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Review

Poly(amidoamine) Dendrimers as a Pharmaceutical Excipient. Are We There yet?

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ABSTRACT

Drug solubility could affect the therapeutic use of a drug because the biological activity of a drug is only possible if some fraction of a dissolved drug can permeate and overcome biological membranes to reach its site of action. The solubility-permeation interplay is therefore, probably the most important factor in determining a successful therapeutic outcome of any drug because more than 40% of marketed drugs and more than 70% of pipeline drugs show poor water solubility. Several solubilization techniques are used and include, balancing of pH-pK_a properties, employment of cosolvents, and the solubilization by host-guest carriers. A relatively new addition to the polymer plethora of solubilizers are the poly(amidoamine) dendrimers. These highly branched, "tree-like" nanocarriers have a significant solubilization capacity for drugs in their cavities and also potentially via their terminals. Despite their successful solubilization capability, they are still plagued by some undesired properties such as cytotoxicity. Poly(amidoamine) however, seems to be a very lucrative target to develop into a pharmaceutical excipient, which will ultimately be confirmed by an official pharmacopeial monograph.

Introduction

The aim of this article is to review the solubilization enhancing potential of poly(amidoamine) (PAMAM) dendrimers from a physicochemical perspective to judge its suitability as a pharmaceutical excipient. In addition, the challenges that are encountered by these nanocarriers are discussed from a cytotoxicity perspective. It has to be emphasized that literature commonly indicates that PAMAM is used as and excipient. However, all pharmaceutical excipients are ultimately officially recognized by a pharmacopeial monograph. An official monograph is still not published for PAMAM dendrimers, although their development into a pharmaceutical excipient has seen significant progress.

The name dendrimer is derived from the Greek words dendron meaning "tree" and meros meaning "part." Inspired by the beauty and efficiency with which nature controls macroscopic 3-dimensional space by the use of branching networks in trees, corals, and physiological structures, a scientist named Tomalia, tried to mimic such branched assemblies at the molecular level and was able to successfully synthesize these 3-dimensional macromolecular polymers called dendrimers.[1,2]

Dendrimers are highly branched and reactive 3-dimensional macromolecules with all bonds emanating from a central core. Since their introduction in the mid 1980s, this novel class of polymeric materials has attracted considerable attention because of their unique structure and properties. Compared to traditional linear polymers, dendrimers have much more accurately controlled structures, with a globular shape, a single molecular weight rather than a distribution of molecular weights, and a large number of controllable peripheral surface functionalities. Although dendrimers had their inception approximately 35 years ago,[3] they can still be considered as new drug solubilizers. Some commonly used solubilizers have been known a very long time. PVP for example was patented in 1939.[4] Cyclodextrins were first described in 1891,[5] and the first description of α-, β- and γ-CD was reported in 1911.[6] The synthesis of PEG was already described in 1859.[7]

Many families of dendrimers with various core molecules and building monomers have been synthesized and are commercially available now. But, the family of dendrimers most investigated for drug delivery is the PAMAM dendrimers. PAMAM dendrimers are biocompatible, nonimmunogenic, water soluble and possess terminal-modifiable amine functional groups for binding various

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targeting or guest molecules. The internal cavities of PAMAM dendrimers can host metals or guest molecules due to the unique functional PAMAM dendrimer architecture, which contains tertiary amines and amide linkages. PAMAM dendrimers were first synthesized by Tomalia[1] and the first publication on these starburst molecular structures was reported in 1985 following the filing of some patents earlier in the 1980s.

A so-called divergent (inside-out) synthesis was followed to construct the dendrimer. First, ethylenediamine was employed as the core moiety from which short amidoamine arms were extended by Michael addition of methyl acrylate. This methacrylate ester was subsequently extended by addition of ethylenediamine to produce amino terminals on the arms.[8] Subsequently, PAMAM dendrimers which terminated in ester groups were designated as "half generation" dendrimers, whereas "full generation" PAMAM dendrimers terminated via amino groups.[1] A convergent (outside-in) method of synthesis was described in 1990 by which the arms were synthesized by reaction of pre-synthesized dendron units with the core functional groups which enabled follow-up structure expansion.[9]

PAMAM Dendrimer Structure and Physicochemical Properties

To better discuss the capability of PAMAM dendrimers to solubilize drugs, a discussion of the structural and physicochemical properties is necessary. All dendrimers comprise 3 structural components. In the case of PAMAM dendrimers, these 3 components are the diamine core, arms extending from the core by substitution of both amine groups of the core, and finally the terminals which are situated on the periphery of the arms (Fig. 1).[9] The most common terminals of PAMAM dendrimers are carboxylic acid groups, hydroxyl groups, and amine groups. These afford, under the appropriate pH conditions of the surrounding medium, negative, neutral, or positive charges, respectively. Owing to the tree-like branched structure of these molecules, cavities are present in the structure which could potentially host some guest molecules.

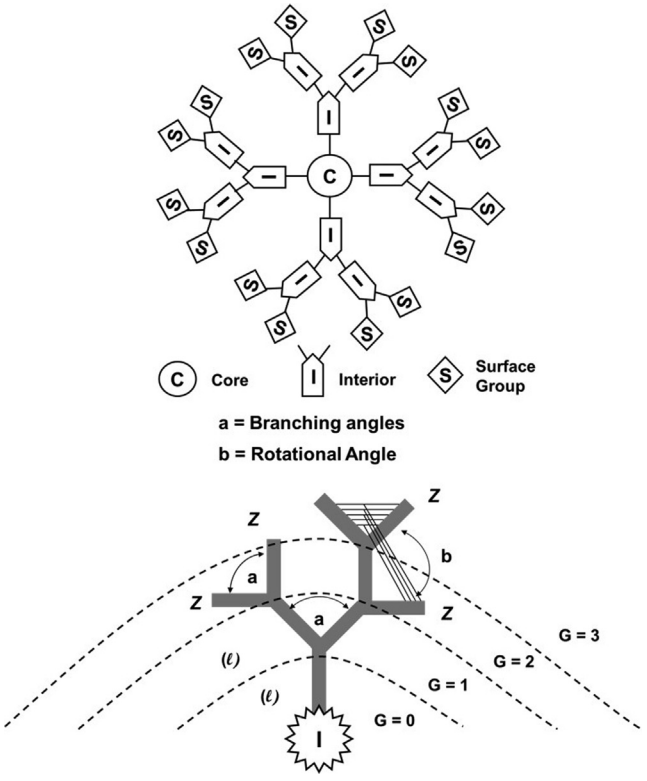

Figure 1. Top: Basic architectural components of dendrimers. Bottom: branch cell structural parameters (a) branching angles, (b) rotation angles, (Z) terminal groups, and (l) repeat unit lengths.[9]

It is known that the pH-pK_a balance determines the extent by which drug molecules would find them in the ionized or neutral state. PAMAM dendrimers also show this pH-pK_a balance due to their amide and amine functional groups in the arms and peripheral structures. The reported pK_a values of the primary amine terminals are 7.0-9.0 and that of the interior tertiary amines 3.0-6.0.[1,10-13] At physiological pH 7.4, most of the primary amines are protonated, and at pH 4.0 all of the tertiary amines are protonated. Therefore, the protonation level of the PAMAM could be altered by changing the solution pH. In turn, the extent of amine protonation significantly affects the ability of the PAMAM dendrimer to interact with guest molecules.

Dendrimers were initially seen to be a type of micellar structure, although in a "solid" type of structure due to the covalent bonds of the structure holding the "micelle" together instead of the spontaneous formation of micelles at the critical micelle concentration of a specific amphiphile in a specific medium as they are classically known to form. However, due to their pK_a-pH protonation properties, they exhibited additional unique "micellar" properties which were not usually observed for common micelles which did not possess functional group ionization potential.[8,14]

The physical structure of PAMAM dendrimers is also a dynamic property of these nanocarriers. For lower generation dendrimers, up to around generation 3.0, they assume almost planar structures. For most PAMAM dendrimers, a globular structure is formed when they exceed a structural extension between generation 3.0 and 4.0 and beyond.[15-17] Planar or globular structures significantly affect for example the cavity volumes and exposure of peripheral terminal groups. Subsequently, one can easily imagine that drug encapsulation and complexation can be affected significantly by the conformation of the PAMAM structure. For PAMAM dendrimers of generation 5 and higher, a significant cavity volume could be observed and provided a probable explanation for the marked increase in gene transfection seen for larger dendrimers due to the increased cavity loading capacity if compared with smaller dendrimers. The effect of pH again demonstrated that electrostatic repulsion will increase dendrimer size due to amine protonation compared to unprotonated condition at pH exceeding 10.[18]

The structure of dendrimers is probably more globular than is commonly recognized under physiological conditions. Owing to salt ions in the body, charge neutralization can take place and therefore condenses the structure due to dampening of the electrostatic repulsion between terminals and arms. Despite the assumption that planar structures are assumed for low-generation dendrimers, G1 and G2-PAMAM dendriplexes with DNA showed significantly more compact and globular shapes than would be assumed if pure dendrimer molecules were examined. Although the effect of salts on structural compaction seemed universal for the G1 and G2 dendrimers, more effective condensation could be seen for the G1 dendrimer since less steric hindrance was present. The structural effect of pH could eventually be compromised by the addition of salt. Exceeding a critical salt concentration resulted in the disruption of the dendrimer-DNA interaction which gave rise to significant expansion of the free PAMAM dendrimer. Subsequently, a markedly looser conjugation between PAMAM and DNA was observed.[19]

Owing to the functional groups in the arms and periphery of the dendrimers, dendrimers also have pK_a values associated with these groups. Therefore, one has to not only consider the drug pK_a in a specific pH environment but also the PAMAM pK_a values. It was proposed that the pK_a values of the PAMAM moieties are shifted once they are bound by guest molecules.[19] More detail of the effect

of pH relative to pK_a is illustrated under the section dealing with the PAMAM dendrimer solubilization mechanism.

To process PAMAM dendrimers from a pharmaceutical technology perspective, some additional properties may affect the process. The glass transition temperature(s), T_g, of full generation PAMAM G0- G5 dendrimers were found to range from −25°C to −34°C.[20] As with other polymers, it was proven that T_g scales with molecular weight for benzylic dendrimers. The T_g ranged from approximately 255-316 K as molecular weight increased from 320-13,646 g/mol (G1 to G6) for hydroxyl-terminated phenolic dendrimers. For bromine-terminated phenolic dendrimers, a similar trend was seen with T_g increasing from 271-325 K as generation increase from G1 to G4. Cyano group terminals increased T_g from 287-349 K for G1 through G4 generations. In all these cases, T_g reaches a plateau as the molecular weight increases above a certain limit.[21] The local packing, therefore density, in the dendrimer structure could also be affected by T_g. If ambient temperature approaches T_g, the dendrimer structure becomes more compact. The flexibility of the dendrimer structure also decreases as the dendrimer melt is gradually cooled toward its T_g. Considering that an amorphous drug-PAMAM dispersion could form, one has to account for the T_g of the components and the employed processing temperature to produce the dispersion.[22,23] Similar findings could be anticipated regarding hydrogels as an example of a type of semi-solid dispersions.[24]

Solubilization Mechanism

Classic solubilization theory conclude that partitioning of the drug between water and oil is one the most important predictors of permeation through a membrane.[25,26] The same principle applies to the drug-loading mechanism into a specific drug carrier. Therefore, during exposure of the carrier system to a drug, the drug may or may not partition into the dendrimer depending on environmental properties. If the drug molecules were poorly soluble in water, but the dendrimer provided a more hydrophobic environment, the drug would prefer partitioning to the dendrimer.[27] Figures 2 and 3 demonstrate the potential interactions that could take place between the dendrimer and drug under different conditions.

As with many hydrophobic drugs, the so-called hydrophobic interaction does not necessarily imply hydrophobic binding forces, but rather the propensity of hydrophobic molecules to avoid water. Therefore, the drug molecules would tend to self-associate in an environment where they could avoid water. In the case of PAMAM dendrimers, in their neutral state, the fraction of neutral drug molecules prefers to be located in the cavities of the dendrimers. Hydrophobic steroids, including testosterone have for example demonstrated hydrophobic capturing in cavities between the arms of PAMAM dendrimers. Subsequently, the cavities between the arms expanded to capture the guest molecules.

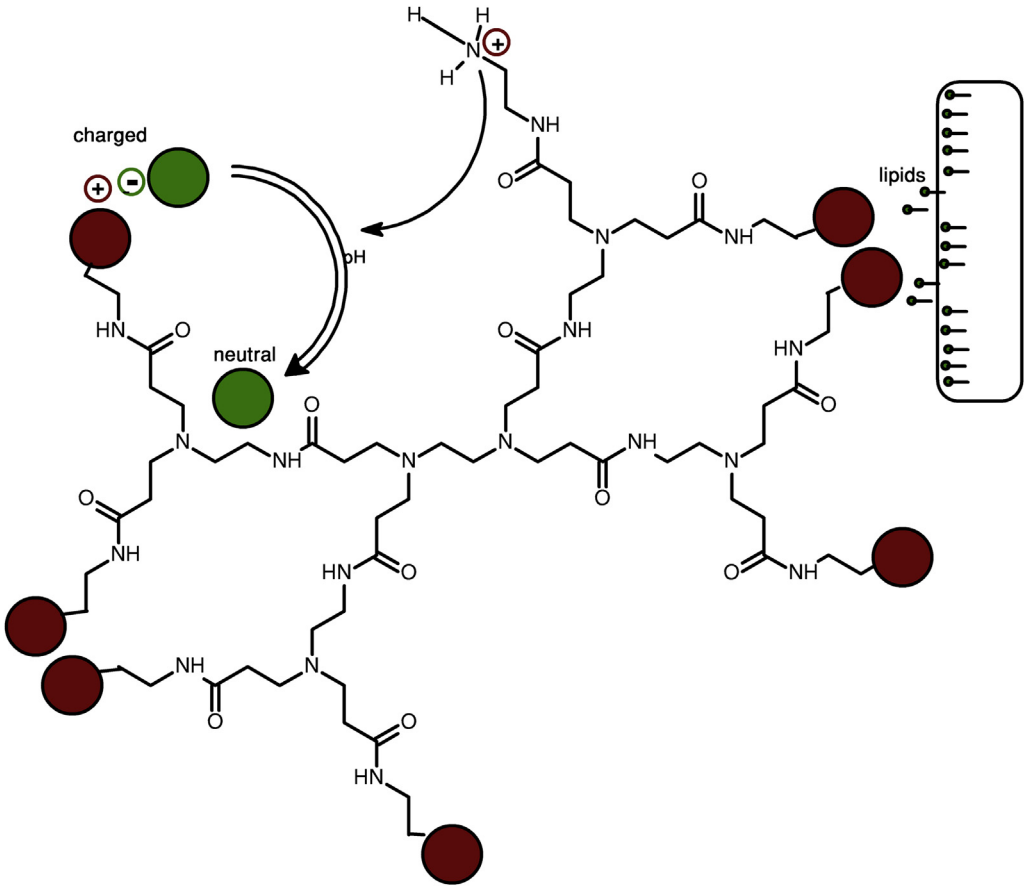

Figure 2. The full terminal PAMAM dendrimers, in unmodified form, will show charged amine terminals at low pH values. If a drug molecule was encapsulated in the cavity under neutral conditions (terminals also neutral), a change in pH could also trigger drug release. The neutral molecule becomes charged (in this case negative) and interacts with the positively charged terminals. This electrostatic interaction could result in prolonged release of the charged drug molecules in the aqueous medium. However, as discussed in the text, on downside of cationic dendrimer terminals may disrupt cell membranes which as negatively charged on their outside. Subsequently, the cationic terminal extracts negatively charged lipids from the membrane with subsequent lysis and cytotoxicity.

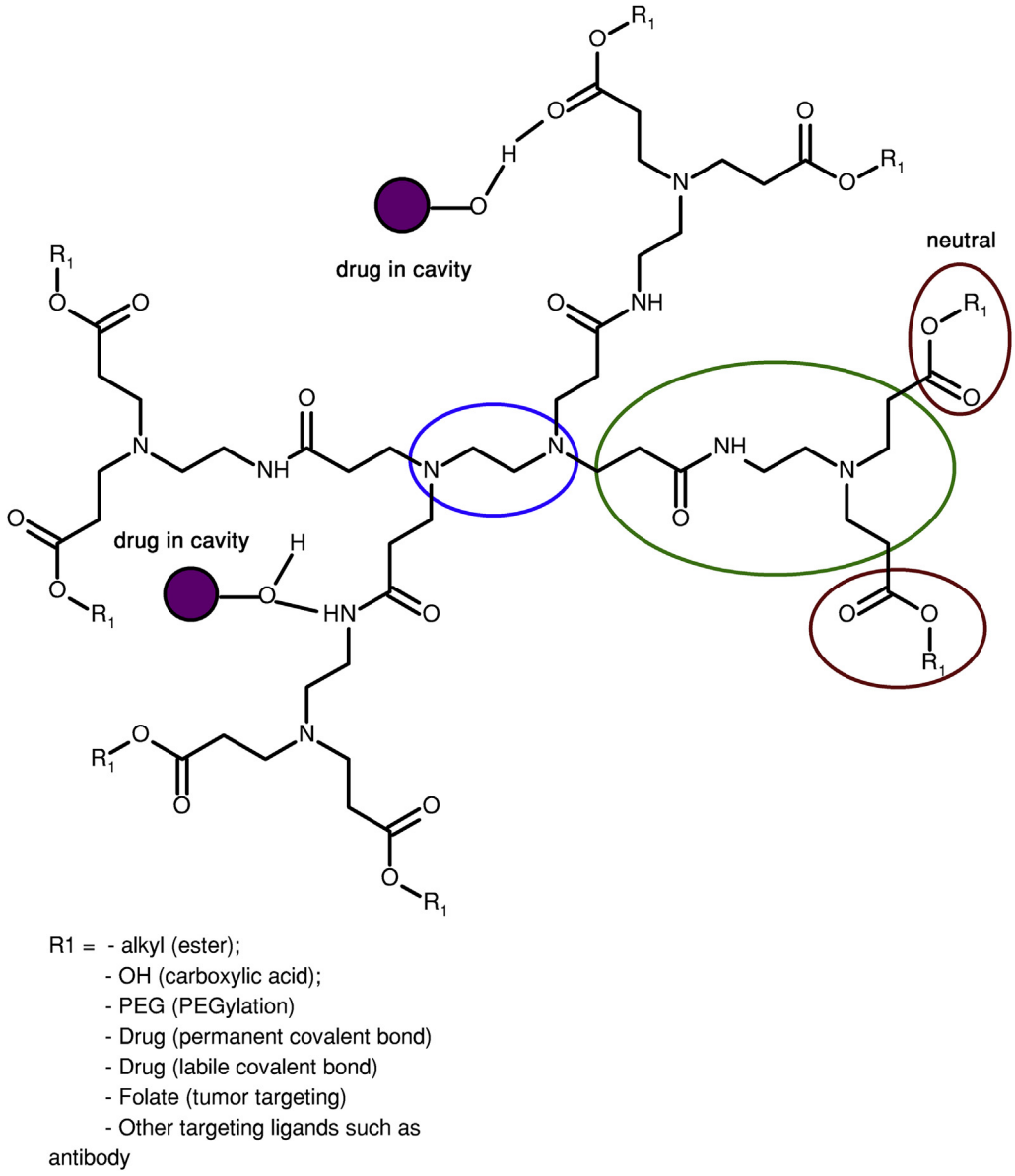

Figure 3. It is suggested that half-generation (G0.5) dendrimers may provide a less cytotoxic delivery system than their cationic counterparts. The structure of a PAMAM dendrimer is elucidated as the ethylenediamine core (blue circle), the amide arms (green circle), and the terminals (red circle). It has been proven that for example hydrogen bonding plays a major role in addition to encapsulate drugs in the PAMAM cores as indicated by the purple particles. Some additional modifications of dendrimer terminals could also lessen cytotoxicity as indicated in the potential substitution of the terminals (R1).

Nifedipine was also captured inside ester-terminated PAMAM dendrimer. In this case hydrogen bonding was the predominant interaction that facilitated encapsulation of the very hydrophobic nifedipine.[28]

Neutral furosemide molecules were captured inside PAMAM dendrimer cavities at neutral pH values. However, electrostatic interactions with the dendrimer terminals and arms were illustrated depending on the pH value of the medium in relation to the pK_a values of both the drug and dendrimer amine and amide groups.[29]

Even though drug molecules could be hosted in the dendrimer cavities, a limited amount of space exists in the cavities. Consequently, a limited number of drug molecules would partition to the cavities. Owing to the limited loading capacity, a percolation limit exists for PAMAM denrimers, as is commonly observed with many other drug-polymer delivery systems. Since drug molecules are captured inside the dendrimer cavities, their cohesiveness is disrupted compared with the bulk state. Therefore, an apparent increase in solubility is observed during the initial phases of drug release up to the point where a critical number of drug molecules can self-associate to result in phase separation. Once sufficient numbers of molecules associate outside of the dendrimer structure, the thermodynamic solubility equilibrium is reached again due to the increase in the extent of drug molecule cohesion that eventually reaches the bulk cohesion value.

A similar limit is seen for loading capacity of charged molecules. A dendrimer will have a certain number of charge groups available, mostly on the periphery, which could interact with oppositely charged drug molecules. Exceeding a certain critical molar limit, not enough charged dendrimer terminals will be available to interaction with all charged drug molecules. Therefore, again a percolation threshold is observed in charged systems. One could,

however argue that in charged systems, a smaller fraction of the neutral drug molecules exist and therefore, a lower degree of phase separation would manifest as for example drug crystallization.

The effect of pH is therefore very complex since the PAMAM dendrimers and drugs could have significantly different pK_a values and show significantly different degrees of ionization. Furthermore, during drug loading, the pH of the loading medium could differ significantly from the physiologically encountered media.[29] In the loading stage, one could tune the pH of the loading medium to selectively facilitate hydrophobic interaction. However, under physiological conditions, both PAMAM and drug could be totally ionized. Therefore, the true number of molecules encapsulated in the dendrimer cavity, could be different from the number of molecules interacting with the dendrimer under physiological conditions. It also implies that some interactions for example electrostatic interactions only take place in the release medium and not in the loading stage.

Some examples which showed these loading-release dependencies on the ionization state of both drug and dendrimer are discussed next. Electrostatic interaction is possible on the terminals of the dendrimers at relatively acidic pH values at which the amine terminals are protonated. Salicylic acid and L-alanine, as water-soluble drugs, and phenylbutazone and primidone, as poorly water-soluble drugs, were used in a computational simulation with G5 PAMAM dendrimers.[30] At a neutral pH in the medium, these guests were all hosted inside the dendrimer cavities. The study illustrated that the drug candidates are better encapsulated in the dendrimer interior at high pH values of around 10, however are released when the pH is lowered to approximately 7. The implication of these observations is that encapsulation of the drug in the dendrimer is facilitated by the hydrophobic interactions at pH 10 and that at pH 7.4, as is the case in for example in blood, electrostatic interaction between the charged terminals and drug molecules result in a sustained release effect.[31] It was proven earlier that the drugs phenobarbital, primidone, sulfamethoxazole, and trimethoprim showed very little encapsulation in PAMAM dendrimer cavities at acidic pH values. Electrostatic interactions, under these conditions, prevailed as the main mechanism by which solubility of the drugs was enhanced.[31]

Yet another effect of pH on drug encapsulation was illustrated by cycling pH values during loading of various estrogens into the PAMAM cavities. Initial loading was performed at pH 8.5 and the amount of captured estrogen was determined. Then the pH was changed to more acidic conditions at pH 4.5 which mimics the environment in endosomes or lysosomes inside cells. As expected, some release of the estrogens took place with observed hormonal action in the cells. After release was determined, pH was raised to 6.5 and then back to 8.5. It was found that some drug was still captured in the interior cavities of the PAMAM G6 which showed a seemingly irreversible bonding interaction on lowering pH to 4.5 again. This demonstrates that although a certain loading value could be achieved for the estrogen, full release from the cavities was not possible. It is suggested that the PAMAM-estradiol conjugate structure is affected by the release of the estradiol in such a way that remaining drug molecules in the cavities are not capable of escaping from the interior of the dendrimer even though an electrostatic interaction with the dendrimer terminal is expected.[32]

The core of the PAMAM dendrimer also plays a role in the accommodation of guest molecules as illustrated by a simulation experiment in which ethylene diamine (EDA), 1,5-diaminohexane (DAH) and bis(3-aminopropyl) ether was used as cores for PAMAM G3 and G4 dendrimers. The DAH cores facilitated the most significant increase in cavity size for the dendrimers solvated in water. The bis(3-aminopropyl) ether core facilitated some increase, however not as effectively as for DAH. DAH resulted in less back-folding of the branching chains with subsequent larger cavities remaining in the dendrimer structure. The increase in core chain length ensured that the dendrimer arms could extend further compared to the shorter EDA core. In the DAH core chain, the 3 lipophilic central methylene groups were capable of repelling the relatively polar arms stronger than the EDA core.[33]

The Toxicity and Biodistribution of PAMAM Dendrimers

As with almost any other polymeric delivery system, some extent of toxicity is observed with dendrimers. The type of dendrimers also markedly affects their toxicity as well as the model that was used to determine the toxicity.

The toxicity of cationic dendrimers has been shown to depend on the surface charge density and structure of the dendrimer due to interaction and disruption of negatively charged membranes.[34-37] The surface charge density of the amine-terminated PAMAM dendrimers are proportional to their size that is generation[38] since it implies that more terminal cationic groups could interact and disrupt with negatively charged cell membranes.[39-41] An example of the effect of generation of the PAMAM dendrimer was observed for bilayer removal from a membrane which was proportional to increasing full PAMAM generation in the order G6 > G4 > G2. Conversely, smaller dendrimers were more easily absorbed in the membrane bilayers in the order G2 > G4 > G6.[42]

These dependencies have generally been observed for *in vitro* test models. An *in vivo* model such as zebrafish may provide more realistic insight into cytotoxicity since cell lines could be less sensitive to toxic effects than that of a whole organism.[43] The general trend of higher cationic than anionic toxicity was proven by showing that anionic PAMAM dendrimers were tolerated in up to 10-fold higher oral doses in CD-1 mice.[44] Hemotoxicity is another undesired type of cytotoxicity of PAMAM dendrimers. However the observed effect is also affected by the experimental conditions and models. It was found that the cationic PAMAM dendrimers resulted in blood platelet activation which resulted in prothrombotic effects.[45] It has also been shown that G3 and G4 full generation amine dendrimers are also prone to cause vascular endothelium lesions in addition to promoting blood clotting.

Chemical modification of the surface terminals of dendrimers to afford negative or neutral charge to the terminals resulted in significantly less cytotoxicity as observed for neutral hydroxyl- and biodegradable ester-terminated dendrimers[46] and for negatively charged carboxylate dendrimer terminals.[47] Anionic G6.5 dendrimers showed good tolerability in CD-1 mice with no signs of toxicity up to 500 mg/kg.[48] The tolerability to PAMAM dendrimers compared well to other polymers such as chitosan that demonstrated a lethal dose of approximately 1.5[49] and 2 g/kg for acrylate polymers[50] in rats.

PEGylation is yet another popular approach to decrease the cytotoxicity of PAMAM dendrimers. The neutral PAMAM dendrimers are seemingly the least toxic type since they do not expose charged cytotoxic amine terminals to cells under physiological conditions.[51,52]

A comparative toxicity study was performed using various polycations[53] using the standard cytotoxicity model, L929 mouse fibroblast cultures (ISO). Different concentrations of the polycation test substances namely PAMAM-G3.0-dendrimer, a polyelectrolyte poly(diallyldimethylammonium chloride), poly(vinylpyridinium bromide), poly(L-lysine hydrobromide) and a common gene transfection vector that is 600-1000 kDa poly(ethyleneimine) (PEI) were investigated, using the mouse fibroblast model. PAMAM dendrimers showed less than 10% cell membrane damage at concentrations between 0.01-1 mg/mL over a 60-min period of incubation. Poly(L-lysine hydrobromide) and PEI showed at least

50% cell death after the 60-min incubation period at the same concentrations as the PAMAM dendrimer. Mitochondrial MTT damage studies on fibroblast cell culture models, confirmed that the PAMAM dendrimer was the least cytotoxic over a 24-h period at the same concentration range as test for cell membrane damage.

Polycationic amino-derivatives of dextrans were also toxic after the 24-h period, with virtually no cell survival after the test at the highest concentration. These results also suggested that more globular structures caused less toxicity than the linear structures which have a higher probability to expose peripheral cationic charge groups.[53]

PEI was also proven to be cytotoxic in human cell lines for example Jurkat T cells which is umbilical vein endothelium model and in the THLE3 liver cell model.[54] However, by modification of the terminals of branched PEIs to produce negative and neutral moieties, the cytotoxicity was reduced.[55] As means of neutralization of the cationic terminal, acetylation of polypropylenimine dendrimers was undertaken.[56] At a level of acetylation that exceeded 80%, virtually full cell viability in MCF-7 and A549 cell lines were achieved over 48 h. In comparison, unmodified polypropylenimine dendrimers demonstrated only 53% and 18% cell viability in MCF-7 and A549 cell lines respectively over 48 h.[57]

Another potential form of toxicity issue that is only observed after *in vivo* administration is the accumulation of dendrimers in the body in a nonspecific way in numerous organs and tissues.[58] Although clearance from the blood is almost complete after intravenous administration of both negatively and positively charged PAMAM dendrimers, they all showed accumulation in the liver, with little elimination via the kidneys as found in rats for gadolinium-PAMAM contrasting agents.[59]

Brain and muscle tissue were also permeated in Wistar rats after intravenous administration of dendrimeric sulfadiazine.[60] Of potential concern is also the fact that PAMAM dendrimers showed transport across human fetal membranes. Topical intravaginal administration of dendrimer-drug conjugates however, curbed the transport to the fetus which was otherwise seen after direct exposure to placental membrane exposure studies.[61] Accumulation of PAMAM dendrimers in mouse brains was also observed after a single intranasal instillation.[62]

Generally, it seems that unmodified, lower generation PAMAM dendrimers, up to G5, are less toxic with a marked increase in toxicity seen from G6 upwards.[34,62] Furthermore, the choice of negative or neutral dendrimers seems warranted since they proved to be virtually nontoxic[34,63] One should also recognize that PAMAM dendrimers may cause environmental toxicity once they end up in for example water and soil. Oxidation reactions and irradiation have been shown to result in degradation products which could be even more toxic than the actual parent dendrimer.[64]

Are We There Yet?

PAMAM dendrimer research is an extremely active field of research. Numerous patents have been filed for PAMAM dendrimers in the field of biomedical applications. However, only a handful have been granted. Most of these patents have also provided some *in vitro* data for their claims with almost *no in vivo* studies and no first-in-human studies. In addition, only a few recent attempts have been published where pharmaceutical formulation of PAMAM drug delivery systems have been demonstrated. In Table 1, some recent examples are shown.

Despite the fact that a limited commercial production capacity exists, the first steps toward large scale synthesis have been taken. The US company Dendritech Inc. was the first company to produce PAMAM dendrimers and can now produce dendrimers in kilogram quantities. Weihai CY Dendrimer Technology is one of the first companies in Asia to produce PAMAM dendrimers in sizable quantities.

Although it seems that commercial PAMAM dendrimer production capacity is increasing, dendrimers are still expensive. The most successful pharmaceutical excipient are the ones that are

Table 1
Selected Examples of Granted PAMAM Dendrimer Patents and Published Formulations of PAMAM Dendrimers for Drug Delivery

Application	Summary	Patent/Formulation	Ref
Cancer diagnosis and treatment	PAMAM dendrimer used as to carry imaging material to image tumors via radiolabelled metals, fluorescein isothiocyanate or 6-TAMARA and transfer of genetic materials/derivatives i.e.5- fluorouracil.	US20090053139A1	65
Delivery of RNA and drugs into cells	Noncationic PAMAM dendrimers which may or may not be extended by PEG branches to provide a micellar vesicle for delivery of for example RNA and doxorubicin delivery	WO2014025795A1	66
Delivery of immunosuppressive drugs	PAMAM dendrimers with PEGylation or terminal modification with specific antigens	US7534449B2	67
Platinum delivery to tumors	PAMAM dendrimer with COO^- terminals conjugated to Pt for treatment of melanoma.	US6585956B2	68
Antimicrobial or antiprotozoan compositions	Both anionic and cationic PAMAM used to form different compositions with melarsoprol, pentamidine and suramin to treat the stated conditions.	US6464971B1	69
Antimicrobial/biocide surface coating applications	PAMAM dendrimer conjugates with silver to be used on skin. Biocompatible with skin.	US20020022012A1	70
Cancer and immunotherapy	Cationic PAMAM dendrimers are conjugated to nucleic acids to carry RNAm plasmids and numerous antineoplastic drugs.	US8491914B2	71
Prophylaxis of eye infections	PAMAM dendrimers used to carry numerous antibiotics such as clindamycin, metronidazole, chloramphenicol and so forth	EP2895161B1	72
Transdermal delivery of ketoprofen	PAMAM dendrimers containing ketoprofen used in conjunction with ultrasound to facilitate *in vitro* release and *in vivo* release in mouse skin	Formulation	73
Retinal drug delivery	Dexamethasone was complexed with PAMAM dendrimers to establish *in vitro* and *in vivo* transport across ocular tissues of rats.	Formulation	74
Pulmonary delivery	A nebulizer formulation of PAMAM dendrimers with beclomethasone dipropionate was delivered into lungs with 35% delivery in 8 h to treat asthma	Formulation	75
Pulmonary delivery	Pressurized metered dose inhaler delivery of doxorubicin for potential anticancer treatment	Formulation	76
Controlled delivery	Liposomes were formulation to encapsulate PAMAM dendrimers which were loaded with doxorubicin. Potentially augmenting toxicity and efficacy of the drug.	Formulation	77
Antimicrobial cream	PAMAM dendrimers formulation with silver sulfonamide in a novel cream for treatment of burn wounds and skin infections	Formulation	78
Corneal disease treatment	PAMAM dendrimer conjugated with dexamethasone was formulated in a cross-linked hyaluronic acid gel for subconjunctival injection	Formulation	79

commonly available in large quantities, have a proven safety record, have the desired technological properties, and those that have a low cost.

In that regard, it has to be said that PAMAM dendrimers have not developed into a feasible pharmaceutical excipient yet. PAMAM dendrimers demonstrate the capacity to solubilize a large number of therapeutic agents under various conditions. However, these compounds have only been around for approximately 30 years—a short time for development into a thoroughbred pharmaceutical excipient.

In general, it is clear that more studies should be performed to establish biodistribution of PAMAM dendrimers. One of the few long-term biodistribution studies conducted in mice showed that a gadolinium-PAMAM G5 lymphatic imaging agent was slowly cleared from the liver. However, the toxicity was monitored only over a period of 90 days, with no evidence of undesired effects. Significant PAMAM accumulation in the liver and spleen was observed. A study of at least 1 year could be performed in future to ensure that 10 elimination half-life times were reached to ensure full *in vivo* clearance of the dendirmers and to establish if any toxicity could be observed over this period.[80]

Another issue which has garnered significant attention is the fact that PAMAM dendrimers are known to be cytotoxic, especially the cationic dendrimers. In context, one should bear in mind that polycationic peptides are especially renowned for their transfection capabilities.[81,82] These peptides cross cell membranes as well as show the ability to enter cell nuclei. These peptides are lauded for these properties and the potential for intracellular delivery of therapeutics. However, as with many other polycations, these peptides exhibit cytotoxicity. It would seem unfair to single out any of these compounds for an undesirable property and abolish their development totally. Therefore, currently one has to admit that the PAMAMs have not developed into a totally non-toxic substance. However we have discussed that there are tolerable doses for these compounds and that specialized delivery systems could be produced which contain low amounts of dendrimers and drugs. One should however, take inspiration from the development that was performed for the cyclodextrins. The naturally occurring CDs were also considered too toxic for use at some stage. Over a long period of time, these were developed to such an extent that some safe derivatives were finally synthesized and they now have pharmacopeial monographs.

To decrease the toxicity of cationic PAMAM dendrimers, numerous modifications have been made to the chemical structure of the dendrimers. These include using half-generation dendrimers which do not display the cationic terminals[83]; the attachment of PEG or lauroyl chains to the dendrimer terminals[38] and the modification of the amine terminals to neutral acetamide terminals.[84] Esterification of hydroxyl groups of the PAMAM dendrimer have also alleviated the cytotoxicity of PAMAM dendrimers, while improving gene delivery in KB and HepG2 cells.[85]

Biodegradable esters have also been created and which show resistance to hydrolysis in the endosomal pH range of pH 5.1-7.4. After hydrolysis of arginine on release of the gene, the relatively safe PAMAM-OH was reformed.[46] The evidence therefore points to the fact that negatively or neutrally charged PAMAM dendrimer is the most likely way in which dendrimer technology should develop in future. In addition, modification seems to benefit significantly from PEGylation which not only renders the PAMAM dendrimer neutral but also prevents accumulation in the liver and spleen.[86]

Considering drug delivery in conjunction with cytotoxicity, it has been suggested that covalent modification of dendrimer terminals with drug conjugated to the terminal via a linker that can degrade because of a specific trigger, will be a more feasible drug delivery strategy than the passive encapsulation mechanisms that take place via dispersive and electrostatic interactions. Unfortunately, this could result in the drug-dendrimer conjugate that will require re-registration as a new chemical entity.[87] Conversely, if toxicity for these conjugates were not significant, the long-term treatment of a chronic disease with a single dose might become feasible since the elimination of the conjugate takes very long.

Conclusion

In many regards, PAMAM has certainly proven itself as an effective solubulizer of poorly water-soluble drugs. In addition, it also facilitates gene transfection very efficiently. These are strongly desired properties for an excipient that can both increase solubility and also ensure intracellular delivery of a therapeutic substance. Some inroads have been made to achieve a more acceptable toxicity profile of PAMAM dendrimers via chemical modification, however still too few *in vivo* biodistribution studies have been conducted to determine the pharmacokinetic fate of the dendrimers themselves.

Long-term toxicity and biodistribution in the *in vivo* situation is still unclear, not only in test animals but also in humans. Recently, it has also been shown that cationic polymeric nanoparticles could interfere in clinical chemistry tests by affecting for example serum protein interaction with test reagents. False positive or negative values could be inferred depending on the type of dendrimer in the circulation. Therefore, false-positive indications for pathology could be indicated in patients who have normal serum levels of the biomarker that is studied but have values close to the upper border. This poses another challenge in the design of dendrimers as pharmaceutical excipients in that they are not passive excipients and actually show some bioactivity themselves. This type of bioactivity is not unique to dendrimers and can occur for all kinds of nanoparticles.[88]

We can therefore say that we are not there yet. PAMAM dendrimers do not have a pharmacopeial monograph yet and cannot be recognized as a pharmaceutical excipient yet. However, progress has been made to a large extent and significant progress has been made toward establishing PAMAM dendrimers as pharmaceutical excipients. Considering that these nanocarriers are newcomers, these dendrimers may yet prove their worth in gold yet.

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