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Digital Object Identifier (DOI)

<https://doi.org/10.1016/j.jpba.2014.04.003>

Notes/Citation Information

Published in *Journal of Pharmaceutical and Biomedical Analysis*, v. 97.

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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 quantify 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 ²⁶Al, ¹⁴C, and their ratio in the dosing material and tissue samples. For CENPs detected in the liver, the rare isotope ratio, ¹⁴C/²⁶Al, 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 CENP components in biological milieu or environments.

23 **Highlights**

- 24 • The core and coating components of engineered nanoparticles were labeled with rare isotopes.
- 25 • These complex nanoparticles were injected into the rat.
- 26 • Dose and tissue samples were analyzed for both rare isotopes.
- 27 • The rare isotope ratio ($^{14}\text{C}/^{26}\text{Al}$) demonstrated the relative stability of the two CENP components

28

29 **Keywords**

30 complex engineered nanoparticles, nanoalumina, accelerator mass spectrometry, biodistribution

31

32 **1. Introduction**

33 Complex engineered nanoparticles are being developed for a variety of applications such as
34 biomacromolecule receptors [1], biosensors [2], imaging indicators [3] and drug carriers [4]. The core
35 nanoparticles include the metal oxides, such as alumina (Al_2O_3) [5]; ceria (CeO_2) [6, 7]; titania (TiO_2) [8];
36 zirconia (ZrO_2) [9]; and carbon-based nanomaterials, such as non-functionalized graphene [10, 11], single,
37 and multi-walled carbon nanotubes [10, 12]. These core materials have extremely low aqueous solubility
38 and therefore persist in biological media with potential to cause delayed toxicity [13-16]. In previous
39 studies, we have found that, in the rat, a single intravenous administration of 30 nm ceria engineered
40 nanoparticles distributed to specific organs within 24 hours; the ceria levels in these organs did not
41 significantly decrease up to 90 days [17]. The coating materials, such as organic acids, silane coupling
42 agents, proteins, or polymers, can control the dispersion and agglomeration of nanoparticles in fluids; they
43 can also interact with solids and solutes in organisms and in the environment [11, 18, 19]. Citric acid, a
44 tridentate carboxylic acid, has been widely applied on stabilizing metal oxide nanoparticles [20, 21]. The

45 fate of the citrate coating on these nanoparticles was not known. Therefore, the fate and toxicology of
46 CENPs in biological media depends not only on the physico-chemical attributes of the core nanoparticle
47 (size, size distribution, shape), but also their surface-bound molecular coatings. Material balances need to
48 be performed on both the core and coatings materials in order to properly interpret their transport and
49 transformations over the product life cycle.

50 The common characterization methods for bio-distribution and bio-persistence of CENPs are high-
51 resolution transmission electron microscopy (HR-TEM) [22] and inductively coupled plasma mass
52 spectrometry (ICP-MS) [23, 24]. The former can give good morphology information but may not provide
53 sufficient chemical analysis. The latter provides good inorganic chemistry information but may not
54 provide sufficient analysis of organic components. Some methods based on radioactive isotopes have been
55 proposed. Perez-Campana et al. [25] utilized ^{13}N -labeled nanoalumina formed by proton beam activation
56 to show bio-distribution in different organs. It verified that nanoalumina accumulated in the liver. As the
57 half-life of ^{13}N is 9.97 min, it is useful for short time periods only. Rojas et al. [26] used the ^{18}F isotope to
58 label the amino coating on ceria nanoparticles and showed that ceria accumulated mainly in lungs, spleen,
59 and liver. However, labeling only one component of CENPs is not enough; the components might
60 differentially dissociate, degrade, or transport in biological media.

61 Therefore, we used one isotope tracer for the core material and another for the coating material.
62 Accelerator mass spectrometry (AMS), the most sensitive form of isotope ratio mass spectrometry, was
63 used to characterize the two tracers. The AMS ion source produced negatively charged cesium ions to
64 sputter the surface atoms of samples. A beam of negative ions, some of which were the radioactive tracer,
65 were produced, and then accelerated to very high speed in a tandem accelerator. At the positive terminal
66 of the tandem accelerator, the negative ions will undergo recharging to positive via a gas or carbon foil
67 electron stripper,. Almost all molecular ions were dissociated in the procedure, since a beam with a
68 positive charge of 4 or greater is typically selected (i.e. at least 5 electrons are removed) and molecular

69 ions dissociate. The ions of the rare isotope were easily selected using electric and magnetic fields and
70 counted using nuclear detection techniques. One of the abundant stable isotopes is measured on the high
71 energy side of the accelerator (after destruction of interfering molecular isobars) in a faraday cup and this
72 provides the second part of the ratio (oftentimes denoted as rare/stable) [27, 28]. Therefore, this analysis
73 tool can separate rare isotopes with high selectivity and sensitivity, detecting such species at levels 10^3 to
74 10^9 times lower than other methods [29, 30]. It has been applied in pharmaceutical and toxicological
75 studies to investigate metabolism of drugs [31], covalent bonding of metabolite to RNA/ protein [32] and
76 imaging of radioactive label [33, 34].

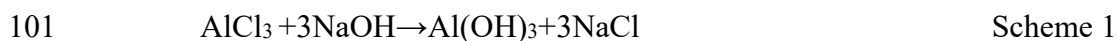
77 [This is not a complete sentence]Using AMS techniques to find and quantity low levels of complex
78 nanoparticle components in biological systems where transporting or transformations might take place.
79 Moreover, it should be possible to identify changes in the molar ratio (coating:core) after biological
80 exposures of CENPs. Dual tracer technology should provide an understanding of the biodistribution and
81 transformation of CENPs in various milieus. In this study, nanoalumina was used as the core material. It
82 has very low solubility in aqueous systems and is relatively common in the environment; it has potential
83 to enter biological tissue and persist there. The nanoalumina was synthesized using a hydrothermal
84 system. ^{26}Al was introduced in the synthesis as the core material tracer. Hydroxyl groups on the
85 nanoalumina surface can react with ^{14}C -labeled citric acid, used as a coating material. The citric acid was
86 either covalently bound to the nanoparticle or self-crosslinked on the surface. The CENP, ^{26}Al -labeled
87 nanoalumina core with ^{14}C -labeled coating, was infused into rats. The dosing material and selected tissues
88 were analyzed by AMS in Purdue Rare Isotope Measurement Laboratory (PRIME lab) to quantify ^{26}Al
89 and ^{14}C .

90

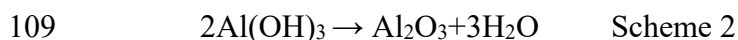
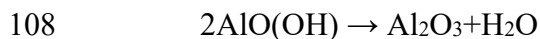
91 2. Experiments

92 2.1 Synthesis of neat nanoalumina

93 The synthesis route was modified from Chuah's work [35]. 0.001 mol of anhydrous aluminum chloride
 94 (AlCl_3 , Acros) was dissolved in 10 mL 1 M HCl solution to form 0.1 mol/L AlCl_3 solution. Anhydrous
 95 AlCl_3 has a very high tendency to hydrolyze so it was dissolved in an acidic solution at a pH of about 2.5
 96 to avoid precipitation. 1 mL ^{26}Al -HCl solution (16.5 nCi/mL, provided by the PRIME Lab) was diluted
 97 10-fold. 600 μL of the diluted solution (1 nCi ^{26}Al) was added to the AlCl_3 solution. 0.5 M NaOH solution
 98 was added dropwise into the aluminum chloride solution with stirring until the pH was 9.5. $\text{AlO}(\text{OH})$ and
 99 $\text{Al}(\text{OH})_3$ are formed in the process (scheme 1).



102 The obtained white opaque mixtures were transferred to PTFE containers. The containers were inserted
 103 into a metal container (Parr Instrument Company, Models 4746). They were put in a furnace at 190 °C for
 104 24 h, and then cooled to room temperature. The products were washed with distilled water three times and
 105 ultracentrifuged to remove the remaining Al^{3+} ion. The solid samples were dried at 90°C for 2 h to remove
 106 the adsorbed water and then heated to 600°C for ½ h. The $\text{AlO}(\text{OH})$ and $\text{Al}(\text{OH})_3$ nanoparticles
 107 decomposed to form γ -alumina nanoparticles via calcination (scheme 2) [36].



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

112 **2.2 Formation of citrate-coated nanoalumina**

113 For coating nanoalumina with citric acid, 400 mg citric acid (MW=192, citric acid: alumina=10:1 w/w)
114 was dissolved in 4 mL water. 50 μ L citric acid with ^{14}C (0.05 mCi/mL, Amersham Bioscience UK limited,
115 CFA263) was diluted into 5 mL, to 0.5 μ Ci/mL. 500 μ L of this diluted solution (250 nCi ^{14}C) was added
116 to the citric acid solution. Thermo-gravimetric analysis showed that the adsorbed citric acid was 0.32%
117 of that added. The adsorbed ^{14}C citric acid should have 0.8 nCi if the adsorbed/total ratio didn't change.
118 40 mg of neat nanoalumina was added to the citric acid solution, then the mixture was stirred for 24 h. The
119 sample was washed by distilled water, ultracentrifuged and recovered three times to remove the free citric
120 acid, and then was dried at 90 $^{\circ}\text{C}$ for 2 h. The dried sample was named "citrate-coated nanoalumina" with
121 an expected radioactivity of 0.02 nCi/mg.

122 **2.3 Characterization of nanoalumina**

123 The shape and morphology of neat nanoalumina were observed by scanning electron microscopy (SEM,
124 Hitachi 4300, University of Kentucky). Quantitation of hydroxyl and citrate groups on the surfaces of neat
125 and citrated-coated nanoalumina was done via thermo gravimetric analysis (TGA) (Perkin Elmer, TGA-7
126 Thermo gravimetric Analyzer). In a nitrogen environment, the neat and citrate-coated nanoalumina were
127 heated from room temperature to 110 $^{\circ}\text{C}$, kept at 110 $^{\circ}\text{C}$ for $\frac{1}{2}$ h to remove physically-adsorbed water,
128 then heated to 750 $^{\circ}\text{C}$ at a rate of 10 $^{\circ}\text{C}/\text{min}$. Within the higher temperature range, hydroxyl groups at the
129 metal oxide surface will dehydrate to form water and the citrate coating will decompose to form carbon
130 dioxide and ethylene [37]. To analyze the ability of citrate coating to create a stable dispersion, some neat
131 and citrate-coated nanoaluminas were dispersed in water with ultrasonication. The particle distribution in
132 the dispersion was measured by dynamic light scattering (90 Plus, particle size analyzer, Brookhaven
133 Instrument Corporation).

134 **2.4 Animal infusions**

135 20 mg of the citrate-coated nanoalumina was put into 1 ml water then ultrasonicated; the dispersion was
136 expected to have 0.4 nCi ^{26}Al and 0.4 nCi ^{14}C /ml. One rat was intravenously infused, via a cannula
137 inserted into a femoral vein that terminated in the vena cava, with 0.4 ml of this dispersion (anticipated
138 dose 0.16 nCi ^{26}Al and 0.16 nCi ^{14}C). The dosed animal was terminated 30 days later and tissues,
139 including liver, brain, and bone, were collected. The similar tissues from one un-dosed rat were collected
140 as the control samples.

141 **2.5 Sample preparation and AMS quantification of ^{26}Al**

142 The pathways to analyze ^{26}Al and ^{14}C are described in the following paragraphs and summarized in
143 Scheme-3 and Scheme-4.

144 To quantify ^{26}Al in the dosing material, it was diluted 100-fold to form a 0.2 mg/mL dispersion. 400 μL of
145 the commercial analytical standard Al in HCl solution (Aluminum Standard for ICP, 10,000 mg/L in 5%
146 nitric acid, 41377 Fluka) was added to two 10 μL aliquots of the diluted dosing material (Al_2O_3 -1 and
147 Al_2O_3 -2). This enabled determination of the $^{26}\text{Al}/^{27}\text{Al}$ ratio by introducing a known amount of ^{27}Al (4 mg)
148 that greatly exceeded the ^{27}Al in the sample. This was done to provide enough material for the AMS
149 measurement. The two samples were then dried and ashed at 1000 $^\circ\text{C}$.

150 To quantify ^{26}Al in tissue samples, each sample (from the dosed and control animals) was transferred to a
151 scrupulously cleaned, pre-weighed, 7-ml Teflon screw-cap container and re-weighed to obtain sample
152 weight. Four mg of ^{27}Al from the standard HCl solution was added to each sample. The mixture was dried
153 at 110 $^\circ\text{C}$. 3 ml 2:1 v/v mixture of HNO_3 and H_2O_2 was added to digest the samples. After evaporating the
154 liquid using a heatable, semi-closed system [38], the samples (except brain samples) were ashed at
155 1000 $^\circ\text{C}$. For brain samples, direct ashing will result in some glasslike material believed to be aluminum
156 oxyphosphate. A procedure that separates Al from phosphate was used [39]. A diluted mixture of HNO_3
157 and H_2O_2 was used to solubilize the residue after evaporating the liquid. Two grams of cation exchange

158 resin containing a sulfonic acid functional group (AG 50-X8, 100-200 mesh; Bio-Rad) was used to
159 complex the Al^{3+} . After washing three times with 5 mL 0.2% HNO_3 , 5 mL of 1M hydrofluoric acid (HF)
160 was used to elute Al from the resin. The solution was dried by evaporation then ashed at 1000 °C. The
161 dosing material and tissue samples were sent to the PRIME lab for ^{26}Al quantification by AMS. Upon
162 receipt at the PRIME Lab, the samples were mixed with Ag in an approximately 2:1, Ag: Al_2O_3 weight
163 ratio. After mixing, the sample was inserted into a cavity in a sample holder (cathode) that was 0.040” in
164 diameter and 0.080” deep. The silver greatly increases the current out of the cesium sputter source and
165 increases efficiency of the measurement. The cathode was inserted into the PRIME Lab ion source. A
166 typical sample will produce a current of 500 nA in the source which translates to a count rate of
167 approximately 25 counts per minute for a sample with a $^{26}\text{Al}/^{27}\text{Al}$ ratio of 10^{-12} . Standards of known value
168 were measured before and after the assay of the unknowns and were used to normalize the ratios. Samples
169 were typically measured until they were used up or a precision of 3% was achieved.

170

171 **2.6 Sample preparation and AMS quantification of ^{14}C**

172 To quantify ^{14}C in the dosing material, it was diluted 10-fold to form a 2 mg/mL dispersion. Three 10 μL
173 aliquots of the diluted dispersion were collected (CA-1, CA-2 and CA-3). The CA 1-3 samples were sent
174 to the PRIME lab. For the dose material dilutions, tributyrin was added directly to the sample. Tributyrin
175 has no vapor pressure to speak of and is carbon rich. The $^{14}\text{C}:^{12}\text{C}$ ratio of the tributyrin is almost exactly 5
176 $\times 10^{-14}$. The mixture was placed in a small quartz tube that was nestled in a Pyrex tube with a glass
177 microfiber filter in the top. The Pyrex tube was then placed in a centrifuge tube with another glass
178 microfiber filter in the top. The lid of the centrifuge tube had a few small holes drilled for water vapor
179 removal. This apparatus was then placed in a centrifuge and spun under vacuum for 24 h to remove the
180 water. The sample was then placed in a combustion tube with appropriate reactants, pumped to less than

181 15 microns as recorded by a gauge on the vacuum line. The tube was sealed with a torch, and placed in an
182 oven to be combusted. The CO₂ was then transferred to another tube and graphitized using the method
183 developed by Ognibene et.al [40].

184 The tributyrin method was good for the dosing material, since it had a very low content of carbon
185 material. There was not enough material for an accurate AMS measurement without addition of a carrier.
186 However, the tissue samples provide sufficient carbon content to permit direct measurements of the
187 ¹⁴C/¹²C ratio without addition of a carrier. The tissue sample was located in a combustion tube with
188 appropriate reactants, the tube sealed with a torch, and the contents combusted, as above. The CO₂ was
189 then transferred to another tube and graphitized as above. After graphitization, the sample was transferred
190 into an aluminum sample holder (cathode) and pressed into a 0.040” diameter hole that was 0.040” deep.
191 The sample holder was then placed into the PRIME Lab ion source (reference below) which typically
192 generates ¹³C-currents of 500-750 nA with the corresponding ¹⁴C⁴⁺ ion detection rates of about 200 Hz for
193 a sample ¹⁴C-enrichment of about 2 x 10⁻¹² of total carbon. The ¹⁴C/¹²C ratios were analyzed with no δ¹³C
194 correction. Standards of known ratio are constantly measured to normalize the values of the unknown.
195 Samples were typically measured until they were used up or a precision of 1% was achieved.

196

197 Ion source reference (can be used for both the aluminum and carbon AMS part): G.S. Jackson, D. Elmore,
198 M. Caffee, K.A. Mueller, B. De Bonte, P. Muzikar, B. Alexander, Nuclear Instruments and Methods in
199 Physics Research Section B: Beam Interactions with Materials and Atoms 223-224 (2004) 155.

200

201 **3. Results and Discussion**

202 **3.1 Particle size analysis**

203 Figure 1 shows the morphology of neat nanoalumina. The top surfaces of most nanoalumina were square.
204 It is not very easy to determine if they were cubic or square disks. The typical particle size was 50 to 80
205 nm. However, some smaller (30 nm) and larger particles (100 nm) existed, which may come from
206 sintering and Ostwald ripening in the hydrothermal and calcination processes. Figure 2 shows the volume-
207 averaged particle size distribution of neat and citrate-coated nanoalumina in their dispersion. The
208 dispersion of neat nanoalumina showed three peaks: one centered at 95 nm ($65 \text{ nm} < D < 180 \text{ nm}$); one
209 over the range, 230 nm to 500 nm; and one over the range, 1 μm to 2 μm . The peak centered at 95 nm was
210 consistent with the size observed in SEM. The larger peaks likely represent agglomerates. The dispersion
211 of citrate-coated nanoalumina showed only two peaks: one over the range of 65 to 105 nm with a peak at
212 83 nm and a second over the range, 230 to 360 nm. No larger agglomerates were observed. The results
213 shows the citrate coating helped stabilize the dispersion of nanoalumina.

214 **3.2 Thermogravimetric analysis of nanoalumina**

215 Figure 3 shows the TGA curves of neat and citrate-coated nanoalumina. The weight of the neat
216 nanoalumina decreased 0.68% due to the loss of surface hydroxyls. Based on a method to estimate surface
217 density of functional groups [24], the sample had a surface density of 17 hydroxyl groups/ nm^2 , assuming
218 an average particle was a 60 nm cube. For the citrate-coated nanoalumina, the weight loss was 3.85%. The
219 3.2% difference was attributed to decomposition of the citrate coating. The estimated surface density of
220 citric acid is 3.3[Doesn't this need some unit?]/ nm^2 . Using a molecular diameter of citric acid of 0.57 nm
221 [41], the coverage of citrate-coating was estimated to be 77% [42].

222 **3.3 ^{26}Al in dosing material**

223 Table 1 shows the ratio of $^{26}\text{Al}/^{27}\text{Al}$ obtained by AMS and the calculated fraction of ^{26}Al in samples. The
224 $^{26}\text{Al}/^{27}\text{Al}$ ratio in dosing material sample Al_2O_3 -1 was 1.41×10^{-9} . The number of ^{26}Al in Al_2O_3 -1 was
225 1.26×10^{11} atoms. The $^{26}\text{Al}/^{27}\text{Al}$ ratio in Al_2O_3 -2 was 1.20×10^{-9} . It contained 1.07×10^{11} ^{26}Al atoms. The

226 average number of ^{26}Al atoms in Al_2O_3 -1 and Al_2O_3 -2 was 1.16×10^{11} . Both of them were 10 μL aliquots
227 of 100-fold diluted dosing material. So the dosing material had an average number concentration of
228 1.16×10^{15} ^{26}Al /ml. The treated rat received 0.4 ml of the dosing material, corresponding to 4.65×10^{14} ^{26}Al
229 atoms or 0.383 nCi. It was 2.4 times the planned dose.

230 **3.4 ^{26}Al in tissue samples**

231 The $^{26}\text{Al}/^{27}\text{Al}$ ratio in liver-1 sample (38.7 mg) was 2.41×10^{-9} . The liver-1 sample contained 2.15×10^{11}
232 ^{26}Al atoms. The liver weight from dosed and control rats was around 16.3 grams so the total liver would
233 contain 9.06×10^{13} ^{26}Al atoms or 7.47×10^{-2} nCi, corresponding to 19.5% of the dose. The $^{26}\text{Al}/^{27}\text{Al}$ ratio in
234 liver-2 (35.9 mg) was 7.61×10^{-13} . The liver-2 sample contained 6.78×10^7 ^{26}Al atoms so the total liver of
235 control rat would contain 3.08×10^{10} ^{26}Al atoms or 2.54×10^{-5} nCi, more than 3 orders of magnitude less
236 than the liver from the dosed rat. The weight of liver was about 3.2% of the rat's weight and contained
237 19.5% of dose, showing accumulation of the nanoalumina in the liver.

238 The $^{26}\text{Al}/^{27}\text{Al}$ ratio in the brain-1 sample was 5.99×10^{-11} . Brain-1 sample (757.1 mg) contained 5.35×10^9
239 ^{26}Al atoms. The average weight of a rat's brain is 1.86 gram so the total brain would contain 1.31
240 $\times 10^{10}$ ^{26}Al atoms or 1.08×10^{-5} nCi, about 0.003% of the dosing material. The $^{26}\text{Al}/^{27}\text{Al}$ ratio in brain-2 was
241 5.01×10^{-11} . Brain-2 sample (738 mg) contained 4.47×10^9 ^{26}Al atoms so the total brain from control rat
242 contained 1.13×10^{10} nCi ^{26}Al atoms or 9.27×10^{-6} nCi.

243 The $^{26}\text{Al}/^{27}\text{Al}$ background for samples without ^{26}Al was around 10^{-14} in the AMS measurements. So the
244 high $^{26}\text{Al}/^{27}\text{Al}$ ratio in the control rat suggests that some low-level contamination happened during
245 infusion or surgery. We can't compare the actual difference between the ^{26}Al in (brains?) from dosed and
246 control rats. However, the 0.003% of dosing material can be considered as the upper limit of ^{26}Al in the
247 brain from dosed rats. It indicates only a very small amount (if any) of the ^{26}Al got incorporated into the
248 brain since the nanoalumina did not cross the blood-brain barrier to enter brain parenchyma.

249 The $^{26}\text{Al}/^{27}\text{Al}$ ratio in bone-1 sample was 4.62×10^{-12} . The bone-1 tissue (52 mg) contained 4.12×10^8 ^{26}Al
250 atoms. The total rat skeletal weight is about of 5% of body weight (25 gram). So the total skeletal system
251 would contain 1.98×10^{11} ^{26}Al atoms or 1.63×10^{-4} nCi, 0.043% of the dose. The bone-2 (50.8 mg)
252 contained 7.52×10^8 ^{26}Al atoms, translating to a total rat skeletal content of 3.05×10^{-4} nCi. The control rat
253 bone had higher radioactivity in the bone than the dosed animal. The unexpected result may be from the
254 same low level contamination that affected the brain. The 0.043% of dosing material was used as the
255 upper limit and indicates only small amount (if any) of the ^{26}Al got incorporated into the brain. In the
256 typical mammal, 60% of the body burden of Al is in the skeletal system and only 3% in the liver [43]. The
257 high concentration of ^{26}Al in liver and low concentration in bone suggests that the ^{26}Al from the
258 nanoalumina is difficult to dissolve and redistribute into bone.

259 **3.5 ^{14}C in dosing material**

260 Table 2 shows the ratio of $^{14}\text{C}/^{12}\text{C}$ obtained by AMS and the calculated sample activity. The $^{14}\text{C}/^{12}\text{C}$ in
261 CA-1, CA-2, and CA-3 samples was 10353, 9342, and 8359, giving an average number of 1.1×10^9 ^{14}C
262 atoms in the three aliquots. They were 10 μL aliquots of 10-fold diluted dosing material so the dosing
263 material had number concentration of 1.1×10^{12} ^{14}C atoms/mL. The rat got 0.4 mL of the dosing material,
264 corresponding to 4.40×10^{11} ^{14}C atoms or 4.56×10^{-2} nCi. It was 28.5% of the planned dose.

265 **3.6 ^{14}C in tissue samples**

266 Because ^{14}C is ubiquitous in air, water and food, it inevitably enters the animal to become stored and form
267 the ^{14}C background. The typical ratio $^{14}\text{C}/^{12}\text{C}$ of mammal's tissue sample via previous AMS
268 measurements is around 1250. It is very close to a value reported in literature [44]. However, the precise
269 ratio in each organ does vary, so we used the ratios in the control rat as our background.

270 After subtracting the average $^{14}\text{C}/^{12}\text{C}$ ratio background (1437.5) from Liver-2 and Liver-3 samples, the
271 actual $^{14}\text{C}/^{12}\text{C}$ ratio in liver-1 sample was 607.5. The typical carbon percent in liver is 15 wt%. There are

272 1.23×10^{23} ^{12}C atoms in total liver (16.3 grams). The calculated ^{14}C in the total liver of the dosed rat was
273 7.45×10^{10} or 7.72×10^{-3} nCi, 16.9% of the dose. The ^{14}C of the coating material concentrated in the liver of
274 the dosed rat, but the differences between the dosed rat and the controls was not as large as those for the
275 core material, ^{26}Al .

276 The $^{14}\text{C}/^{12}\text{C}$ ratio (1261) from Brain-2 of the control rat was used as the background. After subtracting
277 background, the $^{14}\text{C}/^{12}\text{C}$ in brain-1 sample is 22. The difference is larger than one standard deviation of
278 the measured value so there was some higher amount of ^{14}C in brain of the dosed rat than the control rat in
279 a statistically significant sense. The typical carbon percent in brain is 15 wt%. There are 1.40×10^{22} ^{12}C
280 atoms in the total brain (1.86 grams). The total brain would contain 3.08×10^8 ^{14}C atoms or 3.19×10^{-5} nCi,
281 0.07% of the dosage. For the Brain-3 sample, the $^{14}\text{C}/^{12}\text{C}$ ratio is much higher than brain-2 sample, even
282 brain-1. The contamination may have come from a previous sample with high ratio of $^{14}\text{C}/^{12}\text{C}$ that was
283 dried in the vacuum centrifuge. This seemed highly likely since a check of the records at the PRIME Lab
284 showed that the one sample was dried with a batch of samples that had $^{14}\text{C}/^{12}\text{C}$ ratios 100 times higher.
285 Thus, it was considered safe to discard this result.

286 After subtracting the average $^{14}\text{C}/^{12}\text{C}$ ratio background (1412.5) from Bone-2 and Bone-3 samples, the
287 actual ratio of $^{14}\text{C}/^{12}\text{C}$ in bone-1 sample was 76.5. The typical carbon percent in bone was around 12%
288 [45]. There are 1.51×10^{23} ^{12}C atoms in total bone (25 grams). The total bone would contain 1.16×10^{10} ^{14}C
289 atoms or 1.20×10^{-3} nCi, 2.63% of the dosage. The bone from the dosed rat had higher ^{14}C than that from
290 the control rat.

291 **3.7 The ratio of coating/core and material balance**

292 The ratio between $^{14}\text{C}/^{26}\text{Al}$ in different organs and the mass distribution are shown in Table 3. In the
293 dosing material, the average numbers of ^{26}Al and ^{14}C atoms were 4.65×10^{14} and 4.40×10^{11} respectively.

294 The $^{14}\text{C}/^{26}\text{Al}$ was 9.46×10^{-4} . The total recovered ^{26}Al and ^{14}C from liver, brain and bone are 19.5% and
295 19.6% of the dosing material respectively.

296 The numbers of isotope atoms in the dosed liver were: $^{26}\text{Al} = 9.06 \times 10^{13}$ and $^{14}\text{C} = 7.45 \times 10^{10}$. The $^{14}\text{C}/^{26}\text{Al}$
297 in liver of the dosed rat was 8.22×10^{-4} , around 87% of the ratio in the dosing material. The citrated coating
298 entering the liver (19.5% of dosage) partially dissociated from the nanoalumina, and would have been
299 available to redistribute into organs such as the brain and bone.

300 The average numbers of isotope atoms in brain were: $^{26}\text{Al} < 1.31 \times 10^{10}$ and $^{14}\text{C} = 3.08 \times 10^8$, for a $^{14}\text{C}/^{26}\text{Al}$
301 ratio $> 2.35 \times 10^{-2}$. The average numbers of isotope atoms in bone were: $^{26}\text{Al} < 1.98 \times 10^{11}$ and $^{14}\text{C} =$
302 1.16×10^{10} , for a $^{14}\text{C}/^{26}\text{Al}$ calculated ratio $> 5.81 \times 10^{-2}$. Both of these ratios are much higher than that of the
303 dosing material. The reason is that ^{14}C preferentially accumulated in brain (0.07%) and bone (2.63%)
304 compared to the levels of ^{26}Al in these two organs (0.003% and 0.043%, respectively). This finding
305 suggests that some of the citrate coating dissociated from the nanoalumina's surface and then redistributed
306 to organs such as the brain and bone.

307

308 **4. Conclusions**

309 The core and surface coatings of a complex engineered nanoparticle have been tracked during biological
310 exposure to the rat using rare isotope labels detected by AMS. The alumina core was tracked using ^{26}Al
311 and the citrate coating was tracked using ^{14}C . Comparison of the rare isotope levels and their ratios,
312 $^{14}\text{C}:^{26}\text{Al}$, in different organs demonstrated the relative stability of the two CENP components. The amount
313 of ^{26}Al in the liver of the dosed rat was higher than that of control rats. The amounts of ^{26}Al in brain and
314 bone of the dosed rat were similar to those of the control rat. It suggests the nanoalumina accumulated,
315 and persisted in the liver 30 days after infusion. The amounts of ^{14}C in the liver, bone, and brain of the
316 dosed rat were also higher than those of the control rats. However, the $^{14}\text{C}/^{26}\text{Al}$ ratios differed between

317 liver, brain, and bone. Slightly less coating material went to the liver compared to the core material and its
318 levels were significantly higher in brain and bone compared to the core material. Some of the citrate
319 coating dissociated from the nanoparticle surfaces and redistributed to organs such as the brain and bone.
320 AMS methodology provides a new opportunity to characterize the biodistribution of complex engineering
321 nanomaterials.

322

323 **5. Acknowledgement**

324 This work was supported by National Science Foundation to “EAGER: Low-level detection of complex
325 nanomaterials in biological media” [Award Abstract #1249123]. Although the research described in this
326 article has been funded wholly or in part by the National Science Foundation, it has not been subjected to
327 the Agency’s required peer and policy review and therefore does not necessarily reflect the views of the
328 National Science Foundation and no official endorsement should be inferred.

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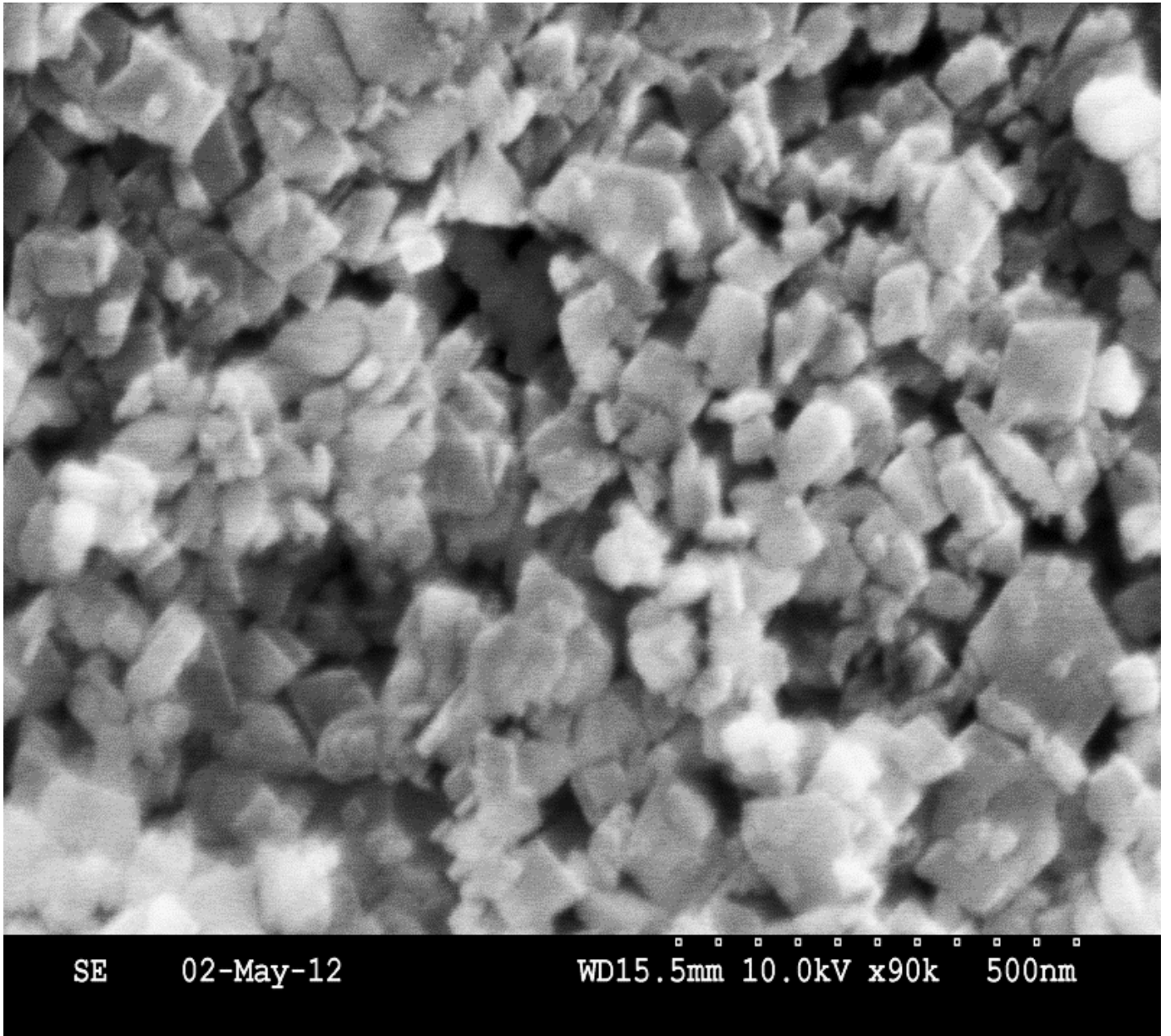
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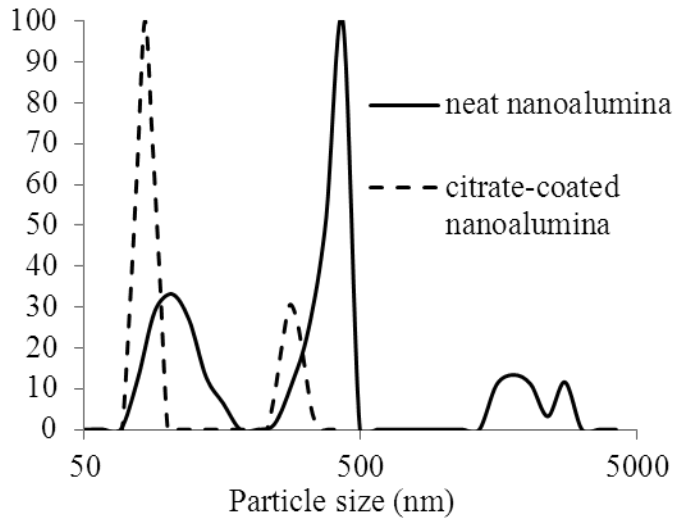
398 Figure 1.



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400 Figure 2.

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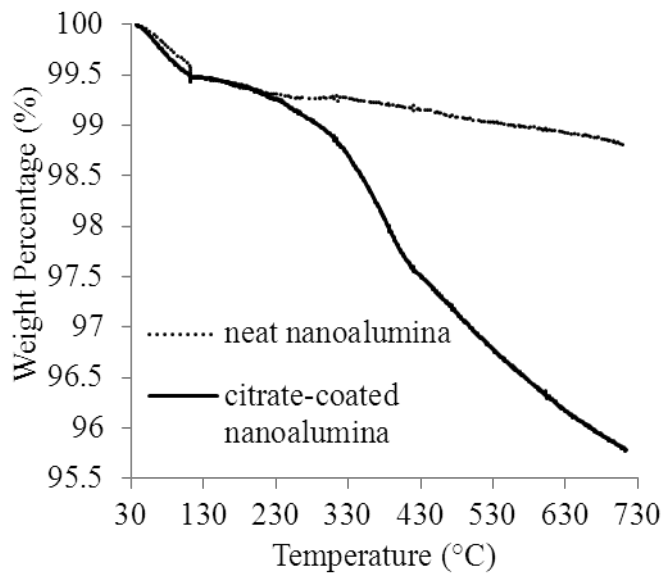


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405 Figure 3.



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