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Reviews

Dendrimers: Analytical characterization and applications

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ABSTRACT

This review focuses on analytical techniques used for separation and characterization of dendrimers and their derivatives. These macromolecules have been attractive material for a development of new drug carriers and imaging agents. They are also interesting for many biological and industrial applications. The review mentions a few of them.

1. Introduction

The development of targeted macromolecular agents with a good blood retention times and selective organ uptake is important task for many scientists. Nowadays it is desired to design a new agent suitable for imaging of small defects at early state disease, for tumor diagnosis and therapy and for other various biological applications too. Precondition for successful tumor radiotherapy is a high specific activity of the radionuclide bonded to a targeting vector which can be a receptor-specific antibody or other type of biologically active compound. Nano-structured polymer capsules could be used to deliver chemotherapy or radioactivity directly to tumors, leaving adjacent tissue intact [1]. Such potential drug carriers and imaging agents might be recently widely studied dendrimers. These polymers discovered in 1980s by Tomalia et al. [2] and Newkome et al. [3] offer a huge opportunity for nanotechnological researches [4,5].

1.1. Synthesis

Dendrimers are symmetric highly branched polymers with a compact spherical structure (diameter ranging from 1.1 nm for the 1st generation (G1) to 9 nm for G8) [6] and unique behavior [7–9]. They are monodisperse if prepared correctly. They are routinely synthesized from a central polyfunctional core by repeated addition of monomers. The core is characterized by a number of functional groups. The following dendrimer generation is created by adding monomers to each functional group, in turn leaving end groups able to react again [10,11]. The structure of the polymer is determined by the number of reactive groups of the core, the branch lengths and surface group dimensions [12,13]. The maximum size is limited to the generation at which dendrimer becomes tightly packed looking like a ball (see Fig. 1).

Their physico-chemical properties are intrinsically dependent on generation number and surface functionalities [6]. Dendrimer syntheses involve divergent or convergent hierarchical assembly strategies [14,15]. Polyamidoamine dendrimers (PAMAMs) are synthesized by divergent approach (reported by Vögtle), in which dendrimer grows outwards from the core molecule [16]. Typically, ethylenediamine (core multiplicity ($N_c = 4$)), ammonia ($N_c = 3$) or cystamine ($N_c = 4$) may be used as cores. The core molecule reacts with monomer molecules giving the first generation dendrimer and the new periphery of the molecule is activated for reactions with more monomers [8,14]. PAMAMs are highly soluble in aqueous solution and show high charge densities restricted to the unique surface of primary amino groups [17,18]. In the convergent approach (pioneered by Hawker and Fréchet [19]), the dendrimer is constructed stepwise, starting from the end groups and progressing inwards [20] (see Figs. 2 and 3).

Size and molecular mass of dendrimers, monodispersity, shape, chirality, density, viscosity, polarity, solubility, flexibility and surface chemistry of the resulting macromolecules can be controlled during synthesis [21]. An outstanding alternative for elaboration and design of dendritic macromolecules is the Diels–Alder reaction. It leads to 3D dendrimer architectures that are structurally different from the traditional dendrimers, for example irreversible polyphenylene dendrimers. Diels–Alder reaction can be employed for both, convergent and divergent methods of synthesis [22].

1.2. Dendrimer as a vehicle

Dendrimer surfaces provide an excellent platform for the attachment and presentation of cell-specific targeting groups,

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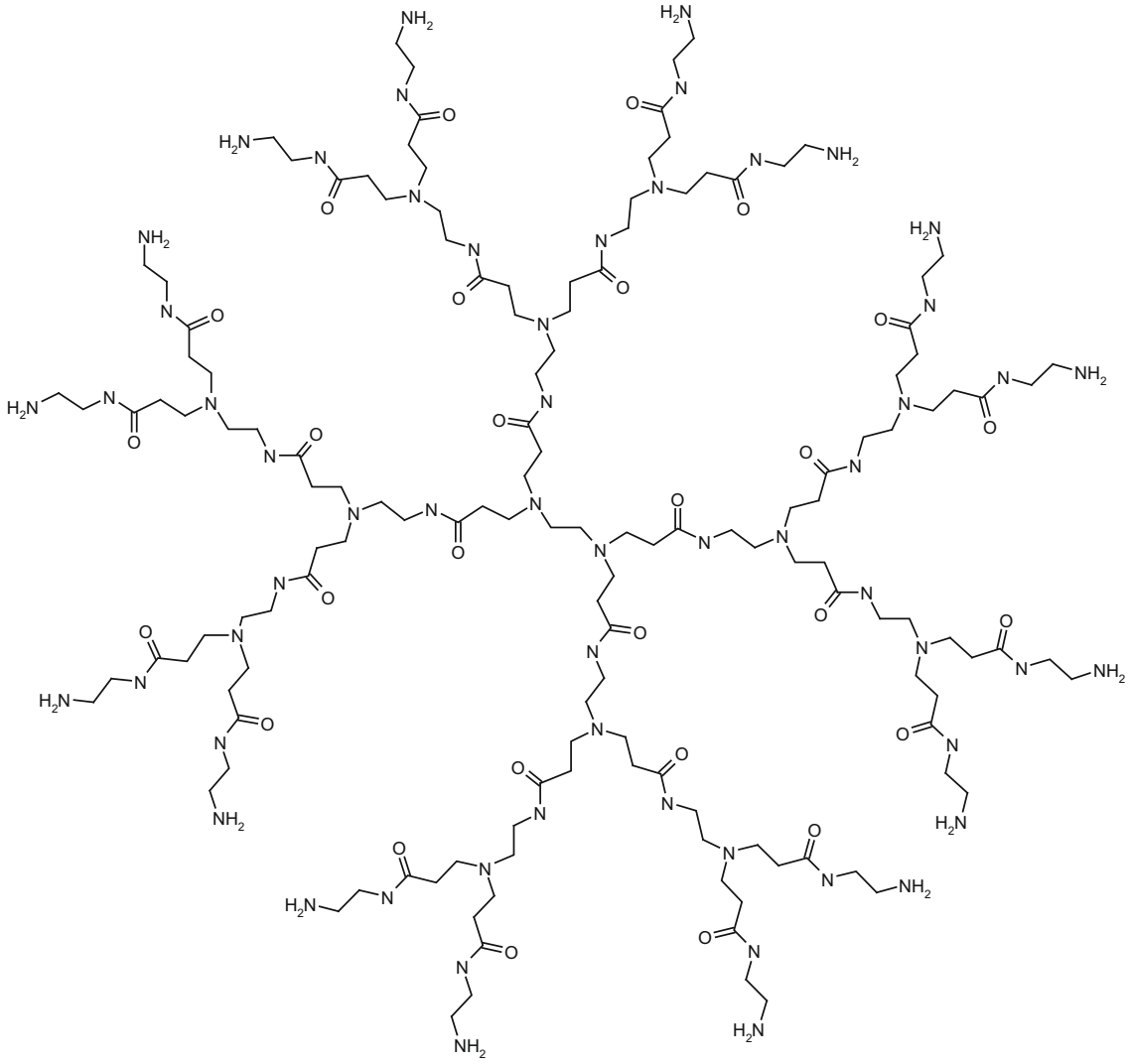

Fig. 1. Polyamidoamine dendrimer of the second generation.

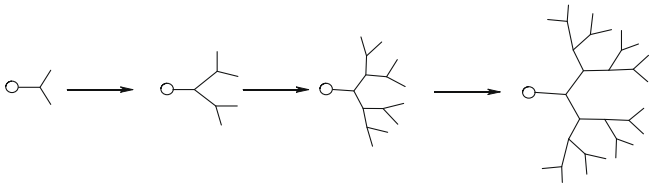

Fig. 2. Divergent synthesis.

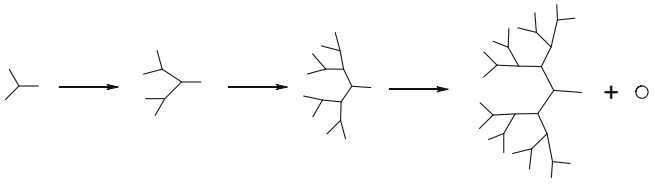

Fig. 3. Convergent synthesis.

solubility modifiers, stealth moieties that reduce immunological interactions and imaging tags, due to the presence of multiple terminal groups on the exterior of the molecule [23,24]. Dendrimers can also encapsulate hydrophobic or hydrophilic drugs inside the non-polar interior cavities present around the focal core of the dendrimer by a host–guest interaction [13,25–27] (see Figs. 4 and 5).

A large number of metal ions for imaging (paramagnetic or radioopaque) and therapy (radioactive particle emitters) can be conjugated to the dendrimer by the application of appropriate metal ion chelates. Using classic organic synthetic techniques a combination of dendrimer conjugates with a targeting vector may potentially site specifically deliver a large amount of these metal ions directly into the body with a dendrimer as the vehicle and the targeting vector directing the modified dendrimer [24,29–31]. To increase their targeting efficiency, interactions of dendrimers with lipid bilayers, DNA and other molecules must be understood. Theoretical and computational modeling methods have been applied in investigation of the atomic-scale insights into the interactions of dendrimers with other molecules [32–34]. Drug molecules can be complexed or conjugated with the surface functional groups through covalent bonds or by electrostatic forces [7,11,27,35]. Dendrimers are particularly attractive as they offer a high drug-loading capacity [36]. They are biochemically inert and non-toxic that makes them an ideal drug carrier system [37].

Four large and 8–10 small guest molecules have been bound to poly (propylene imine) dendrimer (PPI) with protected amino acid groups on the 64 amine terminal groups of dendrimer [25]. After

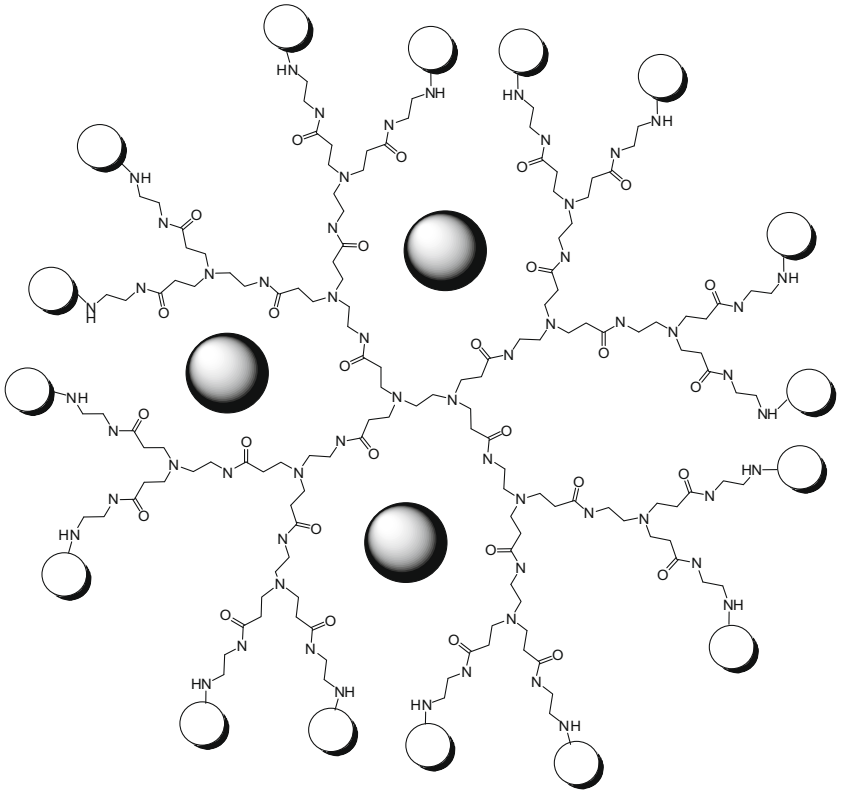

Fig. 4. Example of encapsulation of a guest molecule by functionalised dendrimer.

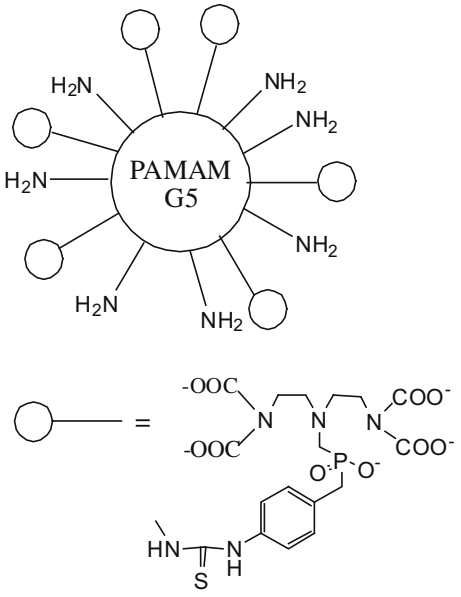

Fig. 5. Example of the dendritic ligand [28].

deprotection of the terminal functionalities, the surface shell opened and the guest molecules were released from the dendrimer.

Poly(benzyl ether) dendrimer with a carboxylic acid surface was able to dissolve hydrophobic guest molecules such as pyrene in water.

Adamantyl urea- and adamantyl thiourea-modified PPIs were reported to be a useful carrier for (Boc)-protected peptides which could be easily released under mild acidic conditions. PAMAMs of the 3rd and 4th generation have created a complex with 78 ibuprofen molecules and in vitro release of ibuprofen was slower in comparison with free ibuprofen drug. The complex has entered carcinoic human alveolar basal epithelial cells (A549 cells) more rapidly than the free drug.

The anticancer drug cisplatin conjugated with PAMAM dendrimer has showed a slower release profile, higher accumulation in solid tumors and lower toxicity compared to non-conjugated cisplatin. Also 5-fluorouracil encapsulated into 4th generation PAMAMs with carboxymethyl PEG_{5000} surface chains had reasonable drug-loading, reduced release rate and decreased hemolytic toxicity in comparison with non-PEGylated dendrimers [25] (see Figs. 6 and 7).

Peptide–dendrimer conjugates have been prepared for an investigation of integrin binding. Interactions between cells are involved in physiological processes and many disease states such as cancer metastasis and different inflammatory conditions. Integrins are cell surface receptors that interact with the extracellular matrix. Integrin VLA-4 ($\alpha_4\beta_1$) is found also in tumor cells. Fibronectin is a cell adhesion protein that contains two major domains that support cell adhesion. Integrin VLA-4 includes both of them while the minimal essential peptide sequence of ligands is LDV (Leu–Asp–Val). However, the affinity of LDV in cell adhesion assays is low in comparison with fibronectin. Dendrimer conjugates have been recognized as a potential alternative to increase the affinity of LDV to the $\alpha_4\beta_1$ integrin. Dendrimer conjugates were prepared in high purity by Monaghan et al. [39] using solid-phase synthesis and evaluated for their ability to inhibit the binding of biotinylated-EILDVPPST-NH_2 to the $\alpha_4\beta_1$ integrin adhesion receptor expressed on cancer cells in a competition Enzyme-Linked ImmunoSorbent Assay (ELISA). Results showed that the presence of dendrimer in EILDVPST sequence increased its affinity. Dendritic compounds bearing LDV showed little or no activity in competition with EILDVPST-NH_2. The solid-phase synthesis seems to be the best

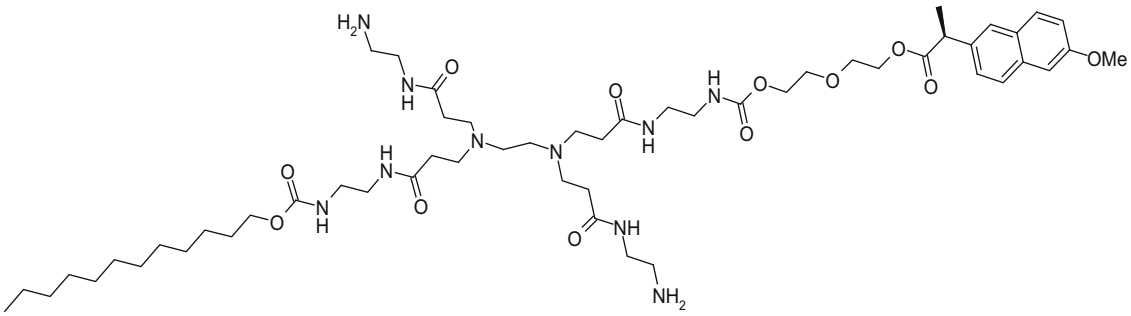

Fig. 6. Example of conjugate of PAMAM dendrimer with a drug molecule (naproxen) – L-G0-deg-NAP [38].

method for preparation of extremely clean monodisperse peptide–dendrimers [39].

Shi et al. [40] modified carbon nanotubes with PAMAM dendrimers for cancer cell targeting and imaging. Carbon nanotubes can release heat in a radiofrequency filed, which may be used to produce thermal cytotoxicity in malignant cells. PAMAMs of the 5th generation modified with fluorescein isothiocyanate or folic acid were covalently linked to acid-treated multiwalled carbon nanotubes. Products were water dispersible, stable and biocompatible. In vitro cell biological assay data show that the folic acid modified conjugates can specifically target to cancer cells overexpressing folic acid receptors [40].

Singh et al. [41] synthesised PAMAM dendrimers of 4th generation, conjugated them with folic acid or folic acid–PEG–NHS {folic acid-poly(ethylene glycol)-N-hydroxysuccinimide} and evaluated for the anticancer drug delivery potential using 5-fluorouracil (31% loading) in tumor-bearing mice. Folate–PEG–dendrimer conjugate was safe and effective in tumor targeting compared to a non-PEGylated formulation. It also reduced hemolytic toxicity and the highest accumulation in the tumor area was observed [41].

1.3. Charge and cytotoxicity

Dendrimers with a hydrophobic interior and hydrophilic surface are called unimolecular micelles because they are able to solubilize hydrophobic molecules in aqueous solutions. The surface charge of dendrimer colloid play a major role in mobility and migration, as its properties are often dominated by electrostatic interactions in aqueous solution. According to the studies of electrophoretic mobility (EM) the PPIs behave as charged spheres and surface charges play the role in influencing their EMs [6]. Their resemblance to biocomponents such as viruses, enzymes, DNA duplexes, bioassemblies as lipid bilayer membranes of biological cells and proteins makes them interesting for theoretical studies and biomedical applications [19,42].

At higher pH (pH > 10) PAMAM dendrimer adopts the most globular shape as the charge of the molecule becomes neutral. The low pH region leads to extended conformations due to electrostatic repulsion between the positively charged ammonium groups [43]. Dendrimers have to be non-toxic and non-immunogenic to be usable in drug delivery applications [44]. Cationic dendrimers with positively charged surface groups as the other cationic macromolecules including liposomes and micelles destabilize cell membranes and cause cell lysis. PAMAM and PPI dendrimers with amino-surface groups are cytotoxic on human intestinal adenocarcinoma cells and the cytotoxicity is generation dependent. Higher generation dendrimers are the most toxic. They also have hemolytic effect observed on a solution of blood cells [44]. Anionic dendrimers with carboxylate surface are not cytotoxic and their incubating with human red blood cells in plasma formate cell aggregates [45].

Dendrimers of first and second generation with amine surface functional groups were modified either with protons, tert-butoxycarbonyl or benzyloxycarbonyl protecting groups, Boc-protected or unprotected natural amino acid residues, ethylene-diamine ligands, and dansyl fluorescence labels by Fuchs et al. [46]. Cytotoxicity of products was determined in vitro using the human MCF-7 breast cancer cells. Surface functionalities influences dendrimer toxicity. The internal structure of dendrimers does not play a main role in toxicity; however, the surface decoration is crucial. Noncharged dendrimers and all diamino propionic acid decorated dendrimers are non-toxic. Noncharged dendrimers are clearly bioavailable. Positive charges on a dendrimer surface do not automatically cause cell toxicity, but it is not clear whether the bidendate nature of ethylenediamine plays a role here, for example by chelating metal ions. Dansylated dendrimers are internalized by HeLa cells and remain intracellularly present over a 20-h incubation period according to confocal fluorescence microscopy studies [46].

Dendrimer surface modifications result in a change in total surface charge, an increase in molar mass of the product, changes in the generational, skeletal and substitutional distributions. As highly positively charged macromolecules are often cytotoxic, the dendrimer surface charges have to be reduced and partially modified as intermediate materials to enhance the biocompatibility of these dendrimers [44]. Charge and charge distribution of partially modified dendrimers play an important role in determining physico-chemical properties of the final products and influences their interactions with biologic entities [6].

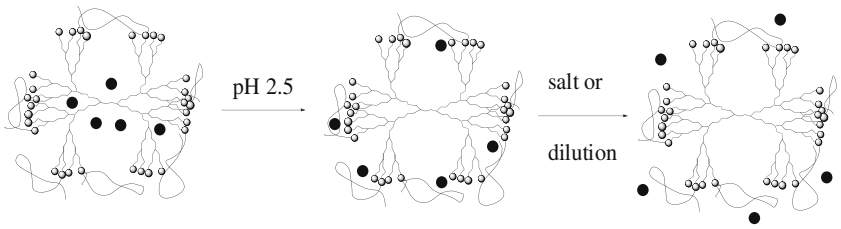

Fig. 7. Schematic representation of stepwise probe release of a drug molecule from dendrimer by addition of NaCl solution at a low pH.

2. Electrophoretic characterization

The terminal amine groups of PAMAM dendrimers can be modified with different functionalities and can be linked with various biomolecules [47]. Such dendrimer surface modifications often create new conjugate structure complexity that characterization requires analytical methodologies [48].

PAMAM dendrimers possess good water solubility and may carry multiple charges. Their solutions may be polycationic or polyanionic in nature, depending on their structure, terminal modifications and solution conditions, such as solvent, pH, temperature and concentration. They can be analyzed by electrophoretic methods. Polyacrylamide gel electrophoresis (PAGE) of them provides semiquantitative information about their purity and electrophoretic mobility [49].

Sedláková et al. [50] have developed a new electrophoretic method based on the dynamic coating of the capillary for the separation of seven generations of PAMAM dendrimers at pH 7.4. The separation of compounds possessing amino groups by capillary zone electrophoresis (CZE) suffers from the interaction of solutes with the capillary wall which results in the absence or incomplete separation and retention in the capillary. Positively charged PAMAM dendrimers strongly bind to the silica surface of the capillary so they cannot be separated at neutral pH. The best separation was obtained with the system containing polyethylenimine (PEI) at a concentration of 0.05% (w/v) for the lower generations. Comparing two buffers, phosphate and Tris–phosphate, better resolution was obtained with the Tris–phosphate buffer. The strong influence of PEI as a dynamic capillary wall modifier was confirmed as it had a great impact on the separation/resolution of individual generations. It also improves the separation at acid pH [50].

Shi et al. [49] synthesized surface-modified ethylenediamine-core PAMAM succinamic acid dendrimers (PAMAM-SAHs) and a core–shell tecto(dendrimer) carrying succinamic acid termini and analyzed them by PAGE, size-exclusion chromatography (SEC), potentiometric acid–base titration and CZE. Native PAGE and sodium dodecyl sulfate PAGE (SDS–PAGE) were performed. PAGE shows that the relative mobilities of generation two to generation seven dendrimers decreased with the increasing number of generations. Native (gradient) PAGE can separate various generations due to the gel filtration effect, thus purity and homogeneity can be assessed. If SDS–PAGE is used, it is possible to estimate the molecular weight of PAMAM-SAHs based on a protein standard. The electrophoretic mobilities of individual generations of PAMAM polyanions are similar, indicating that the separation mainly depends on their approximately identical charge/mass ratio. A CZE method was established for PAMAM-SAHs and applied to a core–shell tecto(dendrimers) too. The similar electrophoretic mobilities for all generations indicate that the polyanionic PAMAMs are not adsorbed on the surface of the quartz capillary thus only the charge/mass ratio and the electroosmotic flow influence the separation. The E5(E3.SAH)n tecto-dendrimer (numbers by *E* are referring to the generation of dendrimers) had a lower electrophoretic mobility, which was consistent with its lower charge/mass ratio [49].

Sharma et al. [51] developed a simple electrophoresis technique for evaluating purity of PAMAM dendrimers. Simple modifications of analytical conditions allowed separating any charged, water-soluble dendrimers of varied shapes and dimensions during one analysis. PAMAM dendrimer separation was studied under basic and acidic conditions. Electrophoresis under acidic conditions increased resolution and sensitivity. The use of low temperature (4 °C) for separation and post-electrophoresis manipulations led to improved dendrimer separation [51].

Castagnola et al. [52] performed capillary zone electrophoresis measurements of dendrimers at different pH values. The results indicated a sensible increase of dendrimer hydrodynamic radius at pH values lower than 2.5 probably due to the Coulombic repulsion of charged amine groups of the inner dendrimer shells [52].

Dendriplexes, complexes of dendrimers and nucleic acids or short oligodeoxynucleotides, were characterized by gel electrophoresis. Charge and molar ratio, formation and stability of the complex and the shape of DNA in the dendriplex are possible to study using agarose gel electrophoretic method. Dendriplex migration is stopped in contrast to free DNA that is negatively charged at neutral pH. Data are analyzed in terms of the presence or absence of DNA in lanes containing dendrimer-nucleic acid complexes at different charge/molar ratios [53].

Cheng et al. [26] studied the formation of aggregates between dendrimers and surfactants and evaluated their potential in new drug formulations, especially in transdermal delivery routes. One of the used analytical methods to determine the size and stability of the drug-loaded formulations was agarose gel electrophoresis. After addition of anionic surfactant and drugs into dendrimers, the surface amine groups of dendrimers were reduced, which causes the reduced mobility of the aggregates in the electrical field. The dendrimer-surfactant aggregates seemed to be stable even in electrical field. The drug-loaded aggregates proved lower stability than the free aggregates [26].

Weber et al. [54] characterized amino-terminated carbosilane dendrimers developed to protect and transport small interfering RNA (siRNA) bind to dendrimer via electrostatic interactions. Stability and the strength of the complex were tested performing heparin competition assays on agarose gel electrophoretic system and PAGE system. Complex is resistant to degradation by RNase and carbosilane dendrimer has a protective effect on siRNA in the presence of RNase [54].

A combination of capillary electrophoresis and mass spectrometry (CE/MS) seems to be an ideal technique for the separation and identification of basic dendrimers. Stöckigt et al. [10] used the online coupling of capillary electrophoresis with a sector mass spectrometer via an electrospray ionization (ESI) source to separate and identify polydisperse dendrimeric diaminobutane (DAB)-based polynitriles (DAB-dendr-$(CN)_8$) and by-products resulting from the synthesis. The samples consisted of two main fractions characterized by different electrophoretic mobilities. Increasing the pH from 3 to 7 changed the selectivity. CE/MS offers the possibility to detect closely related compounds and isomers [10].

Shi et al. [6] have synthesized and characterized acetylated and carboxylated ethylenediamine-core PAMAM dendrimers with defined surface charges to investigate the effect of charge and the influence of surface modifications on electrophoretic mobility and molecular distribution. The surface-modified dendrimers were characterized by size-exclusion chromatography, nuclear magnetic resonance (^{1}H NMR), matrix assisted laser desorption/ionization-time of flight–mass spectrometry (MALDI-TOF–MS), PAGE and capillary electrophoresis (CE). Partially modified dendrimers have a broader migration peak in CE analysis then fully modified or unmodified due to variations in surface substitution. EM decreased nonlinearly with increases in surface acetylation for PAMAM acetamides and PAMAM succinamic acids, indicating a complex migration activity in CE separations that is not solely due to charge/mass ration changes [6].

3. Analytical characterizations

Analytical characterization of dendrimers is a difficult task and since now following techniques have been reported in the literature: nuclear magnetic resonance–mass spectroscopy (NMR–MS), gel permeation chromatography (GPC), high-performance liquid chromatography (HPLC) and ultra-performance liquid chromatog-

raphy (UPLC), fast-atom bombardment–mass spectrometry (FAB–MS) and electrospray ionization mass spectrometry (ESI-MS), UV–VIS spectroscopy [51], Fourier transform infrared spectroscopy (FT-IR), MALDI-TOF–MS, electron and atomic force microscopy, small-angle X-ray scattering (SAXS), small-angle neutron scattering (SANS), dynamic light scattering, potentiometric titration [29], size exclusion chromatography (SEC) and electrophoresis; polyacrylamide gel electrophoresis (PAGE) and capillary electrophoresis (CE) [47,51]. It has been shown that the combination of CE with other techniques is important to understand the structural characteristics of dendrimer derivatives and nanodevices. HPLC has been used for the analysis of PAMAMs and derivatives and other dendritic polymers such as poly (propylene imine) and polyether dendrimers [47]. PAMAM dendrimers were first presented as monodisperse molecules. After closer examination and improved analytical techniques it has been shown that they possess a small percentage of defects [55].

Nourse et al. [12] studied physico-chemical properties of two 5th generation PAMAM dendrimers with different terminal groups, hydroxyl-surface polymer G5-OH and amino-surface polymer G5-NH_2, using the techniques of absorption spectroscopy, SEC, SDS–PAGE, density measurement, measurement of sedimentation velocity and sedimentation equilibrium. Size-exclusion chromatography shows that G5-OH is homogenous and has a matrix exclusion radius close to that of a globular protein of identical mass. Measurements of density increment, sedimentation and diffusion coefficients suggest that dendrimer has more swollen, porous structure then a globular protein. G5-NH_2 did not elute at all on the column in sodium phosphate buffer (NaP) at concentrations lower than 0.6 M, indicating strong electrostatic interaction with the column packing material. At 0.6 M NaP this polymer eluted well displaying a shoulder at a smaller eluting volume than the main peak occurs. The profile indicates that G5-NH_2 is heterogeneous and forms oligomeric aggregates in aqueous solution also in the presence of high salt concentrations. At lower salt concentrations dendrimer adheres tightly to HPLC silica gel particles in the size-exclusion column and fails to penetrate cross-linked polyacrylamide gels [12].

Tsutsumiuchi et al. [56] investigated the formation of ion complexes between PAMAM dendrimer HCl salt and poly(L-glutamic acid) sodium salt in water and phosphate buffers by pH, turbidity and viscosity measurements and electrophoresis analysis. The highest turbidity and neutralization was observed around 1:1 charge ratio, which demonstrates that not only primary amines on the surface of dendrimer but also internal tertiary amines participate in the ion complex formation [56].

HPLC has traditionally been utilized to separate and characterize the purity of various PAMAM dendrimer generations or conjugates as well as to evaluate the polydispersity, surface heterogenity and solubility of multifunctionalized dendrimers and to study the interactions between biomolecules and dendrimers [48]. Cason et al. [48] used UPLC analysis for monitoring PAMAM dendrimer surface transformations and product quality. Results compared to HPLC and found that the application of UPLC increased the average number of theoretical plates and reduced retention times of analytes, while improving the resolution capability to discriminate surface variances in dendrimers. Amine terminated PAMAMs tend to readily adsorb to a variety of substrates, therefore reverse ion pair chromatography was conducted using trifluoroacetic acid (TFA). While PAMAM dendrimer populations are relatively monodisperse, they still exhibit minor defect levels as generational or skeletal dispersity. The polydispersity of them can be estimated qualitatively by assessing the peak width at half height ($W_{H/2}$). Results showed that generational defect levels increase with dendrimer generation. There is enhancement in retention time as a function of PAMAM generation, due to the geometrically increased surface density of the terminal amine groups and TFA ion pairs. Generations 4 and 5 amine terminated PAMAMs exhibited a small shoulder to the right of the main band, which closely overlaps with the next higher generation peak. That indicated the presence of dimers. In both HPLC and UPLC a peak is present to the left of the main band, associated with unreacted G4 PAMAM (6% of the G4 PAMAMs were unmodified) [48].

Shi et al. [47] separated and analyzed PAMAM dendrimers of different generations with carboxyl, acetyl and hydroxyl terminal groups and a folic acid dendrimer conjugate (dendrimer–FA) using reverse-phase HPLC. The separation was achieved using a linear gradient 0–50% acetonitrile (ACN) within 40 min. Also PAMAMs with defined acetylation and carboxylation degrees can be analyzed using HPLC. Dendrimer–FA conjugate of generation 5 (G5.75Ac-FA_4) was analyzed and its specific binding with a bovine folic acid binding protein (FBP) was monitored. The formation of three complexes after the binding of G5.75Ac-FA_4 with FBP occurs. HPLC seems to be an effective technique for characterization and separation of functionalized PAMAM dendrimers and conjugates and also for investigation of the interaction between dendrimers and biomolecules [47].

Ornelas et al. [57] synthesized giant redox dendrimers of seven generations, using the phenoltriallyl dendron as a building block for organic dendrimers, with ferrocenyl and pentamethylferrocenyl terminal groups in order to obtain well-defined macromolecules with interesting physical properties. Redox metallodendrimers were characterized by a variety of analytical techniques. The two first generations of ferrocenyl dendrimers show the corresponding molecular peaks with calculated values in MALDI-TOF mass spectra which confirmed the structure of the products. Above the 6th generation the carbon analysis showed structural defects and increased ability to encapsulate external impurities. The expected structure of pentamethylferrocenyl dendrimers was obtained for the 0th generation according to MALDI-TOF mass spectra. The number of defects in dendritic structures was investigated by UV–VIS spectroscopy using The Lambert–Beer law to determine total number of metallocene groups in the dendrimers. It confirmed that number of the defects in structure of metallodendrimers becomes higher for 6th and 7th generations. Metallodendrimers reach 20–30% of the theoretical number of ferrocenyl termini at the 7th generation. They have high redox stability and can be isolated and characterized in oxidized, reduced and mixed-valence forms. The large amount of defects in seventh generation does not perturb its electrochemical reversibility. These nanomolecules are potential in nanoelectronics applications because of their color and ability to transfer a large number of electrons at once [57].

Majoros et al. [58] synthesised new POMAM hybrid dendrimers constructed from poly(propylenimine) core and poly(amidoamine) shells and characterized them by HPLC, GPC, NMR and noncontact atomic force microscopy (AFM). High symmetry of the products gave very simple spectra in ^{13}C NMR spectroscopy. Any deviations were caused by errors in synthesis or degradation of the material. GPC was used to determine experimental molecular weights of products. POMAM hybrid dendrimers are monodisperse with very narrow distribution. For the higher generations bimodal narrow distribution was observed. HPLC analysis showed that dendrimers are relatively pure with a very small amount of higher molecular weight material present probably due to aggregation. This was observed at lower generations in the HPLC eluograms, but not in the GPC eluogram, which suggests that aggregation behavior is highly influenced by the environment of the molecules. AFM imaging showed relatively uniform size of the particles [58].

Tulu et al. [59] synthesized water-soluble dendrimers based on poly (propylene oxide) amines and characterized them by elemental analysis, GPC, Fourier transform infrared spectroscopy (FT-IR), ^1H and ^{13}C NMR. The first generation dendrimers were relatively

monodisperse according to GPC analysis results. Although molecular weights compared to the reference molecules (polystyrene) were smaller than expected value. Because of the steric repulsion of the voluminous dendritic side chains, dendrimers are expected to be much more stiff then the used calibration standards. This leads to an increased hydrodynamic volume which causes GPC molar mass to become smaller than the true molar mass [59].

Similar issue has been discussed by Koyama et al. [60] who prepared photo-responsive carbosilane dendrimers bearing 4-phenylazo-benzonitrile units. The molecular sizes of the *cis*- and *trans*-isomers of the products were analyzed by GPC using linear polystyrene standards to determine the calibration curve. The weight-average molecular weights calculated from the retention times were not in an agreement with the theoretical values because of the complicated interactions arising from polarities between the gel and the azobenzene unit in the GPC column [60].

Myc et al. [61] synthesised and characterized PAMAM nanodevice in which folic acid is conjugated as a targeting molecule and a caspase-specific FRET-based agent (PhiPhiLux G_1D_2) as the apoptosis-detecting agent to analyze the degree of apoptosis in targeted cells. The number of tertiary and primary amino groups in PAMAM of the 5th generation was determined by potentiometric titration. GPC was used to define defects in analyzed structure. HPLC analysis showed the removal of the free folic acid after membrane filtration purification of the acetylated PAMAM with attached folic acid [61].

Hong et al. [62] synthesised dendrimer nanodevices which characterized spectroscopically using ^{1}H NMR and UV/VIS and chromatographically using GPC and HPLC. They become more polydisperse and gave a bimodal distribution as the number of attached folic acid increased. The number of folic acid per dendrimer was calculated from GPC data and compared to UV/VIS measurements. Because of the higher local concentration of folic acid confined to the dendrimer surface the data deviate from Beer's law using free molecular folic acid to obtain the standard curve [62].

Islam et al. [63] performed HPLC analysis on PAMAM dendrimer based multifunctional devices synthesized by conjugating partially acetylated PAMAMs of the 5th generation with fluorescein isothiocyanate, folic acid and methotrexate. The use of a common gradient allowed determining the purity of the conjugates and stability of all compounds under the experimental conditions. Important information about the surface properties of conjugates can be obtained since elution of analytes occurs as a result of counter-ion binding and surface interaction between the conjugates and the stationary phase [63].

4. Applications

Commercially available dendrimers, such as PAMAMs and PPIs, with different terminal groups have been applied in magnetic resonance imaging [55,64–66], protein/enzyme mimicking/modeling [48], gene delivery systems (siRNA carriers [54]) [34,67], neuroendocrinological studies [68], adhesion, catalysis [69,70], sensors, batteries [42], optics [71,72], electronics [73], cosmetics and personal care products [74], environmental remediation (decontamination agents [48]), material science [75], biology [75] and also as antiviral agents [29,76,77]. Diaminobutane dendrimers (DABs) are also available on market [65]. Dendrimers are appealing to scientists as nanobuilding blocks for materials because of their monodisperse nanoscale sizes, guest–host encapsulation properties, diverse surface chemistries, and low toxicity/nonimmunogenicity features [48].

Dendrimers have been used as boron carriers for antibody conjugation in boron neutron capture therapy [25,34]. One of the newest applications of dendrimers is oriented to the treatment of solid tumors by photodynamic therapy using a novel class of photosensitizers such a newly developed dendrimer phthalocyanine (DPc)-encapsulated polymeric micelle [34,78–81].

5. Conclusion

Dendrimers separation and characterization is important in their synthesis and for studies of their interactions with the other molecules. Various techniques have been utilized to analyze dendrimers and their derivatives. Further work is needed to improve analytical methods for purification of products of synthesis and conjugation with biomolecules or drug molecules. It is also important to characterize physico-chemical properties and biocompatibility of the novel dendrimer conjugates.

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