Chemical and ecotoxicological assessment of poly(amidoamine) dendrimers in the aquatic environment

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Poly(amidoamine) (PAMAM) dendrimers are attracting great interest as a consequence of their unique properties as carriers of active molecules in aqueous media, as we expect their presence in the environment to be widespread in the future.

In this article, we focus on the analytical methods to characterize and to determine these polymeric materials in waters and on their ecotoxicity for aquatic organisms. We review physical characterization techniques (e.g., light scattering, electron microscopy, atomic force microscopy) and analytical techniques, mainly based on liquid chromatography, so as to consider their main capabilities, advantages and drawbacks. We assessed the toxicity of certain PAMAM dendrimers for the green alga *Pseudokirchneriella subcapitata* by determining the EC_{50} and correlating it with the ζ-potential.

Keywords: Aqueous environment; Atomic force microscopy; Dendrimer; Ecotoxicity; EC_{50}; Electron microscopy; Light scattering; Liquid chromatography; Poly(amidoamine); ζ-potential

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1. Introduction

Dendrimers are a new class of polymeric materials, generally described as macromolecules and characterized by an extensively branched 3D structure providing a high degree of surface functionality and versatility. The unique properties associated with dendrimers (e.g., uniform size, high degree of branching, water solubility, multivalency, defined molecular weight and available internal cavities) make them attractive for biological and drug-delivery applications.

Dendrimers are perfectly branched building block polymers that emerge radially from a central core [1]. The poly(amidoamine) (PAMAM) dendrimers have diameters of 1.5–13.5 nm, so they can be viewed by direct techniques {e.g., electron microscopy [2–6], atomic force microscopy (AFM) [3,7,8]} or indirect techniques {e.g., light scattering [9–12]}.

Structurally, the dendrimer comprises three fundamentals parts:

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(1) the core;
(2) the dendrons; and,
(3) the terminal groups.

The core must have reactive centres to which the dendrons (branches) are attached. Fig. 1 shows the cores more commonly used. The dendron has terminal groups that become the new centre of support. Each completed dendron layer around the core is called a generation, the first layer being generation zero. Generation n is usually denoted as Gn, where G is generation, and n is the number of layers. Dendrimers obtained in an intermediate step, before completing layer n + 1, are denoted Gn.5. The extension .5 is refers to intermediate syntheses (or half generation) [13]. The assembly of dendrons is usually carried out in two steps, using a Michael addition synthesis [14]. The first report of these kinds of molecules was made by Vögtle et al. [15], who described a series of synthetic cascade molecules (see references in Vögle's book [1].

In the case in this work, when dendrons are amide-amine structures, the dendrimer is PAMAM. Tomalia reported the first work and took out the first patent on PAMAM dendrimers, with both ammoniac and ethylendiamine cores, some of the most used in research [13,16,17]. Fig. 2 represents the synthesis and divergence of PAMAM, as reported by Tomalia [13], and shows an example of third-generation PAMAM. These PAMAM dendrimers generally have $–NH_2$, –OH, –COOH, $–COOCH_3$ terminal groups located on the external layer, and, in all cases, they have tertiary nitrogens in the inner structure, positioned on the branching points [18]. In general, with respect to PAMAM dendrimers, when the name of the terminal group is not specified, the intention is that the PAMAM referred too has $–NH_2$ as the terminal group. As a consequence, these dendrimers have two pKa corresponding to the terminal group and the tertiary amine {i.e. 9.52 and 6.30, respectively, in the case of PAMAM [19–21]}. At neutral pH, most of the primary amines are protonated, and, at pH < 4, all of the tertiary amines are also protonated, as reported by van Duijvenbode et al. [22]. Similar results with PAMAM dendrimers were also reported by Diallo et al. [23,24].

The branched structures of dendrimers have been studied from a fractal point of view [25–27]. Farin found that PAMAM dendrimers have a fractal dimension of 2.4, similar to enzymes, and suggested that these materials can act as biomolecular mimics with important physiological interactions [26].

PAMAM dendrimers can be used as:

(1) additives in the pharmaceutical and cosmetic industries;
(2) drug carriers (molecular vector) [28–30];
(3) analytical sensors [31–33] because one of the main reasons for the interest in PAMAM dendrimers is their capability in trapping specific molecules thus faciliting their water solubility [13,16];
(4) a chelating agent of metals for remediation of wastewater [23,24]; and,
(5) a carrier of Pt metal in hydrogenation catalysts [34].

PAMAM dendrimers of high generations (above generation 4) have structures that exhibit densely packed near-spherical topologies with many nm-sized cavities [35] (e.g., which can act as organic molecule reservoirs or nanoscale templates to produce metal nanoparticles (NPs) [36–39].

Because we expect PAMAM dendrimers to have widespread future use in human consumption, and then be waste after release to environment, our main objective in this work is to review and to show results on the characterization and the analysis of PAMAM dendrimers in aqueous media and their effect on ecotoxicity.

2. Characterization in the aquatic environment

In aqueous media, PAMAM dendrimers with functional groups ($-NH_2$, -OH, -COOH) are very soluble [20,23]. Table 1 shows the amounts of water bound inside PAMAM at different pH levels and generations: in some cases the molar ratio of water:dendrimers is between 138 (generation 4, high pH) and 1731 (generation 6, low pH), as reported by Maiti et al. [20].

For the same generation of PAMAM dendrimer, the bound water increases when the pH decreases due to an increased protonation level in which the tertiary amines are protonated together to the primary amine on the shell, facilitating penetration of water inside the particle. Also, at a higher generation, the amount of water increases due to the increase in sites that can be bound.

When a non-polar solvent (e.g., acetonitrile) is used instead, the PAMAM dendrimer aggregates

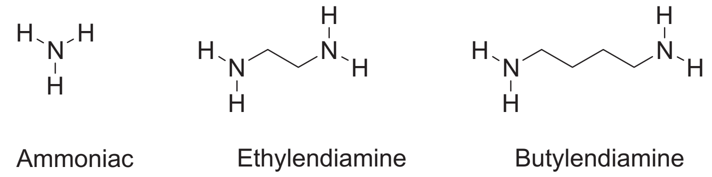

Figure 1. Examples of several types of PAMAM core.