Transformation and toxicity of PAMAM dendrimers under irradiation and ozonation processes

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Abstract

Dendrimers are a class of thoroughly branched polymers characterized by a high chemical versatility. Dendrimers have unique properties such as uniform size, well-defined molecular weight and tunable surface functionality and solubility. Also, the presence of rather large internal cavities makes them interesting for many biological and medical applications [1, 2]. The cytotoxic properties of dendrimers including the influence of surface modification have been the subject of recent scientific publications [3, 4]. Most of the available data found connections between cytotoxicity surface charge and dendrimer generation, which determine cell permeability. In general, cationic PAMAM dendrimers have been associated with higher cytotoxicity than anionic or neutral dendrimers. Also, growing dendrimer generation has been associated with increased toxicity [5]. After therapeutic use dendrimers are excreted. For example, *Nigavekar et al.* reported that neutral PAMAM G5 dendrimer-based organic nanoparticles resulted in a higher urinary excretion (and lower via faeces) than those bearing positive charge [6]. Once released, the dendrimers would undergo oxidation and photolytic transformation processes, either in water treatment plants or under the action of natural processes. This may lead to the production of secondary pollutants which occurrence and toxicity also needs to be addressed. The goal of this work is to identify and to assess the toxicity of the transformation products of G3-PAMAM-$(NH_2)_{32}$ dendrimer during irradiation and oxidative degradation.

Oxidation and irradiation procedures. PAMAM dendrimer G3-PAMAM-$(NH_2)_{32}$ was purchased from Dendritech. The solutions were prepared in pure water (Milipore Mili-Q) with a resistivity of at least 18 Mcm^{-1} at 25 ºC. Ozone was produced by corona discharge and continuously bubbled as described elsewhere [7]. Irradiation experiments were carried out using a 15W Heraeus Noblelight TNN 15/32 low-pressure mercury vapour lamp emitting at 254 nm and a Heraeus TQ Xe 150 Xe-arc lamp with spectral emission in the visible region. The temperature was 25°C and pH was controlled at 7.0 ± 0.1 units. Samples were withdrawn for analysis at prescribed intervals.

Analytical. The analyses have been performed by liquid chromatography using a hybrid quadrupole/time of flight mass analyzer (LC-ESI-QTOF-MS) and a TripleTOF 5600 System (AB SCIEX, Concord, ON) coupled to an HPLC Agilent 1200 (Agilent Technologies, Wilmington, DE, USA) with a ESI source. The AB SCIEX TripleTOF 5600 equipment offers the capability of high resolution detection with resolving power 40000 (FWHM). The LC analyses were performed with a reversed-phase C5 analytical column 150 mm length x 4.6 mm I.D. and 5 µm particle size. The MS was operated in full scan TOF-MS and MS/MS mode through information dependent acquisition (IDA) in a single run analysis. The data acquisition and processing was carried out using Analyst® TF 1.5 and PeakView™.

Toxicity. The toxicity of samples corresponding to partially oxidized and irradiated mixtures was assessed using the following bioassays: multigenerational growth inhibition of the green alga *Pseudokirchneriella subcapitata* (OECD TG 201), the inhibition of the constitutive luminescence of the marine bacterium *Vibrio fischeri* (ISO 11348-3 standard protocol), the 48 h immobilization of the microcrustacean *Daphnia magna* (OECD TG 202 performed with the commercially available text kit Daphtoxkit F™ magna, Creasel, Belgium) and the phytotoxicity based on seed germination tests following US EPA procedures on *Licopersicon esculentum*, *Lactuca sativa* and *Lolium perenne* [8]. The assessment of reactive oxygen species (ROS) generation was performed by loading cultures of control and treated cells with the fluorescents dyes 2,7-dichlorofluorescein diacetate (H_2DCFDA) and C4-BODIPY, the first serving as indicator for hydrogen peroxide and other ROS, such as hydroxyl and peroxyl radicals and the later being used for evaluating lipid peroxidation. Dichlorofluorescein (DCF), chlorophyll and C4-BODIPY fluorescence was visualised using confocal fluorescence microscopy (Leica TCS SP5).

The analytical method developed with LC-ESI-QTOF-MS has allowed the identification of the dendrimer G3-PAMAM-$(NH_2)_{32}$ and the structural elucidation of the transformation products generated in the oxidation and irradiation processes. The multiple-charging phenomenon of G3-PAMAM-$(NH_2)_{32}$, associated with ESI, makes it possible the formation of multiply charged ions and provided key advantages in dendrimer identification by assignation of charge state through high resolution of $^{13}C/^{12}C$ isotopic clusters as well as a mass accuracy below 3.8 ppm for the diagnostic ions. The structures of relevant TP have been elucidated based on mass accuracy, in both full scan and MS/MS modes via IDA, and elemental composition assignation. Fig 1 (left) shows the LC-ESI-QTOF-MS mass spectrum in full scan mode for G3-PAMAM-$(NH_2)_{32}$. G3 was characterized by a charge state distribution up to +10, $^{13}C/^{12}C$ isotopic resolution (peak-spacing and isotopic distribution). This figure also presents an overlay of the TIC (total ion chromatograms) with the gradual formation of two relevant transformation products corresponding to the treatment with O_3 after 130 min.

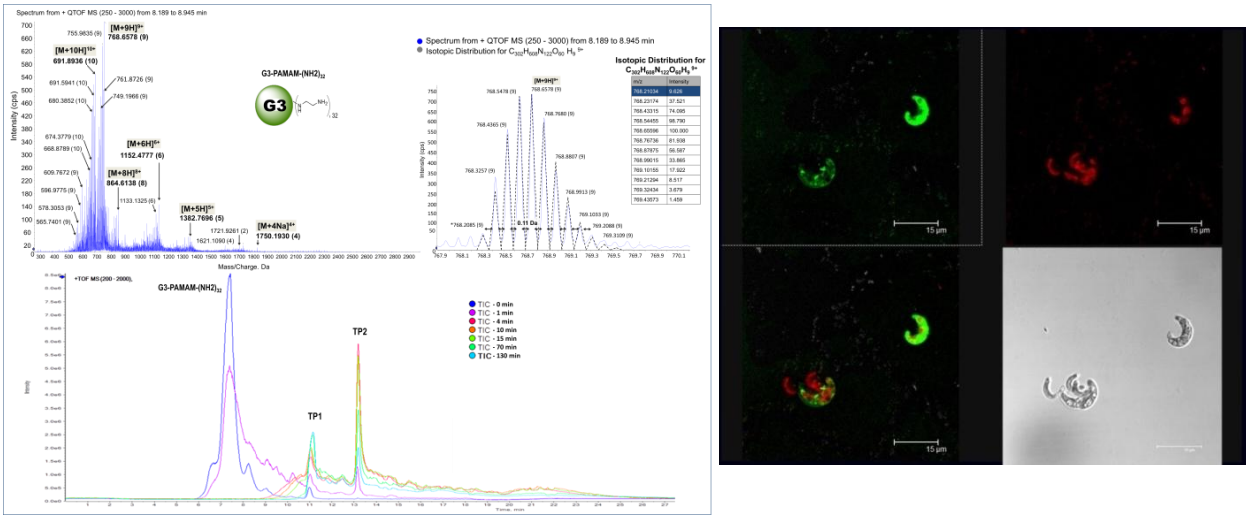

Figure 1. LEFT: LC-ESI-QTOF-MS mass spectrum in full scan mode for G3-PAMAM-$(NH_2)_{32}$ and TIC chromatograms showing the formation of oxidation products. RIGHT: Confocal microscopy images of *P. subcapitata* exposed to 0.5 mg/L G3NH_2 PAMAM during 72 h showing intracellular green BODIPY fluorescence as a result lipid peroxidation. Red fluorescence: chlorophyll autofluorescence. Bottom: overlay image of chlorophyll red autofluorescence and BODIPY green fluorescence and bright field image of the same preparation.

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