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THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

DOSSIER ON DENDRIMERS

**Series on the Safety of Manufactured Nanomaterials
No. 46**

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ENV/JM/MONO(2015)9
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OECD Environment, Health and Safety Publications

Series on the Safety of Manufactured Nanomaterials

No. 46

DOSSIER ON DENDRIMERS



INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among **FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD**

Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
Paris, 2015

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No. 44, *Dossier on Gold nanoparticles (2015)*

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No. 48, *Dossier on Fullerenes (2015)*

No. 49, *Dossier on Multiwalled Carbon Nanotubes (MWCNTs) (2015)*

No. 50, *Dossier on Single-Walled Carbon Nanotubes (SWCNTs) (2015)*

No. 51, *Dossier on Silicon dioxide (2015)*

No. 52, *Dossier on Zinc oxide (2015)*

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PREAMBLE

In November 2007, OECD's Working Party on Manufactured Nanomaterials (WPMN) launched the Sponsorship Programme for the Testing of Manufactured Nanomaterials (hereafter the Testing Programme). The objective was to conduct specific tests, relevant to human health and environmental safety endpoints, on a variety of manufactured nanomaterials (MN). The outcomes of the Testing Programme were intended to assess the applicability of the existing **test guidelines**¹ to nanomaterials, as well as to provide useful information on any intrinsic properties of MNs, which are different from the same bulk material with greater external dimensions. Understanding the properties of NMs is crucial to choose appropriate strategies for hazard identification, risk assessment or risk management measures. The Testing Programme involved delegations from OECD member countries, some non-member economies and other stakeholders. The broad international representation, from a range of delegations enabled the programme to pool expertise and resources without which this programme would not have been possible.

Before launching the Testing Programme, the WPMN first identified a broad list of possible nanomaterials, and the list was later adjusted to a final selection of eleven MNs for testing². This list comprised: i) fullerenes (C60); ii) single-walled carbon nanotubes (SWCNTs); iii) multi-walled carbon nanotubes (MWCNTs); iv) silver nanoparticles; v) titanium dioxide; vi) cerium oxide; vii) zinc oxide; viii) silicon dioxide; ix) dendrimers; x) nanoclays; and xi) gold nanoparticles. One fundamental criterion for selecting these materials was that they should be either in commercial use at the time or expected to be in the near future. At the same time, other considerations were also given attention, such as the production volume of the materials, the likely availability of such materials for testing and the existing information that would readily be available on the materials.

It was also agreed that 59 endpoints would be addressed³ for each material corresponding to the following categories: i) nanomaterial information/ identification; ii) physical-chemical properties and material characterisation; iii) environmental fate; iv) toxicological and eco-toxicological effects; v) environmental toxicology; vi) mammalian toxicology; and vii) material safety. These endpoints were judged to be most important based largely on the general experience of testing chemicals, while taking into account the potentially different or new properties of nanomaterials. It is worth noticing that it was not expected that testing for all of the listed endpoints would be necessary for each of the selected MNs.

To assist with the Testing Programme, the WPMN developed two documents: i) a *Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials* [ENV/JM/MONO(2009)21]; and ii) *Guidance Manual for the Testing of Manufactured Nanomaterials: OECD's Sponsorship Programme* (Guidance Manual) in 2009, which was subsequently updated in 2010

¹ The OECD Test Guidelines are a collection of internationally agreed test methods used by government, industry and independent laboratories. They are used to determine the safety of chemicals.

<http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm>

² Originally Iron nanoparticles, Carbon black, and Polystyrene were suggested but later withdrawn and replaced by gold nanoparticles.

³ As specified in the Guidance Manual, "address" includes the term "completed" which provides that all dossiers will contain the identified endpoint information. Note that for some endpoints (for example, solubility) it is specified that the endpoint must be "completed". In such instances "completed" means that all Dossiers will be providing this endpoint information.

[ENV/JM/MONO(2009)20/REV]⁴. The objective of this *Guidance Manual* was to guide sponsors⁵ in the testing of the materials while ensuring that the information collected was reliable, accurate, consistent and therefore also comparable. The *Guidance Manual* addressed a whole range of issues including the organisation of the work.

The *Guidance Manual* contains detailed information on the selected endpoints for testing and recommendations on sample preparation and dosimetry.

The *Guidance Manual* also described the development of *Dossier Development Plans* (DDPs). These plans were prepared by Lead sponsors, Co-sponsors together with contributors to describe the specific plan for the testing of each nanomaterial including when and where the testing will be undertaken and by whom. The DDPs also included information on the materials to be tested as well as information on issues such as sample preparation and dosimetry. Each of the DDPs was prepared and reviewed by the WPMN before testing work began.

Based on the lessons learned during the Testing Programme, the WPMN also developed *Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials* [ENV/JM/MONO(2012)40]. This latter document is an update of an earlier text first published in 2010.

The work on OECD's Testing Programme was completed by the end of 2013. In June 2014 the WPMN agreed that for each nanomaterial the dataset would be published in IUCLID printed format^{6 7}. The document will include the protocols and methods to allow their wider use (regulators and researchers).

The dataset in this document has been declassified and made publicly available and it is expected regulators and researchers will wish to use it. Due to a broad dissemination of the data and the exploratory setting in which they were developed there are a number of limitations in using the data of which potential users should be aware. The programme focused on answering scientific questions in the field of the OECD test guidelines but not to provide conclusions on the hazard or risk of the materials selected. The absence of data for some endpoints may be a gap for some endpoints but for other end points there may not if the data was not considered necessary. Although the programme ensured a broad participation of many stakeholders it was not intended to arrive at any pre-defined regulatory datasets requirements or risk assessment decisions. It was recognised from the beginning that the exploratory nature of the work would require subsequent follow-up work for example to review the specific needs that may arise when performing risk assessment of nanomaterials. In this context, the programme's ultimate goal, to add to the knowledge of the properties of nanomaterials, would form a cornerstone.

⁴ It is worth noting that while the *Guidance Manual for Sponsors* was primarily intended as a guide to WPMN's Testing Programme, it is also expected that it will be of value to anyone involved in testing NMs.

⁵ The *Guidance Manual* noted, for example, that there could be three levels of participation to the programme. Lead sponsors, who would assume responsibility for conducting or coordinating all of the testing, determined to be appropriate for each of the endpoints for a specific nanomaterial. In some cases, "joint lead" arrangements were developed. Co-sponsors conducted some of the testing determined to be appropriate and feasible to address the endpoints for a specific listed nanomaterial. Contributors provided test data, reference or testing materials or other relevant information to the lead and co-sponsors.

⁶ IUCLID is a software programme for the administration of data on chemical substances. Although it was originally developed to fulfill requirements in the EU for the evaluation and control of the risks of existing chemical substances, it is used by many others.

⁷ SIAR = SIDS Initial Assessment Report (SIDS = Screening Information Data Set)

FOREWORD

As part of its Programme on the Safety of Manufactured Nanomaterials, OECD launched the Sponsorship Programme for the Testing of Manufactured Nanomaterials (hereafter the Testing Programme). The objective was to conduct specific tests, relevant to human health and environmental safety endpoints, on a variety of manufactured nanomaterials (MN). The Testing Programme mainly aimed to assess the applicability of the existing test guidelines to nanomaterials, as well as to provide useful information on any intrinsic properties of MNs, which are different from the same bulk material with greater external dimensions.

This document presents the Dossier of Dendrimers which was prepared under the leadership of Spain. This nanomaterial has been tested for a number of endpoints for: i) Nanomaterials Information / Identification; ii) Physical-Chemical Properties; iii) Environmental Fate; iv) Environmental Toxicology; and v) Material Safety. They have been analysed using OECD Guidelines for the Testing of Chemicals (TG)⁸. The data is presented in an IUCLID⁹ style format and includes the protocols and methods used (see Preamble).

Spain led the Testing Programme on Dendrimers. This included the determination of the tests that were appropriate for Dendrimers, performing a number of tests, as well as coordinating tests performed and inputs provided by the Universidad de Alcalá for Physical-Chemical testing, and by the National Institute for Agricultural and Food Research and Technology (INIA), Department of Environment for the Ecotoxicity testing.

This document presents an overview of the results experimentally obtained for polyamidoamine (PAMAM) Dendrimers in the framework of the OECD Sponsorship Programme. A large amount of the results included have already been published in peer reviewed journals which provides an additional guarantee of the quality and reliability of the data. Data already published in an international journal have been indicated in the text and the complete reference included, as well as a description of what it has been already published without extracting graphs, figures or tables.

It should be noticed that two challenges arisen when determining the toxicity (hazard) and environmental or body levels (exposure) of nanomaterials: i) the low solubility of a number of nanoparticles that lead to important variations in the expected concentrations obtained when applying the tests (hazard) and ii) the lack of an appropriate analytical method that prevents the determination of the real exposure concentrations (exposure). These two problems appear to be solved, at least in part, in the case of PAMAM Dendrimers.

A particular feature of the PAMAM Dendrimers used in this set of experiments is related with their solvation properties that allow these molecules to form stable suspensions in aqueous media. This property has two important consequences for the experimental setup. First, when different concentrations

⁸ <http://www.oecd.org/env/testguidelines>

⁹ IUCLID is a software programme for the administration of data on chemical substances. It was originally developed to fulfil requirements in the EU for the evaluation and control of the risks of existing chemical substances. It is specifically relevant in the context of an international programme for the initial assessment of chemical substances.

are obtained by serial dilutions of a stock suspension, no problems have been faced related to variations in the expected concentrations, because of the very reduced sedimentation of the material in the previous dilutions.

Secondly, as it can be observed from the data about physico-chemical properties, it appears that the level of agglomeration or aggregation of the molecules in the suspensions is minimal. This has also facilitated the establishment of an analytical method (Hernando et al., 2012) that permitted to determine the real concentrations that were applied in the experiments.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology of the OECD.

ACKNOWLEDGMENTS

The OECD Secretariat and the Working Party on Manufactured Nanomaterials wish to thank Spain for leading the Testing Programme for Dendrimers. In particular, Dr. Juan Carlos Flores and Mrs. Marina B. Ramírez-Fernández, from Inorganic Chemistry Department of the Universidad de Alcalá, who led the Physical-Chemical testing, and Dr. José M. Navas and Dr. María-Luisa Fernández-Cruz from the National Institute for Agricultural and Food Research and Technology (INIA), Department of Environment, who coordinated the Eco-toxicological tests. We are also truly indebted to Mr. Emery Scheibert for the collaboration of DENDRITECH, Inc. (USA) providing the valuable Dendrimers samples for the testing.

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4.5 Particle size distribution (Granulometry)

Endpoint study record: *Dendrimers, Particle size distribution (Granulometry), Anonymous, Year, RS, K*

Administrative Data

Purpose flag key study; robust study summary
Study result type experimental result **Study period** No data
Reliability other: no data
Rationale for reliability incl. deficiencies No data

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
review article or handbook	Brauer BJ and Amis EJ.	2001	Dendrimers and other Dendritic Polymers.	Eds. Fréchet MJM and Tomalia DA., John Wiley & Sons, Ltd., 256-307					
publication	Sánchez-Méndez A, de Jesús E, Flores JC and Gómez-Sal P.	2007	Crystal Structures of Poly(aryl ether) Dendrons with Palladium Scorpionate Complexes at their Focal Point.	Inorg Chem. 46(12):4793-5.					

Data access

data published

Cross-reference to same study

No cross-reference

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline followed		

Principles of method if other than guideline

Particle size distribution was studied using Dynamic Light Scattering (DLS) and DOSY NMR.

GLP compliance

no

Test materials**Test material form**

nanomaterial

Details on test material

- Name of test material (as cited in study report): PAMAM dendrimers (G3-PAMAM-(NH₂)₃₂ and G4-PAMAM-(NH₂)₆₄)

Confidential details on test material

No data

Any other information on materials and methods incl. tables**Dynamic Light Scattering (DLS):**

- Experimental data were obtained with a Zetasizer Nano ZS ZEN 3600 (Malvern Instruments Ltd., Worcestershire, UK), with a size range suitable for measurements of 0.3 nm-10 µm (diam), equipped with a helium-neon laser ($\lambda = 633$ nm). Size distributions were measured at 25 °C in standard polystyrene cuvettes with a scattering angle of 173°.

- G3-PAMAM-(NH₂)₃₂ and G4-PAMAM-(NH₂)₆₄ were diluted in high grade purity water (> 18 MΩ cm⁻¹) obtained from a Milli-Q Element A10 Century (Millipore Iberia, ES) at different concentrations (12.5, 25, 50, 100 and 500 mg/L). The commercial water solutions (118 and 85 g/L for G3 and G4, respectively) and a solution of 10 g/L, prepared for each dendrimer from the commercial one, were also measured before testing.

- For the characterization of G3-PAMAM-50% C12 and G4-PAMAM-50% C12 suspensions of both dendrimers at different concentrations (25, 50, 100, 250 and 500 mg/L) were prepared in high grade purity water as described above. In addition, suspensions of a concentration of 100 mg/L in H₂O/MeOH, at 1:1 and 3:1 ratios as solvent systems and the commercial solutions in methanol (263 and 113 g/L for G3 and G4, respectively) were also measured by DLS before testing.

- Their aggregation/agglomeration and mean size have been measured by DLS, and their stability has been followed up during 14 days, with the samples at constant temperature of 25 °C.

DOSY NMR experiments

The hydrodynamic radii, R_H , can be calculated from the diffusion coefficient (D) of a certain molecular species using the Stokes–Einstein equation, where k_B is the Boltzmann constant, T the absolute temperature, and η the viscosity of the solution. NMR spectroscopy is a practical tool for this regard, because self-diffusion coefficients of dendrimers can be measured by using high-resolution diffusion-ordered spectroscopy (DOSY NMR).

$$R_H = k_B T / 6 \pi \eta D$$

The diffusion coefficients were measured at 25 °C, by using the Dbppste (DOSY Bipolar Pulse Simulated Echo) pulse sequence in a Varian NMR System 500 equipped with a high accuracy variable temperature unit (± 0.1 °C), a Performa IV PFG amplifier, and a Z-PFG Triple Resonance 5-mm probe. Fine calibration of the PFG strength (DAC_to_G unit) was performed with an H₂O/HDO (2 Hz) sample as standard

supplied by Varian ($D = 19.04 \times 10^{-10} \text{ m}^2/\text{s}$ at 25°C). The diffusion NMR data were acquired over 64 scans, with settings pw90, an acquisition time of 3 s, a relaxation delay of 2 s, in each one of the 15 steps of the gradient level array between 1 and 50 G/cm (50 ms of diffusion delay and 2 ms of total defocusing time). The experimental data ($32 \text{ K} \times 1 \text{ K}$) was treated with the “DOSY” software from VNMRJ2.1B.

The data was obtained from 5-10 mg/mL solutions of the dendrimers in deuterated water, using Wilmad 5 mm O.D. NMR tubes, and the experiences were performed right after the preparation of the sample and after 3, 7 and 14 days.

Results and discussions

Any other information on results incl. tables

Dynamic Light Scattering (DLS):

- For G3-PAMAM-(NH_2)₃₂ the size distributions in intensity showed aggregates (or polymeric impurity) at any concentration in a range between approx. 100 nm to 200 nm (bigger with increasing concentration; *e.g.*, commercial solution $757.9 \pm 31.1 \text{ nm}$, Figure. 4.5/1) but in number the distribution showed a population virtually free of aggregates.

- For G4-PAMAM-(NH_2)₃₂ the size distributions in intensity showed aggregates at any concentration, ranging between approximately 100 nm to 300 nm (bigger with increasing concentration; *e.g.*, commercial solution $362.8 \pm 18.1 \text{ nm}$; Figure. 4.5/2) but, again, the distribution in number showed a population virtually absent of aggregation. These observations are consistent with the presence of the macromolecules as discrete solvated objects in this solvent accompanied of a negligible amount of aggregates, or an insignificant polymeric impurity formed during the stepwise production process.

- As for the concentration of the solutions, the lowest (50, 25 and 12.5 mg/L) rendered poorly reproducible and bad resolved DLS readings, very likely due to the small size of the solvated molecules. Instead, most of the measurements at higher concentrations (1000, 500 and 100 mg/L) were reproducible, and showed average sizes between 2–4 nm (1.5–3 nm by number) for G3-PAMAM-(NH_2)₃₂ and 3–5 nm (by intensity or by number) for G4-PAMAM-(NH_2)₆₄, with the apparent diameter slightly increasing with the concentration (Fig. 4.5/3 and 4.5/4), in a general trend that seems to be the consequence of the higher ionic strength (and pH) of the medium produced by a growing concentration of terminal $-\text{NH}_3^+/\text{OH}^-$ ion-pairs. Figures 4.5/3 and 4.5/4 also show a small, but steady, general contraction of the dendrimers with the time at any concentration. This feature is consistent with the data collected by DOSY NMR (*vide infra*). Figures 4.5/5 and 4.5/6 show the size changes registered for 10 g/L solutions of G3-PAMAM-(NH_2)₃₂ and G4-PAMAM-(NH_2)₆₄, respectively. Tables 4.5/1 and 4.5/2 summarize the diameters measured by intensity and by number, respectively, for both dendrimers.

- The DLS outcomes for the commercial solutions in methanol indicate the presence of irrelevant amounts of aggregates (60–90 and 220–240 nm for G3 and G4, respectively) –as they are not observable in the distributions plots by number (Fig. 4.5/7 and 4.5/8). Figure 4.5/7 shows a diameter of around 4.5 nm for the most numerous species in the solution freshly prepared ($t = 0 \text{ h}$) of the G3 dendrimer, whereas some molecular association must be present in the solution for the G4 (18–19 nm, Fig. 4.5/8), which is also apparent for the G3 after 1 day (8–9 nm, Fig. 4.5/7).

- The aggregation is very evident in all water suspensions; even visible as they are milky emulsions. Again, at lower concentrations (50 and 25 mg/L) the DLS results were less reproducible than at higher concentrations (500, 250 and 100 mg/L). For the stable solutions, the diameters of PAMAM dendrimer aggregates, measured by intensity at $t = 0 \text{ h}$, were in the range of 230-540 nm in the case of G3, and 200-250 nm for G4.

- The aggregates are also observable in the size distributions plots by number, with highest populations ranging in 100 – 140 nm for G3 and in 120-130 nm for G4 (Fig. 4.5/9 and 4.5/10), meaning that the aggregation phenomenon is relevant and general in the samples. In addition, the size of the most numerous species appears to be fairly constant at any concentration and over the time, particularly for the G4 dendrimer (Fig. 4.5/9 and 4.5/10). When a mixture of water and MeOH was used as solvent medium, bigger aggregates were also observed for the G3 than for the G4 lipophilic dendrimer (*e.g.*, at 100 mg/L in H₂O/MeOH 1:1, 555 and 340 nm, respectively), probably as a consequence of the superior hydrophobic-hydrophilic balance for the former (Fig 4.5/11). The aggregates also seem to be quite stable over the time in the aqueous methanolic medium.

- The zeta potential values obtained for both hydrophobic dendrimers in water at different concentrations and in mixtures of H₂O/MeOH are generally consistent with the formation of stables colloidal systems.

DOSY NMR

The hydrodynamic diameters obtained are in agreement with the apparent size measured by DLS for these dendrimers. As also observed by DLS (*vide supra*), the dendrimers shrink about a 10% of the initial size observed in freshly prepared samples after some time. The contraction of the dendritic structure might be the result of the so called “back-folding” of the flexible arms to the interior of the molecule, caused by a higher affinity for the constituting groups of the interior of the dendrimer than for the medium, leading to a denser structure with a smaller apparent size (Sánchez-Méndez et al., 2007). The “back-folding” of dendrimer is commonly observed by changing the solvent. Here, the effect is observed by NMR and DLS in samples standing over the time.

The formation of emulsions when combining G3- or G4-PAMAM 50% C12 dendrimers with water precludes the determination of their diffusion coefficients by DOSY NMR.

Overall remarks, attachments

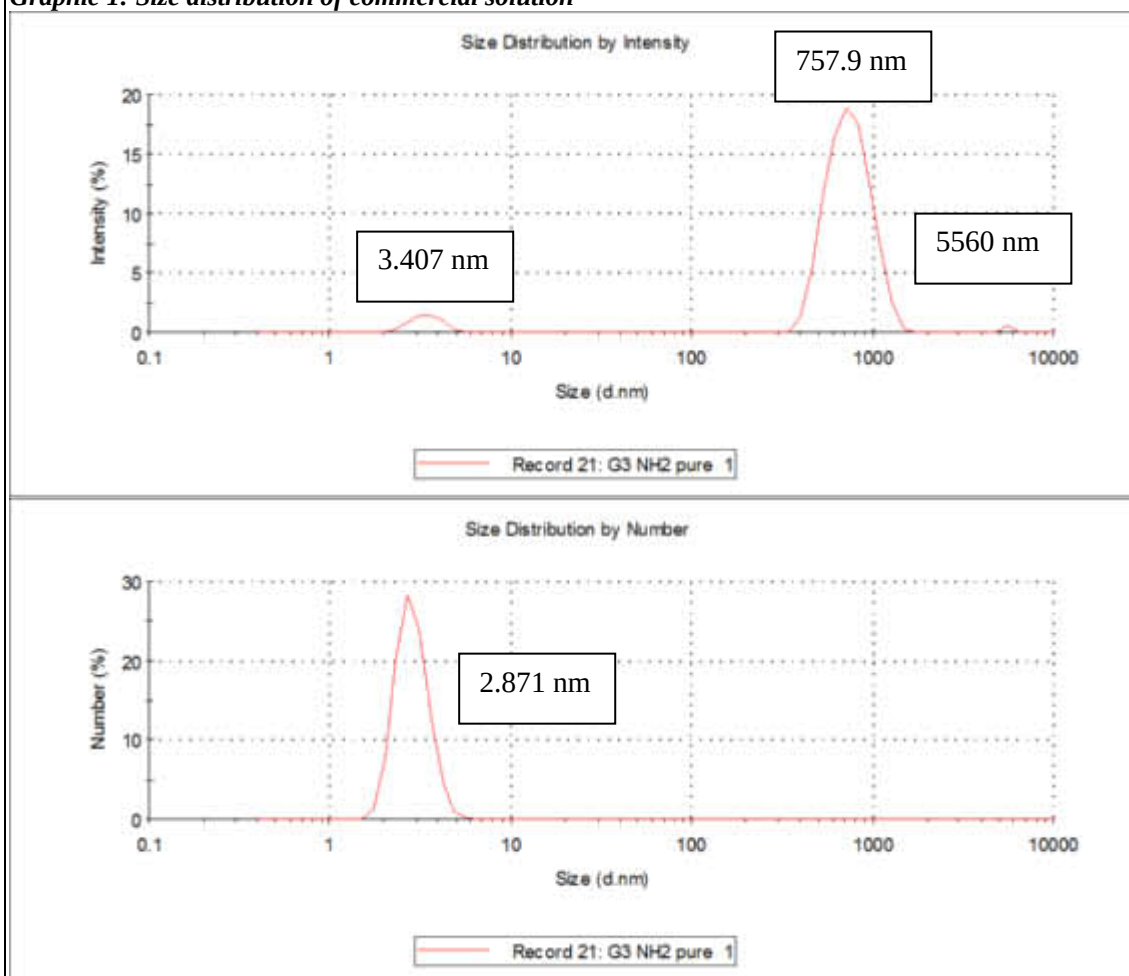
Remarks on results including tables and figures

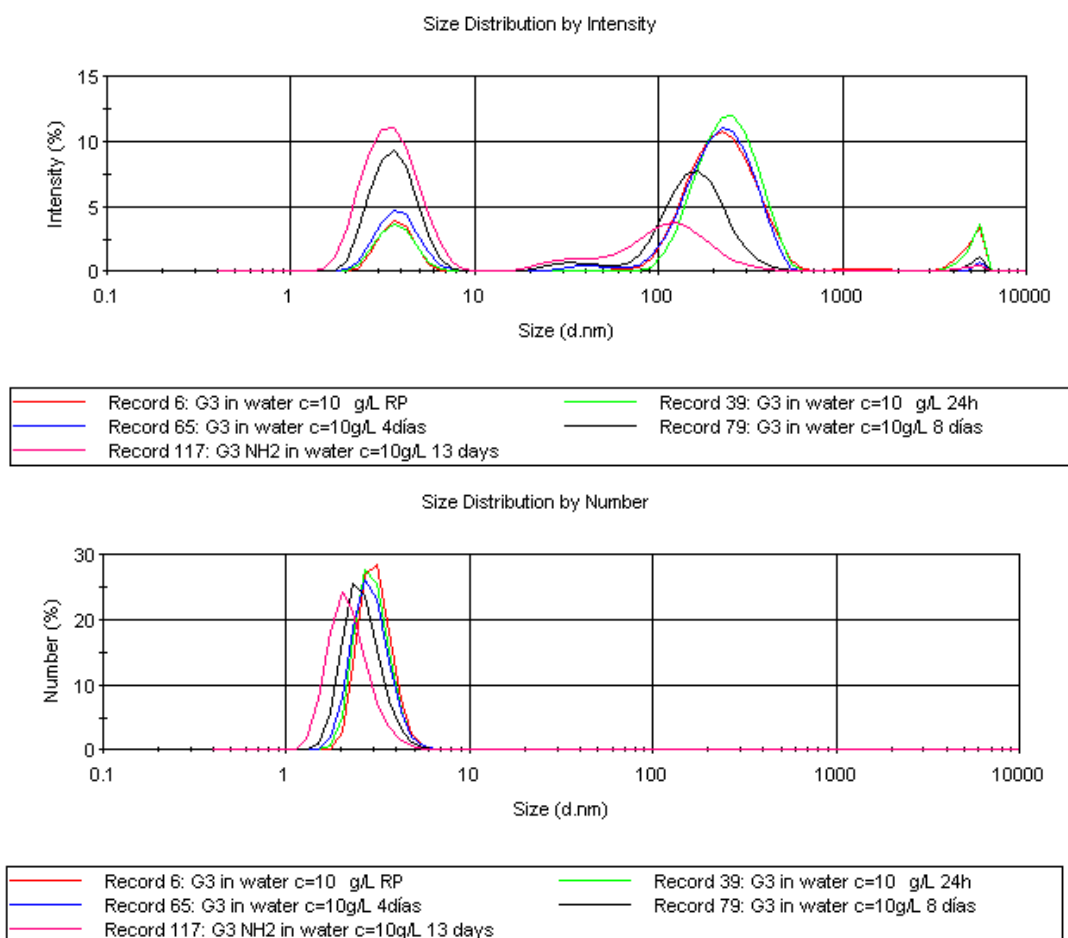
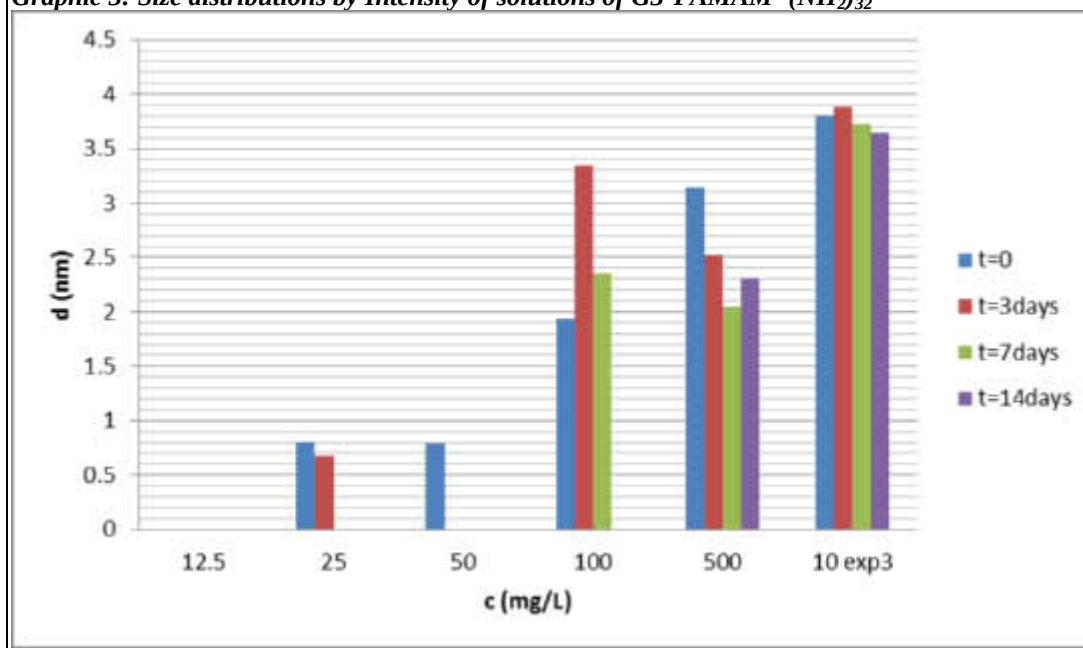
See the attached document for information on results (figures and tables)

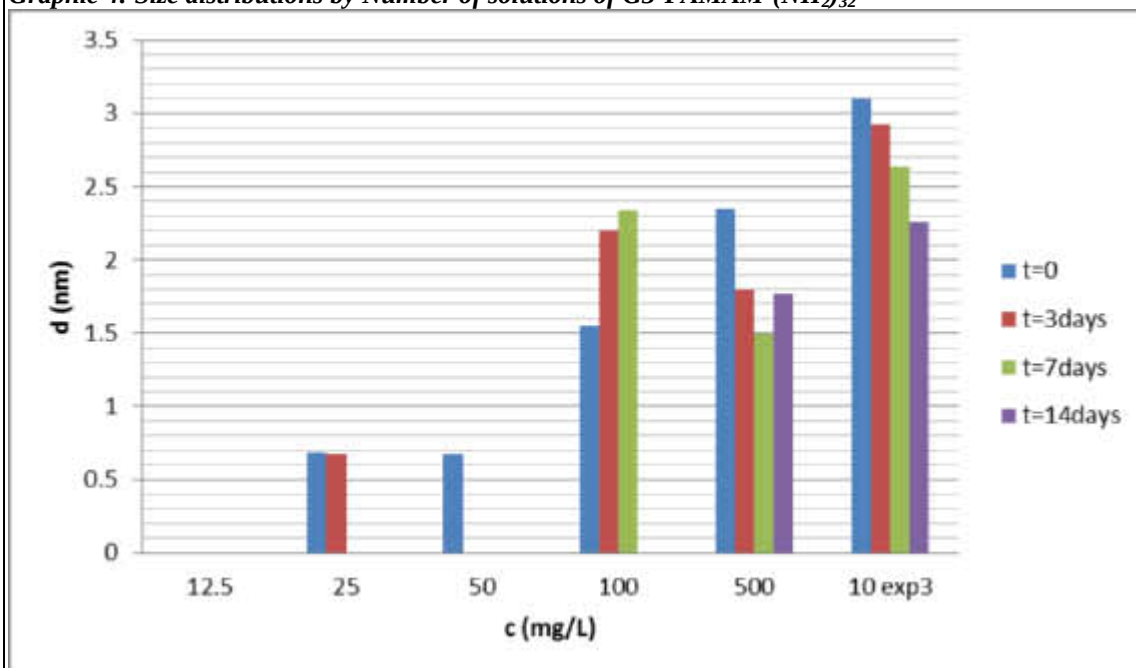
Attached background material

Attached document	Remarks
<u>Particle size distribution - figures and tables.pdf / 432.88 KB (application/octet-stream)</u> <u>Study of Size distributions: G3-PAMAM-(NH₂)₃₂</u> To carry out the characterization of G3-PAMAM-(NH₂)₃₂ we were prepared a solutions in Milli-Q water in different concentrations. An aliquot is taken out of the stock suspension (500 mg/L). This aliquot is diluted by adding solvent (100mg/L, 50 mg/L, 25 mg/L and 12.5 mg/L).The commercial solution and solution of 10 g/L were measured before testing. Their aggregation/agglomeration has been measured by DLS, and their stability has been followed up during 14 days The size distributions in intensity showed aggregates range between approximately 100 nm to 200 nm (bigger in commercial solution 757.9 ± 31.1 nm) but in number the distribution showed a virtually population of aggregation free.	

Graphic 1: Size distribution of commercial solution



Graphic 2: Size distribution during time of solution of 10 g/L**Graphic 3: Size distributions by Intensity of solutions of G3-PAMAM- (NH₂)₃₂**

Graphic 4: Size distributions by Number of solutions of G3-PAMAM-(NH₂)₃₂

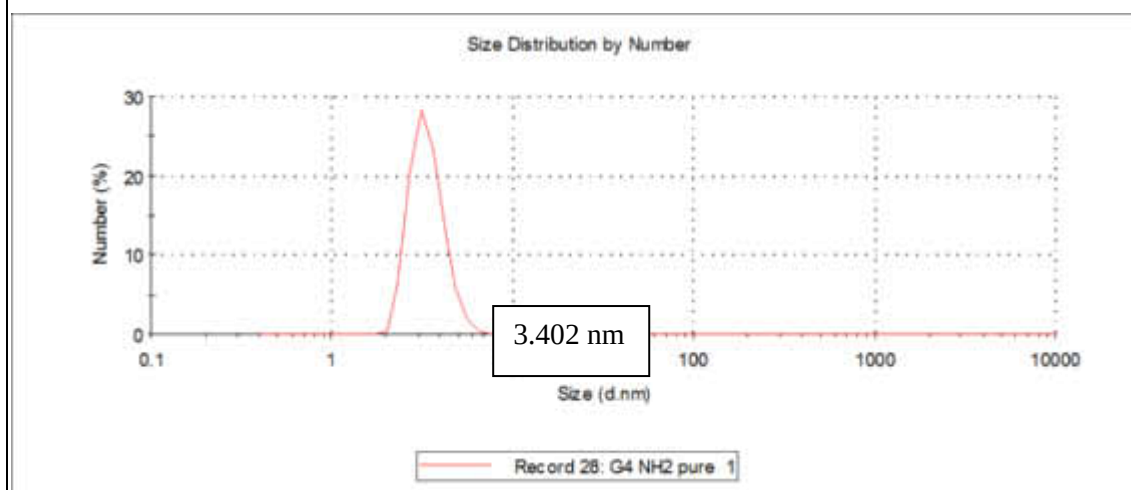
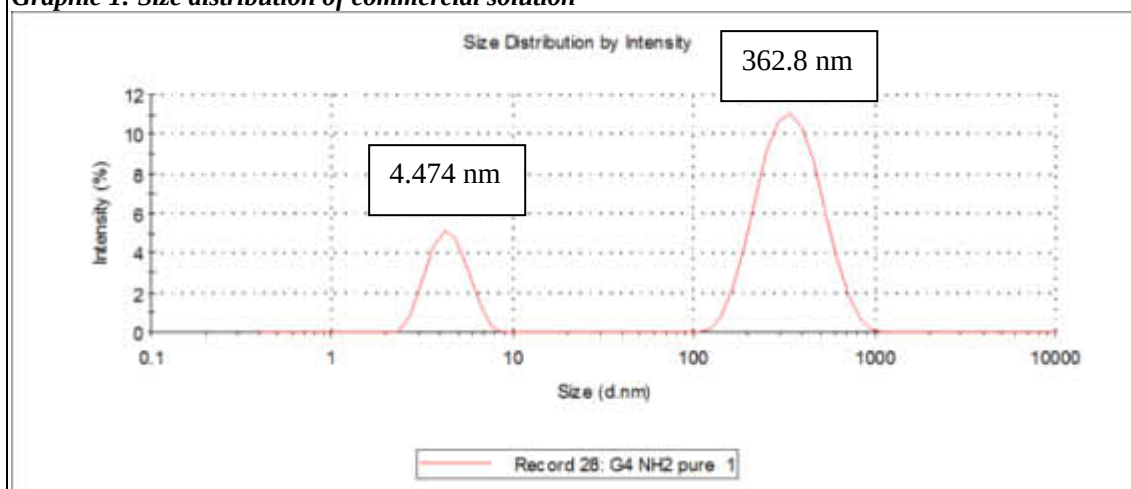
The solutions with minor concentration were not repeatable and showed poor resolution.

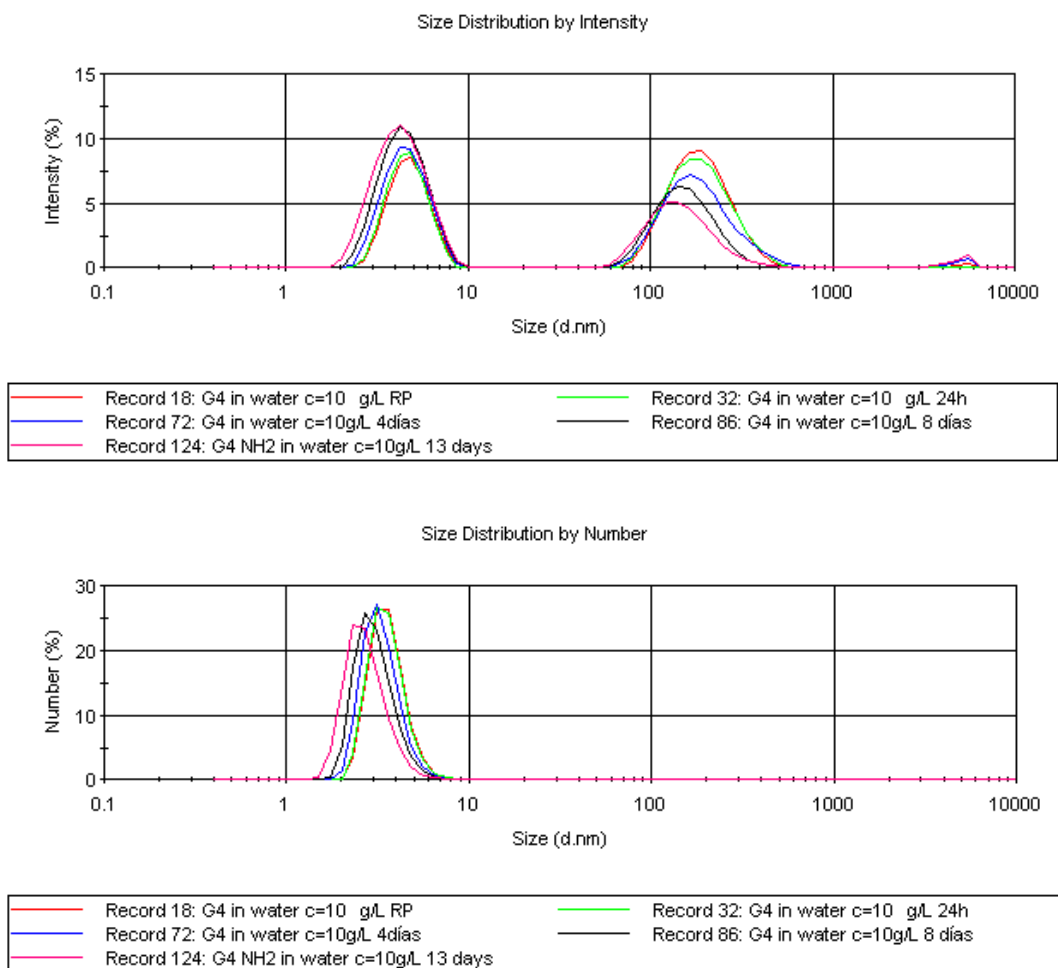
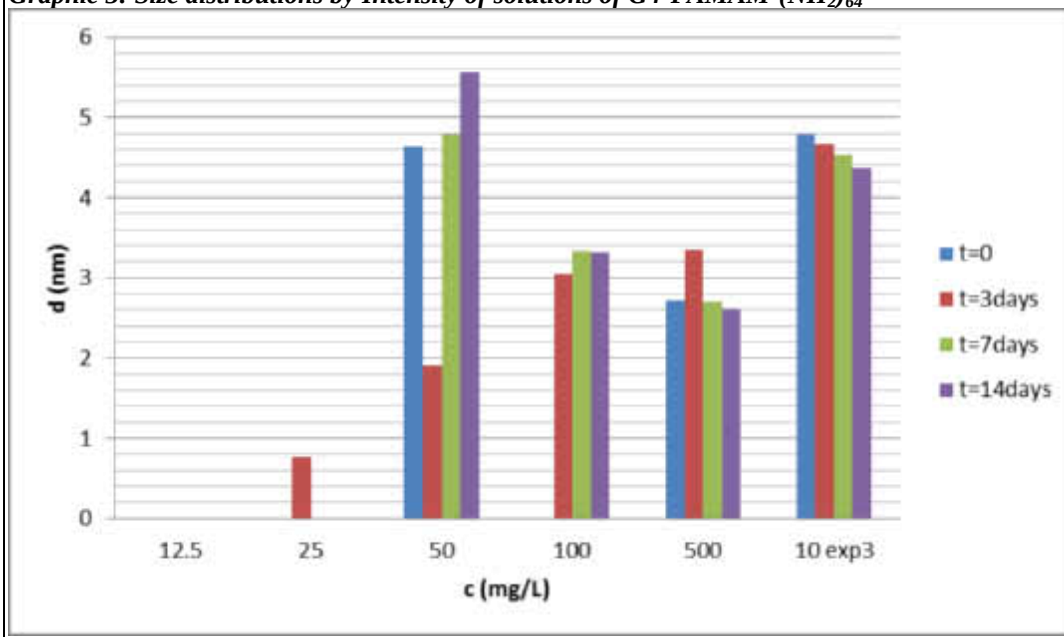
Size distributions: G4-PAMAM-(NH₂)₆₄

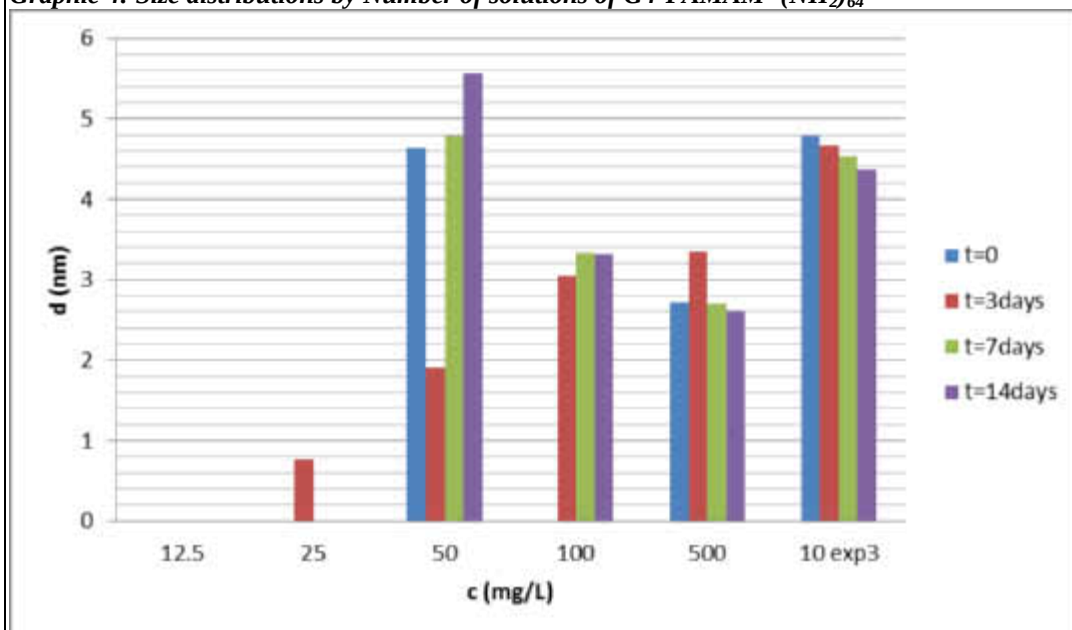
To carry out the characterization of G4-PAMAM-(NH₂)₆₄ were prepared a solutions in Milli-Q water in different concentrations. An aliquot is taken out of the stock suspension (500 mg/L). This aliquot is diluted by adding solvent (100mg/L, 50 mg/L, 25 mg/L and 12.5 mg/L).The commercial solution and solution of 10 g/L were measured before testing.

Their aggregation/agglomeration has been measured by DLS, and their stability has been followed up during 14 days

The size distributions in intensity showed aggregates range between approximately 100 nm to 300 nm (bigger in commercial solution 362.8± 18.1 nm) but in number the distribution showed a virtually population of aggregation free.

Graphic 1: Size distribution of commercial solution

Graphic 2: Size distribution during time of solution of 10 g/L**Graphic 3: Size distributions by Intensity of solutions of G4-PAMAM-(NH₂)₆₄**

Graphic 4: Size distributions by Number of solutions of G4-PAMAM- (NH₂)₆₄

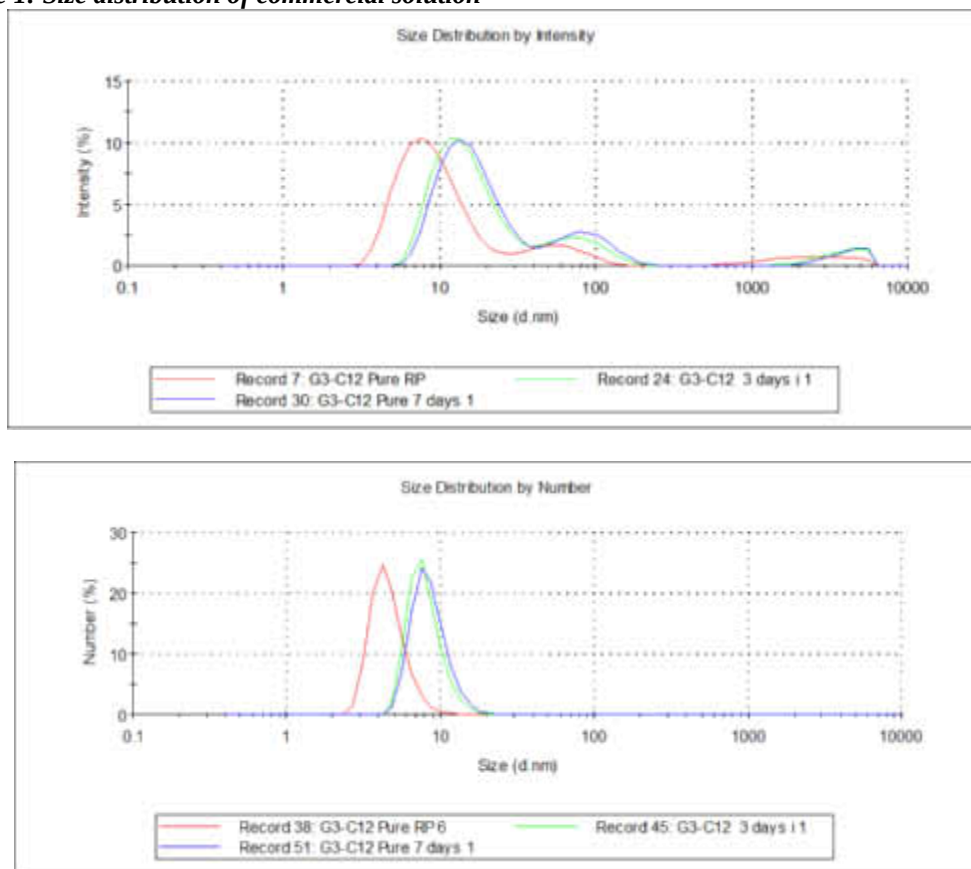
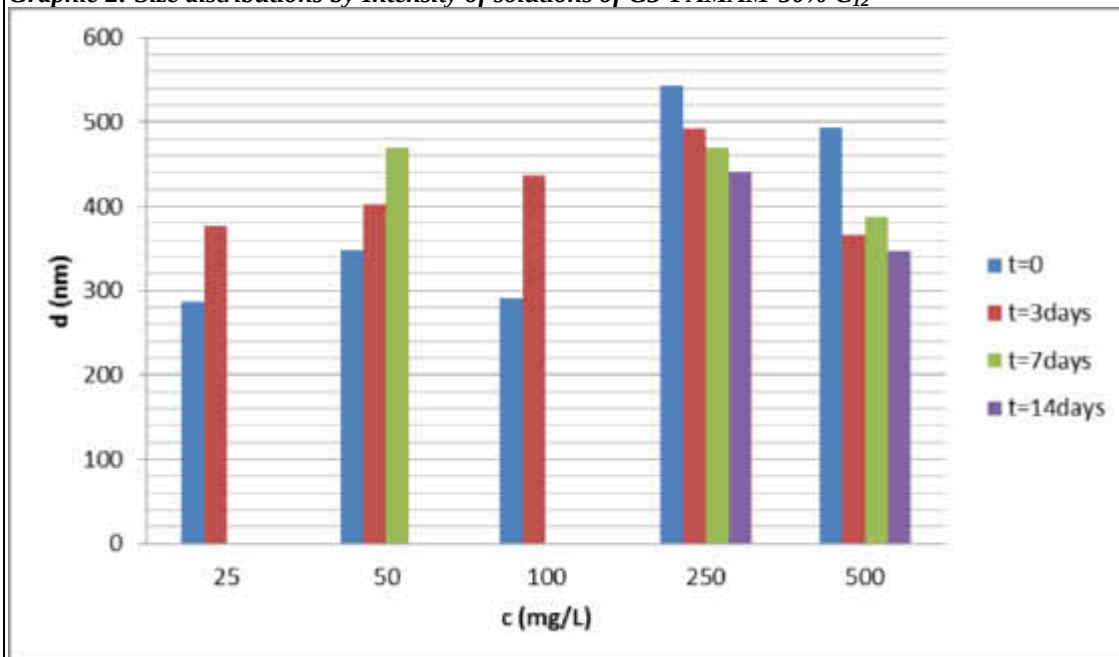
The solutions with minor concentration were not repeatable and showed poor resolution.

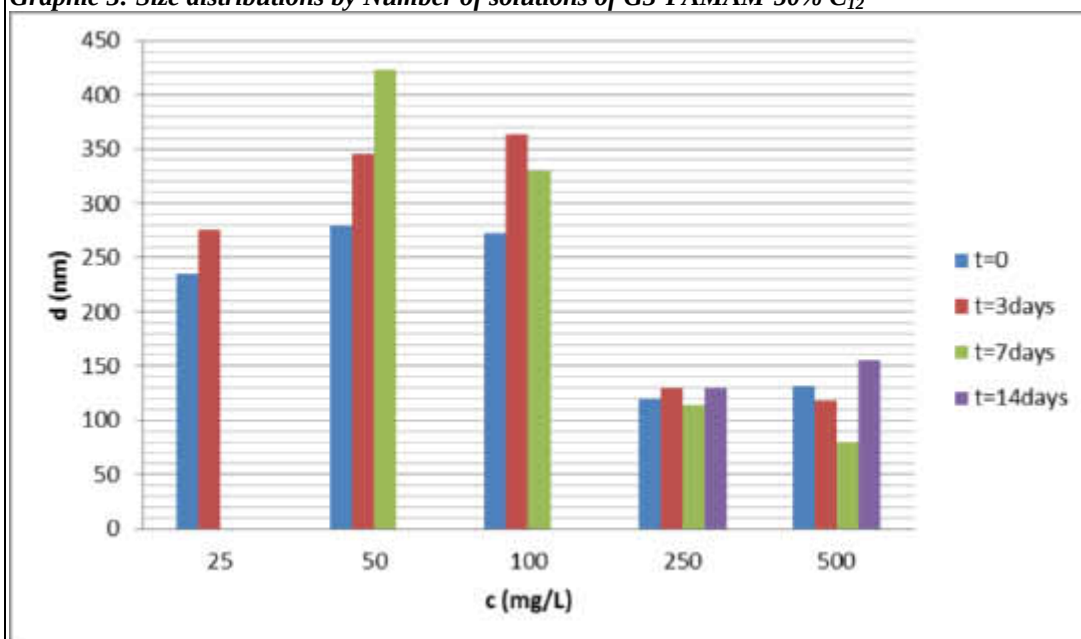
Size distributions: G3-PAMAM-50% C₁₂

To carry out the characterization of G3-PAMAM-50% C₁₂ were prepared solutions in Milli-Q water in different concentrations. An aliquot is taken out of the stock suspension (500 mg/L). This aliquot is diluted by adding solvent (100mg/L, 50 mg/L and 25 mg/L). The commercial solution and solution of 10 g/L were measured before testing.

Their aggregation/agglomeration has been measured by DLS, and their stability has been followed up during 14 days

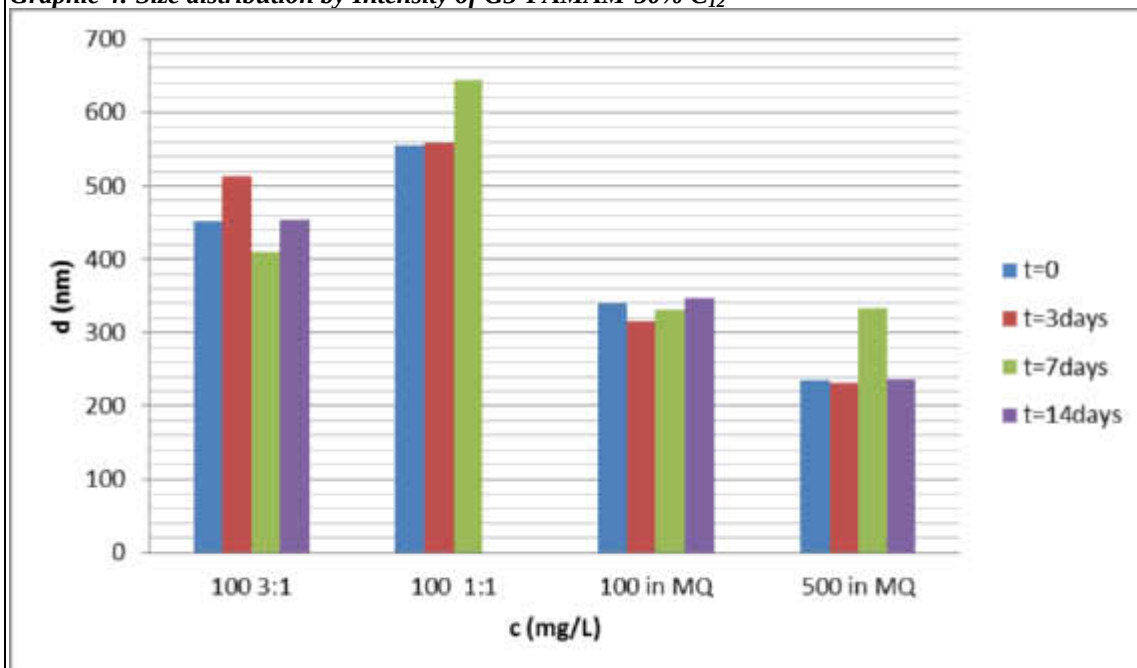
In the case of hydrophobic dendrimers, the aggregation is very evident in Milli-Q water, even visible because they are milky suspensions. At any concentration was observed diameters in the range of 180-400 nm in the case of G3. In general, there is more aggregation in the emulsion of the derivate G3 than G4 (e.g: suspension of 100 mg/L: 332.9±15.4 nm and 267.3±20.1 nm, respectively) consequence the hydrophobic-hydrophilic balance. The zeta potential values obtained for both hydrophobic dendrimers in water at different concentrations and in mixtures of H₂O/MeOH are generally consistent with the formation of stables colloidal systems.

Graphic 1: Size distribution of commercial solution**Graphic 2: Size distributions by Intensity of solutions of G3-PAMAM-50% C₁₂**

Graphic 3: Size distributions by Number of solutions of G3-PAMAM-50% C₁₂

The solutions with minor concentration were not repeatable and showed poor resolution.

In addition, An aliquot is taken out of the stock suspension (10 g/L) This aliquot is diluted by adding solvent (Milli-Q water) (500mg/L and 100 mg/L) and another suspensions in Milli-Q water/MeOH (100mg/L 1:1 and 100 mg/L 3:1)

Graphic 4: Size distribution by Intensity of G3-PAMAM-50% C₁₂

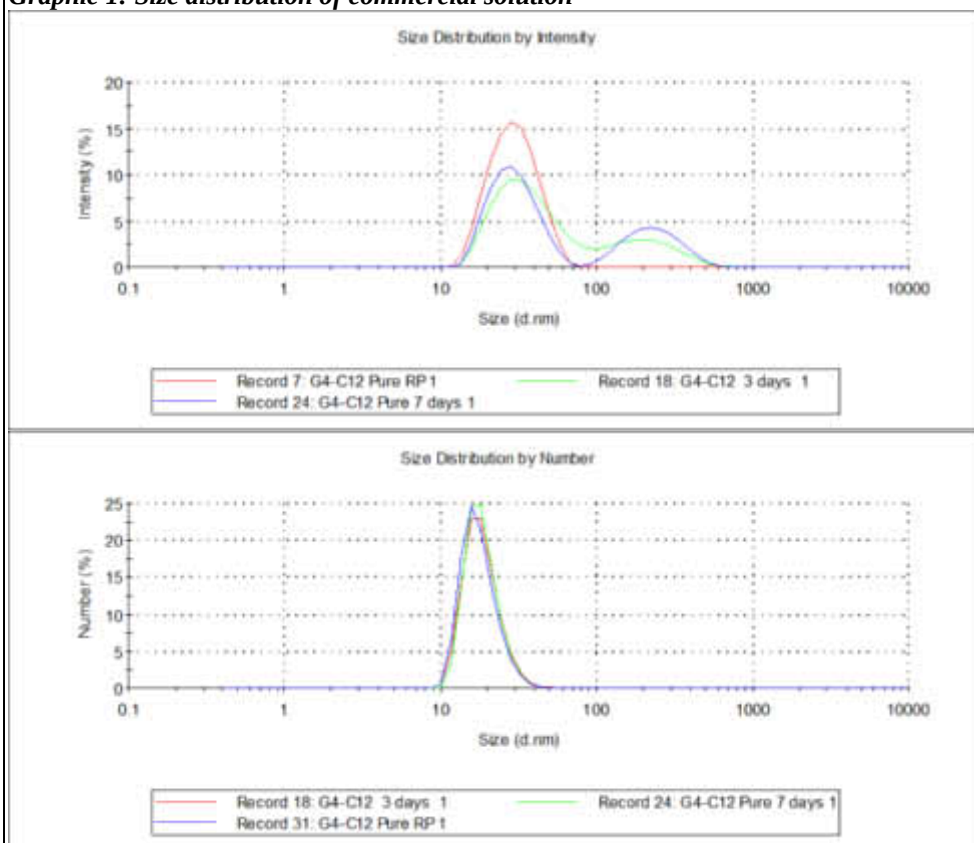
Size distributions: G4-PAMAM-50% C12

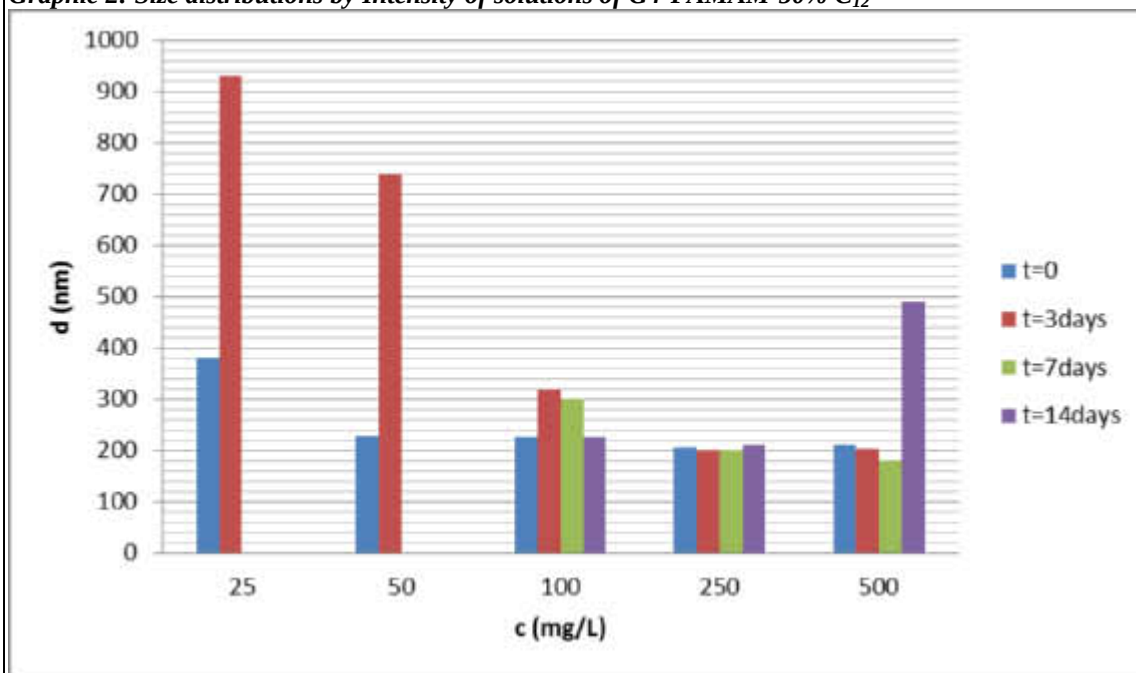
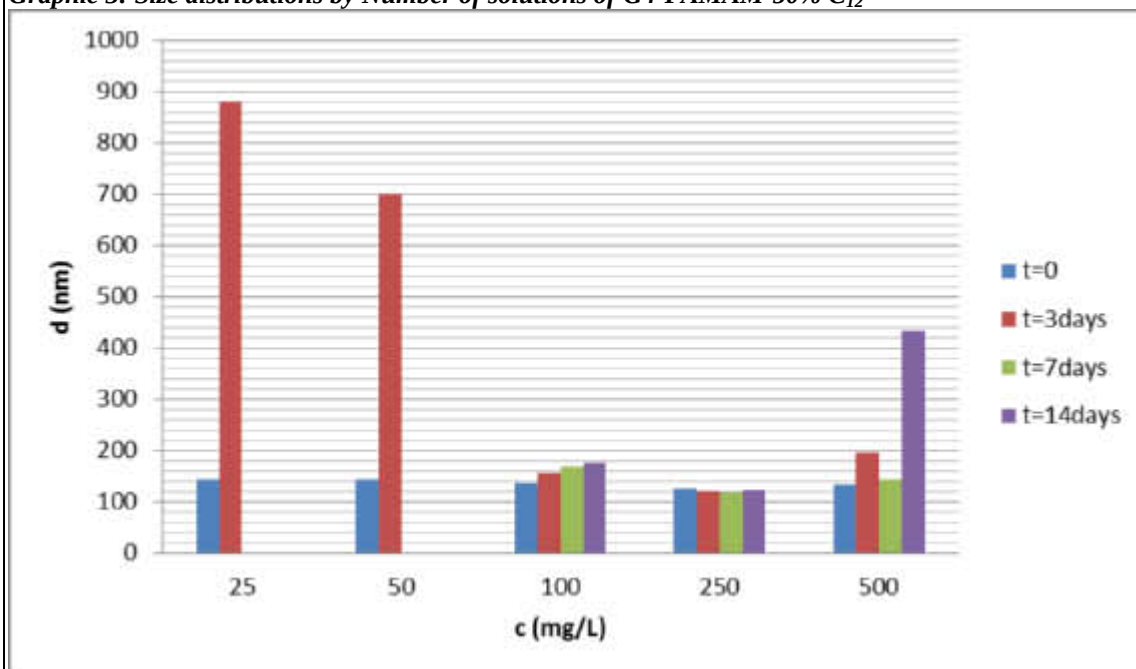
To carry out the characterization of G4-PAMAM-50% C₁₂ were prepared solutions in Milli-Q water in different concentrations. An aliquot is taken out of the stock suspension (500 mg/L). This aliquot is diluted by adding solvent (100mg/L, 50 mg/L and 25 mg/L). The commercial solution and solution of 10 g/L were measured before testing.

Their aggregation/agglomeration has been measured by DLS, and their stability has been followed up during 14 days.

In the case of hydrophobic dendrimers, the aggregation is very evident in Milli-Q water, even visible because we have milky suspensions. At any concentration was observed diameters in the range of 180-300 nm in the case of G4. In general, there is more aggregation in the emulsion of the derivate G3 than G4 (e.g: suspension of 100 mg/L: 332.9±15.4 nm and 267.3±20.1 nm, respectively) consequence the hydrophobic-hydrophilic balance. The zeta potential values obtained for both hydrophobic dendrimers in water at different concentrations and in mixtures of H₂O/MeOH are generally consistent with the formation of stables colloidal systems.

Graphic 1: Size distribution of commercial solution

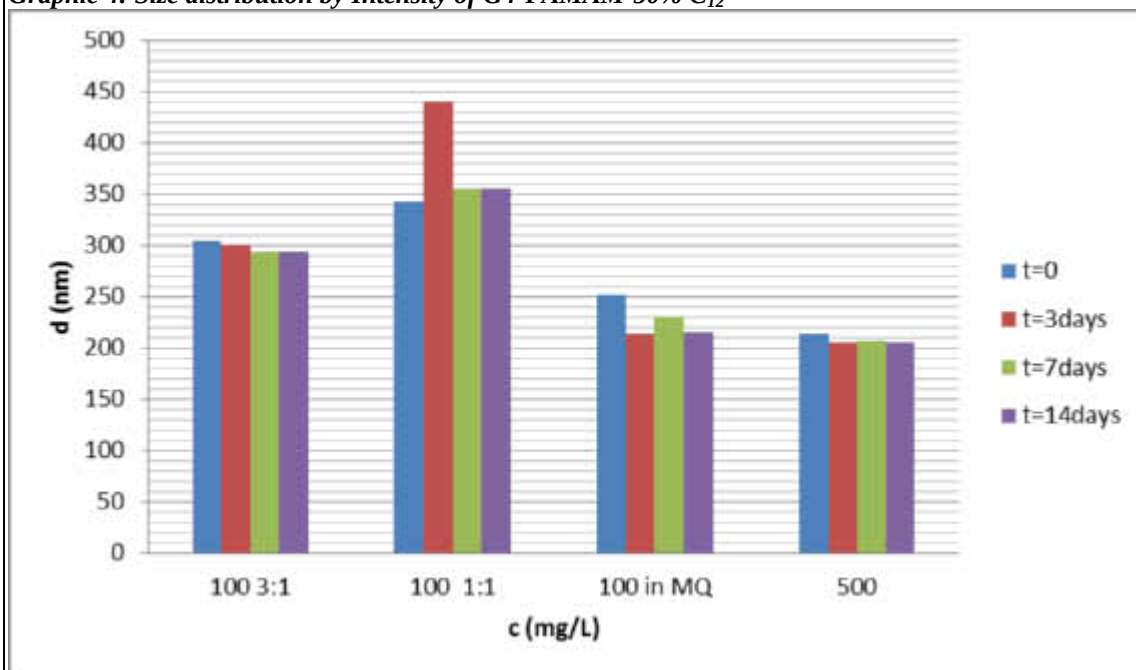


Graphic 2: Size distributions by Intensity of solutions of G4-PAMAM-50% C₁₂**Graphic 3: Size distributions by Number of solutions of G4-PAMAM-50% C₁₂**

The solutions with minor concentration were not repeatable and showed poor resolution.

In addition, An aliquot is taken out of the stock suspension (10 g/L) This aliquot is diluted by adding solvent (Milli-Q water) (500mg/L and 100 mg/L) and another suspensions in Milli-Q water/MeOH (100mg/L 1:1 and 100 mg/L 3:1).

This dendrimer are hydrophobic and soluble in methanol, as different mixtures were tested H₂O/MeOH as solvent medium, in order to obtain true solutions. Even more aggregation is observed for G3 than G4 for (example: 1:1 mixture H₂O/MeOH 557.3 ± 13.1 nm and 350.7 ± 10.1 nm respectively, 100 mg / L).

Graphic 4: Size distribution by Intensity of G4-PAMAM-50% C₁₂

Applicant's summary and conclusion

Conclusions

DLS: The size distributions in intensity showed aggregates at any concentration for G3-PAMAM-(NH₂)₃₂ (in a range between ca. 100-200 nm, but in number the distribution showed a population virtually free of aggregates) and for G4-PAMAM-(NH₂)₃₂ (in a range between ca. 100-300 nm, but the distribution in number showed a population virtually absent of aggregation). At the lowest concentrations (≤ 50 mg/L), the results were poorly reproducible, very likely due to the small size of the solvated molecules. But, at higher concentrations, the results were reproducible. The zeta potential values obtained for both hydrophobic dendrimers in water at different concentrations and in mixtures of H₂O/MeOH are generally consistent with the formation of stable colloidal systems. DOSY NMR: The hydrodynamic diameters obtained are in agreement with the apparent size measured by DLS for these dendrimers. As also observed by DLS (vide supra), the dendrimers shrink about a 10% of the initial size observed in freshly prepared samples after some time. The contraction of the dendritic structure might be the result of the so called “back-folding” of the flexible arms to the interior of the molecule, caused by a higher affinity for the constituting groups of the interior of the dendrimer than for the medium, leading to a denser structure with a smaller apparent size (Sánchez-Méndez et al., 2007). The “back-folding” of dendrimer is commonly observed by changing the solvent. Here, the effect is observed by NMR and DLS in samples standing over the time. The formation of emulsions when combining G3- or G4-PAMAM 50% C₁₂ dendrimers with water precludes the determination of their diffusion coefficients by DOSY NMR.

Executive summary

Particle size distribution of PAMAM dendrimers was studied using Dynamic Light Scattering (DLS) and DOSY NMR.

DLS method: For the characterization of G3-PAMAM-50% C₁₂ and G4-PAMAM-50% C₁₂ (25, 50, 100, 250 and 500 mg/L) and G3-PAMAM-(NH₂)₃₂ and G4-PAMAM-(NH₂)₆₄ (12.5, 25, 50, 100 and 500 mg/L) and commercial solutions were used. Aggregation/agglomeration and mean size have been measured

by DLS, and their stability has been followed up during 14 days, with the samples at constant temperature of 25 °C.

DOSY NMR experiments: The hydrodynamic radii were measured by high-resolution diffusion-ordered spectroscopy (DOSY NMR). In this experiment, 5-10 mg/mL solutions of the dendrimers in deuterated water, using Wilmad 5 mm O.D. NMR tubes, and the experiences were performed right after the preparation of the sample and after 3, 7 and 14 days.

DLS results: The size distributions in intensity showed aggregates at any concentration for G3-PAMAM-(NH₂)₃₂ (in a range between ca. 100-200 nm, but in number the distribution showed a population virtually free of aggregates) and for G4-PAMAM-(NH₂)₃₂ (in a range between ca. 100-300 nm, but the distribution in number showed a population virtually absent of aggregation). At the lowest concentrations (≤ 50 mg/L), the results were poorly reproducible, very likely due to the small size of the solvated molecules. But, at higher concentrations, the results were reproducible. The zeta potential values obtained for both hydrophobic dendrimers in water at different concentrations and in mixtures of H₂O/MeOH are generally consistent with the formation of stables colloidal systems.

DOSY NMR results: The hydrodynamic diameters obtained are in agreement with the apparent size measured by DLS for these dendrimers. As also observed by DLS (*vide supra*), the dendrimers shrink about a 10% of the initial size observed in freshly prepared samples after some time. The contraction of the dendritic structure might be the result of the so called “back-folding” of the flexible arms to the interior of the molecule, caused by a higher affinity for the constituting groups of the interior of the dendrimer than for the medium, leading to a denser structure with a smaller apparent size (Sánchez-Méndez et al., 2007). The “back-folding” of dendrimer is commonly observed by changing the solvent. Here, the effect is observed by NMR and DLS in samples standing over the time. The formation of emulsions when combining G3- or G4-PAMAM 50% C12 dendrimers with water precludes the determination of their diffusion coefficients by DOSY NMR.

Cross-reference to other study

No cross-reference

4.6 Vapour pressure

4.7 Partition coefficient

Endpoint study record: Dendrimers, Partition coefficient, Giri, 2009, RS, K

Administrative Data

Purpose flag key study; robust study summary
Study result type experimental result **Study period** 2009
Reliability other: no data
Rationale for reliability incl. deficiencies No data

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Giri J, Diallo MS, Goddard WA 3rd, Dalleska NF, Fang X and Tang Y.	2009	Partitioning of Poly(Amidoamine) Dendrimers between N-Octanol and Water.	Environ Sci Technol. 43(13): 5123-5129.					

Data access

data published

Cross-reference to same study

No cross-reference

Materials and methods

Partition coefficient type

octanol-water

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 107 (Partition Coefficient (n-octanol / water), Shake Flask Method)	no data
according to	OECD Guideline 117 (Partition Coefficient (n-octanol / water), HPLC Method)	no data

Type of method

other: shake-flask method or HPLC method

Principles of method if other than guideline

Not applicable

GLP compliance

no

Test materials**Test material form**

nanomaterial

Details on test material

- Name of test material (as cited in study report): PAMAM dendrimers

Any other information on materials and methods incl. tables

None

Results and discussions**Partition coefficient**

Type	Partition coefficient	Temp.	pH	Remarks
	1.48 — 2.39		7.4	log Kow for PAMAM dendrimers from 0 to 5 generations at room temperature

Any other information on results incl. tables

Results showed that the log Kow for PAMAM dendrimers from 0 to 5 generations varied between -1.48 and -2.39 at room temperature and a pH of 7.4. PAMAM are soluble both in water and alcohols.

Overall remarks, attachments**Remarks on results including tables and figures**

None

Applicant's summary and conclusion**Conclusions**

Under the test conditions, the log Kow for PAMAM dendrimers from 0 to 5 generations varied between -1.48 and -2.39 at room temperature and a pH of 7.4.

Executive summary

A study was conducted to measure the octanol-water partition coefficients (logK(ow)) of a homologous series of PAMAM dendrimers as a function of dendrimer generation (size), terminal group and core chemistry.

PAMAM are soluble both in water and alcohols. Results showed that the log Kow for PAMAM dendrimers from 0 to 5 generations varied between -1.48 and -2.39 at room temperature and a pH of 7.4. The logK(ow) of PAMAM dendrimers depend primarily on their size and terminal group chemistry. For G1-G5 PAMAM dendrimers with terminal NH₂ groups, the negative values of their logK(ow) indicate that they prefer to remain in the water phase. Conversely, the formation of stable emulsions at the octanol-water (O/ W) interface in the presence of G6-NH₂ and G8-NH₂ PAMAM dendrimers suggest they prefer to partition at the O/W interface. LogK(ow) of a PAMAM dendrimer may not be a good predictor of its

affinity with natural organic media such as the lipid bilayers of cell membranes.

Under the test conditions, the log K_{ow} for PAMAM dendrimers from 0 to 5 generations varied between -1.48 and -2.39 at room temperature and a pH of 7.4.

Cross-reference to other study

No cross-reference

4.8 Water solubility

Endpoint study record: Dendrimers, Water solubility, Anonymous, Year, RS, K

Administrative Data

Purpose flag key study; robust study summary
Study result type experimental result **Study period** No data
Reliability other: no data
Rationale for reliability incl. deficiencies No data

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other: no data									

Data access

other: no data

Cross-reference to same study

No cross-reference

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 105 (Water Solubility)	no data

Type of method

other: no data

Principles of method if other than guideline

Not applicable

GLP compliance

no

Test materials

Test material form

nanomaterial

Details on test material

- Name of test material (as cited in study report): Gn-PAMAM-(NH₂)_m and Gn-PAMAM-50 % C12 dendrimers

Any other information on materials and methods incl. tables

No data

Results and discussions

Any other information on results incl. tables

Gn-PAMAM-(NH₂)_m dendrimers are miscible in water, whereas Gn-PAMAM-50 % C12 are immiscible.

Overall remarks, attachments

Remarks on results including tables and figures

None

Applicant's summary and conclusion

Interpretation of results

other: Gn-PAMAM-(NH₂)_m dendrimers are miscible in water, whereas Gn-PAMAM-50 % C12 are immiscible.

Conclusions

Gn-PAMAM-(NH₂)_m dendrimers are miscible in water, whereas Gn-PAMAM-50 % C12 are immiscible.

Executive summary

A study was conducted to determine the water solubility of Gn-PAMAM-(NH₂)_m and Gn-PAMAM-50 % C12 dendrimers according to OECD TG105.

Under the test conditions, Gn-PAMAM-(NH₂)_m dendrimers are miscible in water, whereas Gn-PAMAM-50 % C12 are immiscible.

Cross-reference to other study

No cross-reference

4.9 Solubility in organic solvents / fat solubility

4.10 Surface tension

4.11 Flash point

4.12 Auto flammability

4.13 Flammability

4.14 Explosiveness

4.15 Oxidising properties

4.16 Oxidation reduction potential

4.17 Stability in organic solvents and identity of relevant degradation products

4.18 Storage stability and reactivity towards container material

4.19 Stability: thermal, sunlight, metals

4.20 pH

4.21 Dissociation constant

4.22 Viscosity

4.23 Additional physico-chemical information

Endpoint study record: Dendrimers, Additional physico-chemical information-Representative TEM, Anonymous, Year, RS, K

Administrative Data

Purpose flag	key study; robust study summary
Study result type	experimental result Study period 2011
Reliability	other: no data
Rationale for reliability incl. deficiencies	No data

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Jackson CL, Chanzy HD, Booy FP, Drake BJ, Tomalia DA, Bauer BJ and Amis EJ.	1998	Visualization of Dendrimer Molecules by Transmission Electron Microscopy (TEM): Staining Methods and Cryo-TEM of Vitrified Solutions.	Macromolecules 31: 6259-6265.					
publication	Suárez IJ, Rosal R, Rodríguez A, Ucles A, Fernández-Alba AR, Hernando MD and García-Calvo E.	2011	Chemical and Ecotoxicological Assessment of Poly(amidoamine) Dendrimers in the Aquatic Environment.	Trends in analytical chemistry 30: 492-506.					

Data access

data published

Cross-reference to same study

No cross-reference

Materials and methods**Endpoint investigated**

other: Representative Electron Microscopy (TEM) picture(s)

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

Principles of method if other than guidelineTEM analysis was performed on G3-PAMAM-(NH₂)₃₂ and G4-PAMAM-(NH₂)₆₂ dendrimers.**GLP compliance**

no data

Test materials**Test material form**

nanomaterial

Details on test material

- Name of test material (as cited in study report): G3-PAMAM-(NH₂)₃₂ and G4-PAMAM-(NH₂)₆₂

Any other information on materials and methods incl. tables

Samples for TEM analysis were prepared by removal of H₂O/MeOH of the commercial dilutions, re-dissolution of the residue in acetone with the help of sonication (10 min), and drop evaporation of the acetone solutions (3 g/L) deposited onto carbon-coated copper grids (3 mm Ø). TEM analyses were performed at the National Center of Electronic Microscopy (Universidad Complutense–Madrid) in a JEOL 2000FX instrument operating at 200 KV. The size analysis was manually by measuring 100 particles on a given grid to obtain a statistical size distribution and the diameter mode value.

Results and discussions

Results

- For G3- and G4-PAMAM-(NH₂)_{2(n+2)} dendrimers, the diameter mode value was of 4.22 ± 1.5 and 5.26 ± 1.3 nm, respectively, superior to the hydrodynamic diameters found in solution (vide infra) indicating that the soft macromolecules are flattened and extended on the grid. Some isolated particles of about 9.2 and 10.5 nm were observed in the respective G3 and G4 samples, which can be attributed to dimers, but no bigger aggregates were detected in any observation field.
- None of the samples of G_n-PAMAM-50 % C12 tested –either in water or in methanol at different concentrations– provided useful pictures for diameter determinations due to the important aggregation already mentioned for the modified dendrimers.

Any other information on results incl. tables

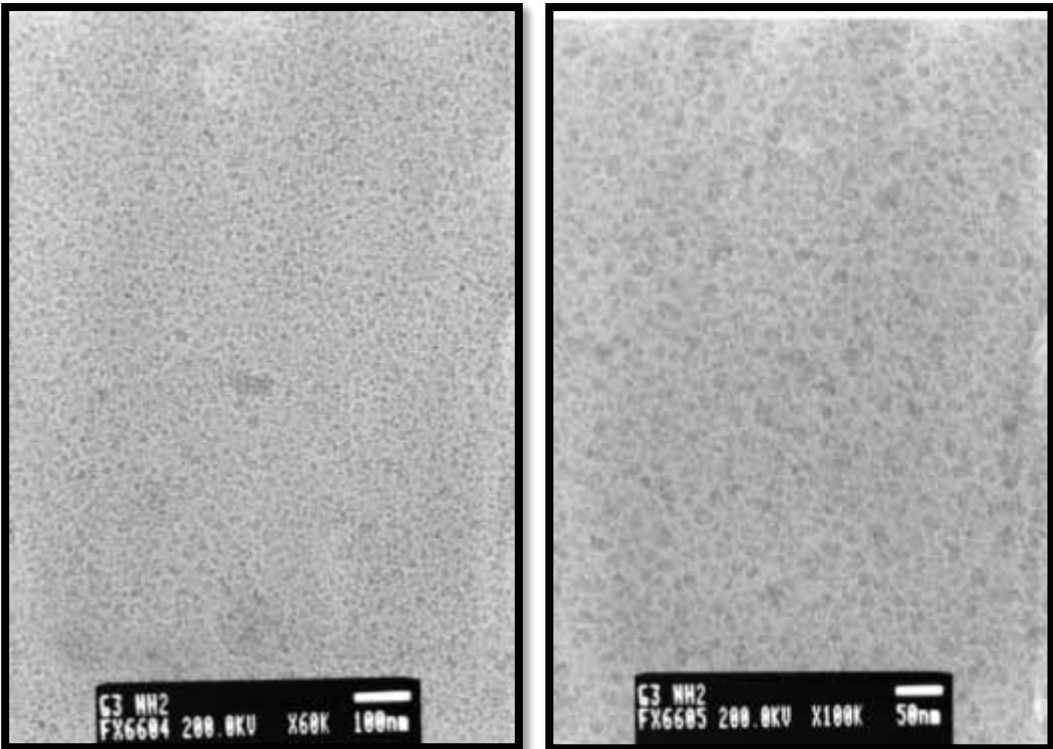
See the attached document for TEM pictures

Overall remarks, attachments

Remarks on results including tables and figures

None

Attached background material

Attached document	Remarks
<u>Representative TEM picture.pdf / 250.21 KB (application/octet-stream)</u> <u>Study by TEM: G3-PAMAM-(NH₂)₃₂</u> Different protocols have been used to prepare the samples, the optimum method to use the dendrimer was to evaporate H₂O/MeOH of the commercial dilutions and dissolve in acetone before drop evaporation phenomena.  Figure 1: TEM pictures of G3-PAMAM-(NH₂)₃₂ The mode values measured in both pictures are 4.22 nm <u>Study by TEM: G4-PAMAM-(NH₂)₆₂</u>	

Different protocols have been used to prepare the samples, the optimum method to use the dendrimer was to evaporate H₂O/MeOH of the commercial dilutions and dissolve in acetone before drop evaporation phenomena.

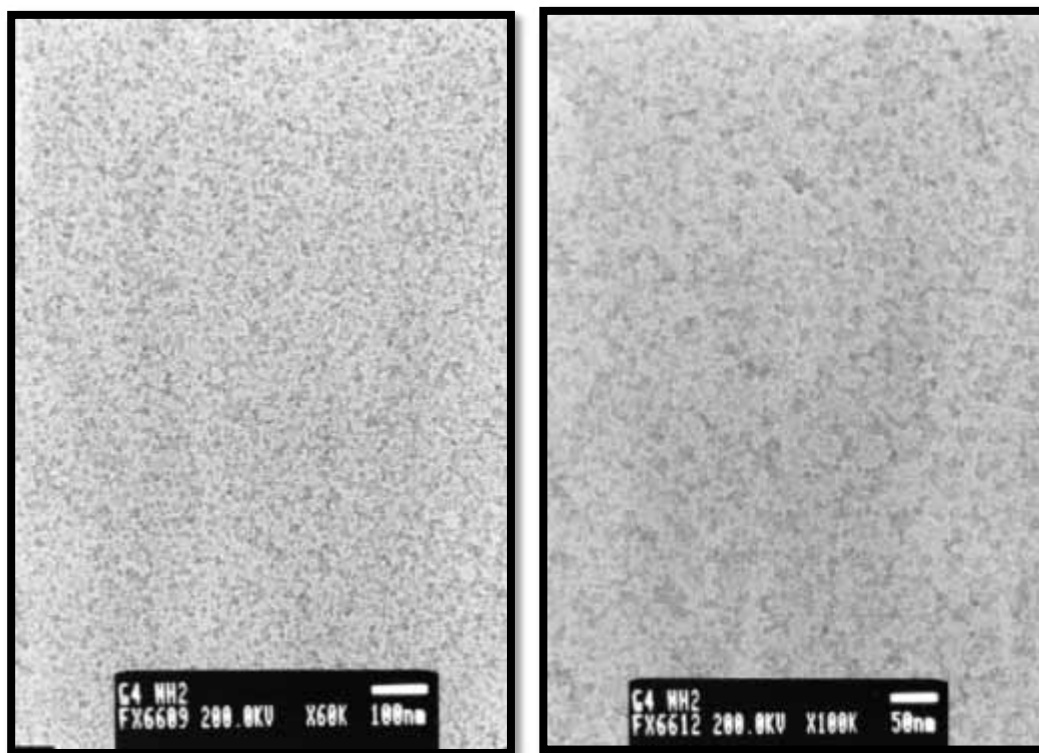


Figure 1: TEM pictures of G4-PAMAM-(NH₂)₆₂

The mode values measured in both pictures are 5.26 nm

Applicant's summary and conclusion

Conclusions

The diameter mode value for G3-PAMAM-(NH₂)₃₂ and G4-PAMAM-(NH₂)₆₂ dendrimers were 4.22 ± 1.5 and 5.26 ± 1.3 nm, respectively.

Executive summary

In a TEM analyses, samples were prepared by removal of H₂O/MeOH of the commercial dilutions, redissolution of the residue in acetone with the help of sonication (10 min), and drop evaporation of the acetone solutions (3 g/L) deposited onto carbon-coated copper grids (3 mm Ø). TEM analyses were performed in a JEOL 2000FX instrument operating at 200 KV. The size analysis was manually by measuring 100 particles on a given grid to obtain a statistical size distribution and the diameter mode value.

For G3-PAMAM-(NH₂)₃₂ and G4-PAMAM-(NH₂)₆₂ dendrimers, the diameter mode value was of 4.22 ± 1.5 and 5.26 ± 1.3 nm, respectively, superior to the hydrodynamic diameters found in solution (*vide infra*) indicating that the soft macromolecules are flattened and extended on the grid. Some isolated particles of about 9.2 and 10.5 nm were observed in the respective G3 and G4 samples, which can be attributed to dimers, but no bigger aggregates were detected in any observation field. None of the samples of G_n-PAMAM-50 % C₁₂ tested –either in water or in methanol at different concentrations– provided useful

pictures for diameter determinations due to the important aggregation already mentioned for the modified dendrimers.

Cross-reference to other study

No cross-reference

4.24 Agglomeration/aggregation

Endpoint study record: Dendrimers, Agglomeration-aggregation, Wang, 2004, RS, K

Administrative Data

Purpose flag key study; robust study summary
Study result type experimental result **Study period** 2004
Reliability other: no data
Rationale for reliability incl. deficiencies No data

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Wang BB, Zhang X, Jia XR, Luo YF, Sun Z, Yang L, Ji Yand Wei Y.	2004	Poly(amidoamine) Dendrimers with Phenyl shells: Fluorescence and Aggregation Behavior.	Polymer 45(25):8395-8402.					
publication	Wang BB, Zhang X, Jia XR, Li ZC, Ji Y, Yang L and Wei Y.	2004	Fluorescence and Aggregation Behavior of Poly(amidoamine) Dendrimers Peripherally Modified with Aromatic Chromophores: the Effect of Dendritic Architectures.	J Am Chem Soc. 126(46):15180-94.					

Data access

data published

Cross-reference to same study

No cross-reference

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline followed		

Method

other: DLS (and TEM of evaporated low-concentrated samples), and DOSY NMR

Principles of method if other than guideline

Agglomeration/aggregation state of PAMAM was tested using DLS (and TEM of evaporated low-concentrated samples), and DOSY NMR.

Details on methods and data evaluation

Agglomeration/aggregation state of PAMAM was tested using DLS (and TEM of evaporated low-concentrated samples), and DOSY NMR.

Sampling

No data

Data gathering

Calibration

No data

Reproducibility

No data

GLP compliance

no

Test materials

Test material form

other: nanomaterial

Confidential details on test material

No data

Any other information on materials and methods incl. tables

None

Overall remarks, attachments

Remarks on results incl. tables and figures

Aggregation is observed, in water or in methanol solutions, for peripherally modified PAMAM with an

amphiphilic nature (e.g., PAMAM-50 % C12), whereas water solutions of the principal materials (Gn-PAMAM-(NH₂)₂⁽ⁿ⁺²⁾) are virtually absent of aggregates, meaning that the macromolecules remain as discrete solvated objects in this solvent. The aggregation in solution of PAMAM dendrimers modified at the periphery with other lipophilic groups has been previously observed.

Remarks on results including tables and figures

None

Applicant's summary and conclusion

Conclusions

Aggregation is observed, in water or in methanol solutions, for peripherally modified PAMAM with an amphiphilic nature (e.g., PAMAM-50 % C12), whereas water solutions of the principal materials (Gn-PAMAM-(NH₂)₂⁽ⁿ⁺²⁾) are virtually absent of aggregates, meaning that the macromolecules remain as discrete solvated objects in this solvent.

Executive summary

Agglomeration/aggregation state of PAMAM was tested using DLS (and TEM of evaporated low-concentrated samples), and DOSY NMR.

Aggregation is observed, in water or in methanol solutions, for peripherally modified PAMAM with an amphiphilic nature (e.g., PAMAM-50 % C12), whereas water solutions of the principal materials (Gn-PAMAM-(NH₂)₂⁽ⁿ⁺²⁾) are virtually absent of aggregates, meaning that the macromolecules remain as discrete solvated objects in this solvent. The aggregation in solution of PAMAM dendrimers modified at the periphery with other lipophilic groups has been previously observed.

Cross-reference to other study

No cross-reference

4.25 Crystalline phase

Endpoint study record: Dendrimers, Crystalline phase, Hernández-Ainsa, 2011, SS, S

Administrative Data

Purpose flag supporting study
Study result type experimental result **Study period** 2011
Reliability other: no data
Rationale for reliability incl. deficiencies No data

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Hernández-Ainsa S, Barberá J, Marcosa M and Serrano JL.	2011	Nanoobjects coming from Mesomorphic Ionic PAMAM Dendrimers.	Soft Matter, 7: 2560-2568.					

Data access

data published

Cross-reference to same study

No cross-reference

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline followed		

Method

other: differential scanning calorimetry (DSC), polarizing optical microscopy (POM), and X-ray diffractometry (XRD)

Principles of method if other than guideline

Crystalline phase of dendrimers was studied using differential scanning calorimetry (DSC), polarizing optical microscopy (POM), and X-ray diffractometry (XRD).

Details on methods and data evaluation

A series of ionic amphiphilic dendrimers constituted by the grafting of poly(amidoamine) (PAMAM) of different generations (G = 0–4) with linear carboxylic acids bearing hydrophobic chains has been prepared.

Their self-assembly tendency both in bulk and in water has been investigated. Almost all of the compounds present liquid crystalline behaviour as shown by differential scanning calorimetry (DSC), polarizing optical microscopy (POM), and X-ray diffractometry (XRD) studies.

Data gathering

Instruments

Differential scanning calorimetry (DSC), polarizing optical microscopy (POM), and X-ray diffractometry (XRD)

Calibration

No data

Reproducibility

No data

GLP compliance

no

Test materials

Test material form

other: nanomaterial

Confidential details on test material

No data

Any other information on materials and methods incl. tables

None

Results and discussions

Remarks on results incl. tables

PAMAM dendrimers are amorphous viscous oils. Derivatized with mesogenic groups they show features of liquid crystals (Hernandez-Ainsa et al., 2011).

Overall remarks, attachments

Remarks on results including tables and figures

None

Applicant's summary and conclusion

Conclusions

PAMAM dendrimers are amorphous viscous oils. Derivatized with mesogenic groups they show features of liquid crystals.

Executive summary

A series of ionic amphiphilic dendrimers constituted by the grafting of poly(amidoamine) (PAMAM) of different generations ($G = 0-4$) with linear carboxylic acids bearing hydrophobic chains has been prepared. Their self-assembly tendency both in bulk and in water has been investigated. Almost all of the compounds present liquid crystalline behaviour as shown by differential scanning calorimetry (DSC), polarizing optical microscopy (POM), and X-ray diffractometry (XRD) studies.

PAMAM dendrimers are amorphous viscous oils. Derivatized with mesogenic groups they show features of liquid crystals.

Cross-reference to other study

No cross-reference

4.26 Crystallite and grain size**4.27 Aspect ratio/shape****4.28 Specific surface area**

Endpoint study record: Dendrimers, Specific surface area, Maiti, 2004, SS, S

Administrative Data

Purpose flag supporting study
Study result type experimental result **Study period** 2004
Reliability other: no data
Rationale for reliability incl. deficiencies No data

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Maiti PK, Cagin T, Wang G and Goddard III WA.	2004	Structure of PAMAM Dendrimers: Generations through 11.	Macromolecules 37: 6236-6254.					

Data access

data published

Cross-reference to same study

No cross-reference

Materials and methods

Principles of method if other than guideline

Specific surface area of PAMAM dendrimers was measured.

Details on methods and data evaluation

No data

Data gathering

Instruments

No data

Calibration

No data

Reproducibility

No data

GLP compliance

no

Test materials

Test material form

other: nanomaterial

Any other information on materials and methods incl. tables

None

Overall remarks, attachments

Remarks on results including tables and figures

PAMAM are viscous oils.

Results and discussions

Remarks on results incl. tables

None

Applicant's summary and conclusion

Conclusions

No data

Executive summary

No data

Cross-reference to other study

No cross-reference

4.29 Zeta potential

Endpoint study record: Dendrimers, Zeta potential, Anonymous, Year, RS, K

Administrative Data

Purpose flag key study; robust study summary
Study result type experimental result **Study period** 2008-2010
Reliability other: no data
Rationale for reliability incl. deficiencies No data

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Maiti PK, Messina R.	2008	Counterion Distribution and Zeta -Potential in PAMAM Dendrimer.	Macromolecules 41: 5002-5006.					
publication	Mukherjee SP, Davoren M and Byrne HJ.	2010	In Vitro Mammalian Cytotoxicological Study of Pamam Dendrimers - Towards Quantitative Structure Activity Relationships.	Toxicol In Vitro. 24(1):169-77.					

Data access

data published

Cross-reference to same study

No cross-reference

Materials and methods

Methods

other: He-Ne laser

Principles of method if other than guideline

A study was conducted to determine the surface charge of dendrimers by measuring the zeta potential

using a Zeta Sizer Nano ZS ZEN 3600.

Details on methods and data evaluation

Zeta potential was determined with a Zeta Sizer Nano ZS ZEN 3600 (Malvern Instruments Ltd., Worcestershire, UK), with a size range suitable for Zeta potentials (Z) measurements of 3.8 nm-100 μ m (diam), equipped with a He-Ne laser (λ = 633 nm). The Z values were measured at 25 °C in polycarbonate cuvettes with gold electrodes and with the detector set at an angle of 17°.

Data gathering

Instruments

Zeta Sizer Nano ZS ZEN 3600 (Malvern Instruments Ltd., Worcestershire, UK)

Calibration

No data

Reproducibility

Yes

GLP compliance

no data

Test materials

Test material form

other: nanomaterial

Confidential details on test material

No data

Any other information on materials and methods incl. tables

None

Results and discussions

Zeta Potential

Zeta -4.07 mV

Potential

Remarks G3-PAMAM-(NH₂)₂ (n+2) - average after 10 independent measurements: : -4.07 ± 2.85 mV

Zeta -5.12 mV

Potential

Remarks G4-PAMAM-(NH₂)₂ (n+2) - average after 10 independent measurements: -5.12 ± 4.12 mV

Remarks on results incl. tables

G3-PAMAM-(NH₂)₃₂ and G4-PAMAM-(NH₂)₆₄

Relatively stable readings of the Z were only possible for the commercial water solutions of G3- and G4-PAMAM-(NH₂)_{2⁽ⁿ⁺²⁾} dendrimers (average after 10 independent measurements: -4.07 ± 2.85 and -5.12 ± 4.12 mV respectively), observing wider dispersion of values with decreasing concentration. Provided that the dendrimers remain as discrete molecules in water solutions, the sizes of these objects are at the lower limit specified for the instrument for Z determinations (3.8 nm). Although the obtained data, consequently, have to be considered cautiously, the low Zs measured are within the classical range of instability (± 30 mV) to aggregation and sedimentation utilized for other nanoparticles, but all water solutions of the amine-terminated dendrimers were stable. Despite of the absent of electrostatic repulsions, their stability must be ascribed to the important contribution of the solvation energy for these monomolecular micelles, which is generally negligible for hard matter nanoparticles.

G3-PAMAM-50 % C12 and G4-PAMAM-50 % C12

The Z values obtained for both hydrophobic dendrimers in water at different concentrations and in mixtures of H₂O/MeOH are generally consistent with the formation of stables colloidal systems. In agreement with the bigger size of the objects in the emulsions compared with that of the G_n-PAMAM-(NH₂)_{2⁽ⁿ⁺²⁾} solutions, the Z readings were much more reproducible –and stable over the time.

Table 4.29/1: Zeta Potential, in mV, G3-and G4-PAMAM-50 % C12 dendrimers in H₂O or in mixtures H₂O/MeOH at different concentrations and time.

Concentrations	G3-PAMAM-50% C12				G4-PAMAM-50% C12			
mg/L	t = 0	t = 3 days	t = 7 days	t = 14 days	t = 0	t = 3 days	t = 7 days	t = 14 days
500	71.0 ± 7.1	69.9 ± 6.3	70.0 ± 5.6	65.3 ± 5.2	-	64.1 ± 7.5	62.4 ± 8.2	62.7 ± 7.6
100	15.9 ± 3.2	25.4 ± 3.6	22.2 ± 3.0	20.8 ± 3.1	49.5 ± 7.1	49.4 ± 6.8	47.9 ± 7.2	-
100 ^a	33.0 ± 3.8	36.5 ± 4.4	6.23 ± 3.2	-	-	-	-	-
100 ^b	40.3 ± 4.1	37.4 ± 4.9	37.0 ± 4.4	39.1 ± 4.0	37.4 ± 5.6	39.8 ± 6.8	34.7 ± 5.3	33.7 ± 5.5

N.B.: a in H₂O/MeOH 1:1; b in H₂O/MeOH 3:1.

Overall remarks, attachments

Remarks on results including tables and figures

None

Applicant's summary and conclusion

Conclusions

Relatively stable readings of the Z were only possible for the commercial water solutions of G3- and G4-PAMAM-(NH₂)_{2⁽ⁿ⁺²⁾} dendrimers (average after 10 independent measurements: -4.07 ± 2.85 and -5.12 ± 4.12 mV respectively), observing wider dispersion of values with decreasing concentration.

Executive summary

A study was conducted to determine the surface charge of dendrimers by measuring the zeta potential

using a Zeta Sizer Nano ZS ZEN 3600. Zeta potential was determined with a Zeta Sizer Nano ZS ZEN 3600 (Malvern Instruments Ltd., Worcestershire, UK), with a size range suitable for Zeta potentials (Z) measurements of 3.8 nm-100 μ m (diam), equipped with a He-Ne laser ($\lambda = 633$ nm). The Z values were measured at 25 °C in polycarbonate cuvettes with gold electrodes and with the detector set at an angle of 17°.

G3-PAMAM-(NH₂)₃₂ and G4-PAMAM-(NH₂)₆₄: Relatively stable readings of the Z were only possible for the commercial water solutions of G3- and G4-PAMAM-(NH₂)_{2⁽ⁿ⁺²⁾} dendrimers (average after 10 independent measurements: -4.07 ± 2.85 and -5.12 ± 4.12 mV respectively), observing wider dispersion of values with decreasing concentration. Provided that the dendrimers remain as discrete molecules in water solutions, the sizes of these objects are at the lower limit specified for the instrument for Z determinations (3.8 nm). Although the obtained data, consequently, have to be considered cautiously, the low Zs measured are within the classical range of instability (± 30 mV) to aggregation and sedimentation utilized for other nanoparticles, but all water solutions of the amine-terminated dendrimers were stable. Despite of the absent of electrostatic repulsions, their stability must be ascribed to the important contribution of the solvation energy for these monomolecular micelles, which is generally negligible for hard matter nanoparticles.

G3-PAMAM-50 % C12 and G4-PAMAM-50 % C12: The Z values obtained for both hydrophobic dendrimers in water at different concentrations and in mixtures of H₂O/MeOH are generally consistent with the formation of stables colloidal systems. In agreement with the bigger size of the objects in the emulsions compared with that of the G_n-PAMAM-(NH₂)_{2⁽ⁿ⁺²⁾} solutions, the Z readings were much more reproducible –and stable over the time.

Cross-reference to other study

No cross-reference

4.30 Surface chemistry

Endpoint study record: Dendrimers, Surface chemistry, Author, Year, RS, K

Administrative Data

Purpose flag key study; robust study summary
Study result type experimental result **Study period** 2012-2013
Reliability other: no data
Rationale for reliability incl. deficiencies No data

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Hernando MD, Rosenkranz P, Ulaszewska MM, Fernández-Cruz ML, Fernández-Alba AR and Navas JM.	2012	In Vitro Dose-Response Effects of Poly(Amidoamine) Dendrimers [Amino-Terminated and Surface-modified with n-(2-hydroxydodecyl) Groups] and Quantitative Determination by Liquid Chromatography-Hybrid Quadrupole/Time-of-Flight Mass Spectrometry based Method.	Analytical and Bioanalytical Chemistry 404: 2749-2763.					
publication	Ulaszewska MM, Hernando MD, Moreno AU, García AV, García Calvo E and Fernández-Alba AR.	2013	Identification and Quantification of Poly(Amidoamine) PAMAM Dendrimers of Generations 0 to 3 by Liquid Chromatography/Hybrid Quadrupole Time-of-Flight Mass Spectrometry in Aqueous Medium.	Rapid Commun Mass Spectrom. 27(7):747-62.					

Data access

data published

Cross-reference to same study

No cross-reference

Materials and methods

Methods

other: no data

Principles of method if other than guideline

No information

Data gathering**Instruments**

No data

Calibration

No data

Reproducibility

No data

GLP compliance

no data

Test materials**Test material form**

other: nanomaterial

Any other information on materials and methods incl. tables

None

Results and discussions**Functionalisation****Remarks on results incl. tables**

Due to the precise and controlled synthesis of dendrimers the whole structure is known at an atomic level – that includes the surface groups and inner part of the molecule. For Gn-PAMAM-(NH₂)₂ (n+2) mainly primary amine groups are at the periphery, together some amide and tertiary amine functions due to back folding of the terminal groups. For the alternate nanomaterial Gn-PAMAM-50 % C12 half of the terminal N–H bonds are substituted by 2-hydroxydodecyl groups at the surface.

In aqueous medium the amines, particularly those at the surface, undergo hydrolysis leading to basic pH solutions. The below tables show the pH values, measured with a Crison pH-Meter Basic 20+, for solutions of the dendrimers under study. The pH values in water solutions of the G3- and G4-PAMAM-(NH₂)₂⁽ⁿ⁺²⁾ dendrimers were quite stable over time at any concentration, whereas the pH slightly change for the lipophilic dendrimers (increases and decreases for the G3-and G4 dendrimers, respectively) after a few days of the constitution of the suspension in any media and concentration tested.

Table 4.30/1: pH measured in water solutions of the G3- and G4-PAMAM-(NH₂)₂⁽ⁿ⁺²⁾ dendrimers at different concentration.

	500 mg/L	250 mg/L	125 mg/L	62.5 mg/L	3.125 mg/L
G3-PAMAM-(NH ₂) ₃₂	9.88	9.78	9.68	9.27	9.04
G4-PAMAM-(NH ₂) ₆₄	9.90	9.81	9.56	9.29	9.02

Table 4.30/2: pH measured in suspensions of G3-and G4-PAMAM-50 % C12dendrimers at different concentration and time.

mg/L (solvent)	G3-PAMAM-50 % C12		G4-PAMAM-50 % C12	
	t = 0	t = 3-14 days	t = 0	t = 3-14 days
500 (H ₂ O)	7.85	8.0 ± 0.2	8.19	6.4 ± 0.1
100 (H ₂ O)	7.51	7.7 ± 0.2	7.88	6.0 ± 0.3
100 (1:1 H ₂ O/MeOH)	6.52	7.8 ± 0.2	7.38	6.8 ± 0.2
100 (3:1 H ₂ O/MeOH)	6.46	7.7 ± 0.1	7.31	6.4 ± 0.2

Overall remarks, attachments

Remarks on results including tables and figures

None

Applicant's summary and conclusion

Conclusions

In aqueous medium the amines, particularly those at the surface, undergo hydrolysis leading to basic pH solutions. The pH values in water solutions of the G3- and G4-PAMAM-(NH₂)₂(ⁿ⁺²) dendrimers were quite stable over time at any concentration, whereas the pH slightly change for the lipophilic dendrimers (increases and decreases for the G3-and G4 dendrimers, respectively) after a few days of the constitution of the suspension in any media and concentration tested.

Executive summary

PAMAM dendrimers were identified and quantitated in aqueous medium using liquid chromatography interfaced to a hybrid quadrupole/time-of-flight mass analyzer. Due to the precise and controlled synthesis of dendrimers the whole structure is known at an atomic level –that includes the surface groups and inner part of the molecule. For G_n-PAMAM-(NH₂)₂(ⁿ⁺²) mainly primary amine groups are at the periphery, together some amide and tertiary amine functions due to back folding of the terminal groups. For the alternate nanomaterial G_n-PAMAM-50 % C12 half of the terminal N–H bonds are substituted by 2-hydroxydodecyl groups at the surface.

In aqueous medium the amines, particularly those at the surface, undergo hydrolysis leading to basic pH solutions. The pH values in water solutions of the G3- and G4-PAMAM-(NH₂)₂(ⁿ⁺²) dendrimers were quite stable over time at any concentration, whereas the pH slightly change for the lipophilic dendrimers (increases and decreases for the G3-and G4 dendrimers, respectively) after a few days of the constitution of the suspension in any media and concentration tested.

Cross-reference to other study

No cross-reference

4.31 Dustiness

4.32 Porosity

Endpoint study record: Dendrimers, Porosity, Maiti, 2004, SS, S

Administrative Data

Purpose flag supporting study
Study result type experimental result **Study period** 2004
Reliability other: no data
Rationale for reliability incl. deficiencies No data

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Maiti PK, Cagin T, Wang G and Goddard III WA.	2004	Structure of PAMAM Dendrimers: Generations through 11.	Macromolecules 37: 6236-6254.					

Data access

data published

Cross-reference to same study

No cross-reference

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline followed		

Principles of method if other than guideline

No data

Details on methods and data evaluation

No data

Data gathering

Instruments

No data

Calibration

No data

Reproducibility

No data

Test materials

Test material form

other: nanomaterial

Any other information on materials and methods incl. tables

None

Results and discussions

Remarks on results incl. tables

No porous are present in dendrimers. Instead, they show cavities and voids of variable dimensions because of their flexible structure. Average dimensions and radial densities are available.

Overall remarks, attachments

Remarks on results including tables and figures

None

Applicant's summary and conclusion

Conclusions

No porous are present in dendrimers. Instead, they show cavities and voids of variable dimensions because of their flexible structure. Average dimensions and radial densities are available.

Executive summary

A study was conducted to determine the porosity of dendrimers.

No porous are present in dendrimers. Instead, they show cavities and voids of variable dimensions because of their flexible structure. Average dimensions and radial densities are available.

Cross-reference to other study

No cross-reference

4.33 Pour density**4.34 Photocatalytic activity****4.35 Radical formation potential****4.36 Catalytic activity****5. ENVIRONMENTAL FATE AND PATHWAYS****6. ECOTOXICOLOGICAL INFORMATION****6.1 Aquatic toxicity****6.1.1 Short-term toxicity to fish**

Endpoint study record: PAMAM dendrimers, Short-term toxicity to fish - zebra fish, Heiden, 2007, Summary level, Study level

Administrative Data

Study result type experimental result Study period No data

Rationale for reliability incl. deficiencies No data

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Heiden TCK, Dengler E, Kao WJ, Heideman W and Peterson RE.	2007	Developmental Toxicity of Low Generation PAMAM Dendrimers in Zebrafish.	Toxicology and Applied Pharmacology, 225: 70-79.					

Data access

data published

Cross-reference to same study

No cross-reference

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

Principles of method if other than guideline

The toxicity of G4-PAMAM-(NH₂)₆₄ on zebra fish embryos has been assessed by Heiden et al., (2007).

GLP compliance

no data

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material form

nanomaterial

Details on test material

- Name of test material (as cited in study report): G4-PAMAM-(NH₂)₆₄

Confidential details on test material

No data

Details on properties of test surrogate or analogue material

No data

Analytical monitoring

no data

Details on sampling

No data

Details on analytical methods

No data

Vehicle

no data

Details on test solutions

No data

Test organisms

Test organisms (species)

Danio rerio

Details on test organisms

No data

Study design

Test type

no data

Water media type

no data

Limit test

no

Total exposure duration

120 h

Remarks 24, 72 and 120 h

Post exposure observation period

None

Test conditions

Hardness

No data

Test temperature

No data

pH

No data

Dissolved oxygen

No data

Salinity

No data

Nominal and measured concentrations

No data

Details on test conditions**TEST SYSTEM**

- Fertilized eggs were collected within 2 h post fertilization (hpf) and distributed in 96-well cell culture plates (1 embryo /well) for exposure.
- Replicates: Initially, three replicate experiments (n= 16 embryos / treatment) were conducted using several concentrations of the dendrimer to observe effects on mortality (24 hpf), sublethal toxicity and hatching rate. Three additional replicate experiments (n=16 embryos/treatment) were carried out to determine the dose-dependent effects of the dendrimer on mortality and toxicity endpoints.
- Renewal rate of test solution (frequency): Medium with appropriate concentrations of dendrimer was renewed daily from 6 hpf until 120 hpf (Heiden et al., 2007).

Reference substance (positive control)

no data

Any other information on materials and methods incl. tables

None

Results and discussions**Effect concentrations**

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
24 h	other: 24 hpf LC50	1 µM	no data	test mat.	mortality	
72 h	other: 72 hpf LC50	0.6 µM	no data	test mat.	mortality	
120 h	other: 120 hpf LC50	0.4 µM	no data	test mat.	mortality	

Details on results

Sublethal toxicity signs were observed after exposure to concentrations as low as 0.2 µM G4-PAMAM-(NH₂)₆₄. Such signs include reduced body growth, bent trunk and smaller head and eyes. They also indicate that there was a tendency for G4-PAMAM-(NH₂)₆₄ exposed embryos to show mild pericardial edema and smaller caudal fin area, although neither effect was statistically significant.

Results with reference substance (positive control)

No data

Reported statistics and error estimates

No data

Any other information on results incl. tables

All the details about the material and methods and the results appear in Heiden et al., (2007) that can be consulted for further information.

Overall remarks, attachments**Remarks on results including tables and figures**

None

Applicant's summary and conclusion**Validity criteria fulfilled**

no data

Conclusions

Not available

Executive summary

Not available

Cross-reference to other study

No cross-reference

Endpoint study record: PAMAM dendrimers, Short-term toxicity to fish - zebra fish, Oliveira, Year, Summary level, Study level

Administrative Data

Study result type experimental result **Study period** No data

Rationale for reliability incl. deficiencies No data

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Oliveira et al.	2007	No data						

Data access

other: data not published

Cross-reference to same study

No cross-reference

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

Principles of method if other than guideline

The toxicity of G3-PAMAM-(NH₂)₃₂ and to G4-PAMAM-(NH₂)₆₄ on zebra fish embryos has been assessed by Oliveira et al.

GLP compliance

no data

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material form

nanomaterial

Details on test material

- Name of test material (as cited in study report): G3-PAMAM-(NH₂)₃₂ and to G4-PAMAM-(NH₂)₆₄

Confidential details on test material

No data

Details on properties of test surrogate or analogue material

No data

Analytical monitoring

no data

Details on sampling

No data

Details on analytical methods

No data

Vehicle

no data

Details on test solutions

No data

Test organisms

Test organisms (species)

Danio rerio

Details on test organisms

TEST ORGANISM

- Common name: Zebra fish

- Method of breeding: Zebra fish fertilized eggs were obtained by natural mating and raised at 28.5 °C with a 12h light: 12 h dark photoperiod in embryo water: 90 µg/ml of Instant Ocean (Aquarium Systems, Sarrebourg, France), 0.58 mM CaSO₄ · 2H₂O, dissolved in reverse osmosis purified water.

Study design

Test type

no data

Water media type

no data

Limit test

no

Total exposure duration

48 h

Post exposure observation period

None

Test conditions

Hardness

No data

Test temperature

No data

pH

No data

Dissolved oxygen

No data

Salinity

No data

Nominal and measured concentrations

No data

Details on test conditions**TEST SYSTEM**

- Test vessel: Fertilized eggs were collected within 1hpf (4 to 128 cell stages) and distributed in 24 well cell culture plates (1 embryo/well).
- Embryos were exposed to a range of concentrations of PAMAM dendrimers. In this case the International Standard 15088 (ISO 2007) for zebrafish eggs was followed.
- In addition a negative control (only medium) was set for each plate.

EFFECT PARAMETERS MEASURED:

- Mortality data was obtained after 4, 24 and 48 h exposure. Live embryos were observed to assess developmental progression as well as alterations in morphology and signs of toxicity.

OTHERS

- In addition embryos were exposed to dendrimers for observing sublethal effects by means of transcriptomic analysis using microarrays. For that a concentration of 1.5 mg/L of each dendrimer was used. Six biological replicates consisting of 50 embryos were made for each nanomaterial treatment.

Reference substance (positive control)

yes (positive control 3.7 mg/L 3,4- dichloroaniline was tested simultaneously)

Any other information on materials and methods incl. tables

None

Results and discussions**Effect concentrations**

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
48 h	LC50	1.835 mg/L	no data	test mat.	mortality	G3-PAMAM-(NH ₂) ₃₂
48 h	NOEC	1 mg/L	no data	test mat.	mortality	G3-PAMAM-(NH ₂) ₃₂
48 h	LOEC	2 mg/L	no data	test mat.	mortality	G3-PAMAM-(NH ₂) ₃₂
48 h	LC50	2.725 mg/L	no data	test mat.	no data	G4-PAMAM-(NH ₂) ₆₄
48 h	NOEC	1 mg/L	no data	test mat.	no data	G4-PAMAM-(NH ₂) ₆₄
48 h	LOEC	2 mg/L	no data	test mat.	no data	G4-PAMAM-(NH ₂) ₆₄

Details on results

- Mortality curves followed a sigmoidal function.

Results with reference substance (positive control)

No data

Reported statistics and error estimates

No data

Any other information on results incl. tables

Transcriptomic analyses showed significant changes on mRNA abundance for 478 genes for G3-PAMAM-(NH₂)₃₂ treated embryos and for 372 genes for G4-PAMAM-(NH₂)₆₄ treated ones. In total 700

transcripts were affected by at least one of the treatments and of them, 150 by both treatments. The significant proportion of genes affected by both treatments suggests a common mechanism of action. Gene ontology analyses revealed the ability of dendrimers to elicit a response in the embryos similar to this triggered by exposure to foreign bodies (for instance bacteria) so that PAMAM dendrimers act as inducers of the natural immune response.

All details about the work, the material and methods and the results obtained have been published elsewhere (Oliveira et al.). Please, consult this reference for further details.

Overall remarks, attachments

Remarks on results including tables and figures

None

Applicant's summary and conclusion

Validity criteria fulfilled

yes Embryo toxicity tests met the standard validity criteria (lower mortality than 10% in the negative controls after 48h and 100% mortality in the positive controls)

Conclusions

Not available

Executive summary

Not available

Cross-reference to other study

No cross-reference

6.1.2 Long-term toxicity to fish

6.1.3 Short-term toxicity to aquatic invertebrates

Endpoint study record: PAMAM dendrimers, Short-term toxicity to aquatic invertebrates (Daphnia magna), Naha, 2009, Summary level, Study level

Administrative Data

Study result type experimental result **Study period** No data

Rationale for reliability incl. deficiencies No data

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Naha PC, Davoren M, Casey A and Byrne HJ.	2009	An Ecotoxicological Study of Poly(amidoamine) Dendrimers-Toward Quantitative Structure Activity Relationships.	Environmental Science and Technology, 43: 6864-6869.					

Data access

data published

Cross-reference to same study

No cross-reference

Materials and methods**Test guideline**

Qualifier	Guideline	Deviations
no guideline available		

Principles of method if other than guideline

Naha et al., (2009) performed acute immobilization tests with G4-PAMAM-(NH₂)₆₄ in *Daphnia magna* according to the British Standard (BS 1996).

GLP compliance

no data

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material form

nanomaterial

Details on test material

- Name of test material (as cited in study report): G4-PAMAM-(NH₂)₆₄

Confidential details on test material

No data

Details on properties of test surrogate or analogue material

No data

Analytical monitoring

no data

Details on sampling

No data

Details on analytical methods

No data

Vehicle

no data

Details on test solutions

No data

Test organisms

Test organisms (species)

Daphnia magna

Details on test organisms

TEST ORGANISM

- Age at study initiation: < 24 h

Study design

Test type

no data

Water media type

no data

Limit test

no

Total exposure duration

48 h

Remarks 24 and 48 h exposure

Post exposure observation period

None

Test conditions

Hardness

No data

Test temperature

No data

pH

No data

Dissolved oxygen

No data

Salinity

No data

Nominal and measured concentrations

No data

Details on test conditions

EFFECT PARAMETERS MEASURED:

- Immobilization (no independent movement after gentle agitation of the test liquid for 15 s) was visually determined at 24 and 48 h exposure.

Reference substance (positive control)

yes (phenol and potassium dichromate)

Any other information on materials and methods incl. tables

None

Results and discussions

Effect concentrations

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
24 h	LC50	1.13 µM	no data	test mat.	mobility	
48 h	LC50	0.68 µM	no data	test mat.	mobility	

Details on results

No data

Results with reference substance

As reference chemicals phenol and potassium dichromate were tested in tandem with the nanoparticles to ensure the validity of the assays.

Reported statistics and error estimates

No data

Any other information on results incl. tables

None

Overall remarks, attachments**Remarks on results including tables and figures**

None

Applicant's summary and conclusion**Validity criteria fulfilled**

yes

Conclusions

For G4-PAMAM-(NH₂)₆₄ the 24h exposure LC₅₀ was 1.13 µM and the 48h LC₅₀ was 0.68 µM.

Executive summary

Not available

Cross-reference to other study

No cross-reference

6.1.4 Long-term toxicity to aquatic invertebrates**6.1.5 Toxicity to aquatic algae and cyanobacteria**

Endpoint study record: PAMAM dendrimers, Toxicity to aquatic algae - Algae growth inhibition test, Suárez, 2011, Summary level, Study level

Administrative Data

Study result type experimental result **Study period** No data

Rationale for reliability incl. deficiencies No data

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Suárez IJ, Rosal R, Rodríguez A, Ucles A, Fernández-Alba AR, Hernando MD and García-Calvo E.	2011	Chemical and Ecotoxicological Assessment of Poly(amidoamine) Dendrimers in the Aquatic Environment.	Trends in analytical chemistry, 30: 492-506.					

Data access

data published

Cross-reference to same study

No cross-reference

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 201 (Alga, Growth Inhibition Test)	no data

Principles of method if other than guideline

The effect of G4-PAMAM-(NH₂)₆₄ on algae growth has been studied by Suárez et al., (2011) following the OECD TG 201 (Algae growth inhibition test) using the green alga *Pseudokirchneriella subcapitata*.

GLP compliance

no data

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material form

nanomaterial

Details on test material

- Name of test material (as cited in study report): G4-PAMAM-(NH₂)₆₄

Confidential details on test material

No data

Details on properties of test surrogate or analogue material

No data

Analytical monitoring

no data

Details on sampling

No data

Details on analytical methods

No data

Vehicle

no data

Details on test solutions

No data

Test organisms

Test organisms (species)

Pseudokirchnerella subcapitata

Details on test organisms

No data

Study design

Test type

no data

Water media type

no data

Limit test

no

Total exposure duration

72 h

Post exposure observation period

None

Test conditions

Hardness

No data

Test temperature

22 °C

pH

No data

Dissolved oxygen

No data

Salinity

No data

Nominal and measured concentrations

No data

Details on test conditions**TEST SYSTEM**

- Test vessel: Exposure was performed in 96 well plates culturing the algae in a total volume of 210 µL
- Aeration: Yes
- No. of replicates per concentration: Three replicates of each toxic concentration or blank

OTHER TEST CONDITIONS

- Light intensity: 100 µE/m²/s

EFFECT PARAMETERS MEASURED (with observation intervals if applicable) :

- Chlorophyll measurement: The growth of *P. Subcapitata* was monitored for 72 h by determining the increase of chlorophyll concentration measured by fluorescence (444 nm / 680 nm, excitation / emission wavelengths, respectively).

TEST CONCENTRATIONS

- Test concentrations: 0.21 and 1.41 mg/L

Reference substance (positive control)

no data

Any other information on materials and methods incl. tables

None

Results and discussions**Effect concentrations**

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
72 h	EC10	EC10 was 0.21 mg/L (15 nM)	no data	test mat.	growth rate	
72 h	EC50	EC50 was 1.41 mg/L (99 nM)	no data	test mat.	growth rate	

Details on results

For G4-PAMAM-(NH₂)₆₄ the EC₅₀ was 1.41 mg/L (99 nM) and the EC₁₀ was 0.21 mg/L (15 nM) (Suárez et al., 2011).

Results with reference substance (positive control)

No data

Reported statistics and error estimates

No data

Any other information on results incl. tables

None

Overall remarks, attachments**Remarks on results including tables and figures**

All the details about the material and methods and the results appear in Suárez et al., (2011) that can be consulted for further information.

Applicant's summary and conclusion**Validity criteria fulfilled**

no data

Conclusions

For G4-PAMAM-(NH₂)₆₄ the EC₅₀ was 1.41 mg/L (99 nM) and the EC₁₀ was 0.21 mg/L (15 nM)

Executive summary

Not available

Cross-reference to other study

No cross-reference

6.1.6 Toxicity to aquatic plants other than algae**6.1.7 Toxicity to microorganisms****6.1.8 Toxicity to other aquatic organisms**

Endpoint study record: PAMAM dendrimers, Toxicity to other aquatic organisms, Naha 2009 and Petit 2010, Summary level, Study level

Administrative Data

Study result type experimental result **Study period** No data

Rationale for reliability incl. deficiencies No data

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Naha PC, Davoren M, Casey A and Byrne HJ.	2009	An Ecotoxicological Study of Poly(amidoamine) Dendrimers-Toward Quantitative Structure Activity Relationships.	Environmental Science and Technology, 43: 6864-6869.					
publication	Petit A-N, Eullaffroy P, Debenest T and Gagné F.	2010	Toxicity of PAMAM dendrimers to Chlamydomonas reinhardtii.	Aquatic toxicology, 100: 187-193.					

Data access

data published

Cross-reference to same study

No cross-reference

Materials and methods**Test guideline**

Qualifier	Guideline	Deviations
no guideline available		

Principles of method if other than guideline

Toxicity of PAMAM dendrimers was studied using Chlamydomonas reinhardtii (Petit et al., 2010), Vibrio fischeri and Thamnocephalus platyurus (Naha et al., 2009). For G3-PAMAM-50% C12 and G3-PAMAM-50% C12 no data were obtained.

GLP compliance

no data

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material form

nanomaterial

Details on test material

- Name of test material (as cited in study report): G4-PAMAM-(NH₂)₆₄ and G4-PAMAM diaminobutane core (NH₂)₆₄

Confidential details on test material

No data

Details on properties of test surrogate or analogue material

No data

Analytical monitoring

no data

Details on sampling

No data

Details on analytical methods

No data

Vehicle

no data

Details on test solutions

No data

Test organisms

Test organisms (species)

other: Chlamydomonas reinhardtii, Vibrio fischeri and Thamnocephalus platyurus

Details on test organisms

No data

Study design

Test type

no data

Water media type

no data

Limit test

no

Total exposure duration

Remarks Vibrio fischeri (5, 15 and 30 minutes); Thamnocephalus platyurus (24 h); Chlamydomonas reinhardtii (72 h)

Post exposure observation period

None

Test conditions

Hardness

No data

Test temperature

No data

pH

No data

Dissolved oxygen

No data

Salinity

No data

Nominal and measured concentrations

No data

Details on test conditions

- *Vibrio fischeri*: The marine bacterium *Vibrio fischeri* is one of the most used organisms for determining the toxicity of PAMAM dendrimers. Naha et al., (2009) used lyophilized bacteria and Microtox® reagents for determining the EC50 of PAMAM dendrimers after 5, 15 and 30 minutes exposure. Results are shown in table 6.1.8/1.

- *Chlamydomonas reinhardtii*: This unicellular green alga has been used as a model organism to study responses to different environmental factors. Petit et al., (2010) exposed exponentially growing cells to G4 polyamidoamine dendrimers and measured chlorophyll and esterase activities after 72 h treatment by means of flow cytometry. However, these authors (Petit et al., 2010) used a G4 PAMAM with a 1,4-diaminobutane core (62 –NH2 terminal groups, C624H1252N250O124, molecular weight 14.243). Since the structure of this dendrimer is almost identical to that of the G4-PAMAM-(NH2)64 used here the results obtained by Petit et a., (2010) are also included in the table 6.1.8/1.

- *Thamnocephalus platyurus*: This freshwater shrimp was also used to study the toxicity of PAMAM dendrimers (Naha et al., 2009). Results are shown in table 6.1.8/1.

Reference substance (positive control)

no data

Any other information on materials and methods incl. tables

None

Results and discussions

Details on results

No data

Results with reference substance (positive control)

No data

Reported statistics and error estimates

No data

Any other information on results incl. tables**Table 6.1.8/1: Summary of toxicity data for G3-PAMAM-(NH₂)₃₂ and to G4-PAMAM-(NH₂)₆₄ in aquatic organisms.**

PAMAM dendrimer	Species (conditions of exposure)	EC50/IC50	Reference
G4-PAMAM-(NH ₂) ₆₄	<i>Vibrio fischeri</i> (5 min)	15.18 µM	Naha et al., 2009
G4-PAMAM-(NH ₂) ₆₄	<i>Vibrio fischeri</i> (15 min)	5.08 µM	Naha et al., 2009
G4-PAMAM-(NH ₂) ₆₄	<i>Vibrio fischeri</i> (30 min)	1.64 µM	Naha et al., 2009
G4-PAMAM-(NH ₂) ₆₄	<i>Thamnocephalus platyurus</i> (24 h lethality)	2.90 µM	Naha et al., 2009
G4-PAMAM diaminobutane core (NH ₂) ₆₄	<i>Chlamydomonas reinhardtii</i> (72 h)	2.97 mg/L	Petit et al., 2010

Overall remarks, attachments**Remarks on results including tables and figures**

None

Applicant's summary and conclusion**Validity criteria fulfilled**

no data

Conclusions

Not available

Executive summary

Not available

Cross-reference to other study

No cross-reference

6.2 Sediment toxicity**6.3 Terrestrial toxicity****6.4 Biological effects monitoring****6.5 Biotransformation and kinetics****6.6 Additional ecotoxicological information**

Endpoint study record: PAMAM dendrimers, Additional ecotoxicological information - In vitro toxicity (cytotoxicity), Hernando, 2012, Summary level, Study level

Administrative Data

Study result type experimental result **Study period** No data

Rationale for reliability incl. deficiencies No data

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Hernando MD, Rosenkranz P, Ulaszewska MM, Fernández-Cruz ML, Fernández-Alba AR and Navas JM.	2012	In Vitro Dose–Response Effects of Poly(amidoamine) dendrimers [amino-terminated and surface-modified with N-(2-hydroxydodecyl) groups] & Quantitative Determination by a Liquid Chromatography–Hybrid Quadrupole/Time-of-Flight Mass Spectrometry based Method.	Analytical and Bioanalytical Chemistry, 404: 2749–2763.					

Data access

data published

Cross-reference to same study

No cross-reference

Materials and methods

Type of information

In vitro toxicity (cytotoxicity)

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

Principles of method if other than guideline

Some in vitro assays were performed to generate initial data about the toxicity and the possible mechanisms of toxicity of PAMAM dendrimers. These in vitro studies also helped to determine the concentrations or quantities of PAMAM dendrimers to be used in higher level ecotoxicity tests.

GLP compliance

no data

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material form

nanomaterial

Details on test material

- Name of test material (as cited in study report): PAMAM dendrimers (G3-PAMAM-(NH₂)₃₂, G4-PAMAM-(NH₂)₆₄, G3-PAMAM-50% C12 and G4-PAMAM-50% C12)

Confidential details on test material

No data

Any other information on materials and methods incl. tables

H4IIE Reuber rat hepatoma cells were obtained from the European Collection of Cell Cultures (ECACC). RTG-2 cells, derived from rainbow trout (*Oncorhynchus mykiss*) gonadal tissue, were obtained from American Type Culture Collection (ATCC). Both cell lines were cultured in Eagle's minimum essential medium (EMEM) (Lonza, Barcelona, Spain.) at 37 °C and 5% CO₂ for the H4IIE cell line and 20 °C for the RTG-2 cell line. For both cell lines, culture medium was supplemented with 10% FBS, 1% antibiotic mixture (Penicillin/Streptomycin) and 2% Ultraglutamine. The medium was replaced approximately every second day.

Cells were exposed to a series of PAMAM dendrimer concentrations for different incubation periods and the toxic responses monitored. Cell treatments were carried out in 96 well-plates. 100 µL of cell suspension at 2.5×10^5 cells/mL for the H4IIE cell line and 1×10^5 cells/mL for the RTG-2 cell line were added to each well and then incubated for 24 h at 37 °C and 5% CO₂ for the H4IIE or 20 °C and 5% CO₂ for the RTG-2 cell line, respectively. After 24 h, cells were exposed to the respective dendrimers by adding 40 µL of dendrimer stock solution to 160 µL of media to create a nominal exposure concentration of 500 mg/mL.

The remaining concentrations were obtained by a 50 % downwards dilution for all assays. All treatments were done in triplicate. A positive control containing sodium dodecyl sulfate (SDS) was also included, ranging from 10 mg /mL to 0.3 mg /mL.

Plasma membrane integrity was evaluated through the lactate dehydrogenase leakage as done by Scholz and Segner (1999). The assay is based on the ability of lactate dehydrogenase (LDH) to catalyze the conversion of pyruvate to lactic acid with simultaneous conversion of NADH to NAD. The pyruvate not converted by LDH, attaches to 2,4-dinitrophenylhydrazine and forms a brown complex. The intensity and therefore the measured absorption are inversely proportional to the LDH concentration. Triton X-100 is used as a positive control since it is assumed to cause 100 % cell death and therefore represents the total releasable LDH.

Alterations of the sensitive lysosomal membrane leading to lysosomal fragility were evaluated by means of neutral red uptake, NRU (Borenfreund and Puerner, 1985). This assay is based on the ability of intact lysosomes to retain the dye NR in living cells.

The integrity of mitochondria and of the respiratory chain will be assessed by measuring, photometrically, the reduction of tetrazolium salts (in this case MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, was used) to formazan (Mosmann, 1983).

The cytotoxicity assays selected in this study were performed at 2 time points (24 h and 72 h) for each cell type line and nanoparticle. At least three independent experiments were done for each assay. NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect Concentration) were calculated. For further details and additional information about the cytotoxicity assays performed and the results obtained see Hernando et al., 2012.

Results and discussions

Any other information on results incl. tables

Three different cytotoxicity assays were performed. Modified from Hernando et al., 2012.

Table 6.6/1: Cytotoxicity of G3-PAMAM-(NH₂)₃₂, G4-PAMAM-(NH₂)₆₄, G3-PAMAM-50% C12, and G4-PAMAM-50% C12 in H4IIE cells after 24 h exposure.

H4IIE cells (24 h exposure)	MTT		NRU		LDH	
	LOEC (µg/mL)	NOEC (µg/mL)	LOEC (µg/mL)	NOEC (µg/mL)	LOEC (µg/mL)	NOEC (µg/mL)
G3-PAMAM-(NH ₂) ₃₂	62.5	31.2	NA	500	NA	500
G4-PAMAM-(NH ₂) ₆₄	15.6	7.8	NA	500	500	250
G3-PAMAM-50% C12	125	62.5	500	250	7.8	3.9
G4-PAMAM-50% C12	125	62.5	500	250	125	62.5

Table 6.6/2: Cytotoxicity of G3-PAMAM-(NH₂)₃₂, G4-PAMAM-(NH₂)₆₄, G3-PAMAM-50% C12, and G4-PAMAM-50% C12 in H4IIE cells after 72 h exposure.

H4IIE cells (24 h exposure)	MTT		NRU		LDH	
	LOEC (µg/mL)	NOEC (µg/mL)	LOEC (µg/mL)	NOEC (µg/mL)	LOEC (µg/mL)	NOEC (µg/mL)
G3-PAMAM-(NH ₂) ₃₂	NA	500	NA	500	62.5	31.2
G4-PAMAM-(NH ₂) ₆₄	NA	500	NA	500	7.8	3.9
G3-PAMAM-50% C12	250	125	250	125	15.6	7.8
G4-PAMAM-50% C12	62.5	32.2	250	125	62.5	31.2

Table 6.6/3: Cytotoxicity of G3-PAMAM-(NH₂)₃₂, G4-PAMAM-(NH₂)₆₄, G3-PAMAM-50% C12, and G4-PAMAM-50% C12 in RTG-2 cells after 24 h exposure.

RTG-2 cells (24 h exposure)	MTT		NRU		LDH	
	LOEC (µg/mL)	NOEC (µg/mL)	LOEC (µg/mL)	NOEC (µg/mL)	LOEC (µg/mL)	NOEC (µg/mL)
G3-PAMAM-(NH ₂) ₃₂	NA	500	NA	500	NA	500
G4-PAMAM-(NH ₂) ₆₄	NA	500	NA	500	NA	500
G3-PAMAM-50% C12	NA	500	NA	500	62.5	31.2
G4-PAMAM-50% C12	NA	500	NA	500	62.5	31.2

Table 6.6/4: Cytotoxicity of G3-PAMAM-(NH₂)₃₂, G4-PAMAM-(NH₂)₆₄, G3-PAMAM-50% C12, and G4-PAMAM-50% C12 in RTG-2 cells after 72 h exposure.

RTG-2 cells (24 h exposure)	MTT		NRU		LDH	
	LOEC (µg/mL)	NOEC (µg/mL)	LOEC (µg/mL)	NOEC (µg/mL)	LOEC (µg/mL)	NOEC (µg/mL)
G3-PAMAM-(NH ₂) ₃₂	125	31.2	NA	500	NA	500
G4-PAMAM-(NH ₂) ₆₄	125	31.2	31.2	15.6	NA	500
G3-PAMAM-50% C12	500	250	NA	500	62.5	31.2
G4-PAMAM-50% C12	NA	500	NA	500	125	62.5

N.B.: MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NRU: Neutral red uptake; LDH: lactate dehydrogenase; NOEC: No Observed Effect Concentration; LOEC: Lowest Observed Effect Concentration; IC₅₀: Inhibitory Concentration 50, i.e. the concentration causing a 50% inhibitory effect; NA: not applicable because the maximal concentration used was 500 µg/mL and no effect was detected at this concentration.

Overall remarks, attachments**Remarks on results including tables and figures**

None

Applicant's summary and conclusion**Conclusions**

Not available

Executive summary

Not available

Cross-reference to other study

No cross-reference

7. TOXICOLOGICAL INFORMATION**8. ANALYTICAL METHODS**

Endpoint study record: PAMAM dendrimers, Analytical methods, Suarez 2011, Hernando 2012, Ulaszewska 2013, Summary level, Study level

Administrative Data

Study result type experimental result **Study period** No data

Rationale for reliability incl. deficiencies No data

Data source**Background information**

For any study about environmental fate or ecotoxicology of PAMAM dendrimers it is essential to have appropriate analytical methods allowing the detection of dendrimers and the estimation of concentrations in a variety of samples.

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Suárez IJ, Rosal R, Rodríguez A, Ucles A, Fernández-Alba AR, Hernando MD and García-Calvo E.	2011	Chemical and Ecotoxicological Assessment of Poly(amidoamine) Dendrimers in the Aquatic Environment.	Trends in analytical chemistry, 30: 492-506.					
publication	Hernando MD, Rosenkranz P, Ulaszewska MM, Fernández-Cruz ML, Fernández-Alba AR and Navas JM.	2012	In Vitro Dose–Response Effects of Poly(amidoamine) dendrimers [amino-terminated and surface-modified with N-(2-hydroxydodecyl) groups] & Quantitative Determination by a Liquid Chromatography–Hybrid Quadrupole/Time-of-Flight Mass Spectrometry based Method.	Analytical and Bioanalytical Chemistry, 404: 2749-2763.					
publication	Ulaszewska MM, Hernando MD, Uclés Moreno A, Valverde García A, García Calvo E and Fernández-Alba AR.	2013	Identification and Quantification of Poly(amidoamine) PAMAM dendrimers of Generations 0 to 3 by Liquid Chromatography/Hybrid Quadrupole Time-of-Flight Mass Spectrometry in Aqueous Medium.	Rapid Commun Mass Spectrom.27(7):747-62.					

Data access

data published

Cross-reference to same study

No cross-reference

Materials and methods

Matrix / medium

other: culture media and analysed in two different cell lines, one rat liver cell line (H4IIE) and one cell line derived from the gonad of rainbow trout (RTG-2)

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

Principles of method if other than guideline

Quantification of PAMAM dendrimers was achieved by means of liquid chromatography-hybrid quadrupole/time-of-flight mass spectrometry. This method showed the existence of defective dendrimers instead of “ideal structures”.

GLP compliance

no data

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material form

nanomaterial

Details on test material

- Name of test material (as cited in study report): PAMAM dendrimers

Confidential details on test material

No data

Details on properties of test surrogate or analogue material

No data

Principles of analytical methods**Instrument / detector**

LC-MS

Details on analytical method

No data

Confirmatory method (if applicable)**Any other information on materials and methods incl. tables**

None

Results and discussions

Recovery results and characteristics of analytical method

Recovery results

No data

Characteristics of analytical method

No data

Independent laboratory validation (if applicable)

Independent laboratory validation

No data

Any other information on results incl. tables

Quantification of PAMAM dendrimers was achieved by means of liquid chromatography-hybrid quadrupole/time-of-flight mass spectrometry. This method showed the existence of defective dendrimers instead of “ideal structures”. The identification was based on accurate mass measurement, assignment of elemental composition and the fully resolved ¹³C/¹²C isotopic clusters of the multiply charged ions of PAMAM dendrimers. Validation of the liquid chromatography-mass spectrometry method made possible reliable and reproducible quantification of the extracellular and intracellular concentration of dendrimers at a micromolar level (limits of detection from 0.14 to 1.34 µM and from 0.43 to 1.82 µM in standard and culture medium, respectively). PAMAM dendrimers were analysed not only in the culture media but also successfully extracted and analysed in two different cell lines, one rat liver cell line (H4IIE) and one cell line derived from the gonad of rainbow trout (RTG-2). Considering the different features of both cell lines, the developed method appears as a promising one for determining concentrations of PAMAM dendrimers in cells and in biological media. In addition, this same methods could be applied (maybe after further refinement) to a variety of environmental samples.

Overall remarks, attachments

Remarks on results including tables and figures

None

Applicant's summary and conclusion

Conclusions

Not available

Executive summary

Not available

Cross-reference to other study

No cross-reference

9. RESIDUES IN FOOD AND FEEDINGSTUFFS

10. EFFECTIVENESS AGAINST TARGET ORGANISMS

11. GUIDANCE ON SAFE USE

12. LITERATURE SEARCH

13. ASSESSMENT REPORTS

14. INFORMATION REQUIREMENTS