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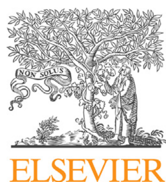

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Applying accelerator mass spectrometry for low-level detection of complex engineered nanoparticles in biological media

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ABSTRACT

Complex engineered nanoparticles (CENPs), which have different core and surface components, are being developed for medicinal, pharmaceutical and industrial applications. One of the key challenges for environmental health and safety assessments of CENPs is to identify and quantity their transformations in biological environments. This study reports the effects of in vivo exposure of citrate-coated nanoalumina with different rare isotope labels on each component. This CENP was dosed to the rat and accelerator mass spectrometry (AMS) was used to quantify ^{26}Al, ^{14}C, and their ratio in the dosing material and tissue samples. For CENPs detected in the liver, the rare isotope ratio, $^{14}C/^{26}Al$, was 87% of the dosing material's ratio. The citrate coating on the nanoalumina in the liver was stable or, if it degraded, its metabolites were incorporated with nearby tissues. However, in brain and bone where little alumina was detected, the rare isotope ratio greatly exceeded that of the dosing material. Therefore, in the animal, citrate dissociated from CENPs and redistributed to brain and bone. Tracking both the core and surface components by AMS presents a new approach for characterizing transformations of CENPs components in biological milieu or environments.

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1. Introduction

Complex engineered nanoparticles are being developed for a variety of applications such as biomacromolecule receptors [1], biosensors [2], imaging indicators [3] and drug carriers [4]. The core nanoparticles include the metal oxides, such as alumina (Al_2O_3) [5]; ceria (CeO_2) [6,7]; titania (TiO_2) [8]; zirconia (ZrO_2) [9]; and carbon-based nanomaterials, such as non-functionalized graphene [10,11], single, and multi-walled carbon nanotubes [10,12]. These core materials have extremely low aqueous solubility and therefore persist in biological media with potential to cause delayed toxicity [13–16]. In previous studies, we have found that, in the rat, a single intravenous administration of 30 nm ceria engineered nanoparticles distributed to specific organs within 24 h; the ceria levels in these organs did not significantly decrease up to 90 days [17]. The coating materials, such as organic acids, silane coupling agents, proteins, or polymers, can control the dispersion and agglomeration of nanoparticles in fluids; they can also interact with solids and solutes in organisms and in the environment [18,19]. Citric acid, a tridentate carboxylic acid, has been widely applied on stabilizing metal oxide nanoparticles [20,21]. The fate of the citrate coating on these nanoparticles was not known. Therefore, the fate and toxicology of CENPs in biological media depends not only on the physico-chemical attributes of the core nanoparticle (size, size distribution, shape), but also their surface-bound molecular coatings. Material balances need to be performed on both the core and coatings materials in order to properly interpret their transport and transformations over the product life cycle.

The common characterization methods for bio-distribution and bio-persistence of CENPs are high-resolution transmission electron microscopy (HR-TEM) [22] and inductively coupled plasma mass spectrometry (ICP-MS) [23]. The former can give good morphology information but may not provide sufficient chemical analysis. The latter provides good inorganic chemistry information but may not provide sufficient analysis of organic components. Some methods based on radioactive isotopes have been proposed. Perez-Campana et al. [24] utilized ^{13}N-labeled nanoalumina formed by proton beam activation to show bio-distribution in different organs. It verified that nanoalumina accumulated in the liver. As the half-life of ^{13}N is 9.97 min, it is useful for short time periods only. Rojas et al. [25] used the ^{18}F isotope to label the amino coating on ceria nanoparticles and showed that ceria accumulated mainly in lungs, spleen, and liver.

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However, labeling only one component of CENPs is not enough; the components might differentially dissociate, degrade, or transport in biological media. Therefore, we used one isotope tracer for the core material and another for the coating material.

Accelerator mass spectrometry (AMS), the most sensitive form of isotope ratio mass spectrometry, was used to characterize the two tracers. The AMS ion source produced negatively charged cesium ions to sputter the surface atoms of samples. A beam of negative ions, some of which were the radioactive tracer, were produced, and then accelerated to very high speed in a tandem accelerator. At the positive terminal of the tandem accelerator, the negative ions will undergo recharging to positive via a gas or carbon foil electron stripper. Almost all molecular ions were dissociated in the procedure, since a beam with a positive charge of 4 or greater is typically selected (i.e. at least five electrons are removed) and molecular ions dissociate. The ions of the rare isotope were easily selected using electric and magnetic fields and counted using nuclear detection techniques. One of the abundant stable isotopes is measured on the high energy side of the accelerator (after destruction of interfering molecular isobars) in a faraday cup and this provides the second part of the ratio (oftentimes denoted as rare/stable) [26,27]. Therefore, this analysis tool can separate rare isotopes with high selectivity and sensitivity, detecting such species at levels 10^3 to 10^9 times lower than other methods [28,29]. It has been applied in pharmaceutical and toxicological studies to investigate metabolism of drugs [30], covalent bonding of metabolite to RNA/protein [31] and imaging of radioactive label [32,33].

Utilizing AMS techniques to find and quantitate the low levels of CENPs components in biological systems where transporting or transformations might take place was proposed. Moreover, it should be possible to identify changes in the molar ratio (coating/core) after biological exposures of CENPs. Dual tracer technology should provide an understanding of the biodistribution and transformation of CENPs in various milieus. In this study, nanoalumina was used as the core material. It has very low solubility in aqueous systems and is relatively common in the environment; it has potential to enter biological tissue and persist there. The nanoalumina was synthesized using a hydrothermal system. ^{26}Al was introduced in the synthesis as the core material tracer. Hydroxyl groups on the nanoalumina surface can react with ^{14}C-labeled citric acid, used as a coating material. The citric acid was either covalently bound to the nanoparticle or self-crosslinked on the surface. The CENP, ^{26}Al-labeled nanoalumina core with ^{14}C-labeled coating, was infused into rats. The dosing material and selected tissues were analyzed by AMS in Purdue Rare Isotope Measurement Laboratory (PRIME lab) to quantify ^{26}Al and ^{14}C.

2. Experiments

2.1. Synthesis of neat nanoalumina

The synthesis route was modified from Chuah's work [34]. 0.001 mol of anhydrous aluminum chloride ($AlCl_3$, Acros) was dissolved in 10 mL 1 M HCl solution to form 0.1 mol/L $AlCl_3$ solution. Anhydrous $AlCl_3$ has a very high tendency to hydrolyze so it was dissolved in an acidic solution at a pH of about 2.5 to avoid precipitation. 1 mL ^{26}Al-HCl solution (16.5 nCi/mL, provided by the PRIME Lab) was diluted 10-fold. 600 μL of the diluted solution (1 nCi ^{26}Al) was added to the $AlCl_3$ solution. 0.5 M NaOH solution was added dropwise into the aluminum chloride solution with stirring until the pH was 9.5. AlO(OH) and $Al(OH)_3$ are formed by the pathway:

$$AlCl_3 + 3NaOH \rightarrow AlO(OH) + 3NaCl + H_2O \quad (1a)$$

$$AlCl_3 + 3NaOH \rightarrow Al(OH)_3 + 3NaCl \quad (1b)$$

The obtained white opaque mixtures were transferred to PTFE containers. The containers were inserted into a metal container (Parr Instrument Company, Models 4746). They were put in a furnace at 190 °C for 24 h, and then cooled to room temperature. The products were washed with distilled water three times and ultracentrifuged to remove the remaining Al^{3+} ion. The solid samples were dried at 90 °C for 2 h to remove the adsorbed water and then heated to 600 °C for 1/2 h. The AlO(OH) and $Al(OH)_3$ nanoparticles decomposed to form γ-aluminananoparticles via calcination [35]:

$$2\,AlO(OH) \rightarrow Al_2O_3 + H_2O \quad (2a)$$

$$2\,Al(OH)_3 \rightarrow Al_2O_3 + 3\,H_2O \quad (2b)$$

The final samples were named "neat nanoalumina". The weight was 40 mg, 80% of the expected product mass. The expected radioactivity was ^{26}Al 0.02 nCi/mg.

2.2. Formation of citrate-coated nanoalumina

For coating nanoalumina with citric acid, 400 mg citric acid (MW = 192, citric acid:alumina = 10:1 w/w) was dissolved in 4 mL water. 50 μL citric acid with ^{14}C (0.05 mCi/mL, Amersham Bioscience UK limited, CFA263) was diluted into 5 mL, to 0.5 μCi/mL. 500 μL of this diluted solution (250 nCi ^{14}C) was added to the citric acid solution. Thermo-gravimetrical analysis showed that the adsorbed citric acid was 0.32% of that added. The adsorbed ^{14}C citric acid should have 0.8 nCi if the adsorbed/total ratio did not change. 40 mg of neat nanoalumina was added to the citric acid solution, then the mixture was stirred for 24 h. The sample was washed by distilled water, ultracentrifuged and recovered three times to remove the free citric acid, and then was dried at 90 °C for 2 h. The dried sample was named "citrate-coated nanoalumina" with an expected radioactivity of 0.02 nCi/mg.

2.3. Characterization of nanoalumina

The shape and morphology of neat nanoalumina were observed by scanning electron microscopy (SEM, Hitachi 4300, University of Kentucky). Quantitation of hydroxyl and citrate groups on the surfaces of neat and citrated-coated nanoalumina was done via thermo gravimetric analysis (TGA) (Perkin Elmer, TGA-7 Thermo gravimetric Analyzer). In a nitrogen environment, the neat and citrate-coated nanoalumina were heated from room temperature to 110 °C, kept at 110 °C for 1/2 h to remove physically-adsorbed water, then heated to 750 °C at a rate of 10 °C/min. Within the higher temperature range, hydroxyl groups at the metal oxide surface will dehydrate to form water and the citrate coating will decompose to form carbon dioxide and ethylene [36]. To analyze the ability of citrate coating to create astable dispersion, some neat and citrate-coated nanoaluminas were dispersed in water with ultrasonication. The particle distribution in the dispersion was measured by dynamic light scattering (90 Plus, particle size analyzer, Brookhaven Instrument Corporation).

2.4. Animal infusions

20 mg of the citrate-coated nanoalumina was put into 1 mL water then ultrasonicated; the dispersion was expected to have 0.4 nCi ^{26}Al and 0.4 nCi ^{14}C/mL. One rat was intravenously infused, via a cannula inserted into a femoral vein that terminated in the vena cava, with 0.4 mL of this dispersion (anticipated dose 0.16 nCi ^{26}Al and 0.16 nCi ^{14}C). The dosed animal was terminated 30 days later and tissues, including liver, brain, and bone, were collected. The similar tissues from one un-dosed rat were collected as the control samples.

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