

Synthesis, Characterization, and Neutron Activation of Holmium Metallofullerenes

Dawson W. Cagle,[†] Thomas P. Thrash,[†] Michael Alford,[‡] L. P. Felipe Chibante,[‡] Gary J. Ehrhardt,^{*,§} and Lon J. Wilson^{*,†}

Contribution from the Department of Chemistry and Laboratory for Biochemical and Genetic Engineering, Rice University, P.O. Box 1892 MS-60, Houston, Texas 77251-1892, TDA Research, 12345 West 52nd Avenue, Wheat Ridge, Colorado 80033, NanoTechnologies of Texas, P.O. Box 2441, Bellaire, Texas 77402-2441, and Research Reactor Facility, University of Missouri—Columbia, Columbia, Missouri 65211

Received March 14, 1996[⊗]

Abstract: Isolation of the first macroscopic quantities of endohedral holmium metallofullerenes (principally Ho@C₈₂, Ho₂@C₈₂, and Ho₃@C₈₂ by LD-TOF mass spectrometry) has been accomplished by carbon-arc and preparative HPLC methodologies. The detailed procedure for production and isolation of the metallofullerenes includes a new technique whereby holmium-impregnated electrodes are prepared simply by soaking porous graphite rods in an ethanolic solution of Ho(NO₃)₃·xH₂O. Monoisotopic ¹⁶⁵Ho offers a unique combination of advantages for neutron-activation studies of metallofullerenes, and purified samples containing ¹⁶⁵Ho@C₈₂, ¹⁶⁵Ho₂@C₈₂, and ¹⁶⁵Ho₃@C₈₂ have been activated by high-flux neutron irradiation ($\Phi = 4 \times 10^{13} \text{ n cm}^{-2} \text{ s}^{-1}$) to generate ¹⁶⁶Ho metallofullerenes, which undergo β^- decay to produce stable ¹⁶⁶Er. Chemical workup of the irradiated samples, followed by re-irradiation, has been used to demonstrate that observed decomposition of holmium metallofullerenes is due mainly to “fast” neutron damage rather than to holmium atom nuclear recoil ($E_{\text{max}} = 200 \text{ eV}$). This implies that metallofullerene damage can be minimized by using neutron fluxes with the highest possible thermal component. A detailed analysis of the neutron-activation results is presented as a prelude to the possible use of neutron-activated metallofullerenes in nuclear medicine.

Introduction

Since the discovery of fullerenes in 1985¹ and the subsequent incorporation of a metal atom within the carbon cage,² intense interest has focused on these molecules. Improved synthetic methods developed in 1990 by Krätschmer and Huffman led to the availability of macroscopic amounts of fullerenes.³ With production of macroscopic quantities, a vast array of physical and chemical information has been gathered about C₆₀ and, to a lesser extent, the higher fullerenes.^{4–6}

Parallel chemistry on endohedral metallofullerenes has been slower to materialize due to their lower yields, lower solubilities, and greater air-sensitivity in some cases.^{7–11} Nevertheless, these

molecules have been intensely pursued because of their novel structures and potential applications in diverse fields ranging from nuclear medicine to optical materials.¹² Using a modification of the Krätschmer–Huffman method, sufficient quantities of La metallofullerenes were produced in 1991 for the first characterization in bulk, and La@C₈₂ was found to be a soluble, air-stable species.⁷ A wide variety of endohedral metallofullerenes, especially those of the lanthanides, have been detected by mass spectrometry^{12,13} and EPR spectroscopy,^{8,12,13d,14} but purified samples did not become available until milligram quantities of La@C₈₂ were isolated in 1993 by two-step HPLC.¹⁵ Since that time, macroscopic (milligram) quantities of endohedral La,^{10,16–19} Y,^{20–23} Sc,^{21,22,24–27} Gd,^{18,21,28} Pr,²¹ Ce,²⁹ and Er^{27,30} metallofullerenes have all been isolated by variations of the method. Reports of the chemical derivatization,^{31–34} electrochemistry,^{16,20,29,34–38} magnetism,^{18,28,39} X-ray structural properties,^{22,30,40,41} EXAFS,^{42–45} and UV/VIS/IR spectroscopy^{11,15,16,19–21,24,25} of these species now appear regularly.

Herein, we report the isolation of milligram quantities of holmium metallofullerenes for the first time (principally Ho@C₈₂, Ho₂@C₈₂, and Ho₃@C₈₂), their characterization by LD-TOF mass spectrometry, and their study by neutron-activation

* Author to whom correspondence should be addressed.

[†] Rice University.

[‡] TDA Research.

[‡] NanoTechnologies of Texas.

[§] University of Missouri—Columbia.

[⊗] Abstract published in *Advance ACS Abstracts*, August 1, 1996.

(1) Kroto, H. W.; Heath, J. R.; O'Brien, S. C.; Curl, R. F.; Smalley, R. E. *Nature (London)* **1985**, *318*, 162.

(2) Heath, J. R.; O'Brien, S. C.; Zhang, Q.; Liu, L.; Curl, R. F.; Kroto, H. W.; Tittel, F. K.; Smalley, R. E. *J. Am. Chem. Soc.* **1985**, *107*, 7779.

(3) (a) Krätschmer, W.; Lamb, L. D.; Fostiropoulos, K.; Huffman, D. R. *Nature (London)* **1990**, *347*, 354. (b) Krätschmer, W.; Fostiropoulos, K.; Huffman, D. R. *Chem. Phys. Lett.* **1990**, *170*, 167.

(4) Olah, G. A.; Bucsi, I.; Aniszfeld, R.; Prakash, G. K. S. *Carbon* **1992**, *30*, 1203.

(5) Taylor, R.; Walton, R. M. *Nature (London)* **1993**, *363*, 685.

(6) Yang, Y.; Arias, F.; Echegoyen, L.; Chibante, L. P. F.; Flanagan, S.; Robertson, A.; Wilson, L. J. *J. Am. Chem. Soc.* **1995**, *117*, 7801.

(7) Chai, Y.; Guo, T.; Changming, J.; Haufler, R. E.; Chibante, L. P. F.; Fure, J.; Wang, L.; Alford, J. M.; Smalley, R. E. *J. Phys. Chem.* **1991**, *95*, 7564.

(8) Shinohara, H.; Sato, H.; Saito, Y.; Ohkohchi, M.; Ando, Y. *J. Phys. Chem.* **1992**, *96*, 3571.

(9) Bandow, S.; Kitagawa, H.; Mitani, T.; Inokuchi, H.; Saito, Y.; Yamaguchi, H.; Hayashi, N.; Sato, H.; Shinohara, H. *J. Phys. Chem.* **1992**, *96*, 9609.

(10) Shinohara, H.; Yamaguchi, H.; Hayashi, N.; Sato, H.; Inagaki, M.; Saito, Y.; Bandow, S.; Kitagawa, H.; Mitani, T.; Inokuchi, H. *Mater. Sci. Eng. B* **1993**, *B19*, 25.

(11) Yamamoto, K.; Funasaka, H.; Takahashi, T.; Akasaka, T. *J. Phys. Chem.* **1994**, *96*, 2008.

(12) For a review, see: Bethune, D. S.; Johnson, R. D.; Salem, J. R.; de Vries, M. S.; Yannoni, C. S. *Nature (London)* **1993**, *366*, 123.

(13) See for example: (a) Ross, M. M.; Nelson, H. H.; Callahan, J. H.; McElvany, S. W. *J. Phys. Chem.* **1992**, *96*, 5231. (b) Gillan, E. G.; Yeretizian, C.; Min, K. S.; Alvarez, M. M.; Whetten, R. L.; Kaner, R. B. *J. Phys. Chem.* **1992**, *96*, 6869. (c) Moro, L.; Ruoff, R. S.; Becker, C. H.; Lorents, D. C.; Malhotra, R. *J. Phys. Chem.* **1993**, *97*, 6801. (d) Bartl, A.; Dunsch, L.; Fröhner, J.; Kirbach, U. *Chem. Phys. Lett.* **1994**, *229*, 115.

analysis. This report follows our earlier neutron-irradiation study of empty C₆₀.⁴⁶ We also describe a facile new solution method for preparing metal-doped graphite electrodes for

(14) See for example: (a) Suzuki, S.; Kawata, S.; Shiromaru, H.; Yamauchi, K.; Kikuchi, K.; Kato, T.; Achiba, Y. *J. Phys. Chem.* **1992**, *96*, 7159. (b) Johnson, R. D.; de Vries, M. S.; Salem, J.; Bethune, D. S.; Yannoni, C. S. *Nature (London)* **1992**, *355*, 239. (c) Yannoni, C. S.; Hoinkis, M.; de Vries, M. S.; Bethune, D. S.; Salem, J. R.; Crowder, M. S.; Johnson, R. D. *Science* **1992**, *256*, 1191. (d) Shinohara, H.; Sato, H.; Ohkohchi, M.; Ando, Y.; Kodama, T.; Shida, T.; Kato, T.; Saito, Y. *Nature (London)* **1992**, *357*, 52. (e) Suzuki, S.; Kojima, Y.; Nakao, Y.; Wakabayashi, T.; Kawata, S.; Kikuchi, K.; Achiba, Y.; Kato, T. *Chem. Phys. Lett.* **1994**, *229*, 512. (f) Kato, T.; Bandou, S.; Inakuma, M.; Shinohara, H. *J. Phys. Chem.* **1995**, *99*, 856. (g) Dunsch, L.; Bartl, A.; Kirbach, U.; Fröhner, J. In *Fullerenes: Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials*; Kadish, K. M., Ruoff, R. S., Eds.; The Electrochemical Society: Pennington, NJ, 1995; Vol. 2, p 182. (h) Kato, T.; Suzuki, T.; Yamamoto, K.; Funasaka, H.; Takahashi, T. In *Fullerenes: Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials*; Kadish, K. M., Ruoff, R. S., Eds.; The Electrochemical Society: Pennington, NJ, 1995; Vol. 2, p 733.

(15) Kikuchi, K.; Suzuki, S.; Nakao, Y.; Nakahara, N.; Wakabayashi, T.; Shiromaru, H.; Saito, K.; Ikemoto, I.; Achiba, Y. *Chem. Phys. Lett.* **1993**, *216*, 67.

(16) Yamamoto, K.; Funasaka, H.; Takahashi, T.; Akasaka, T.; Suzuki, T.; Maruyama, Y. *J. Phys. Chem.* **1994**, *98*, 12831.

(17) Savina, M. R.; Martin, G.; Xiao, J.; Milanovich, N.; Meyerhoff, M. E.; Francis, A. H. In *Fullerenes: Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials*; Kadish, K. M., Ruoff, R. S., Eds.; The Electrochemical Society: Pennington, NJ, 1994; Vol. 1, 1309.

(18) Funasaka, H.; Sugiyama, K.; Yamamoto, K.; Takahashi, T. *J. Phys. Chem.* **1995**, *99*, 1826.

(19) Yamamoto, K.; Funasaka, H.; Takahashi, T.; Akasaka, T.; Suzuki, T.; Maruyama, Y. *Mater. Res. Soc. Symp. Proc.* **1995**, *359*, 137.

(20) Kikuchi, K.; Nakao, Y.; Suzuki, S.; Achiba, Y.; Suzuki, T.; Maruyama, Y. *J. Am. Chem. Soc.* **1994**, *116*, 9367.

(21) Shinohara, H.; Kishida, M.; Nakane, T.; Kato, T.; Bandow, S.; Saito, Y.; Wang, X.-D.; Hashizume, T.; Sakurai, T. In *Fullerenes: Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials*; Kadish, K. M., Ruoff, R. S., Eds.; The Electrochemical Society: Pennington, NJ, 1994; Vol. 1, p 1361.

(22) Inakuma, M.; Ohno, M.; Shinohara, H. In *Fullerenes: Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials*; Kadish, K. M., Ruoff, R. S., Eds.; The Electrochemical Society: Pennington, NJ, 1995; Vol. 2, p 330.

(23) Shinohara, H.; Ohno, M.; Kishida, M.; Yamazaki, S.; Hashizume, T.; Sakurai, T. In *Fullerenes: Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials*; Kadish, K. M., Ruoff, R. S., Eds.; The Electrochemical Society: Pennington, NJ, 1995; Vol. 2, p 763.

(24) Shinohara, H.; Yamaguchi, H.; Hayashi, N.; Sato, H.; Ohkohchi, M.; Ando, Y.; Saito, Y. *J. Phys. Chem.* **1993**, *97*, 4259.

(25) Shinohara, H.; Inakuma, M.; Hayashi, N.; Sato, H.; Saito, Y.; Kato, T.; Bandow, S. *J. Phys. Chem.* **1994**, *48*, 8597.

(26) Suzuki, S.; Kojima, Y.; Nakao, Y.; Wakabayashi, T.; Kawata, S.; Kikuchi, K.; Achiba, Y.; Kato, T. *Chem. Phys. Lett.* **1994**, *229*, 512.

(27) Dorn, H. C.; Stevenson, S.; Burbank, P.; Sun, Z.; Glass, T.; Harich, K.; van Loosdrecht, P. H. M.; Johnson, R. D.; Beyers, R.; Salem, J. R.; de Vries, M. S.; Yannoni, C. S.; Kiang, C. H.; Bethune, D. S. *Mater. Res. Soc. Symp. Proc.* **1995**, *359*, 123.

(28) Funasaka, H.; Sakurai, K.; Oda, Y.; Yamamoto, K.; Takahashi, T. *Chem. Phys. Lett.* **1995**, *232*, 273.

(29) Suzuki, T.; Kikuchi, K.; Nakao, Y.; Suzuki, S.; Achiba, Y.; Yamamoto, K.; Funasaka, H.; Takahashi, T. In *Fullerenes: Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials*; Kadish, K. M., Ruoff, R. S., Eds.; The Electrochemical Society: Pennington, NJ, 1995; Vol. 2, p 259.

(30) van Loosdrecht, P. H. M.; Johnson, R. D.; Beyers, R.; Salem, R.; de Vries, M. S.; Bethune, D. S.; Burbank, P.; Haynes, J.; Glass, T.; Stevenson, S.; Dorn, H. C.; Boonman, W.; van Bentum, P. J. M.; Meijer, G. In *Fullerenes: Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials*; Kadish, K. M., Ruoff, R. S., Eds.; The Electrochemical Society: Pennington, NJ, 1994; Vol. 1, 1320.

(31) Akasaka, T.; Kato, T.; Kobayashi, K.; Nagase, S.; Yamamoto, K.; Funasaka, H.; Takahashi, T. *Nature (London)* **1995**, *374*, 600.

(32) Suzuki, T.; Maruyama, Y.; Kato, T.; Akasaka, T.; Kobayashi, K.; Nagase, S.; Yamamoto, K.; Funasaka, H.; Takahashi, T. *J. Am. Chem. Soc.* **1995**, *117*, 9606.

(33) Akasaka, T.; Nagase, S.; Kobayashi, K.; Suzuki, T.; Kato, T.; Kikuchi, K.; Achiba, Y.; Yamamoto, K.; Funasaka, H.; Takahashi, T. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2139.

(34) Akasaka, T.; Nagase, S.; Kobayashi, K.; Suzuki, T.; Kato, T.; Yamamoto, K.; Funasaka, H.; Takahashi, T. *J. Chem. Soc., Chem. Commun.* **1995**, 1343.

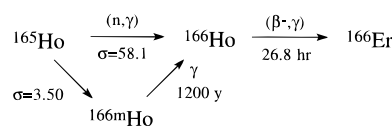


Figure 1. Neutron-activation scheme for holmium-165.

metallofullerene synthesis. Finally, EDS electron microprobe analysis is shown to be a reliable method for detecting holmium metallofullerenes in HPLC fractions, and these results corroborate those of the more sensitive neutron-activation measurements. Holmium has been specifically selected for neutron-activation studies because of its single natural isotope (¹⁶⁵Ho), the promising use of holmium compounds in therapeutic nuclear medicine,^{47,48} and the favorable nuclear properties exhibited by the neutron-activated ¹⁶⁶Ho radioisotope (see Figure 1). Previous documentation of holmium metallofullerenes in the literature is sparse and includes only microscale generation and detection by TOF MS, EPR spectroscopy, and magnetometry.^{13b-d,14g,39} Neutron-activation studies for only three other metallofullerenes (La@C₈₂, Y@C₈₂, and Gd@C₈₂) have been reported.⁴⁹⁻⁵¹

Toxicity data for derivatized, water-soluble C₆₀ is beginning to appear in the literature, and initial results indicate that fullerenes may exhibit an unusual biodistribution.⁵²⁻⁵⁸ A preliminary report has also appeared concerning the biodistri-

(35) Suzuki, T.; Maruyama, Y.; Kato, T.; Kikuchi, K.; Achiba, Y. *J. Am. Chem. Soc.* **1993**, *115*, 11006.

(36) Suzuki, T.; Maruyama, Y.; Kato, T.; Kikuchi, K.; Achiba, Y.; Yamamoto, K.; Funasaka, H.; Takahashi, T. In *Fullerenes: Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials*; Kadish, K. M., Ruoff, R. S.; The Electrochemical Society: Pennington, NJ, 1994; Vol. 1, p 1077.

(37) Suzuki, T.; Maruyama, Y.; Kato, T.; Kikuchi, K.; Nakao, Y.; Suzuki, S.; Achiba, Y.; Yamamoto, K.; Funasaka, H.; Takahashi, T. *Synth. Met.* **1995**, *70*, 1443.

(38) Yamamoto, K.; Funasaka, H.; Takahashi, T.; Akasaka, T.; Suzuki, T.; Maruyama, Y. *Mater. Res. Soc. Symp. Proc.* **1995**, *359*, 137.

(39) Diggs, B.; Zhou, A.; Silva, C.; Kirkpatrick, S.; Nuhfer, N. T.; McHenry, M. E.; Petasis, D.; Majetch, S. A.; Brunett, B.; Artman, J. O.; Staley, S. W. *J. Appl. Phys.* **1994**, *75*, 5879.

(40) Beyers, R.; Kiang, C.-H.; Johnson, R. D.; Salem, J. R.; de Vries, M. S.; Yannoni, C. S.; Bethune, D. S.; Dorn, H. C.; Burbank, P.; Harich, K.; Stevenson, S. *Nature (London)* **1994**, *370*, 196.

(41) Suematsu, H.; Murakami, Y.; Kawata, H.; Fujii, Y.; Hamaya, N.; Shimomura, O.; Kikuchi, K.; Achiba, Y.; Ikemoto, I. *Mater. Res. Soc. Symp. Proc.* **1994**, *349*, 213.

(42) Soderholm, L.; Wurz, P.; Lykke, K. R.; Parker, D. H.; Lytle, F. W. *J. Phys. Chem.* **1992**, *96*, 7153.

(43) Park, C.-H.; Wells, B. O.; DiCarlo, J.; Shen, Z.-X.; Salem, J. R.; Bethune, D. S.; Yannoni, C. S.; Johnson, R. D.; de Vries, M. S.; Booth, C.; Bridges, F.; Pianetta, P. *Chem. Phys. Lett.* **1993**, *213*, 1996.

(44) Kikuchi, K.; Nakao, Y.; Achiba, Y.; Momura, M. In *Fullerenes: Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials*; Kadish, K. M., Ruoff, R. S., Eds.; The Electrochemical Society: Pennington, NJ, 1994; Vol. 1, p 1300.

(45) Kajiyama, H.; Tomioka, Y.; Ishibashi, M.; Tamiguchi, Y. *Mol. Cryst. Liq. Cryst.* **1995**, *267*, 347.

(46) Lawrence, K. L. S.; Ehrhardt, G. J.; Cagle, D. W.; Thrash, T. P.; Wilson, L. J. In *Fullerenes: Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials*; Kadish, K. M., Ruoff, R. S., Eds.; The Electrochemical Society: Pennington, NJ, 1995; Vol. 2, p 66.

(47) Mumper, R. J.; Ryo, U. Y.; Jay, M. *J. Nucl. Med.* **1991**, *32*, 2139.

(48) Dadachova, E.; Mirzadeh, S.; Lambrecht, R. M.; Hetherington, E. L.; Knapp, F. F., Jr. *Anal. Chem.* **1994**, *66*, 4272.

(49) Sueki, K.; Kobayashi, K.; Kikuchi, K.; Tomura, K.; Achiba, Y.; Nakahara, H. *Fullerene Sci. Technol.* **1994**, *2*, 213.

(50) Kikuchi, K.; Kobayashi, K.; Sueki, K.; Suzuki, S.; Nakahara, H.; Achiba, Y.; Tomura, K.; Katada, M. *J. Am. Chem. Soc.* **1994**, *116*, 9775.

(51) Kobayashi, K.; Kuwano, M.; Sueki, K.; Kikuchi, K.; Achiba, Y.; Nakahara, H.; Kananishi, N.; Wantanabe, M.; Tomura, K. *J. Radioanal. Nucl. Chem.* **1995**, *192*, 81.

(52) Sijbesma, R.; Srdanov, G.; Wudl, F.; Castoro, J. A.; Wilkins, C.; Friedman, S. H.; DeCamp, D.; Kenyon, G. L. *J. Am. Chem. Soc.* **1993**, *115*, 6510.

(53) Tokuyama, H.; Yamago, S.; Nakamura, E.; Shiraki, T.; Sugiura, Y. *J. Am. Chem. Soc.* **1993**, *115*, 7918.

(54) Schinazi, R. F.; Sijbesma, R.; Srdanov, G.; Hill, C. L.; Wudl, F. *Antimicrob. Agents Chemother.* **1993**, *37*, 1707.

bution of underivatized Gd@C₈₂.⁵¹ For metallofullerenes to realize their full potential in nuclear medicine they must be made readily water soluble, and their toxicity, biodistribution, neutron-activation, and nuclear-decay properties systematically documented and understood. The present paper supplies a detailed analysis of neutron activation of holmium inside fullerenes as a reasonable working model for fullerene-based radiotracers and radiopharmaceuticals in general.

Experimental Section

Synthesis. Holmium-impregnated graphite electrodes were prepared by evacuating (1 Torr) 0.25 in. diameter 40% porosity graphite rods (Poco Graphite, Decatur, TX). The evacuated rods were then soaked in a saturated absolute ethanolic solution of Ho(NO₃)₃·xH₂O (4 ≤ x ≤ 6) (Alfa Aesar, Ward Hill, MA) for 10 min. The solution-saturated rods were air dried and resistively heated under vacuum with an arc welder for 10 min to convert the metal nitrate to the oxide or carbide. The average holmium content in the rods after heating was 0.40 mol %. The holmium-impregnated graphite rods were vaporized in a previously described carbon arc furnace at 150 Torr of He.⁵⁹ Empty fullerenes and holmium metallofullerenes were separated from the crude soot by overnight Soxhlet extraction with CS₂. A typical yield of extractable material was approximately 0.2% by weight after drying under vacuum.

HPLC Separation. The crude extract was dissolved in toluene to make a 3 mg/mL solution and filtered through a Nylon Acrodisc syringe filter (13 mm diameter, 0.2 mm pore size). All HPLC solvents were freshly distilled from Na or CaH₂ and filtered through a filter/degasser (EM Science, 0.2 μm Teflon pore size) before use.

Chromatographic separation was performed on an Hitachi HPLC system with a coupled L-6200A "intelligent pump" and L-6000 pumping system. The detector was a Hitachi model L-3000 photodiode array detector (200–520 nm). Data acquisition and analysis was accomplished with Hitachi Model D-6000 chromatograph data system software.

In a typical separation scheme, 2 mL of the filtered fullerene extract was injected into a Buckyclutcher I column (Regis, 50 cm × 21.1 mm i.d.) protected by a Phase 1010 Buckyclutcher guard column (Regis, 5 cm × 10.0 mm i.d.). The eluent was 1:1 toluene/n-hexane (10.0 mL/min). Fractions were collected every minute for 65 min using an Isco Retriever IV automated fraction collector system. These fractions were then combined to correspond to the resolved peaks or groups of peaks. Combined fractions collected were the 0–18, 18–21, 21–25, 25–30, 30–36, 36–43, 43–49, and 49–65 min fractions.

The fractions were analyzed for holmium by electron microprobe analysis (Cameca SX-50 with a SUN-based data control and reduction system). Concentrated toluene solutions of the combined fractions were spotted repeatedly on Al foil to make a thin film and assayed in the EDS mode using a 15 keV accelerating potential, 15 nA beam current, 20 mm electron beam size, and 100 s. live count time. Detection was based on the 6.72 keV holmium α X-ray peak. Holmium was detected in only the 43–49 and 49–65 min fractions.

(55) Schinazi, R. F.; McMillan, A.; Juodawlkis, A. S.; Pharr, J.; Sijbesma, R.; Srdanov, G.; Hummelen, J.-C.; Boudinot, F. D.; Hill, C. L.; Wudl, F. In *Fullerenes: Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials*; Kadish, K. M., Ruoff, R. S., Eds.; The Electrochemical Society: Pennington, NJ, 1994; Vol. 1, p 689.

(56) Yamago, S.; Tokuyama, H.; Nakamura, E.; Kikuchi, K.; Kananishi, S.; Sueki, K.; Nakahara, H.; Enomoto, S.; Ambe, F. *Chem. Biol.* **1995**, *2*, 385.

(57) Schinazi, F. F.; Bellavia, C.; Gonzalez, R.; Hill, C. L.; Wudl, F. In *Fullerenes: Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials*; Kadish, K. M., Ruoff, R. S., Eds.; The Electrochemical Society: Pennington, NJ, 1995; Vol. 2, p 696.

(58) Chiang, L. Y.; Lu, F.-J.; Lin, J.-T. In *Fullerenes: Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials*; Kadish, K. M., Ruoff, R. S., Eds.; The Electrochemical Society: Pennington, NJ, 1995; Vol. 2, p 699.

(59) Yang, Y.; Arias, F.; Echegoyen, L.; Flanagan, S.; Robertson, A.; Wilson, L. J. In *Fullerenes: Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials*; Kadish, K. M., Ruoff, R. S., Eds.; The Electrochemical Society: Pennington, NJ, 1995; Vol. 2, p 306.

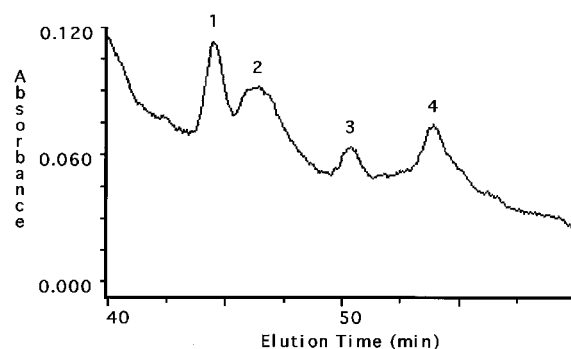


Figure 2. HPLC trace at 280 nm showing separation of the Ho_x@C_{2n}-containing fractions (1–3).

Holmium-containing fractions were re-injected into the HPLC under identical conditions to those described above, except only 0.3 mL of a concentrated solution was injected each time. Individual peaks from the target region were collected manually at 43–45, 45–48, 49.5–52, and 53–55.8 min, and are labeled peaks 1, 2, 3, and 4, respectively, in Figure 2. Only peaks 1, 2, and 3 gave substantial assays for holmium by neutron-activation analysis.

Mass Spectrometry. Peaks 1, 2, and 3 in Figure 2 were each analyzed by LD-TOF mass spectrometry and by a Finnigan Mat 95 mass spectrometer operating in the EI, CI, and FAB positive and negative ion modes. Laser desorption for TOF MS was performed with 532 nm light from a Q-switched Nd-YAG laser, and ionization was accomplished at 193 nm with an ArF excimer laser. Samples were coated on to a stainless steel target disk by allowing a toluene solution of the metallofullerene to evaporate. The equipment has been previously described.^{1,2}

Neutron-Activation Analysis. Neutron-activation studies and related experiments were performed at the Missouri University Research Reactor (MURR). In a typical experiment, a small solid sample of Ho_x@C_{2n} (50–500 μg) was sealed inside a high-purity polyethylene vial (ambient atmosphere) or Suprasil (T-21) high-purity quartz vial (1 Torr vacuum). Quartz vials were used for the long-term irradiations (> 30 min) because the routinely-used polyethylene vials degrade under the long-term conditions due to neutron damage. For irradiations lasting between 1 and 30 min, the samples were irradiated at 49–60 °C inside polyethylene "rabbits" inserted into the reactor via a pneumatic tube. Samples requiring longer irradiations were irradiated in light-water flooded aluminum "cans" which maintained the samples at 43 °C. Solvent was removed from all samples before irradiation by overnight vacuum centrifugation (Savant) at 30 °C. Long-term irradiations were performed in a neutron flux of $\Phi = 4.0 \times 10^{13} \text{ n cm}^{-2} \text{ s}^{-1}$ with a high thermal-neutron component ($\Phi_{\text{thermal}} = 3.03 \times 10^{13} \text{ n cm}^{-2} \text{ s}^{-1}$) ($E < 0.1 \text{ eV}$) and relatively small epithermal- ($\Phi_{\text{epithermal}} = 2.59 \times 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$) ($E \sim 0.1 - 1000 \text{ eV}$) and fast- ($\Phi_{\text{fast}} = 7.12 \times 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$) ($E > 1000 \text{ eV}$) neutron components, respectively. Short-term (1–30 min) irradiation fluxes were $\Phi_{\text{thermal}} = 4 \times 10^{13} \text{ n cm}^{-2} \text{ s}^{-1}$ and $\Phi_{\text{epithermal}} = 1 \times 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$.

Irradiated samples were analyzed using a γ-ray spectrometer with an intrinsic germanium detector and multichannel analyzer. The detector efficiency was 4.04%. ¹⁶⁶Ho analysis was measured using the integrated intensity of the 80.5 keV ¹⁶⁶Ho γ-emission (6.2% abundant).⁶⁰ Irradiated samples were treated as follows: The untreated vial was placed in the detector at a uniform position, counted for 5 min, and then sequentially extracted with 2 × 0.5 mL of toluene, 2 × 0.5 mL of dilute HCl, and an additional 2 × 0.5 mL of toluene. The acid extract, combined toluene extracts, and the "extracted" vials were all placed in glass scintillation vials of uniform geometry and counted as above. All activity measurements for extracted solid samples were performed on the liquid toluene or aqueous acid extracts.

Re-irradiation and chemical workup was performed on the decayed long-term irradiation aqueous acid extracts to determine the source of activated ¹⁶⁶Ho. The acid solutions were placed in irradiation vials, the solvent evaporated, and the solid re-irradiated for the length of the original irradiation time period. The irradiated samples were redissolved

(60) Erdtman, G.; Soyka, W. *The Gamma Rays of the Radionuclides*; Verlag Chemie: Weinheim and New York, 1978; p 160.

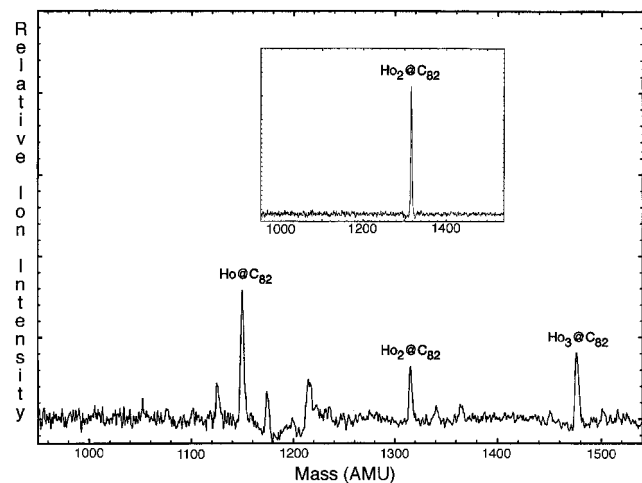


Figure 3. Laser-desorption time-of-flight mass spectrometry data for HPLC fraction 2 and fraction 3 (inset) showing parent-ion masses of the $\text{Ho}_n\text{@C}_{82}$ ($n = 1-3$) metallofullerenes.

in 3 M HCl and excess $^{165}\text{Ho}^{3+}$ scavenger was added. The pH was raised to 14 with aqueous NaOH and the $\text{Ho}(\text{OH})_3$ precipitate was collected by decanting the supernatant liquid. The precipitate was washed three times with deionized H_2O , redissolved in 3 M HCl, and counted for 5 min in a uniform geometry. Chemical workup was necessary to remove interfering ^{24}Na introduced by Na^+ leached from the glass scintillation vials over time. Measured activities were decay-corrected back to the time of the end of irradiation using the half-life of ^{166}Ho . Samples were repeatedly counted over several days to verify that the 80.5 keV peak was decreasing with the half-life of ^{166}Ho .

Results

Peaks 1, 2, and 3 in Figure 2 showed a variety of holmium metallofullerene parent ions by LD-TOF MS. Peak 1 displayed parent ion masses for Ho@C_{80} , Ho@C_{82} , $\text{Ho}_2\text{@C}_{82}$, and $\text{Ho}_2\text{@C}_{84}$. Peak 2 contained Ho@C_{82} , $\text{Ho}_2\text{@C}_{82}$, and $\text{Ho}_3\text{@C}_{82}$. LD-TOF MS also indicated some residual C_{60} and C_{70} in these fractions, but residual higher fullerenes were not present in amounts above background levels. Peak 3 showed only $\text{Ho}_2\text{@C}_{82}$. The LD-TOF MS data for fractions 2 and 3 (inset) are shown in Figure 3. Parent ion mass peaks for the holmium metallofullerenes were not observed under any conditions with the Mat 95 MS. However, empty fullerenes C_{2n} ($n = 44-50$) were indicated in peak 2 along with residual C_{70} and other higher empty fullerenes using the CI-negative ion mode (methane reagent gas) when the sample was desorbed from a filament previously coated with C_{60} .

Initial neutron-activation analyses were performed by irradiating 5–10 min HPLC fractions from a typical holmium metallofullerene burn for 10 min to confirm the electron microprobe results. Significant holmium was detected only in HPLC fractions eluting at greater than 40 min, paralleling the electron microprobe data.

HPLC fractions 1 through 4 were irradiated for 1 min and analyzed for ^{166}Ho (data available as supporting information). The activities for these samples were 470, 2300, 620, and 150 (Cts/min/mg sample), respectively, with error margins no larger than 25%. Since the activity of **2** was approximately four times greater than that of any other fraction, efforts were focused on it. Fraction 2 was analyzed to determine the percent survivability of the neutron-activated ^{166}Ho metallofullerenes by measuring the activity distribution between the toluene extract (survival) and aqueous acid extract (decomposition). The percent survivability was determined by dividing the toluene extract activity by the total activity in the vial before extraction. Irradiation of **2** for 1 min was found to give 27% survival of

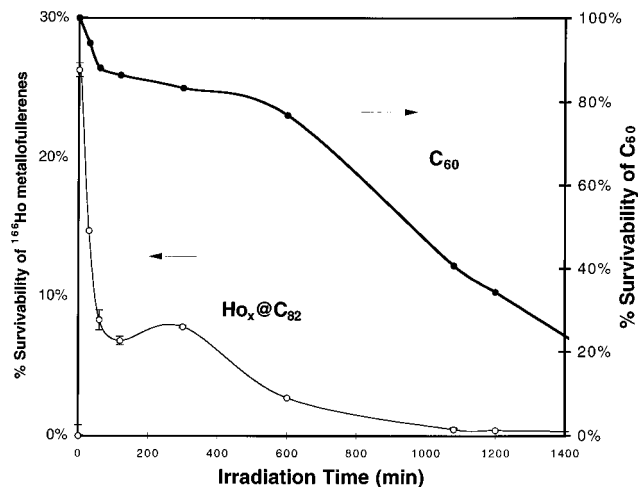


Figure 4. Percent survivability of neutron-activated $^{166}\text{Ho}_n\text{@C}_{82}$ and of C_{60} (ref 46) as a function of neutron irradiation time ($\Phi = 4 \times 10^{13}$ $\text{n cm}^{-2} \text{s}^{-1}$). The line through the holmium data points is a nonlinear, least-squares fit, with resulting error bars as shown.

activated holmium metallofullerenes. Another 10% of the activity was found in the aqueous acid phase, and the residual activated material was unextractable from the vial. Irradiation for 30 min lowered the survivability of holmium metallofullerenes to about 14%, with another 19% of the ^{166}Ho activity in the aqueous acid extract. Even though the short-term irradiations indicated relatively high survivability, the overall percent activation was low. The longer-term survivability data displayed a maximum activity in the toluene extract at about 5 h of irradiation (8% survival), followed by reduced survivability thereafter, as shown in Figure 4.

Re-irradiation of the chemically-treated acid extracts showed essentially the same activity obtained from the initial irradiation.

Discussion

Figure 1 displays the neutron-activation scheme for monoisotopic ^{165}Ho . The pathway involving $^{166\text{m}}\text{Ho}$ is insignificant because of the small neutron-capture cross section and long half-life of $^{166\text{m}}\text{Ho}$. Thus, neutron activation is principally by $^{165}\text{Ho}(n,\gamma)^{166}\text{Ho}$ with its large capture cross section and subsequent β^- decay to stable ^{166}Er . Unlike the holmium atom, the carbon atoms of the fullerene cage are not activated because of the very small capture cross section of ^{12}C ($\sigma = 0.0035$ b). The “simplicity” of the ^{165}Ho activation/decay scheme makes holmium an especially appealing candidate for neutron-activation studies of metallofullerenes. Furthermore, the favorable neutron-capture cross section and $t_{1/2}$ of holmium make the isotopic decay pathway “easy” to interpret. Previous reports of neutron-activated metallofullerenes of La@C_{82} , Y@C_{82} , and Gd@C_{82} have relied on irradiation of only crude soot extracts, or in one instance on isolated trace quantities of La metallofullerenes from defined volume HPLC aliquots.^{49–51} The current study utilizes macroscopic quantities of HPLC-purified metallofullerene samples of defined composition, since it is likely that neutron activation will need to be performed on pre-purified materials if metallofullerenes are to find a specialty niche in nuclear medicine.

During neutron activation of metallofullerenes, sample decomposition can occur by three independent mechanisms. Firstly, fast and epithermal neutrons can cause fullerene carbon atom translocations from (fast neutron)–(carbon atom) collisions. In an earlier study involving neutron irradiation of C_{60} , we have shown that this process produces serious damage for long-term irradiation (> 12 h) at the same neutron-flux and

composition used in the present study; a portion of the original C_{60} decomposition data is reproduced in Figure 4.⁴⁶ Secondly, decay of activated ^{166}Ho produces an energetic β^- particle ($E_{\beta^-} = 1.78, 1.86$ MeV) and a γ -ray ($E_{\gamma} = 80.5$ keV) which cause the holmium nucleus to recoil with maximum energies of 17 and 0.2 eV, respectively. These recoil energies compare to carbon-carbon bond energies of only 5-10 eV/bond in the fullerene cage. Finally and most likely, decomposition can occur during the very energetic (n, γ) activation step where $E_{\gamma} \approx 8$ MeV and E_{max} (Ho recoil) ≈ 200 eV. A recoil energy of 200 eV is an obvious possible source of metallofullerene decomposition due to "breakout" of recoiling ^{166}Ho . Damage from ionizing γ -radiation in the reactor also remains a possible source of degradation,⁵¹ but we have previously shown that this radiation is non-destructive toward C_{60} .⁴⁶

Figure 4 displays the percent survivability of the neutron-activated $\text{Ho}_x@C_{82}$ sample as a function of irradiation time from 1–1200 min. For the metallofullerene samples, "survivability" was determined by extracting the post-irradiated solid sample with toluene and determining the solution activity of solubilized metallofullerene (see Experimental Section). After 1 min of irradiation, only 27% of the metallofullerene sample survived, whereas nearly 100% of the C_{60} survived the same conditions. This behavior continued with time, and in general, the percent survivability of the metallofullerene sample decreased much more rapidly as a function of irradiation time than did C_{60} . In addition, it can be seen from Figure 4 that the activated metallofullerene survivability displayed a maximum after approximately 5 h of irradiation (8% survivability) which is probably the combined result of decreasing survivability and increasing ^{166}Ho activation with irradiation time. At long irradiation times (1200 min) the metallofullerene sample survived to only 1–2%, while C_{60} survived to 30%. Thus, for a given neutron dosage, the metallofullerene sample suffered greater damage than did C_{60} . At first glance, this result seems quite reasonable since metallofullerenes have additional energetic (n, γ) and (β^-, γ) processes associated with neutron activation of the metal atom. Previous studies on crude fullerene extracts containing $\text{Gd}@C_{82}$ ⁵⁰ and $\text{La}@C_{82}$ ^{49,51} have concluded that metallofullerenes surviving initial neutron activation (≈ 10 –30%) did not degrade over time by (β^-, γ) recoil, but that observed initial degradation ($\approx 90\%$) was probably due to recoil from the prompt (n, γ) emission.^{49,51} However, the latter conclusion is not entirely justified for the present holmium case, as discussed below.

If damage to the holmium metallofullerenes was caused primarily by (n, γ) recoil, it would be expected that only ^{165}Ho atoms absorbing a neutron would be affected, and thus that the acid-extractable, non-fullerene-contained ^{166}Ho would be of very high specific activity, perhaps even carrier-free. Indeed, the product might be expected to contain some carrier (stable) ^{165}Ho

due to collateral metallofullerene damage adjacent to "breakout" ^{166}Ho fullerene molecules, but this should be a small effect because only a minuscule fraction of the ^{165}Ho atoms actually absorb a neutron. However, re-irradiation of the decayed ^{166}Ho acid extracts showed them to contain carrier holmium equivalent to the bulk specific activity of ^{166}Ho produced in the first, equivalent irradiations. Thus, the dominant mechanism for release of holmium from the fullerene cage completely failed to discriminate between activated ^{166}Ho and non-activated ^{165}Ho , and therefore could not have been primarily a consequence of the (n, γ) recoil process. Rather, fast neutron damage seems the most likely cause for the observed massive release of holmium from the metallofullerenes, and if (n, γ) degradation occurs, it is being completely overshadowed by fast neutron damage. Further experiments in a neutron flux containing the absolute minimum of high energy neutrons are necessary to resolve this point. Nothing can be said here about the (β^-, γ) recoil process because the ^{166}Er daughter product is stable and not observable, but the relatively small magnitude of the recoil energy makes destruction of the fullerene cage by this mechanism less likely than by other causes. In any event, the fate of non-radioactive ^{166}Er daughter product is of no consequence, due to the minuscule quantities of ^{166}Er produced.

In conclusion, this study has shown that macroscopic quantities of pre-purified samples of holmium metallofullerenes ($\text{Ho}_x@C_{82}$ with $x = 1$ –3) survive neutron activation with up to 8% survivability after 5 h of irradiation at $\Phi = 4 \times 10^{13}$ n cm^{-2} s^{-1} ($\approx 75\%$ thermal; $\approx 25\%$ epithermal and fast). More importantly, however, it has also been demonstrated for the first time that observed metallofullerene decomposition occurs principally (and surprisingly!) from neutron-irradiation damage and not from metal atom recoil associated with the energetic (n, γ) and (β^-, γ) nuclear properties of holmium. This finding suggests that metallofullerene degradation can be minimized using a neutron flux with a higher thermal component. Experiments designed to test this hypothesis, as well as experiments to activate individual $\text{Ho}@C_{82}$, $\text{Ho}_2@C_{82}$, and $\text{Ho}_3@C_{82}$ species, are in progress.

Acknowledgment. The Robert A. Welch Foundation (Grant C-0627) and DARPA (Army Grant DAAL-03-92-G-0186) are gratefully acknowledged for support of this project (L.J.W.), as well as support of the US Department of Energy for reactor fuel (G.J.E.).

Supporting Information Available: A listing of the neutron-activated samples, sample weights, ^{166}Ho activities, calculated masses in toluene and aqueous acid, and statistical errors (Tables S-I and S-II) (1 page). See any current masthead page for ordering and Internet access instructions.

JA960841Z