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COLORIMETRIC DETERMINATION OF BORON-10 IN MACROMOLECULAR DELIVERY AGENTS

Maria A.P. Camillo[1], Eduardo Moura[1], Alvaro A.A.A.de Queiroz[2]

[1]Centro de Biologia Molecular, IPEN/CNEN-SP. Av. Prof. Lineu Prestes, 2242. Cidade Universitária
05508-900, São Paulo, SP
E-mail: mcamillo@ipen.br

[2]Departamento de Física e Química/Instituto de Ciências Exatas - Universidade Federal de Itajubá
Av. BPS. 1303, 37500-903, Itajubá-MG
alencar@unifei.edu.br

ABSTRACT

A polyglycerol with dendritic structure (PGLD) was synthesized by the ring opening polymerization of deprotonated glycidol using a polyglycerol as core functionality in a step-growth process denominated divergent synthesis. After PGLD reaction with ^{10}B-enriched boric acid there was a marked increase in the bulk viscosity of the PGLD dendrimer evidencing the polyester formation. Gel permeation chromatography (GPC) analysis was used to characterize the molecular weight and the polydispersivity of the synthesized PGLD dendrimer. A dendritic polyglycerol structure with M_n value of 16.7 kDa and a narrow polydispersity (M_w/M_n = 1.05) was obtained in this work. ^{1}H-NMR and ^{13}C-NMR measurements were employed to assess the degree of branching (DB) in PGLD. The DB of 0.85 indicates the tendency of a dentritic structure for the PGLD synthesized in this work. The boron-10 concentration was dependent of the PGLD generation. A selective reagent, curcumine, was studied for spectrophotometric determination of boron in polyglycerol dendrimers. Boron reacts with curcumine to form a complex, which has a maximum absorption peak at 552 nm. Under the optimal conditions, Beer´s law was obeyed over the range 0~20 μg of boron in 25 mL of solution. The biological assays indicate the PGLD-B with boron-10 concentration of 25 mg^{10}B/gPGLD as the most promising macromolecule enriched with boron-10 for the BNCT therapy.

1. INTRODUCTION

Boron Neutron Capture Therapy (BNCT) is a non-invasive therapy used against tumoral brain affections and against melanomas. BNCT make use of neutrons capture by boron-10 (^{10}B) nucleus present in carriers drugs as sodium mercaptododecaborate ($Na_2H_{11}{}^{10}B_{12}SH$, BSH) and 4-borono-L-phenylalanine ($(HO)_2{}^{10}B$-C_6H_4-$CH(NH_2)CO_2H$, BPA) [1].

In tumor tissue selectively accumulated ^{10}B followed by irradiation with thermal neutrons generates high-energy $^4He^{2+}$ and $^7Li^{3+}$ particles. Due to their high linear-energy transfer (LET) and the short-range emission of these particles the radiation damage is confined to the vicinity of the boron-accumulating tissue [2].

The lower water solubility of BSH or BPA as well as their clinical complications at sub-therapeutic or therapeutic doses have incentive the development of better tumor-seeking

boron rich agents which could be selectively delivered to cancer cells either directly, or by use of targeting strategies.

Over the past decade, highly branched regular three-dimensional monodisperse macromolecules denominated dendrimers have received immense interest from academics as well as industrial researchers due to their great potential for future clinical use [3].

Currently, polyglycerols have received a considerable attention for coating biomaterials and because of some of their properties, such as nontoxicity and good biological compatibility have became an interesting alternative for biomedical applications. Polyglycerol (PGL) is a fully water-soluble polymer that contains both pendant hydroxyl groups and ether linkages. The lower cytotoxicity and the FDA approval use of PGL as emulsifiers in the pharmaceutical and food industries [4] make the polyglycerol a promising polymer for use in the BNCT therapy.

Our research has been engaged in the development of boron 10-enriched polyglycerol dendrimers (^{10}B-PGLD). In view of the great importance of boron-10 concentration in the BNCT therapy, it is of interest to consider the analytical development for the rapid and efficient determination of boron in the boronated PGLD (^{10}B-PGLD). A modified curcumine method was used for the assay. It is accurate, safe and sensitive enough to be used to determining boron levels also in biological samples.

2. EXPERIMENTAL

2.1. Dendrimer synthesis

In this work the polyglycerol dendrimers (PGLD) were synthesized by the ring opening polymerization of glycidol in a step-growth processes denominated divergent synthesis. The nucleophilic attack of the epoxy group by alkoxide ions at 230^{o}C and 50 mmHg in an argon atmosphere was used as new approach to obtain a polyglycerol dendrimer (PGLD). After purification, the nature of the PGLD dendritic structure was characterized by GPC (HPLC 510, Waters), ^{13}C-NMR (Bruker 500 MHz) and MALDI-TOF spectroscopy (Bruker, BIFLEXTM, Bremen).

The dendritic PGLD borate, PGLD-^{10}B, was prepared by charging PGLD dendrimer to the reactor and warming to 70^{o}C. The appropriate ratio of boric acid (^{10}B) was added and the reaction mixture is heated to 160^{o}C under vacuum. After about 3 hrs at this temperature the reaction will be almost complete, indicated by a reduced production of water of reaction.

2.2. Analytical method for ^{10}B in PGLD

The analytical method was chosen based on the reaction of a boron with curcumine in acid medium, resulting in a complex of red coloration called “rosocyanine”. It is stable for at least 1 hour. Stock solution of boron (0.1 mg/mL in water) was used for the standard curve. Serial dilutions between 0.25 and 20 μg B/mL, were prepared. In each dilution was added 2 mL of 2-etil-1,3-hexanodiol 10% in chloroformium; stirring for 3 minutes and centrifuged at 200*g*

for 2 minutes. The aqueous suspension was discarded by aspiration. For the colorimetric reaction, 0.5 mL of chloroformic extract, 1 mL of curcumine solution (0.375 % p/v in glacial acetic acid) and 0.3 mL of concentrated sulfuric acid. The mixture was incubated for 15 minutes at room temperature and diluted to 30 mL with 95% ethanol. The absorbance was measure at 552 nm. For the standard curve, the mathematics adjust was made by linear regression using the software GraphPad Prism.

The PGDL in different boron concentrations were injected into the mouse peritoneum. After 2 hours the peritoneal exsudate was collected. For this purpose, 5 mL of cold PBS were injected in the same local, aspirated and thus obtained the macrophages. After these, cells were centrifuged at 200g for 5 minutes at 4^0C and the aqueous suspension was discarded. The cellular pellet was lised. by ultrasom and the boron content was determined.

3- RESULTS AND DISCUSSION

The ^{13}C-NMR spectroscopy was used to verify the relation between the architecture and the degree of branching of the dendritic polyglycerol (PGLD) synthesized in this work. The ^{13}C-NMR spectrum of polyglycerol dendrimer (PGLD) is given in Fig. 1. The degree of branching (DB) calculated on the basis of integration of ^{13}C-NMR signals was 0.85. The degree of branching (DB) measures the suitability of a hyperbranching reaction to create dendritic structures. The linear polymers not have a degree of branching (DB=0) while in the perfect dendrimer the degree of branching is unitary (DB=1).

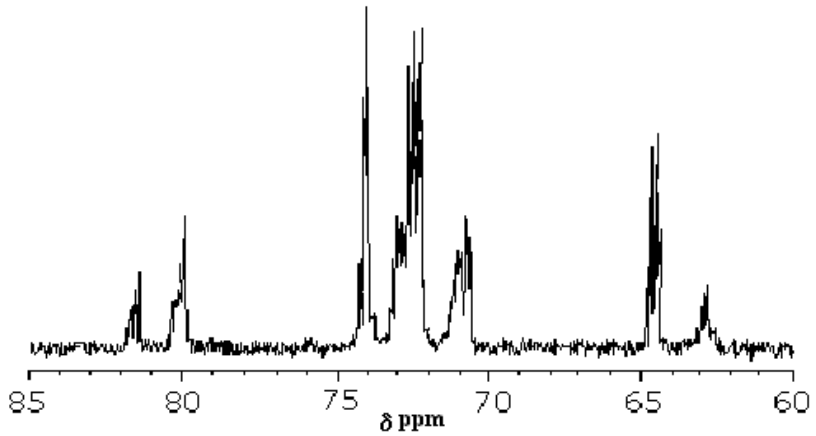

Figure 1. ^{13}C-NMR spectrum of PGLD dendrimer.

The high hydroxyl functionalities of the PGLD could be favorable to the acid-base/esterification reactions between boric acid and the PGLD macromolecule providing the formation of the cyclic boron esters as shown by Fig. 2.

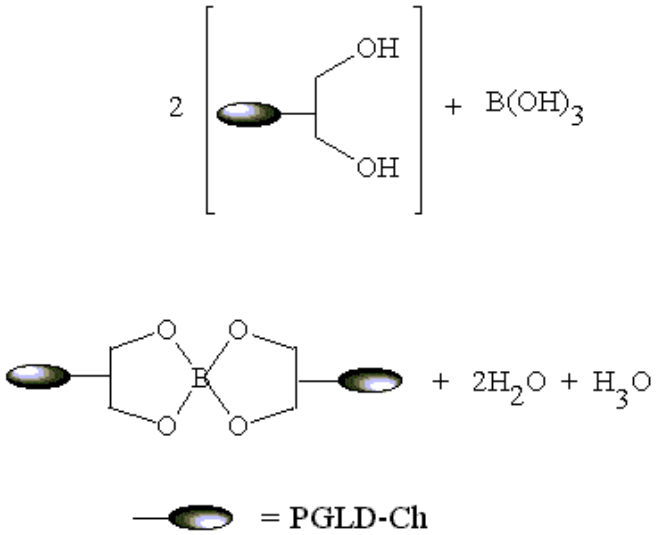

Figure 2. Molecular structure of boronated PGLD ^{10}B-PGLD).

The knowledge of the molar mass distribution is fundamental to the characterization of the PGLD dendritic structure. Thus, the PGLD dendrimer was characterized by gel permeation chromatography (GPC) with respect to their molecular weight and polydispersity. As shown in Fig. 3, a monomodal molecular weight distribution and polydispersity (M_w/M_n) of 1.05 were attained in this work.

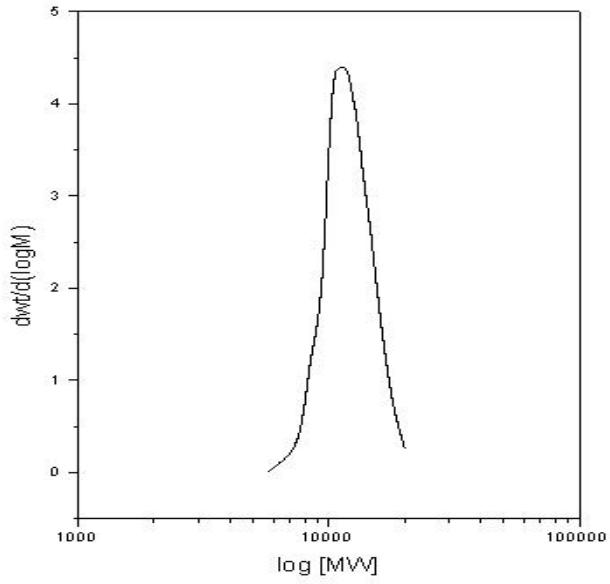

Figure 3. GPC analysis of PGLD dendrimer.

The molecular weights of the samples measured by gel permeation chromatography (GPC) analyses indicate values of 18.6 kDa, larger than those given by MALDI-TOF spectroscopy

(16.7 kDa). The hydrodynamic volume and the presence of PGLD agglomerates may be the principal reasons for deviation of GPC data from MALDI-TOF data.

One usual method for planning new drugs by BNCT is binding the boron and the ligand in the same molecule. The level of boron incorporation per molecule is a determinant factor for real application of this compound in the treatment. The therapeutic dose of boron-10 in tumorals cells is about 10^9 boron atoms per cells or analytical concentration 10-30 ppm inside the tumoral tissue. This concentration correspond 1-3x10^3 g atoms/L of boron-10 in the tumoral cells at the moment of neutrons irradiation.

The standard curve of boron was: $Y = 0.1161\ X + 0.0260$; r^2 0.9991 e $P < 0.0001$ and used to calculate the values for concentration of incorporated boron-10 in PGLD-B of different generations.

The bulk viscosities were measured in function of various boron concentrations and the results are shown in Fig. 5 (B). When the PGLD was reacted with boric acid, there was a marked increase in the bulk viscosity of the PGLD dendrimer evidencing physically that boron can react with PGLD dendrimer forming polyesters.

The PGLD-B with higher levels of boron incorporation have a higher viscosity, the samples with 140 mg B/g showed inappropriate for manipulation *in vivo* due to the fact that this compound is very viscous. The physical chemistry characteristic (viscosity and ^{10}B components) of dendrimer of 2.5 generation with boron concentration equivalent a 25 mg/g PGLD seems to be more adequate for the continue of this research.

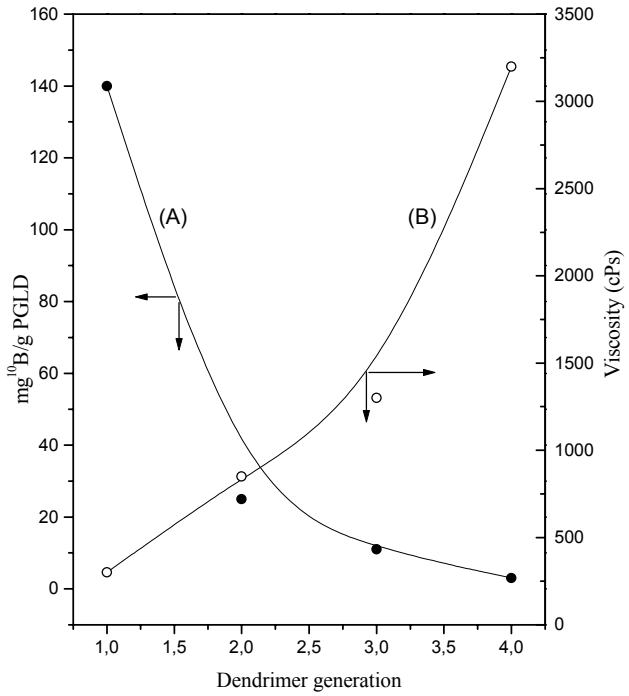

Figure 5. Influence of the dendrimer generation on the boron-10 content (A) and bulk viscosity at 25^{o}C (B).

3. CONCLUSIONS

Enriched-^{10}B polyglycerol dendrimers are especially attractive candidates for BNCT because they may deliver boron-10 to the nuclei of tumor cells. The experiment demonstrated that the proposed method has adequate sensitivity and accuracy for determination of boron in PGLD dendrimers and biological sample. The results show that the proposed method can provide results with considerably low cost and it is easily available in most laboratories for routine determination of boron, especially in developing countries.

REFERENCES

1. J.H. Morris, *Chem.*Brit., **April**, pp. 331-43 (1991).
2. R.G. Fairchild, V.P. Bond, *Int.J.Radiat.Oncol.Biol.Phys.*, **11**, pp. 831-38 (1985).
3. J.M.J. Fréchet, *Science*, **263,**pp. 1710-14 (1994).
4. W.R. Michael, R.H. Coots, *Toxicol.Appl.Pharmacol.* **20**, pp. 334-45 (1971).