

Review

Dendrimers: properties and applications

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Dendrimers are a new class of polymeric materials. They are highly branched, monodisperse macromolecules. The structure of these materials has a great impact on their physical and chemical properties. As a result of their unique behaviour dendrimers are suitable for a wide range of biomedical and industrial applications. The paper gives a concise review of dendrimers' physico-chemical properties and their possible use in various areas of research, technology and treatment.

Polymer chemistry and technology have traditionally focused on linear polymers, which are widely in use. Linear macromolecules only occasionally contain some smaller or longer branches. In the recent past it has been found that the properties of highly branched macromolecules can be very different from conventional polymers. The structure of these materials has also a great impact on their applications.

First discovered in the early 1980's by Donald Tomalia and co-workers [1], these hyperbranched molecules were called **dendrimers**. The term originates from 'dendron' meaning a tree in Greek. At the same time, Newkome's group [2] independently reported synthesis of similar macromolecules. They called them **arborols** from the Latin word 'arbor' also meaning a tree. The

term **cascade molecule** is also used, but 'dendrimer' is the best established one.

SYNTHESIS

Dendrimers are generally prepared using either a divergent method or a convergent one [3]. There is a fundamental difference between these two construction concepts.

In the **divergent methods**, dendrimer grows outwards from a multifunctional core molecule. The core molecule reacts with monomer molecules containing one reactive and two dormant groups giving the first generation dendrimer. Then the new periphery of the molecule is activated for reactions with more monomers. The pro-

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Abbreviations: 5FU, 5-fluorouracil; BDA, butylenediamine; DTPA, diethylenetriaminepentaacetic acid; EDA, ethylenediamine; PAMAM, polyamidoamine; THF, tetrahydrofuran

cess is repeated for several generations and a dendrimer is built layer after layer (Fig. 1A). The

'starburst' is a trademark of the Dow Chemicals Company. Ammonia is used as the core molecule.

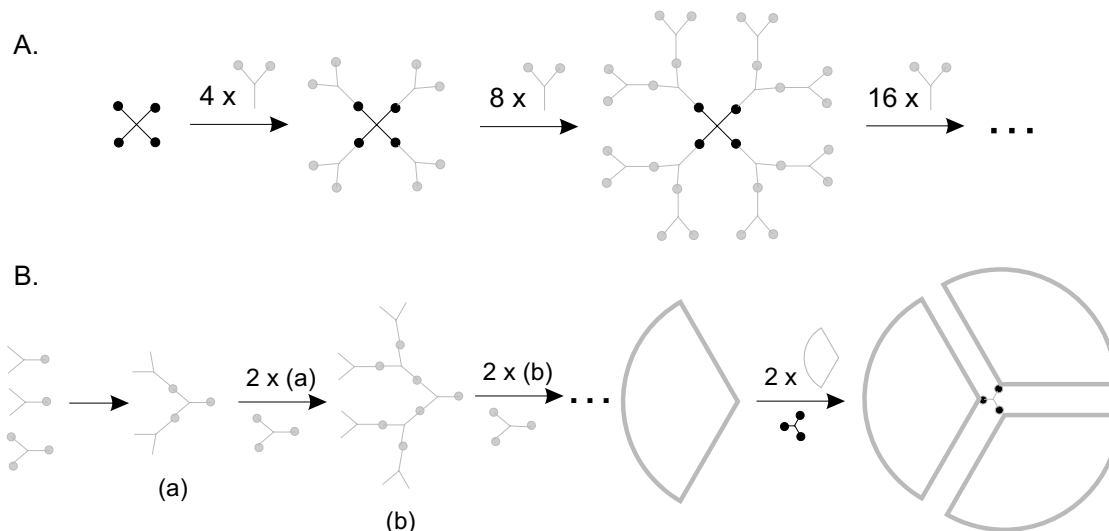


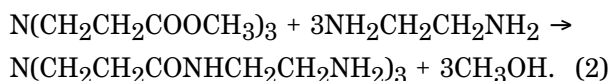
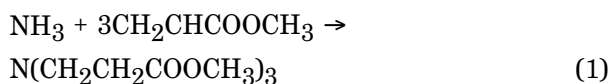
Figure 1. A. The divergent growth method; B. The convergent growth method [3].

divergent approach is successful for the production of large quantities of dendrimers. Problems occur from side reactions and incomplete reactions of the end groups that lead to structure defects. To prevent side reactions and to force reactions to completion large excess of reagents is required. It causes some difficulties in the purification of the final product.

The **convergent methods** were developed as a response to the weaknesses of the divergent synthesis [4]. In the convergent approach, the dendrimer is constructed stepwise, starting from the end groups and progressing inwards. When the growing branched polymeric arms, called dendrons, are large enough, they are attached to a multifunctional core molecule (Fig. 1B). The convergent growth method has several advantages. It is relatively easy to purify the desired product and the occurrence of defects in the final structure is minimised. It becomes possible to introduce subtle engineering into the dendritic structure by precise placement of functional groups at the periphery of the macromolecule. 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.

The first synthesised dendrimers were polyamidoamines (PAMAMs) [5]. They are also known as starburst dendrimers. The term

In the presence of methanol it reacts with methyl acrylate and then ethylenediamine is added:



At the end of each branch there is a free amino group that can react with two methyl acrylate monomers and two ethylenediamine molecules. Each complete reaction sequence results in a new dendrimer generation. The half-generations PAMAM dendrimers (e.g., 0.5, 1.5, 2.5) possess anionic surfaces of carboxylate groups. The number of reactive surface sites is doubled with every generation (Table 1). The mass increases more than twice (Fig. 2).

The molar mass of the dendrimer can be predicted mathematically [6]:

$$M = M_c + n_c \cdot \left[M_m \cdot \left(\frac{n_m^G - 1}{n_m - 1} \right) + M_t \cdot n_m^G \right], \quad (3)$$

where: M_c – is the molar mass of the core, M_m – the molar mass of the branched monomer, M_t – the molar mass of the terminal groups, n_c – the

Table 1. Theoretical properties of PAMAM dendrimers

Generation	Ammonia core		EDA core	
	molecular mass	number of terminal groups	molecular mass	number of terminal groups
0	359	3	516	4
1	1043	6	1428	8
2	2411	12	3252	16
3	5147	24	6900	32
4	10619	48	14196	64
5	21563	96	28788	128
6	43451	192	57972	256
7	87227	384	116340	512
8	174779	768	233076	1024
9	349883	1536	466548	2048
10	700091	3072	933492	4096

core multiplicity, n_m – the branch-juncture multiplicity, G – the generation number.

The increase of the number of dendrimer terminal groups is consistent with the geometric progression:

$$Z = n_c \cdot n_m^G \quad (4)$$

Nowadays dendrimers are commercially available. DendritechTM (U.S.A.) manufactures PAMAM dendrimers. They are based on either an ethylenediamine (EDA) core or ammonia core and possess amino groups on the surface. They are usually sold as a solution in either methanol or water. DSM (Netherlands) has developed production of **poly(propylene imine) dendrimers**. They are currently available under the name AtramolTM. Butylenediamine (BDA) is used as the core molecule. The repetitive reaction sequence involves Michael addition of acrylonitrile to a primary amino group followed by hydrogenation of nitrile groups to primary amino groups [7].

MOLECULAR STRUCTURE

Dendrimers of lower generations (0, 1, and 2) have highly asymmetric shape and possess more open structures as compared to higher generation

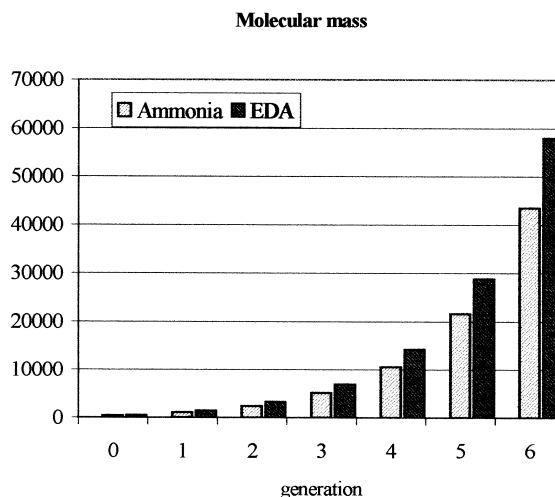


Figure 2. Molecular mass of PAMAM dendrimers with ammonia and EDA core.

dendrimers. As the chains growing from the core molecule become longer and more branched (in 4 and higher generations) dendrimers adopt a globular structure [8]. Dendrimers become densely packed as they extend out to the periphery, which forms a closed membrane-like structure. When a critical branched state is reached dendrimers cannot grow because of a lack of space. This is called the ‘starburst effect’ [9]. For PAMAM dendrimer synthesis it is observed after tenth generation. The rate of reaction drops suddenly and further reactions of the end groups cannot occur. The tenth generation PAMAM contains 6141 monomer units and has a diameter of about 124 Å [6].

The increasing branch density with generation is also believed to have striking effects on the structure of dendrimers. They are characterised by the presence of internal cavities and by a large number of reactive end groups (Fig. 3).

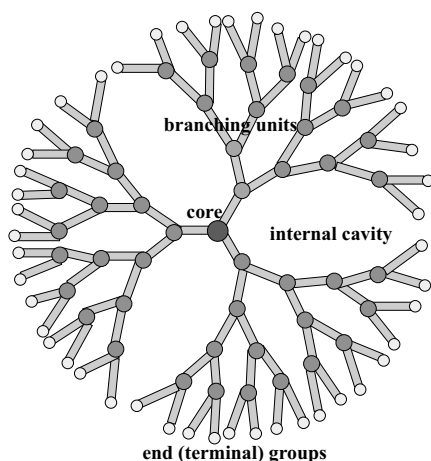


Figure 3. Representation of a fourth generation dendrimer.

Dendritic copolymers are a specific group of dendrimers. There are two different types of copolymers (Fig. 4). **Segment-block dendrimers** are built with dendritic segments of different constitution. They are obtained by attaching different

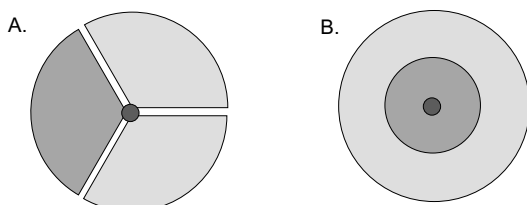


Figure 4. Copolymers: A. segment-block dendrimer; B. layer-block dendrimer.

wedges to one polyfunctional core molecule. **Layer-block dendrimers** consist of concentric spheres of differing chemistry. They are the result of placing concentric layers around the central core. Hawker and Fréchet [10] synthesised a segment-block dendrimer which had one ether-linked segment and two ester-linked segments. They also synthesised a layer-block dendrimer. The inner two generations were ester-linked and the outer three ether-linked.

The multi-step synthesis of large quantities of higher generation dendrimers requires a great effort. This was the reason why Zimmerman's group [11] applied the concept of self-assembly to

dendrimer synthesis. They prepared a wedgelike molecule with adendritic tail in such a manner that six wedge-shaped subunits could self-assemble to form a cylindrical aggregate. This hexameric aggregate is about 9 nm in diameter and 2 nm thick. It has a large cavity in the centre. The six wedges are held together by hydrogen bonds between carboxylic acid groups and stabilised by van der Waals interactions. However, the stability of the hexamer is affected by many factors. The aggregate starts to break up into monomers when the solution is diluted, when the aggregate is placed in a polar solvent like tetrahydrofuran (THF), and when the temperature is high. The hexamer's limited stability is due to its noncovalent nature.

PROPERTIES

Dendrimers are monodisperse macromolecules, unlike linear polymers. 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.

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. Dendrimer solutions have significantly lower viscosity than linear polymers [12]. When the molecular mass of dendrimers increases, their intrinsic viscosity goes through a maximum at the fourth generation and then begins to decline [13]. Such behaviour is unlike that of linear polymers. For classical polymers the intrinsic viscosity increases continuously with molecular mass.

The presence of many chain-ends is responsible for high solubility and miscibility and for high reactivity [12]. Dendrimers' solubility is strongly influenced by the nature of surface groups. Dendrimers terminated in hydrophilic groups are

soluble in polar solvents, while dendrimers having hydrophobic end groups are soluble in nonpolar solvents. In a solubility test with tetrahydrofuran (THF) as the solvent, the solubility of dendritic polyester was found remarkably higher than that of analogous linear polyester. A marked difference was also observed in chemical reactivity. Dendritic polyester was debenzylated by catalytic hydrogenolysis whereas linear polyester was unreactive.

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 (up to $1000 \text{ m}^2/\text{g}$) [5].

Dendrimers have some unique properties because of their globular shape and the presence of internal cavities. The most important one is the possibility to encapsulate guest molecules in the macromolecule interior.

Meijer and co-workers [14, 15] trapped small molecules like rose bengal or *p*-nitrobenzoic acid inside the 'dendritic box' of poly(propylene imine) dendrimer with 64 branches on the periphery. Then a shell was formed on the surface of the dendrimer by reacting the terminal amines with an amino acid (L-phenylalanine) and guest molecules were stably encapsulated inside the box (Fig. 5). Hydrolysing the outer shell could liberate the guest molecules. The shape of the guest and the architecture of the box and its cavities determine the number of guest molecules that can be entrapped. Meijer's group described experiments in which they had trapped four molecules of rose bengal or eight to ten molecules of *p*-nitrobenzoic acid in one dendrimer.

Archut and co-workers [16] developed a method in which boxes could be opened photochemically. A fourth generation polypropylene imine dendrimer with 32 end groups was terminated in azobenzene groups (Fig. 6). The azobenzene groups undergo a fully reversible photoisomerization reaction. The E isomer is switched to the Z form by 313 nm light and can be converted back to the E form by irradiation with 254 nm light or by heating. Such dendrimers can play the role of photoswitchable hosts for eosin Y. Photochemical modifications of the dendritic surface cause encapsulation and release of guest molecules.

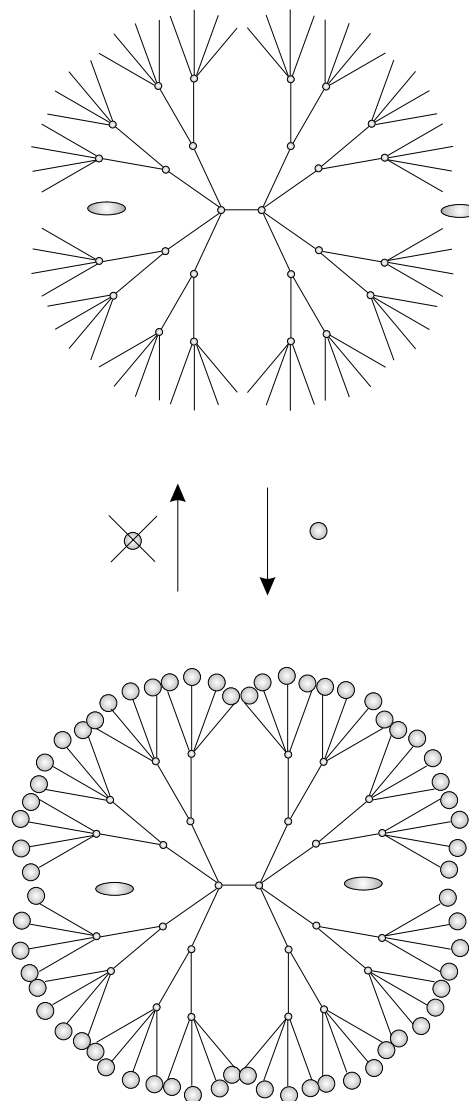
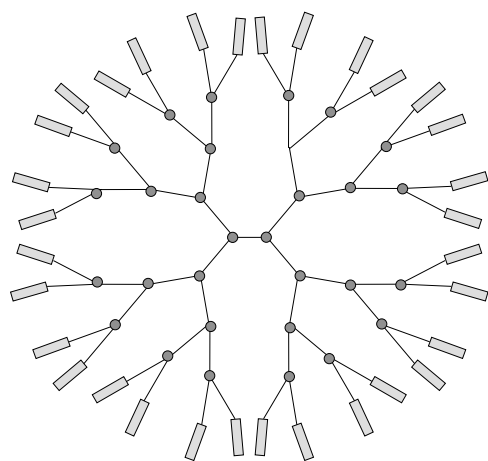


Figure 5. 'Dendritic box' encapsulating guest molecules.

Archut's experiment demonstrated that the Z forms of the fourth generation dendrimers are better hosts than the E forms.

It is possible to create dendrimers which can act as extremely efficient light-harvesting antennae [17, 18]. Absorbing dyes are placed at the periphery of the dendrimer and transfer the energy of light to another chromophore located in the core. The absorption spectrum of the whole macromolecule is particularly broad because the peripheral chromophores cover a wide wavelength range. The energy transfer process converts this broad absorption into the narrow emission of the central dye (Fig. 7). The light harvesting ability increases with generation due to the increase in the number of peripheral chromophores.

Biological properties of dendrimers are crucial because of the growing interest in using them in biomedical applications. "Cationic" dendrimers (e.g., amine terminated PAMAM and poly(propylene imine) dendrimers that form cationic groups at low pH) are generally haemolytic and cytotoxic. Their toxicity is generation-dependent and increases with the number of surface groups [19]. PAMAM dendrimers (generation 2, 3 and 4) interact with erythrocyte membrane proteins causing changes in protein conformation. These changes increase with generation number and the concentration of dendrimers. The interactions between proteins and half-generation PAMAM dendrimers



(2.5 and 3.5) are weaker¹. Anionic dendrimers, bearing a carboxylate surface, are not cytotoxic over a broad concentration range [20]. Incubation of human red blood cells in plasma or suspended in phosphate-buffered saline with PAMAM dendrimers causes the formation of cell aggregates. No changes in aggregability of nucleated cells such as Chinese hamster fibroblasts are observed².

APPLICATIONS

There are now more than fifty families of dendrimers, each with unique properties, since

the surface, interior and core can be tailored to different sorts of applications. Many potential applications of dendrimers are based on their unparalleled molecular uniformity, multifunctional surface and presence of internal cavities. These specific properties make dendrimers suitable for a variety of high technology uses including biomedical and industrial applications.

Dendrimers have been applied in *in vitro* diagnostics. Dade International Inc. (U.S.A.) has introduced a new method in cardiac testing. Proteins present in a blood sample bind to immunoglobulins which are fixed by dendrimers to a sheet of glass. The result shows if there is any heart

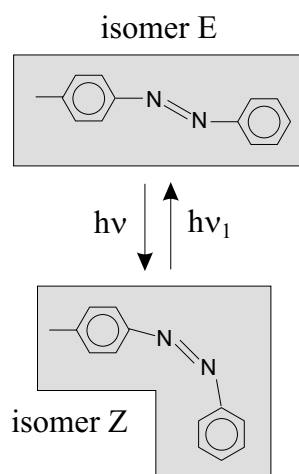


Figure 6. Dendrimer terminated in azobenzene groups [16].

muscle damage. This method significantly reduces the waiting time for the blood test results (to about 8 min). When a randomly organised solution of immunoglobulins is used the test lasts up to 40 min. Conjugates of dendrimer and antibody improve also precision and sensitivity of the test.

Dendrimers have been tested in preclinical studies as **contrast agents** for magnetic resonance. Magnetic resonance imaging (MRI) is a diagnostic method producing anatomical images of organs and blood vessels. Placing a patient in a generated, defined, inhomogeneous magnetic field results in the nuclear resonance signal of water, which is assigned to its place of origin and converted into pictures. Addition of contrast agents

¹ Faber, M.A., Domański, D.M., Bryszewska, M. & Leyko, W. (1999) 1st International Dendrimer Symposium, Frankfurt/Main; Book of Abstracts p. 61.

² Domański, D.M., Faber, M.A., Bryszewska, M. & Leyko, W. (1999) 1st International Dendrimer Symposium, Frankfurt/Main; Book of Abstracts p. 63.

(paramagnetic metal cations) improves sensitivity and specificity of the method. Gadolinium salt of diethylenetriaminepentaacetic acid (DTPA) is used clinically but it diffuses into the extravascular area due to its low molecular mass [9]. Dendrimers due to their properties are highly suited for use as image contrast media. Several groups have prepared dendrimers containing gadolinium ions chelated on the surface [21, 22]. Preliminary tests show that such dendrimers are stronger con-

trast agents than conventional ones. They also improve visualisation of vascular structures in magnetic resonance angiography (MRA) of the body. It is a consequence of excellent signal-to-noise ratio [23].

There are attempts to use dendrimers in the targeted **delivery of drugs** and other therapeutic agents. Drug molecules can be loaded both in the interior of the dendrimers as well as attached to the surface groups.

Sialylated dendrimers, called sialodendrimers, have been shown to be potent inhibitors of the haemagglutination of human erythrocytes by influenza viruses. The first step in the infection of a cell by influenza virus is the attachment of the virion to the cell membrane. The attachment occurs through the interaction of a virus receptor haemagglutinin with sialic acid groups presented on the surface of the cell [24]. Sialodendrimers bind to haemagglutinin and thus prevent the attachment of the virus to cells. They can be useful therapeutic agents in the prevention of bacterial and viral infections. Attaching α -sialinic acid moieties to the dendrimer surface enhances the thera-

peutic effect and allows the dendrimer to attain a higher activity in inhibiting influenza infection [25, 26]. A larger effect occurs with an increase in the number of sialinic acid groups.

The therapeutic effectiveness of any drug is strictly connected with its good solubility in the body aqueous environment. There are many substances which have a strong therapeutic activity but due to their lack of solubility in pharmaceutically acceptable solvents have not been used for

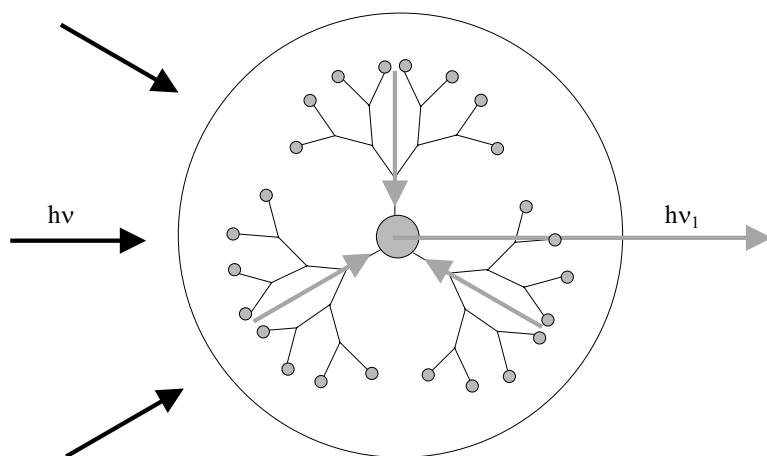


Figure 7. Light-harvesting dendrimer.

therapeutic purposes. Water soluble dendrimers are capable of binding and solubilising small acidic hydrophobic molecules with antifungal or antibacterial properties. The bound substrates may be released upon contact with the target organism. Such complexes may be considered as potential drug delivery systems [27, 28].

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. 5-Fluorouracil (5FU) is known to have remarkable antitumour activity, but it has high toxic side effects. PAMAM dendrimers after acetylation can form dendrimer-5FU conjugates [29]. The dendrimers are water soluble and hydrolysis of the conjugates releases free 5FU. The slow release reduces 5FU toxicity. Such dendrimers seem to be potentially useful carriers for antitumour drugs.

Therapeutic agents can also be attached to a dendrimer to direct the delivery. A good example of such application is using dendrimers in **boron neutron capture therapy (BNCT)**. Boron neutron capture therapy is an experimental approach

to cancer treatment which uses a two-step process. First, a patient is injected with a non-radioactive pharmaceutical which selectively migrates to cancer cells. This component contains a stable isotope of boron (^{10}B). Next, the patient is irradiated by a neutral beam of low-energy or thermal neutrons. The neutrons react with the boron in the tumour to generate alpha particles, which destroy the tumour leaving normal cells unaffected [30]. In order to sustain a lethal reaction a large number of ^{10}B atoms must be delivered to each cancer cell. Dendrimers with covalently attached boron atoms have been prepared and first tests on these compounds have given positive results [31–33].

Dendrimers can act as carriers, called **vectors, in gene therapy**. Vectors transfer genes through the cell membrane into the nucleus. Currently liposomes and genetically engineered viruses have been mainly used for this. PAMAM dendrimers have also been tested as genetic material carriers [34, 35]. They are terminated in amino groups which interact with phosphate groups of nucleic acids. This ensures consistent formation of transfection complexes. A transfection reagent called SuperFectTM consisting of activated dendrimers is commercially available. Activated dendrimers can carry a larger amount of genetic material than viruses. SuperFect–DNA complexes are characterised by high stability and provide more efficient transport of DNA into the nucleus than liposomes. The high transfection efficiency of dendrimers may not only be due to their well-defined shape but may also be caused by the low pK of the amines (3.9 and 6.9). The low pK permit the dendrimer to buffer the pH change in the endosomal compartment [36].

Besides biomedical applications dendrimers can be used to improve many **industrial processes**.

The combination of high surface area and high solubility makes dendrimers useful as nanoscale catalysts [37]. They combine the advantages of homogenous and heterogeneous catalysts. Homogenous catalysts are effective due to a good accessibility of active sites but they are often difficult to separate from the reaction stream. Heterogeneous catalysts are easy to separate from the reaction mixture but the kinetics of the reaction is lim-

ited by mass transport. Dendrimers have a multifunctional surface and all catalytic sites are always exposed towards the reaction mixture. They can be recovered from the reaction mixture by easy ultrafiltration methods. The first example of a catalytic dendrimer was described by the group of van Koten [38]. They terminated soluble polycarbosilane dendrimers in diamino arylnickel (II) complexes. Such dendrimers can be used in addition reactions of polyhaloalkanes.

An alternative application of dendrimers that has gained some attention is based on nanostructures which can find use in environment friendly industrial processes. Dendrimers can encapsulate insoluble materials, such as metals, and transport them into a solvent within their interior. Cooper and co-workers [39] synthesised fluorinated dendrimers which are soluble in supercritical CO_2 and can be used to extract strongly hydrophilic compounds from water into liquid CO_2 . This may help develop technologies in which hazardous organic solvents are replaced by liquid CO_2 .

It has been a progressing field of research and at present all these industrial applications are under study.

SUMMARY AND PROSPECTS

A rapid increase of interest in the chemistry of dendrimers has been observed since the first dendrimers were synthesised. At the beginning work concentrated on methods of synthesis and investigations of properties of the new class of macromolecules. Soon first applications appeared. Despite two decades since the discovery of dendrimers the multi-step synthesis still requires great effort. Unless there is a significant break through in this field, only few applications for which the unique dendrimer structure is crucial will pass the cost-benefit test.

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