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Quantitative detection of carbon nanotubes in biological samples by an original method based on microwave permittivity measurements

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ABSTRACT

Due to their nanoscale, morphology, and chemical composition, the tracking and the quantitative analysis of carbon nanotubes (CNTs) in biological samples still represent huge challenges. A new technique for the quantitative and accurate detection of CNTs in various biological samples at different scales (whole organisms to organs) was developed using amphibian larvae exposed to double-walled CNTs (DWCNTs). This technique is based on the dielectric relaxation of ultra-low volume suspensions under a microwave electromagnetic field. CNT concentrations were consequently extracted from complex permittivity measurements at 5 GHz, making possible to quantitatively assess the animal exposure to CNTs. Our results indicate a detection threshold of 0.02 μg of DWCNTs, which is the lowest achieved in the literature to date.

1. Introduction

The ingestion and the excretion of carbon nanotubes (CNTs) by aquatic organisms, such as amphibian larvae, daphnia, copepods, and fish exposed to CNTs have been widely reported. These phenomena could be observed simply by the naked eye or under a light microscope [1–7]. In previous works [1,8,9], the ingestion and excretion by the amphibian *Xenopus laevis* larvae of double-walled CNTs (DWCNTs) and multi-walled CNTs (MWCNTs) were observed during their exposure (semi-static conditions) to a large range of CNT concentrations (0.1–50 mg/L). According to the darkening intensity of the intestine, the CNT amount in this organ seemed to rise with the CNT concentration in the exposure medium. Nevertheless, we could not assume, for example, that the CNT amount ingested by larvae exposed to 10 mg/L was less than after exposure to 50 mg/L based only on visual inspection. Both individual and agglomerated CNTs were evidenced in the gut lumen of exposed aquatic organisms, such as *Xenopus* larvae, by Transmission Electron Microscopy (TEM), which is commonly used to characterize CNT powders or CNT composites [1,10]. Other techniques such as Raman

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spectroscopy [8] and confocal microscopy [6,11] were also used. However, ecotoxicological studies using these techniques are limited to qualitative observations. Finally, isotopic labeling (radioactivity measurements for ^{14}C [4,12,13], or isotopic ratio by GC–MS for ^{13}C [14,15]) can be used and have the advantage of being quantitative, but are rather expensive because the amount of ^{13}C or ^{14}C to be included in the samples during their synthesis should be as high as possible to increase the chances of detection of the CNTs when they are finally diluted in a matrix (soil, water, organism). Moreover, ^{14}C radiolabeling is restricted to accredited laboratories authorized to handle this radioactive isotope. Among other quantitative techniques that are currently in development, near infrared fluorescence and photoluminescence [16,17] are unfortunately only suitable for unbundled semiconducting SWCNTs (tracking and semi-quantitative analysis), and thermogravimetry [18], even if potentially interesting, is often extremely difficult in very complex matrices such as biological samples. It is thus necessary to develop a method more accessible than the latter in order to quantify the presence of CNTs in environmental samples such as for example aquatic organisms and potential accumulating organs.

To reach this target, we took advantage of the intrinsic high conductivity of CNTs at microwaves frequencies. They feature notably high shielding properties against electromagnetic (EM) interference (dissipation of the incident EM radiation as heat) [19], which make CNTs good candidates for the development of composite materials suitable for industrial EM applications (for example microwave absorption devices). They are generally incorporated in polymers, such as epoxy resins, [20–24], to design materials with improved electronic, thermal and mechanical properties compared with their components. The electrical properties of CNTs are often studied through their complex permittivity determined from transmission and/or reflection measurements in microwave range [20,24]. Although the values obtained vary widely according to the nature of the polymer and the analysis device [25], dielectric relaxation phenomena were observed when CNTs were submitted to an EM field in microwave frequencies [20,26].

In addition, research activities have consequently emerged from the convergence of high-frequency (HF) microsystems and microfluidics to develop new analytical and biological, medical and environmental diagnostic systems. HF biosensor which exploits the near-field interaction between EM waves and biological fluids, such as suspensions of cells in their culture medium has already been demonstrated [27]. A microfluidic channel designed to load biological fluids was integrated perpendicularly to a coplanar waveguide (coplanar line CPW) which propagates the EM field. When the EM waves that propagate through the channel interact with the fluid, a modulation of the EM signal (amplitude and phase) is recorded according to the dielectric characteristics of the fluid. Using this device, Grenier et al. [27] showed that the addition of (biological) cells in suspension in the culture medium created a decrease of the relative permittivity correlated with the increase of the cell density. Moreover, they observed a significant difference in the values of relative permittivity (both the real part and the imaginary part) between living and dead cells [28]. Finally, by working on cancer cell suspensions, Chen et al. [29] reported that for low cells concentration the sensor response is proportional to the number of cells contained in the sensing area. The analysis technique has therefore many advantages. It allows non-invasive analysis of a very small volume of biological materials/liquids (microliters [28] or even nanoliters [29]), the detection and quantification of cells in suspensions, and their distinction according to their status (alive/dead or non-cancerous/cancerous).

We employed the developed technique and devices to examine the relationship between the CNT concentration in amphibian *X. laevis* larvae and the dielectric signature of the sample in the HF range in order to develop a quantification methodology suited to ecotoxicological studies made in laboratory conditions. The biological matrix consisted in samples of either entire larvae exposed to DWCNTs or only their intestines, where CNTs were mainly observed under classical binocular inspection of the larvae and appeared more concentrated. As the biological samples were analyzed in suspensions, the design of a dispersion and measurement protocol was required. The detection limit and the measurement accuracy were also estimated. The DWCNT concentrations in samples of larvae exposed to DWCNTs (whole larvae and intestines) measured with the HF device were compared to those obtained by the quantification of the catalytic by-products of the DWCNT synthesis (Co and Mo) by classical chemical analysis. Finally, the comparison between the content of DWCNTs in whole larvae and in the intestine only of larvae exposed to the same DWCNT concentration, but also of whole larvae exposed to different DWCNTs concentrations (10 or 50 mg/L) provides some interesting quantitative data in terms of accumulation in the body, and especially saturation in the intestine above some critical concentration.

2. Experimental

2.1. Material

DWCNTs were produced by the catalytic chemical vapor deposition (CCVD) of methane on a $Mg_{0.99}(Co_{3/4}Mo_{1/4})_{0.01}0$ solid solution, as described earlier [30]. CNT batch was composed of 80% of DWCNTs, 15% of SWCNTs and 5% of MWCNTs, with external diameter and length ranging respectively from 1 to 3 nm and from 1 to 100 μm or more (bundles) [31]. DWCNTs had a purity greater than 92% (carbon content of dry DWCNTs measured by flash combustion; heating up to 1000 °C during about 1 s, after preheating at 925 °C; measurement accuracy $\approx \pm 2\%$). They contained only $3.00 \pm 0.15\%$m Co and $0.90 \pm 0.04\%$m Mo. For the elemental analysis of metals [40], a few milligrams of sample were weighed in a platinum crucible and placed in a quartz tube specially designed for our open system which is called a matra (proprietary design, French CNRS Central Service of Analysis). A 2 mL mixture of 1:1 HNO_3/H_2SO_4 was added. The matra was heated at 250 °C for 12 h. Sonication (bath, Bransonic 1510, VWR) during a few minutes was then necessary to unstuck black residual deposit. The matra was then introduced for a few seconds in an electric bunsen (VWR) pre-heated at 600 °C to enhance dissolution of residual particle and was finally heated again at 250 °C for 12 h. Metals quantification was performed by ICP AES, ICAP 6300 model

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