

Published in final edited form as:

Environ Pollut. 2011 May ; 159(5): 1334–1342. doi:10.1016/j.envpol.2011.01.018.

Detection of fullerenes (C₆₀ and C₇₀) in commercial cosmetics

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Abstract

Detection methods are necessary to quantify fullerenes in commercial applications to provide potential exposure levels for future risk assessments of fullerene technologies. The fullerene concentrations of five cosmetic products were evaluated using liquid chromatography with mass spectrometry to separate and specifically detect C₆₀ and C₇₀ from interfering cosmetic substances (e.g., castor oil). A cosmetic formulation was characterized with transmission electron microscopy, which confirmed that polyvinylpyrrolidone encapsulated C₆₀. Liquid-liquid extraction of fullerenes from control samples approached 100% while solid-phase and sonication in toluene extractions yielded recoveries of 27–42%. C₆₀ was detected in four commercial cosmetics ranging from 0.04 to 1.1 μg/g, and C₇₀ was qualitatively detected in two samples. A single-use quantity of cosmetic (0.5 g) may contain up to 0.6 μg of C₆₀, demonstrating a pathway for human exposure. Steady-state modeling of fullerene adsorption to biosolids is used to discuss potential environmental releases from wastewater treatment systems.

Keywords

Fullerenes; Cosmetics; Environmental transport; Nanomaterials; Liquid chromatography-mass spectroscopy; Radical Sponge[®]; Polyvinylpyrrolidone (PVP)

1. Introduction

Manufacturers are incorporating nanosized carbon particles, including fullerenes (e.g., C₆₀ and C₇₀), into consumer products such as cosmetics for their suggested “anti-aging” or radical scavenging properties (Halford, 2006; Woodrow Wilson International Center for Scholars, 2009; Xiao et al., 2006). Despite this commercial use, research has shown that fullerenes can cause adverse biological effects (Johansen et al., 2008; Kai et al., 2003; Miyata et al., 2000; Tsuchiya et al., 1996; Usenko et al., 2008), and uncertainty remains in regards to the environmental and human health effects of these molecules (Spohn et al., 2009). Fullerenes in cosmetics will increase human exposure through product use, and like

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Fullerenes were detected in cosmetics with liquid chromatography-mass spectrometry up to 1.1 μg/g, demonstrating a source for human/environmental exposure.

Author disclosure statement

No competing financial interests exist.

other chemicals found in personal care products, can potentially be released into the environment after being rinsed “down the drain” (Benotti et al., 2009). In fact, a probabilistic study has predicted an increase in the fullerene concentration of sewage treatment plant effluent between 4 and 33 ng/L as a result of their use in new technologies (Gottschalk et al., 2009). The use of fullerenes in cosmetics is not trivial, as consumer products can be a means to introduce nanotechnologies to the public, who in turn make decisions whether or not to accept new technologies and to support nanotechnology development. An adequate evaluation of the environmental and human health impacts of nanoparticles (e.g., C₆₀) in consumer products would be useful information for decision-making, and could therefore help prevent public distrust (Halford, 2006) and the slow economic development of nanotechnology (National Science and Technology Council, 2006). Such evaluations must use validated analytical techniques for fullerene detection in complex matrices like cosmetics.

Although some patent applications for the use of fullerenes in cosmetic formulations report fullerene contents of up to 9% by weight (Burangulov et al., 2005; Ito and Matsubayashi, 2006; Ziolo, 1993), the fullerene contents of off-the-shelf products need to be quantified. The “fullerenes” listed in cosmetic ingredient lists could encompass C₆₀, C₇₀, higher-order fullerenes, fullerene derivatives (i.e., C₆₀-PVP), and fullerene-based molecules (i.e., C₆₀O, C₆₀O₂, etc.), each of which might require separate and specific detection and quantification. Detection of fullerenes in cosmetics will indicate a possible environmental emission source of engineered fullerenes. One recent study has reported the occurrence of μg/L concentrations of fullerenes associated with the suspended solids of treated wastewater effluents (Farré et al., 2010). Although a correlation between increased effluent fullerene concentration and highly populated and industrialized areas was suggested, the source of these fullerenes was not determined. Detection of fullerenes in cosmetics would confirm commercial applications as fullerene emission sources and support the hypothesis that wastewater systems provide an environmental exposure route for these engineered nanomaterials.

Fullerenes can be modified in many ways to promote dispersion in water and subsequent integration into cosmetic matrices. In a formulation called Radical Sponge[®], fullerenes are complexed with polyvinylpyrrolidone (PVP) (Xiao et al., 2007, 2006, 2005). It is hypothesized that PVP disperses C₆₀ in water by forming a non-covalently bound C₆₀-PVP complex or by encapsulation of C₆₀ in the hydrophobic core of a PVP micelle. Previous work indicates that pure C₆₀ can be qualitatively recovered from the Radical Sponge[®] aqueous formulation using a salt or non-polar solvent (toluene) (Yamakoshi et al., 1994). Quantifying recovery of C₆₀ from this formulation will validate the potential detection of fullerenes in cosmetics.

High-performance liquid chromatography (HPLC) techniques have been used to detect fullerenes in various matrices (Becker et al., 1994; Benn et al., 2010; Chen et al., 2008; Fortner et al., 2005; Heymann et al., 1995; Santa et al., 1995) and are extensively reviewed elsewhere (Isaacson et al., 2009; Pycke et al., 2011). Briefly, HPLC coupled to mass spectrometry (LC-MS) has been used to quantify C₆₀ in aqueous samples at environmentally relevant, ng/L concentrations (Chen et al., 2008). Additionally, MS can be used to distinguish between fullerene oxidation products (Lebedkin et al., 1995; Smith et al., 1995). Solvent extraction has been employed to recover fullerenes from complex geologic matrices (Heymann et al., 1995, 1994), although quantitative recovery has proven difficult (Jehlicka et al., 2005). Solvent extraction and LC-MS analysis have yet to be applied to detection of C₆₀ in consumer products such as cosmetic matrices.

The goal of this research is to apply common extraction approaches and analytical techniques towards separating and detecting C₆₀ in cosmetic products. First, the recovery of fullerenes from cosmetic matrices is investigated using three common extraction methods (liquid-liquid, toluene-sonication, and solid phase extraction). Second, the recovery of C₆₀ from a common cosmetic fullerene formula (C₆₀-PVP) is quantified using liquid-liquid (LLE) and solid phase (SPE) extractions. Third, LC-MS chromatograms are analyzed to confirm the detection of fullerenes in cosmetics as well as to identify possible fullerene-based molecules. Next, five commercial cosmetic samples are subjected to the extraction procedures to quantify C₆₀ and qualitatively detect C₇₀. Finally, a steady-state mass balance is used to model the fate of fullerenes released into a wastewater treatment plant (WWTP).

2. Methods and materials

2.1. Cosmetic and fullerene sources

Five cosmetics from four manufacturers were purchased based on labeling that indicated that the products contained “fullerenes” or “Radical Sponge[®]” (Table 1). The matrices of the cosmetics varied considerably: two were watery serums (medium water content), two were dense creams (low water content), and one was a water-based lotion (high water content). A sunscreen (SkinCeuticals, Garland, TX) that did not identify the use of fullerenes was used as a control to represent a common cosmetic cream matrix. A common cosmetic ingredient, castor oil (USP 100%, CVS Pharmacy, Inc., Woonsocket, RI), was tested for interference with fullerene detection at specific mass-to-charge ratios.

C₆₀ and C₇₀ (99% purity) were purchased from MER Corp. (Tucson, AZ). Standard fullerene solutions for LC-MS calibration and spike/recovery experiments were prepared by adding 13 mg of fullerene powder to 100 mL of HPLC-grade toluene (Mallinckrodt, Phillipsburg, NJ) and sonicating in a water bath (Branson Model 2510, Danbury, CT) for 15 min.

The C₆₀-PVP suspension was prepared in nanopure water as described elsewhere (Xiao et al., 2006). Five mL of concentrated C₆₀-toluene solution (920 mg/L) was mixed with 10 mL of 50,000 mg/L PVP solution (K30, Sigma Aldrich, St. Louis, MO) in chloroform (Mallinckrodt, Phillipsburg, NJ), and the solvents were evaporated under purified nitrogen. The residual solid was sonicated in 10 mL nanopure water (Millipore, Billerica, MA), and particulates were filtered (GF/F, Whatman, Kent, UK). A carbon-coated, copper TEM grid was immersed in the C₆₀-PVP solution and air-dried in preparation for imaging using a JEOL 1200EX electron microscope (JEOL Ltd., Tokyo, Japan). TEM images of the C₆₀-PVP solution indicate a dominant presence of spherical agglomerates around 100 nm in size (Fig. 1A) in which C₆₀ appears to be wrapped in PVP (Fig. 1B). These agglomerates are similar to those found by SEM (Xiao et al., 2006).

2.2. Extraction methods

Fullerenes were extracted from cosmetics using three methods. 1) Liquid-liquid extraction was performed patterned after previous research (Xia et al., 2006) by sonicating 0.5–1.5 g of the cosmetic in 5 mL of nanopure water followed by addition of 2 mL of 100 mM Mg(ClO₄)₂ (Fisher, Fair Lawn, NJ) and 11.3 mL of glacial acetic acid (GAA) to control emulsions. Five mL of toluene were added, and the two-phase solution was mixed on an orbital shaker table (New Brunswick Scientific, Edison, NJ) at 35 RPM for at least 1 h. The two-phase solution was allowed to separate for at least 20 min. The toluene phase was sampled (1 mL) and evaporated under purified (Ultrapure) nitrogen to dryness to remove GAA, which interferes with mass spectrometric detection using atmospheric pressure chemical ionization (APCI). The sample was reconstituted in 1 mL of toluene and briefly sonicated (3–5 min) to dissolve fullerenes. 2) The toluene-sonication extraction method was

performed by sonicating 0.5–1.5 g of cosmetic in 10 mL of toluene for at least 4 h. The toluene extract was centrifuged at $F = 12,000$ G (Thermo IEC, Waltham, MA) for 10 min and filtered (Qualitative 2 or GF/F, Whatman, Kent, UK) to remove particulates. Control experiments showed negligible removal of fullerenes from pure toluene during filtration and centrifugation. The toluene filtrate was then evaporated to a measured volume less than 2 mL prior to LC-MS quantification. 3) SPE was performed after sonicating 0.5–1.5 g of cosmetic in nanopure water for at least 4 h. Highly particulate samples were filtered (GF/F) prior to SPE. The aqueous solutions were passed through SPE cartridges using a vacuum pump (Buchi Labortechnik, Flawil, Switzerland) at approximately 1 mL/min. Strata C-18 E SPE cartridges (Phenomenex, Torrance, CA) were conditioned with 5 mL of HPLC-grade methanol (Fisher, Fair Lawn, NJ) followed by 5 mL of nanopure water. The sample was loaded and the column allowed to air-dry prior to washing with 5 mL of methanol. Samples were eluted with 10 mL of toluene and evaporated to a measured volume less than 2 mL prior to LC-MS quantification.

2.3. Quantification of fullerenes

Fullerenes (C_{60} and C_{70}) were quantified in the toluene extracts using LC-MS with APCI. The method is a variation of a procedure reported elsewhere (Chen et al., 2008). An LC-MS system was used consisting of 2 Varian Prostar 210 pumps coupled to a Varian 1200 triple quadrupole mass spectrometer (Varian, Inc., Palo Alto, CA). Samples were eluted isocratically using toluene and acetonitrile at a ratio of 55/45 on a Nova-Pak C-18 column (3.9×150 mm) (Waters, Milford, MA) with a constant flow rate of 1 mL/min. Using a 200 μ L sample loop, 50 μ L of sample was loaded followed by 75 μ L of acetonitrile to bring the sample matrix close to the eluent mixture and facilitate a resolved C_{60} peak. The temperatures for the APCI housing, N_2 drying gas, and APCI torch were set at 50 $^{\circ}C$, 200 $^{\circ}C$, and 350 $^{\circ}C$, respectively. Shield voltage was set at -200 V, and the mass detector was operated at a fixed voltage of -1200 V. Negatively-charged m/z 720 and 840 were used for the identification and quantification of C_{60} and C_{70} , respectively, with a scan width of 0.70 amu and scan time of 0.3 s. Calibration was achieved by using dilutions from stock solutions of solid C_{60} and C_{70} dissolved in toluene. Fig. 2 shows representative calibration curves for C_{60} and C_{70} , which elute at 4 and 6 min, respectively. Instrument detection limits based on $3 \times$ the background signal were 3 and 12 μ g/L for C_{60} and C_{70} in toluene, respectively.

Standard addition was used to determine any cosmetic matrix interference on LC-MS detection using fullerene-toluene solutions. C_{60} was introduced at 55 μ g/L into the toluene extractions (LLE and SPE) of cosmetic samples. The percentage decrease from the expected signal was compensated for in the spike/recovery of C_{70} and the quantification of C_{60} in cosmetic samples.

2.4. Spike/recovery experiments

Spike-recovery experiments were used to evaluate the efficiency of three methods for extracting fullerenes. Cosmetic samples #2 and #4 were chosen as representative serum and cream matrices, respectively. Because these sample matrices were expected to contain C_{60} , C_{70} was chosen as a surrogate to spike fullerene quantities relevant to the C_{60} concentration of the cosmetics. C_{70} (4.2 μ g) was administered in toluene (1 mL) to the serum (~ 0.6 g of cosmetic #2) and the cream (~ 1.1 g of cosmetic #4), the toluene was evaporated, and C_{70} was recovered using SPE, LLE, and toluene-sonication extraction methods. The spiked quantity (4–7 μ g- C_{70} /g-cosmetic) was within one order of magnitude of the C_{60} detected in the cosmetic samples (see Results).

It has been hypothesized that allowing fullerene-toluene solutions to evaporate to dryness may inhibit recovery in trace analysis owing to adsorption to glassware (Perez et al., 2009;

Xia et al., 2006). To test this possibility, triplicate samples of the C₇₀ spike alone were evaporated in a glass vial under N₂ and subsequently recovered in toluene with sonication. Nearly complete (94 ±14%) recovery of the spike (4.1 μg-C₇₀) suggests that adsorption of C₇₀ to glassware for this spiked concentration is not inhibiting recovery in these experiments.

2.5. Modeling fullerene transport within a WWTP

A steady-state mass balance with non-linear adsorption can be applied to model the transport of nanomaterials in a WWTP (Benn and Westerhoff, 2008) as,

$$C=C_0-\left(\frac{X\tau KC^{1/n}}{\theta}\right) \quad (1)$$

where C is the effluent concentration of fullerenes, C_0 is the influent fullerene concentration, and K and $1/n$ are the Freundlich adsorption parameters. Variables X , τ , and θ , are operational parameters of a WWTP (mixed liquor suspended solids, hydraulic and solids retention time, respectively), and were set as 2000 mg/L, 720 min, and 7200 min, respectively, for a one million gallon per day (MGD) WWTP. Freundlich adsorption parameters were estimated from previous research where the adsorptive removal of n-C₆₀ (3 mg/L) and C₆₀-PVP (2 mg/L) with 50 mg/L of biosolids was 36 and 6%, respectively (Kiser et al., 2010). From prior work $1/n$ was set at 0.5 while K was estimated for C₆₀-PVP as 1/6th (6%/36%, $K = 0.17$ (μg-C₆₀/g-biomass) (L/μg-C₆₀)^{1/n}) of the relative capacity of n-C₆₀ which has an experimental K value of 1 (μg-C₆₀/g-biomass)(L/μg-C₆₀)^{1/n}.

3. Results

3.1. Evaluating extraction method efficiencies

C₇₀ was spiked into a serum and a cream cosmetic matrix and recovered using SPE, LLE, and sonication in toluene (Fig. 3). LLE was used to recover 96 and 107% of the C₇₀ from the serum and cream, respectively. SPE and sonication in toluene achieved much lower fullerene recovery efficiencies than LLE, ranging from 27 to 42%. These lower recoveries could be due to inefficient extraction from the cosmetic or matrix interferences on LC-MS detection.

Fullerene standard addition was applied to determine any matrix interference on LC-MS detection. The LLE of the cream (sample #4) caused a 47% reduction in fullerene detection signal. When this interference was accounted for, recovery of the C₇₀ spike was 107%. In contrast to the cream matrix, no LC-MS interference was observed for the LLE of the serum, indicating the role that specific components in cosmetic formulations play in suppressing the LC-MS detection of fullerenes under these experimental conditions. No matrix interferences on LC-MS detection were observed for the SPEs of the serum or cream. SPE produced a toluene extract that did not interfere with LC-MS detection unlike LLE, but SPE was less efficient than LLE at recovering fullerenes.

It is important to note that this spike/recovery experiment was designed only to compare extraction methods and identify matrix interferences, not to quantify actual fullerene recovery from cosmetics. Evaporating fullerenes into a cosmetic matrix from an organic solvent does not necessarily represent how fullerenes are incorporated into commercial cosmetics. For example, fullerenes are often added to cosmetics with a dispersant (i.e., PVP), which may influence fullerene recovery by these methods. Therefore, the recovery of C₆₀ from an aqueous C₆₀-PVP formulation using LLE and SPE was assessed.

3.2. Recovery of C₆₀ from C₆₀-PVP with LLE and SPE

Since three of the cosmetic samples (1, 2 and 3) contained fullerenes in the Radical Sponge[®] formulation (Xiao et al., 2007, 2006, 2005), the recovery of C₆₀ from a C₆₀-PVP formula prepared in-house was investigated. C₆₀ was recovered in triplicate from 5 mL of a stock solution of C₆₀-PVP. The concentration of C₆₀ in the C₆₀-PVP stock solution as determined by LLE and SPE was 800 ± 30 and 860 ± 20 $\mu\text{g/L}$, respectively. When C₆₀-PVP was exposed to a cosmetic cream sample not advertised to contain fullerenes (sunscreen) C₆₀ recovery was $50 \pm 13\%$ and $70 \pm 13\%$ for LLE and SPE, respectively. The presence of cosmetic cream impairs the recovery of fullerenes compared to extraction of C₆₀-PVP in water.

The cosmetic matrix can interfere with the extraction and/or the detection of fullerenes via LC-MS. Standard addition was conducted to investigate the interference in LC-MS detection. Toluene solutions from LLE and SPE of blank sunscreen samples (i.e., containing no C₆₀-PVP) were spiked with 110 $\mu\text{g/L}$ of a C₆₀-toluene standard. The detection of this standard in the LLE and SPE toluene solution was impaired by 28 and 33%, respectively. Accounting for these interferences, recoveries of C₆₀ from C₆₀-PVP spiked into the sunscreen using LLE and SPE could be as high as $78 \pm 13\%$ and $103 \pm 13\%$, respectively.

These results suggest that SPE is more efficient than LLE at recovering C₆₀ from the C₆₀-PVP formula in contrast to the recovery of C₇₀ spiked into cosmetics. Therefore, multiple extraction methods were used to quantify C₆₀ in commercial cosmetics where the matrices vary.

3.3. Detection of C₆₀ extracted from cosmetic formulations

Fig. 4 shows the chromatograms from SPEs of A) aqueous PVP, B) a cosmetic cream (sunscreen) without C₆₀, C) aqueous C₆₀-PVP, and D) cosmetic #2 with Radical Sponge[®]. SPE of aqueous PVP results in a low LC-MS response between 1 and 2.5 min retention times for -720 m/z (Fig. 4A), but chromatography efficiently separates these materials from times at which C₆₀ or C₇₀ elute (4 and 6 min., respectively). This small signal (<8 kCts) is considered background noise from SPE and is not attributed to fullerenes. SPE of a cosmetic matrix without C₆₀ (sunscreen) does not yield a -720 m/z peak at 4 min (Fig. 4B), indicating that these methods yield no false-positive C₆₀ detection. However, significant responses occur at m/z ratios -720 , -736 , and -752 between 1 and 4 min, which inhibits the identification of fullerene-based compounds such as C₆₀O and C₆₀O₂ in fullerene-containing cosmetics. Castor oil, a common triglyceride ingredient in other cosmetic samples, was found to increase background signal at fullerene mass-to-charge ratios -720 and -840 (Fig. 5). While these background signals from cosmetic matrices inhibit the identification of fullerene-based compounds that would be retained in the column for less than 4 min, they do not interfere with quantification of pure C₆₀. The chromatogram of a SPE of aqueous C₆₀-PVP (Fig. 4C) shows insignificant background noise at 1–2.5 min similar to Fig. 4A, and C₆₀ is detected at 4 min. This indicates that these methods can recover and identify fullerenes from cosmetics containing the Radical Sponge[®] formulation. Finally, the peak at -720 m/z in the chromatogram from SPE of cosmetic #2, reported to contain the Radical Sponge[®] formulation, demonstrates the extraction of pure C₆₀ (Fig. 4D). The chromatogram also shows peaks for -720 , -736 , and -752 m/z between 1 and 2.5 min, which are potentially explained by 1) background interferences in the cosmetic matrix, 2) fullerene-epoxide molecules, or 3) functionalized/derivatized C₆₀ molecules. More research using standard solutions of fullerene-based molecules would be necessary to investigate any chemical change to the fullerenes used in cosmetics.

3.4. Quantification of C₆₀ extracted from cosmetics

C₆₀ was detected in four out of five cosmetic samples using LLE, toluene-sonication, and SPE (Table 2). Fullerene concentrations in the cosmetics ranged from 0.04 to 1.1 ppm (μg-C₆₀/g-product). Standard additions of C₆₀ were performed on LLE and SPE samples. No matrix interference on detection was observed for LLE of cosmetics 1, 2, and 3. No matrix interference was observed for SPE of cosmetics 2 and 4. SPE resulted in the highest detection of fullerenes for samples 2 and 4, while LLE yielded the highest detection for sample 1 and 3. Neither LLE nor SPE yielded the highest recoveries for both serum and cream matrices. This suggests that varied cosmetic compositions (serum versus cream) and/or cosmetic ingredients require specific extraction techniques to achieve the highest fullerene recoveries.

C₆₀ was not detected in the water-based lotion (sample 5). The fullerenes of this cosmetic may be functionalized differently (i.e., not with PVP) such that recovery of the C₆₀ molecule by these extraction/detection methods is inefficient. C₇₀ was also detected in samples 1 and 2 (Fig. 6) but was below quantification limits (<70 ng-C₇₀/g-cosmetic). These small quantities of C₇₀ could be impurities from the manufacturing process of C₆₀ (Taylor et al., 1990).

3.5. Modeling environmental releases through WWTP

The experimental results confirm that cosmetics 1–4 contain fullerenes (sample 5 remains unconfirmed) detectable as C₆₀. These products are intended for skin application and consequently would result in dermal exposure to fullerenes. Using the highest concentration of C₆₀ detected of 1.1 μg-C₆₀/g-cosmetic, an approximate single-use quantity (0.5 g) of cosmetic might contain up to 0.6 μg of C₆₀. This type of usage information can be used for estimating human exposures and releases of engineered C₆₀ into wastewater systems.

Assuming that 10,000 people release 0.6 μg-C₆₀/day into a 3.79 million liter per day capacity WWTP, the averaged influx of C₆₀ would be 1.6 ng/L (assuming 379 L/capita/day of wastewater flow). The fate of these fullerenes in a WWTP is primarily a function of their adsorption to biosolids, which then settle and are removed from the water prior to discharge of the treated effluent to streams, rivers or lakes. Biosolids adsorption of n-C₆₀ can be six times more efficient than that of C₆₀-PVP (Kiser et al., 2010). Using these adsorption data, and methodology described in the methods section, for a steady-state mass balance model for a 3.79 million liter per day WWTP, the predicted effluent concentration for n-C₆₀ fullerenes is 6×10^{-8} ng/L compared to 2×10^{-6} ng/L if the fullerenes are in C₆₀-PVP form. These effluent concentrations are at least 8 orders of magnitude lower than predicted no effect concentrations previously calculated for fullerenes (Gottschalk et al., 2009, Supporting Information). The concentration of n-C₆₀ or C₆₀-PVP in the biosolids would be 9×10^{-4} mg/kg-biosolids, more than 4 orders of magnitude lower than the predicted no effect concentration (1 mg/kg-soil) for fullerenes in soils (Gottschalk et al., 2009, Supporting Information). While this analysis started with a conservative assumption that everyone in a population uses cosmetics containing fullerenes, the model suggests that environmental release of fullerenes through WWTP effluent and biosolids disposal will be minimal as a result of their use in cosmetics. The model also illustrates that hydrophilic fullerene formulations (i.e., C₆₀-PVP) used in some products may be more difficult to remove during activated sludge wastewater treatment than n-C₆₀ aggregates. Therefore, the use of fullerenes in a wide range of applications in addition to cosmetics may lead to higher concentrations in WWTPs. In fact, modeling efforts that account for many applications of fullerenes have predicted ng/L and μg/kg concentrations in WWTP effluent and biosolids, respectively (Gottschalk et al., 2009).

4. Summary and implications

It is important to note that these methods quantify individual C₆₀ molecules (via extraction into toluene) whereas human and environmental exposures to C₆₀ may occur as fullerene agglomerations (such as in Fig. 1) and/or as other fullerene-based molecules following chemical transformation processes such as oxidation. This possibility must be considered when evaluating fullerene exposure and toxicity studies.

Similar to other chemicals in cosmetics or personal care products that can be found in treated wastewater and downstream water sources, fullerene molecules currently used in cosmetics could be released into wastewater by rinsing the skin after product use. The increased water solubility of some commercial fullerenes may increase their environmental persistence. For example, C₆₀-PVP is more difficult to remove by adsorption to biosolids during wastewater treatment than is aqueous n-C₆₀ (Kiser et al., 2010). The results presented here suggest that commercial applications provide a pathway for engineered fullerenes to enter the environment.

If some commercial fullerenes are likely to be released and persist in aquatic environments, products can be designed to use fullerenes with discretion. With the ability to quantify fullerenes in products and various environmental matrices (e.g., water, soil, biological samples), research can evaluate the minimum quantity of fullerenes necessary to produce a desired function in products. This will help minimize the excess environmental release of fullerenes.

Acknowledgments

This work has been funded through the Urban Ecology IGERT at Arizona State University (NSF Grant #0504248), the Paul L. Busch Award from the Water Environment Research Foundation (Westerhoff recipient), and the EPA STAR Grant (#RD833322). This work was also supported by the NIH Grand Opportunities (RC2) program through NIEHS grant DE-FG02-08ER64613. TEM images were acquired by David Lowry at the Bioimaging Facility within the School of Life Sciences at Arizona State University. James Hutchings provided support with LC-MS operation. The authors thank Frank von der Kammer for useful discussions during the development of these experiments. The work presented here reflects the views of the authors only.

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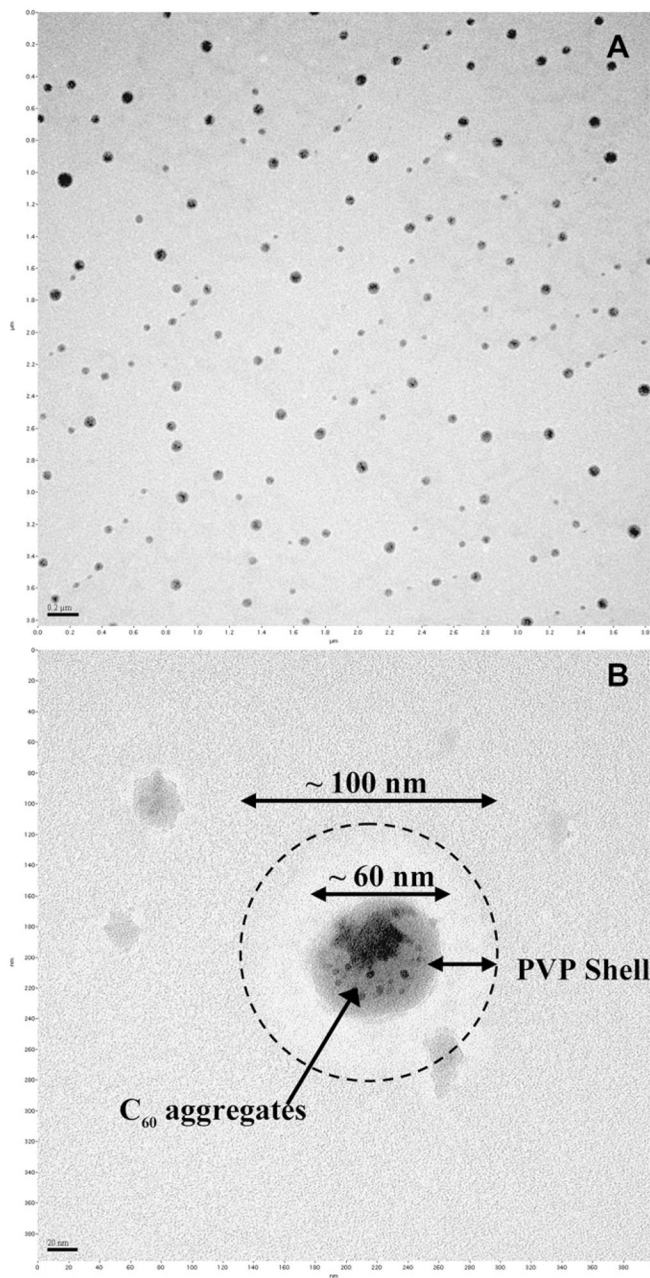


Fig. 1.
A) TEM images of spherical C₆₀-PVP agglomerations 50–100 nm in diameter (scale bar = 0.2 μm). B) Higher magnification shows that fullerenes (dark particles) appear to be wrapped within a PVP structure (scale bar = 20 nm).

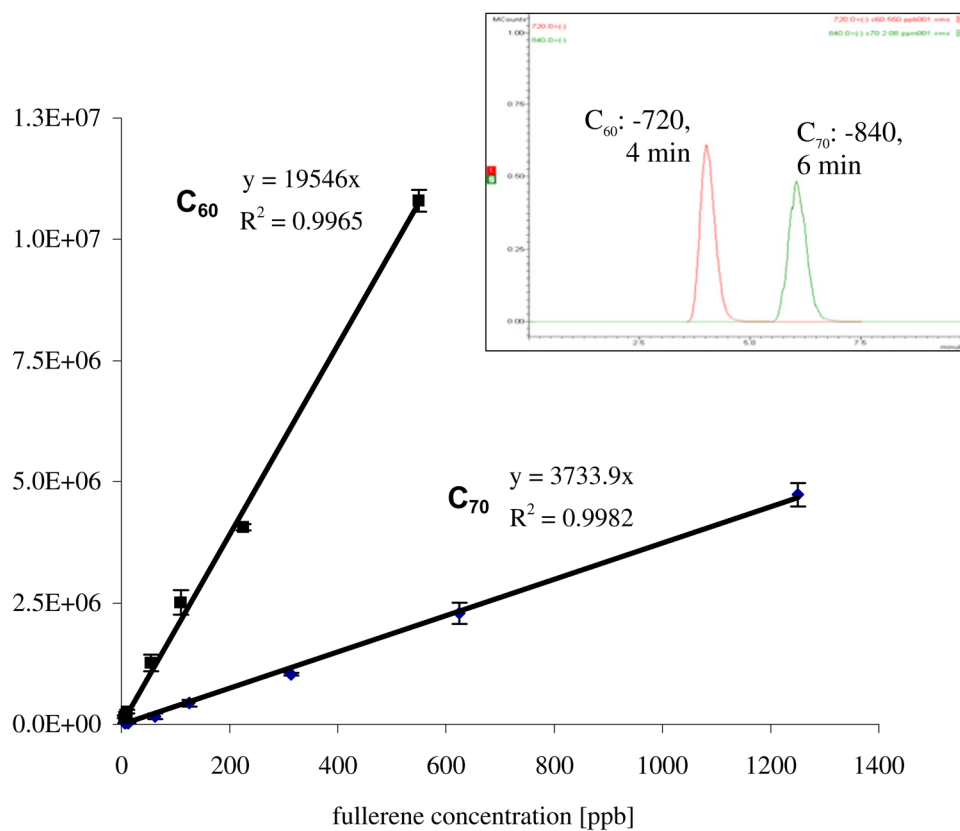


Fig. 2. LC-MS calibration curves for C₆₀ and C₇₀ standards in toluene. Instrument detection limits based on 3× the background signal are 3 ppb and 12 ppb for C₆₀ and C₇₀, respectively. Error bars represent three analyses of each standard. Inset: C₆₀ (−720 *m/z*) and C₇₀ (−840 *m/z*) elution peaks at 4.0 and 6.0 min., respectively.

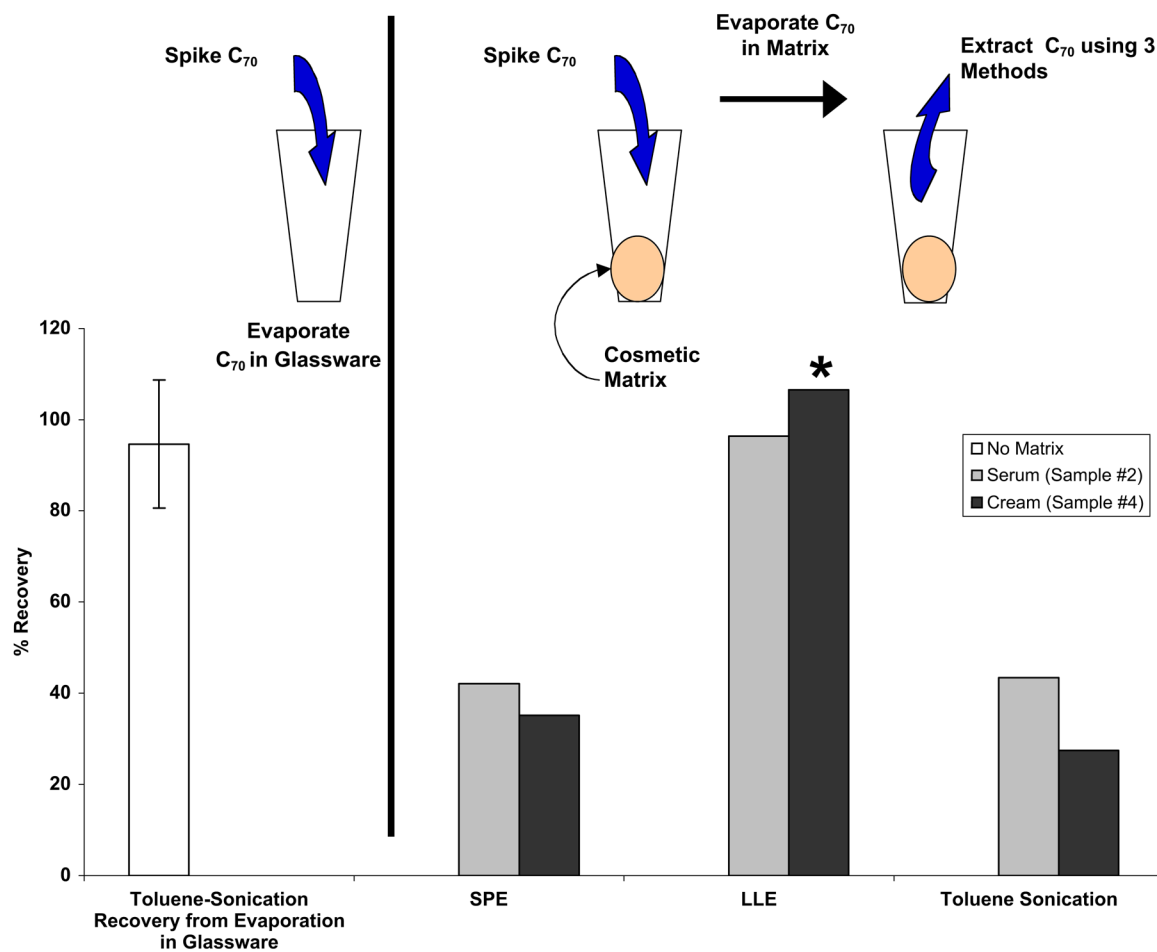


Fig. 3.

Comparison of three extraction methods to recover C_{70} ($4-7 \mu\text{g-C}_{70}/\text{g-cosmetic}$) spiked into two types of cosmetic matrices (serum and cream). C_{70} was administered in toluene, allowed to deposit in the matrix, then extracted. The first column represents triplicate recoveries of C_{70} allowed to deposit in a glass vial with no matrix. * Recovery adjusted for 47% matrix inhibition of LC-MS detection of fullerenes.

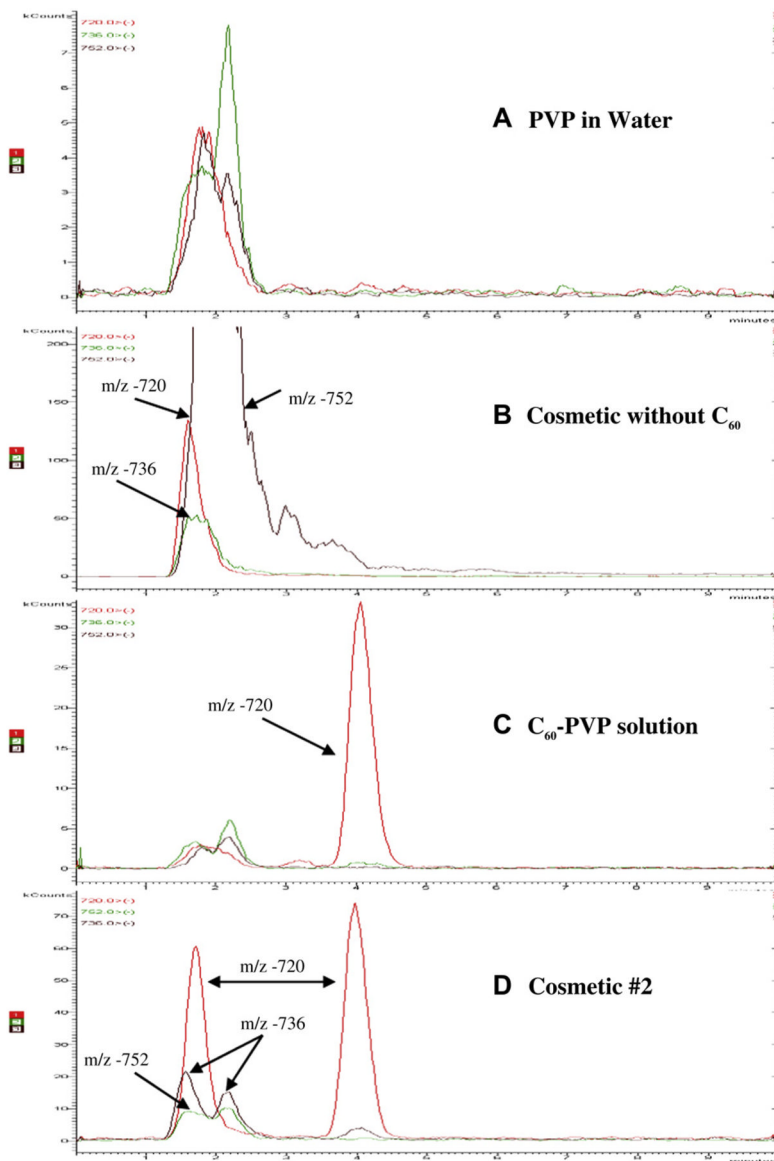


Fig. 4. Chromatograms of m/z -720, -736, and -752 for solid phase extractions of A) PVP aqueous solution, B) a cosmetic (sunscreen) without C_{60} , C) C_{60} -PVP aqueous solution, and D) cosmetic #2 with Radical Sponge[®]. Elution time is plotted on the x -axis up to 10 min. The y -axis (MS signal in kilocounts) maximum is 8, 200, 35, and 75 for A, B, C, and D, respectively.

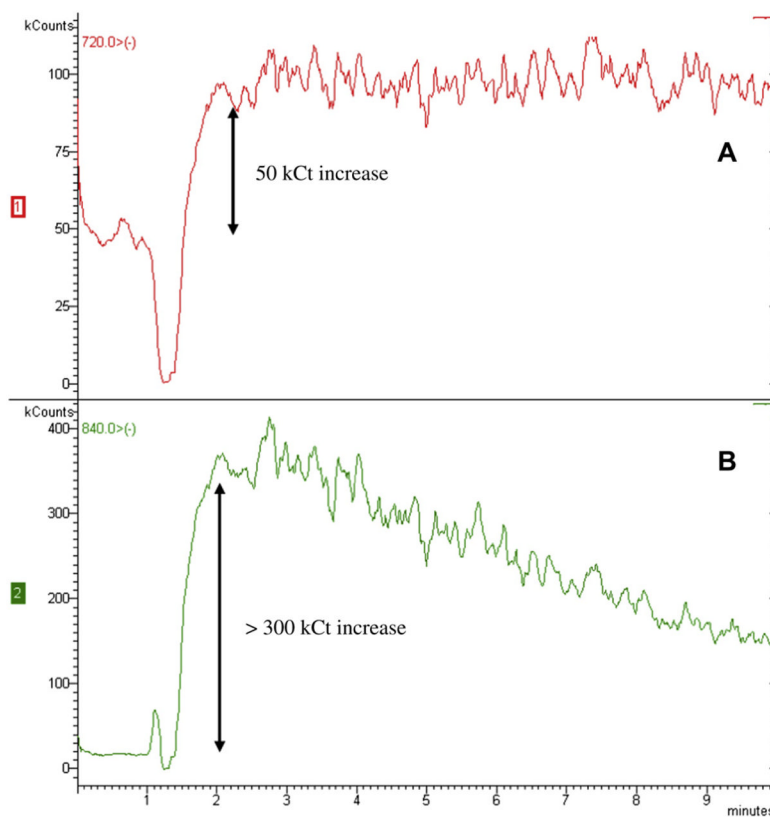


Fig. 5. Chromatograms showing the increase in background signal at A) –720 (50 kCt) and B) –840 m/z (>300 kCt) due to toluene sample containing 1% castor oil.

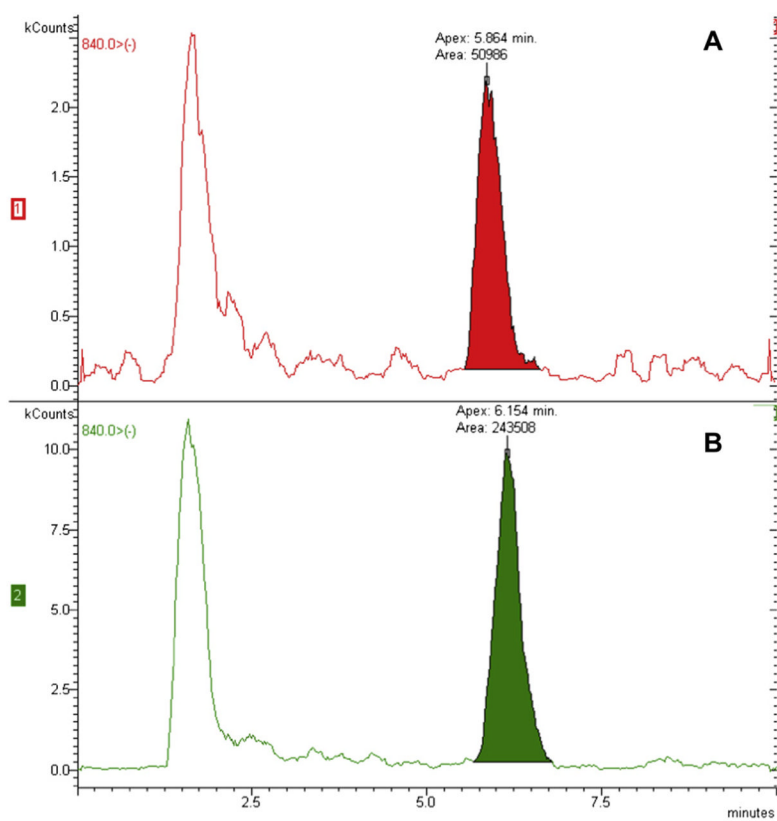


Fig. 6. Chromatograms of A) cosmetic #2 and B) cosmetic #1 showing qualitative detection of C₇₀ at ~6 min for m/z -840.

Table 1

Cosmetic sample characterization.

Sample ID	Commercial name	Intended use	Fullerene ingredient	Matrix appearance
1	Dr. Wu Whitening System	Antioxidant whitening face serum	“Radical Sponge®”	Clear, water-based serum
2	Jeudisurpris Essence	Clinical lift face serum	“Radical Sponge®”	Clear, water-based serum
3	Derma Science DP EST Charge Plus	Face cream	“Radical Sponge®”	Dense, opaque cream
4	Dr. Brandt Laser Lightning Day Lotion	Anti-aging face cream	“fullerene”	Dense, opaque cream
5	Dr. Brandt Laser Lightning Toner	Anti-aging skin toner	“fullerene”	Water-based lotion

Table 2Mass of C₆₀ detected in cosmetics [$\mu\text{g-C}_{60}/\text{g-product}$].

Sample ID	Matrix	LLE	Toluene sonication	SPE
1	Serum	1.0 \pm 0.3 ^a	0.40	0.42 ^a
2	Serum	ND	0.04	0.24 ^a
3	Cream	1.1 \pm 0.2	0.48	0.04
4	Cream	ND	ND	0.04
5	Water	ND	NA	ND

ND – No Detection.

NA – Not Analyzed.

^aC₇₀ also qualitatively detected at a retention time of 6 min but was below quantification limits.