, and can be a test for the purity of PPI dendrimers having azobenzene as end groups, for phosphorus dendrimers having azobenzenes within the branches or double-layered carbosilane dendrimers. However, a deviation from the Beer–Lamber law is observed for G4 and G5 PPI dendrimers having methyl orange as end groups .UV–Vis has been used also to define morphological information.

**Fluorescence**

The high sensitivity of fluorescence has been used to quantify defects during the synthesis of dendrimers. A progressive isolation of the fluorescent core of PBzE or ARB dendrimers is also detected, whereas the fluorescence depolarization technique allowed the determination of the hydrodynamic volume of PBzE dendrimers having a rubicene core[9]. Finally, the large free space available inside dendrimers was shown by the formation of excimers for pyrene linked to the internal branches of PMMH dendrimers.

**X-ray diffraction**

This technique should allow precise determination of the chemical composition, size and shape of dendrimers, but even if most dendrimers are solids, they are amorphous and lack long-range order in the condensed phase. Thus, their molecular structure is generally impossible to determine by X-ray diffraction, except for the first generation. The structure of a few second generation dendrimers was also determined, but due to the generally small number of linkages per arm.

**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 b3000. For higher molecular weights, techniques developed for the characterization of proteins and polymers have to be applied. Electro-Spray Ionisation (ESI) can be used for dendrimers able to form stable multi charged species. It has been applied dendrimers up to generation 10. The capability of this technique has been extended using Fourier transform ion

cyclotron resonance (FT-ICR MS) applied to PAMAM dendrimers.

**Scattering techniques**

**Small angle X-ray scattering (SAXS)**

The SAXS technique is often used for the characterization of polymers; applied to dendrimers it gives 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, hence on the segment density distribution within the molecule. This technique was applied to fluorinated carbosilane dendrimers and PAMAM dendrimers to afford their Rg values.

**Small angle neutron scattering (SANS)**

The SANS technique also gives access to the radius of gyration, but may also reveal more accurate information than SAXS about the internal structure of the entire dendrimer. In particular, SANS may indicate the molecular weight; such experiments have been conducted with PPI, PAMAM and PBzE dendrimers.

**Laser light scattering (LLS)**

In most cases, the Laser Light Scattering technique is used as a detector coupled to size exclusion chromatography apparatus, to determine the hydrodynamic radius of dendrimers. However, in some cases, it has been used for the direct analysis of samples of dendrimers in solution; for instance, the molecular weight (Mw) of PPI dendrimers has been evaluated with low-angle LLS.

**Microscopy**

Two types of microscopy 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" at a few angstroms of a sensitive can till ever arm with the sample. Transmission Electron Microscopy (TEM) allowed observing only aggregates of small ARB, but images of individual dendrimer molecules from G3 to G10 were obtained for PMMH dendrimers having gold covalently attached to each end groups. Atomic Force Microscopy (AFM) has been mainly used for the characterization of dendritic macromolecular films. However, the spin coating technique applied to generations 5 to10 of PAMAM dendrimers allowed the visualization of isolated dendrimer molecules [(10)].

**Size exclusion chromatography (SEC)**

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 is connected to the SEC apparatus for the determination of the poly dispersity, which is generally very close to unity. Most types of dendrimers were characterized by

SEC. The calibration generally uses linear polymers but, due to the very different morphology, an increased deviation is observed between the experimental mass values obtained by SEC and the theoretical mass values of dendrimers, when the generation number increases, as shown for PBzE or PMMH dendrimers; thus well characterized PAMAM dendrimers were used as standards for polyether dendrimers. SEC was also used to monitor size changes of ARB dendrimers with pH variance.

**Rheology, physical properties**

**Intrinsic viscosity**

Rheology, and particularly dilute solution viscosimetry studies, can be used as analytical probe of the morphological structure of dendrimers. Dendrimers should exhibit a maximum in the dependence of the intrinsic viscosity 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 behaviour is experimentally observed for several series of dendrimers. The maxima of the intrinsic viscosity occurs at different generation, G2 or G3 for PbzE dendrimers, depending on the density of branches, G3 phosphorus dendrimers with two types of end groups , G4 for PAMAM ,G5 for PPI with two types of end groups.

**Differential Scanning Calorimetry (DSC)**

The 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 the molecular mass for PBzE dendrimers. DSC and Temperature Modulated Calorimetry (TMC) were also used to detect physical aging of PMMH dendrimers.

**Dielectric spectroscopy (DS)**

Dielectric spectroscopy gives information about molecular dynamic processes in polymers (a-, h-, g-, and y-relaxation). This technique was applied to various types of dendrimers, and it was generally found that the a-relaxation values obtained by DS agree well with those obtained in Differential Scanning Calorimetry measurements. Carbosilane, ARB, poly (ether amide), PMMH, and carbosilazane dendrimers were analyzed by DS; in most cases, both the alpha and beta relaxations were obtained and identified.

**E) APPLICATIONS FOR CANCER TREATMENT**

**Targeted and controlled release drug delivery**:

The targeted delivery of chemotherapeutics to tumour cells significantly reduce the side effects compared to the systemic delivery where healthy tissue such as liver, spleen, kidneys and bone marrow can accumulate toxic levels of drug. The two strategies of targeting include [11].

1. The passive targeting of cancer cells.

2. The active targeting of tumour cells.

**Passive targeting of cancer cells**: Passive targeting of tumour cells can be achieved by increasing the hydrodynamic radius of dendrimer through PEGylation leading to the accumulation of dendrimer in the tumour tissue via the enhanced permeability and retention effect (EPR) effect which is a result of tumour induced angiogenesis leading to neo vasculature that is irregular, leaky or defective with disorganized endothelial cells [(12)].

E.g. 1.The anticancer drug doxorubicin was covalently bound to the dendrimer via an acid labile hydrazone linkage .The cytotoxicity was significantly reduced (80-98%) and the drug was successfully taken up by the cancer cell lines and there is high encapsulation efficiency. The encapsulating efficiency depends on the PEG length and size of the dendrimer [(13)].

2. The anticancer drug 5-fluorouracil which is a pyrimidine analogue which belongs to the family of anti-metabolites when encapsulated into the PEGylated PAMAM dendrimers reasonable drug loading, reduced release rate and haemolytic toxicity compared to the non PEGylated dendrimer [(14)].

3. Methotrexate an anti metabolite and antifolate drug used in the treatment of many cancers, acts by inhibiting the metabolism of folic acid when encapsulated into PEGylated dendrimers showed high encapsulating efficiency, reduced release rate and significant reduction in hepatotoxicity.

**Active targeting of tumour cells:** Active targeting of dendrimers relies on the conjugation of one or more targeting moieties to the dendrimer to facilitate cell-receptor-mediated interactions. The various targeting moieties include folic acid, peptides, monoclonal antibodies etc [(15)].

- **Folic acid**: The folic acid conjugated dendrimers target tumour cells that over expresses folic acid receptors. Binding avidity to folic acid molecules increased with the additionally bound folic acid molecule conjugated to the dendrimer [(16)].

E.g.1. DNA-assembled PAMAM dendrimer clusters were prepared by linking two dendrimer components with single but different functionalities for concurrent delivery of therapeutic, imaging and targeting agents. Complexes were formed between a folic acid modified dendrimer and a FITC –modified dendrimer connected by a 34 base-pair long oligonucleotide that effectively targets KB cells expressing folic acid receptors and were internalization by the cells.

2. The Baker group has investigated several variations of the folic–acid conjugated dendrimers for targeted drug delivery. Surface conjugated folic acid G5-PAMAM dendrimers were prepared where the remaining free amino groups were capped

with the glycidol to neutralize the positive charges and linked with methotrexate via ester linkage .This complex shows higher affinity for KB cells over expressing the folic acid receptors and the complex was found to be sustained release.

- **Peptides:** A doubly cyclised RGD peptide and Alexa Fluor 488 fluorescent label were conjugated to G5 PAMAM dendrimer for targeting tumour neovasculature via uniquely expressed $\alpha_v\beta_3$ integrins showed that dendrimers dissociated approximately 522 times slower, suggesting a synergistic effect of multiple peptide conjugation on binding avidity in mice with human SK-RC-52 tumours.

- **Monoclonal antibodies:** Monoclonal antibody conjugation to PAMAM dendrimers is used for specific targeting of tumour cells that over expresses certain antigens[17].

E.g. Methotrexate was covalently bounded to G5 PAMAM dendrimer biconjugates containing cetuximab, a monoclonal antibody that acts as an epidermal growth factor receptor (EGFR) inhibitor which is used for the treatment of EGFR-positive brain tumours.

- **Glycosylation:** Glyco peptide dendrimers conjugated to the anti mitotic agent colchicine showed that the dendrimers were 10-20 times more effective at inhibiting proliferation of HeLa cancer cell lines. These glycol dendrimers are a class of dendrimers that can incorporate sugar moieties such as glucose, galactose, mannose, and di or mono saccharides into their structure.

**Solubility enhancers**:

Dendrimers having a hydrophobic core and a hydrophilic surface layers are termed a uni molecular micelles. They do not have critical micelle concentration. This feature helps to soluble poorly soluble drugs by encapsulating them within the dendritic structure at all concentrations of dendrimers [18].

E.g.1. A hydrophobic –hydrophilic core shell dendrimer with PAMAM dendrimer and long alkane chain exterior was bound to 5-flurouracil, a water soluble anti-tumour drug. The bioavailability in rats was found to be nearly twice the level of the free 5-flurouracil[19].

2. Camptothecin an anti cancer drug that damages DNA, leading to cell destruction whose therapeutic efficiency is limited by very low water solubility and adverse side effects such as inflammation of the urinary bladder. This drug when conjugated to dendrimer composing of natural metabolites, glycerol and succinic acid showed 16-fold increase in cellular uptake and increase in drug retention within the cell in cancer cell lines.

3. Cisplatin anticancer drug acts by forming stable DNA-cisplatin complexes through intrastrand cross links resulting in the

alteration of the DNA structure that prevents replication and initiates apoptosis. Its therapeutic activity is limited by its poor water solubility, low lipophilicity and the development of résistance. Encapsulation of cisplatin within PAMAM dendrimers resulted in complexes with slow release, higher accumulation in solid tumours and lower toxicity than free cisplatin was observed in tumour induced mice.

4. Curcumin and dimethoxycurcumin has the ability to induce apoptosis in cancer cells without effect on healthy cells. Their water solubility can be improved by conjugation with the dendrimers

5. Etoposide an inhibitor of topoisomerase II which is used for the treatment of malignancies such as lung cancer, testicular cancer, lymphoma and leukaemia. Its poor water solubility can be improved by conjugating it with PEGlyated PAMAM-OH dendrimer.

6. Paclitaxel an anticancer drug is a mitotic inhibitor used in the treatment of patients with lung, ovarian, breast and neck cancers. The drug works by interfering with normal microtubule growth during cell divison.To enhance the water solubility of the drug is encapsulated into polyglycerol dendrimers resulted in 400 fold improved water solubility compared to the free drug.

**Photodynamic therapy:**

Photodynamic therapy (PDT) relies on the activation of a photosensitising agent with visible or near infrared (NIR) light. Upon excitation, a highly energetic state is formed which upon reaction with oxygen affords a highly reactive singlet oxygen capable of inducing necrosis and apoptosis in the tumour cells. The tumour selectivity of leaky vasculature is due its characteristic leaky vasculature, compromised lymphatic drainage and high degrees of newly synthesized collagen and lipid content for which porphyrins have an affinity. PDT has shown to reduce tumours by direct killing of tumour vasculature and triggering of an acute inflammatory response that attracts leucocytes to the tumour. Dendritic delivery of PDT agents showed improved tumour selectivity and retention [(20)].

E.g.1.Dendrimers composed in part of a multiple 5-aminolevulinic acid for improved delivery and enhanced intracellular accumulation of porphyrins.

2. Negatively charged G3-poly (benzyl ether) dendrimer with carboxylate periphery groups and a zinc porphyrin at the focal core, surrounded by positively charged linear PEG-lysine micelle system resulted in 280 fold increase in photo toxicity in Lewis lung cells in vitro when compared to the free dendrimer.

**Boron neutron capture therapy**:

Boron neutron capture therapy (BNCT) is based on a lethal $^{10}B$ (n, α)$^{7}Li$ capture reaction that occurs when $^{10}B$ is irradiated with low energy thermal neutrons to produce high energy α particles and $^{7}Li$ nuclei which are toxic to the tumour cells.

When a PAMAM dendrimer carrying 1100 boron atoms at its surface is attached to a monoclonal antibody cetuximab which is specific for the EGF receptor showed that the conjugate was present at an almost 10-fold higher concentration in brain tumours than in normal brain tissue. Human gliomas have been targeted with the boronated G5 PAMAM dendrimer conjugated to anti-EGF receptor monoclonal antibodies that work against over expressed tumour cell receptors [21]..

**Photo thermal therapy:**

Gold based nanoparticles have been developed that strongly absorb light in the near IR region facilitating deep optical penetration into the tissues, generating a localized dose of heat at the site of tumour that kills the tumour cells. Dendrimer encapsulated gold nanoparticles in which amine terminated G5 PAMAM dendrimers covalently conjugated to fluorescein and folic acid for targeted delivery to tumour cells over expressing the folic acid receptors [22].

**Cancer Diagnosis and Imaging:**

Labelled Dendrimers are Important Research Tools for Bio distribution Studies:

The synthetic ability to attach both a tumour targeting antibody and a potent payload of anti cancer drugs to the same dendritic molecule provides a plat form for the multifunctional nano scale drug delivery devices. Radioisotopes like $^{3}H$,$^{14}C$,$^{88}Y$,$^{111}In$ and $^{125}I$ have been conjugated to dendrimers so that the physical and chemical properties can be tuned to favour distribution to or away from the specific organs and finally to achieve favourable distribution to the tumour tissues[23].

MRI Imaging Agents:

Imaging modalities can be used in oncology to diagnose, locate, stage, plan treatment and potentially find recurrence.Coumputed topography (CT) and magnetic resonance imaging are the two standard methods of imaging associated with cancer diagnoses. Gadolinium (Gd) paramagnetic contrast agents for MRI have been complexed with the dendrimer for visualizing both tumour vasculature and lymphatic involvement. Changes in tumour vasculature were visualized by magnetic resonance imaging using G8-Gd-PAMAM contrast agent after a large dose of radiation treatment. Iodinated contrast agents for computer topography (CT) could benefit from dendrimer conjugation with improved retention time and potential targeted delivery [24].

**CONCLUSION**

Dendrimers hold a promising future in various pharmaceutical applications and diagnostic field as they possess unique properties like high degree of branching, multivalency, well defined molecular weight. Dendrimer can also work as useful tool for optimizing drug delivery of drugs facing problems of poor solubility, bioavailability and permeability. Recent success in simplifying and optimizing the

synthesis provides a large variety of structures with reduced cost. As research progresses increased application of dendrimers will emerge and the future should witness an increasing number of commercialized dendrimer based drug delivery systems.

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