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Determination of fullerenes (C60) in artificial sediments by liquid chromatography

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ABSTRACT

In this new century, nanotechnology has evolved from a novel concept to an integral aspect of product advancement. With an increasing presence of nanomaterials in commercial products, more concern about the impact of nanomaterials on human health and also the environment has been considered and evaluated. Fullerenes (C60), have been studied in several different areas and applied widely. Wider application of fullerenes into different products in the recent decades has increased the potential of fullerene releases into the environment. Fullerene research involves physical and chemical characteristics, toxicity, environment fate, and interaction with other pollutions. However, few studies have addressed fullerene quantification in solid matrices. Standardized artificial sediment was prepared following OECD guideline 225, and extracted C60 was quantified by HPLC–UV. A normal shaking method was employed for extraction for two times. Extracts were concentrated and analyzed. Recovery results revealed up to 90.7 ± 4.5%, 90.0 ± 3.8%, 93.8 ± 5.4%, respectively for 1.62, 0.65, and 0.32 μg/g C60 in dry sediment, which shows no significant difference between different concentration levels. Furthermore, extraction efficiency did not show significant difference while using Telfon^{TM} tubes (96.5 ± 6.0%) or silanized glass vessels (90.7 ± 4.5%). This indicated that relative low cost is required for the method to be initially started in any lab. This technique has also been applied in the determination of C60 in sediment samples collected after a 10 day benthic exposure study. Extraction precision has been increased from 4.5% (S.D.) as the validation value up to 15.4% (RSD%) or more. The increased inhomogeneity by bioturbation and matrix complexity of the sediment after the toxicity test could both lower the extraction precision.

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1. Introduction

In this new century, nanotechnology has matured from a novel concept to an integral aspect of product advancement. Mass production of nanomaterials has been involved in important industrial applications, including textiles, cosmetics, tires, electronics, aerospacial materials, and also the medical products. [1–4] With an increasing presence of nanomaterials in commercial products, more concern about the impact of nanomaterials on human health and also the environment has been considered and evaluated. [1,5] In nanomaterials related regulations, dose responses of nanomaterials are considered to be the same as macromaterials. For this reason, human and ecological responses are often assumed to be the same for macromaterials and nanomaterials. [6–8] However, it is a challenge to perform such an analysis with insufficient empirical data. In fact, it is difficult to investigate the environmental fate of nanomaterials due to their variable aggregation, suspension and reactivity when released into the environment. [1,9,10] Therefore, an analysis of organismal exposure to nanomaterials in the environment and their related environmental transport warrants serious evaluation.

Fullerenes (C60), have been studied in several different areas and applied widely. This nano-sized group of carbon allotropes has important physical properties that depend upon their chemical composition [1]. Wider application of fullerenes into different products in recent decades has increased the potential of fullerene release into the environment [1,9,10]. Fullerene research involves physical and chemical characteristics [11–14], toxicity [14–19], and environment fate of fullerenes [20–23]. Interaction with other pollutions in the environment [24–26] have been evaluated in some instances. However, few published methods require no sample pretreatment while using low solvent volume for extraction and allowing simple instrumental process for fullerene extraction and quantification in solid matrices. [27–30]. The critical review by Isaacson et al. [31] summarized the existing method that has been applied or developed to extract fullerenes from different matrices.

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Shareef et al. [28] used an Accelerated Solvent Extraction Method to extract fullerenes from soil sample, which requires advanced instrumental process, cautious pretreatment and sample preparation. Heymann et al. [29] described two approaches of extracting fullerenes. One of them used sonication procedures, and the other one used the Soxhlet method. The Soxhlet method is effective with 80 ± 9% recovery but inefficient with long time and large solvent volume consumption. The sonication method did not indicate the extraction recovery which also requires sample demineralization. Jehlicka et al. [30] applied Soxhlet method in recovering fullerenes from geological materials as well. However, the reported recovery is not stable with a high deviation.

Natural sediments have complex characteristics that complicate efficient extraction methods. Different percentage organic matter in solid matrices controls sorption of organic contaminants to sediment [32]. Various sediment compositions could alter extraction efficiency. In this study, artificial sediment is used to develop a simple and effective analytical method for fullerene quantification. Artificial sediments are often employed for method development to allow better control and characterization of the sediment components. It was also used in the latter C60 uptake experiment to validate the applicability of the method and to evaluate the consistency of the test media.

In this paper, we present a validated method of fullerene extraction with consideration of major components in solid matrices using simple but high recovery procedures with an alternative HPLC column for UV detection. This method has less solvent, instrument and supply requirements which allow a potentially wider application in fullerene extraction and quantification.

2. Experimental

2.1. Chemical and reagents

Fullerenes (99.5%) were purchased from Aldrich Sigma. Toluene (HPLC grade) was purchased from JT Baker. Sand was obtained from Fisher Scientific (standard Ottawa), and kaolin clay was from MP (CAS 1332-58-7). Peat moss was obtained from Lowes (Lubbock, TX). Water (>18 MΩ) was obtained from a Barnstead NANOpure infinity system (Dubuque, IA, USA). Teflon syringe filters in size of 0.2 μm was obtained from Fisher Scientific.

Artificial sediment was prepared by the description in OECD Method 225 [33]. Artificial sediment was characterized by Midwest Laboratory. Sediment contained 85.5% sand, 4.5% slit, and 10% clay by average. Sand (0.5 g) was spiked with toluene that contained 17.5 μg C60 and mixed thoroughly with 10 g dry sediment in a tumbler for 3 h. Spiked sediment was air-dried at least 48 h to evaporate toluene.

2.2. Normal shaking extraction (NSE) procedure

Normal shaking extraction (NSE) was used to extract fullerenes from sediment. Toluene (40 mL) was added, and the slurry was mechanically shaken for 60 min at 350 rpm. The mixture was centrifuged allowing supernatant to be collected and filtered through 0.2 μm PTFE filter. Extraction was repeated, and 8 mL ultra-pure water was added before the centrifugation. Because of the solvent loss of 5–10 mL after the first cycle of extraction, ultra-pure water is added to segregate toluene from the sediment matrix for a full solvent recovery. The upper solvent layer was filtered and combined with the first extract. Total extracts were sonicated, and 10 mL of the total extract was evaporated using nitrogen evaporator. Final volume was adjusted to 2 mL and transferred into an autosampler vial for HPLC analysis.

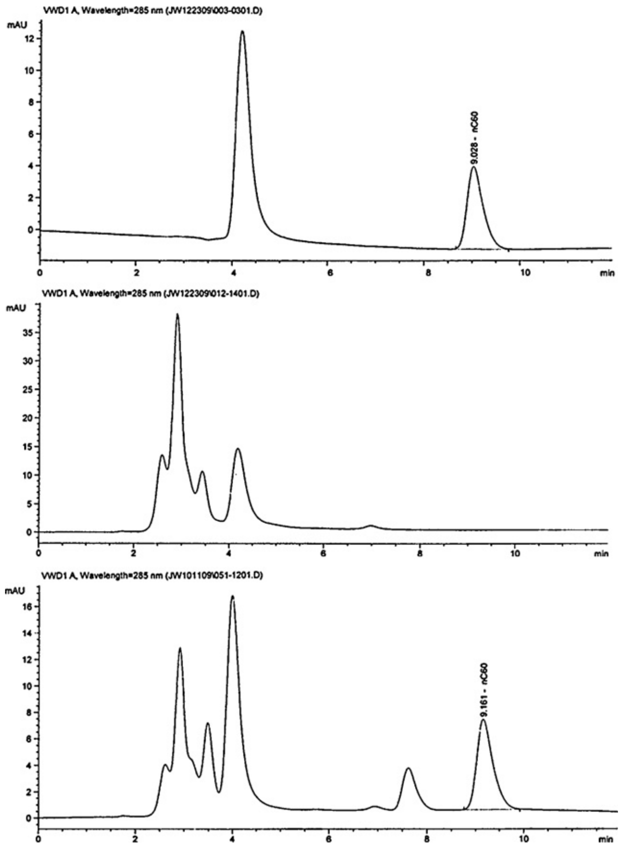

Fig. 1. Representative liquid chromatograms of: (a) C60 standard in toluene; (b) a blank sample of artificial sediment; (c) C60 extracted from the spiked sediment.

2.3. LC–UV analysis

HPLC–UV (Aglient series 1100) was used to quantify fullerene concentrations in sediment extracts. Chromatographic separation was achieved at room temperature using a gel column from Waters (COSMOSIL 5PBB, 250 mm × 4.6 mm, 5 μm packing). The isocratic mobile phase was 100% toluene, flowing at a rate of 1 mL/min. The injection volume was 25 μL. UV absorbance was monitored at 285 nm. In this LC–UV analysis program, fullerenes have a retention time at 9.0 ± 0.2 min at room temperature (Fig. 1a).

2.4. Method optimization

Fullerene standards were prepared in toluene without filtration. Calibration was linear with excellent correlation coefficients (>0.99) for each analysis in the range of both low concentration range of 36–100 μg/L and high concentration range of 0.1–15 mg/L.

Four different groups of five replicate sediment aliquots were prepared at each of three concentrations to determine method recovery, precision and reproducibility. These experiments, used silanized glass vials as extraction vessels. Precision was represented by the standard deviation (S.D.), and standard error (S.E.). Both variance measurements are presented to allow evaluation of overall method performance (S.D.) and comparison of means for different extraction procedures (S.E.). To evaluate the effect of the C60 sorption to extraction apparatus, a further validation was performed using Teflon^{TM} (PTFE) tubes. This test was limited to three groups of five replicates at one concentration. Different people extracted the C60 from the sediment for each group of sediments within this method validation. This is designed to predict a recovery difference in method application. The instrumental detection limit for fullerenes was 18 μg/L C60 (S/N ≥ 3), and the quantification limit

Table 1
Recovery of fullerenes extraction in sediment using silanized glass extraction vessels.

Groups	Concentration of nC60 spiked into artificial sediment [μg/g]								
	1.62			0.65			0.32		
	Mean	S.D.	S.E.	Mean	S.D.	S.E.	Mean	S.D.	S.E.
1	90.2	2.6	1.1	$90.7^{a,b}$	2.8	1.3	99.4^{c}	2.9	1.3
2	87.7	2.9	1.3	85.7^{a}	4.2	1.9	86.8^{d}	0.8	0.4
3	90.7	6.9	3.1	93.0^{b}	2.1	0.9	95.0^{c}	3.4	1.5
4	94.3	2.7	1.2	$90.5^{a,b}$	2.3	1.0	94.0^{c}	3.9	1.7
Average	90.7	4.5	1.0	90.0	3.8	0.7	93.8	5.4	1.2

Each group has 5 replicates for extraction and quantification. a,b: means in 0.65 μg/g group with the same letter shows no significant difference by Tukey HSD test. c,d: means in 0.32 μg/g group with the same letter shows no significant difference by Tukey HSD test.

was 36 μg/L C60 ($S/N \geq 6$). These threshold limits translate to an instrument quantification limit of 0.15 mg/kg C60 for 10 g of dry sediment.

To investigate matrix effects, spiked components of the sediment (sand, clay, and peat moss) were extracted according to the same procedure described above. Recoveries of each component were calculated and compared.

Blank sediment was extracted applying the protocol as well. HPLC analysis was to ensure the extracts from sediment would not influence the elution of fullerenes.

2.5. Application to toxicity tests

To further test the developed method, sediment samples were obtained from a 10 day benthic toxicity test involving the exposure of (*Lumbriculus variegatus*) to fullerenes. Fullerenes (C60) were spiked into four replicate sediments and conditioned in water for 14 days. Samples were collected and air dried after the exposure and stored at −20 °C. Dry sediment (10 g) from each replicate was collected after exposure and followed the extraction procedures for quantitative determination.

2.6. Statistics analysis

Extraction efficiency was compared using one way ANOVA between and among groups of three concentrations and two types of extraction vessels using SPSS software.

3. Results and discussion

Within the respective retention window, a blank sediment extract shows no interference peaks. Blank extracts indicate that chemicals extracted from the sediment neither coelute with nor alter the resolution of C60 (Fig. 1b and c). Fullerene recoveries from individual replicates of the three tested concentrations, ranged from 85.7% to 99.4% (Table 1). Mean recoveries were 90.7 ± 4.5%, 90.0 ± 3.8%, and 93.8 ± 5.4% from sediments containing 1.62, 0.65, and 0.32 μg/g C60, respectively. No significant differences in recovery were determined between sediments of different concentrations ($p = 0.368$), demonstrating the reliability and reproducibility of our extraction method within the tested concentration range. However, some intra-group differences were found ($p = 0.009$, $p < 0.001$) for 0.65, and 0.32 μg/g, respectively. Relatively low recoveries of 85.7 ± 4.2% and 86.8 ± 0.8% were observed within the concentration of 0.65 and 0.32 μg/g, respectively. Differences in operators' techniques on different days are possibly the main reason that demonstrates the variance of method performance. In application of the method, skilled operators are needed to reduce the variance of recovery.

Table 2
Recovery of fullerenes[a] from sediment using Teflon ™ extraction vessels.

Groups	Extraction apparatus types					
	Teflon™ (PTFE) tube			Silanized Glass Vial		
	Mean	S.D.	S.E.	Mean	S.D.	S.E.
1	104	2.7	1.2	90.2	2.6	1.1
2	91.2	2.0	0.9	87.7	2.9	1.3
3	94.5	1.8	0.8	90.7	6.9	3.1
4	–	–	–	94.3	2.7	1.2
Average	96.5	6.0	1.5	90.7	4.5	1.0

[a] 1.62 μg/g fullerenes in spiked sediment.

Table 3
Recovery of fullerenes[a] from sand, clay, and peat.

Components	Extraction efficiency %		
	Mean	S.D.	S.E.
Sand	101	1.6	0.9
Clay	77.6	1.2	0.7
Peat	72.3	1.3	0.7

Each group has 3 replicates for extraction and quantification.
[a] 1.7 μg/g fullerenes in spiked sediment.

Validation using Teflon™ containers was performed for 1.62 μg/g C60 in sediment. Mean recovery of C60 using Teflon™ tubes was 96.5 ± 6.0% (91.2–104%). It shows no significant differences ($p = 0.166$) in recovery as compared to the mean value using silanized glass vessels (90.7 ± 4.5%). Similar recoveries using two vessel types demonstrate the cost savings that can be realized if glass extraction containers are used to perform this method. Glass vials are available in most analytical labs, and are far less expensive than Teflon™ if materials are being purchased for initial method use. For our sample analysis, 10 samples were extracted for each day extraction. The cost of 40 mL glass vials from I-Chem is about $40 per 10 items, while 45 mL centrifuge Teflon™ tube is about $450 per 10 items. It would be convenient to have 20 vessels available to perform the extraction procedure for batches. Therefore, using glass vials to conduct the extraction procedure would reduce initial costs $820 for processing samples with this extraction process (Table 2).

Since natural sediments have a wide range of chemical and bulk characteristics, a matrix effect investigation was performed. Average recovery for the individual components of artificial sediment, sand, clay and peat decreased from 100%, 78% to 72%, respectively (Table 3). Recoveries from each component are also significantly different from each other ($p < 0.001$). These respective results indicate the applicability of the method in handling different types of solid matrix and its potential influences with different percentage of organic matters in real samples. Additionally, extraction efficiency with each component was used to project a theoretical recovery of C60 from artificial sediment (Table 4). Computations estimated a C60 recovery of 97.0 ± 1.4%, which is higher than the experimental results as of 90.7 ± 4.5%. The possible reason for this difference could be due to the complexity of sediment as a mixture of different components in the matrix. Quartz sand surface

Table 4
Computation of nC60 recovery from artificial sediment upon the recovery efficient of each component in the sediment matrix.

Components	Mean composition%	Mean recovery %	Predicted recovery %	Experimental recovery %
Sand	85.5 ± 4.7	101 ± 1.6	97.0 ± 1.4	83.9–102
Clay	4.5 ± 3.0	77. 6 ± 1.2		
Peat	10 ± 4.3	72.3 ± 1.3		

Table 5
Concentration of fullerenes in the spiked sediment after exposure determined by application of extraction protocol in a preliminary 10-day fullerene exposure test.

nC60 concentration [mg/kg dw]		
Nominal	Determined after exposure[a]	RSD (%)[b]
0.05	0.03 ± 0.02	71.4
0.13	0.08 ± 0.04	50.6
0.44	0.47 ± 0.07	14.7
1.51	1.27 ± 0.15	11.8
3.67	2.53 ± 0.39	15.4
11.33	9.36 ± 1.25	13.4

Each group has 4 replicates for extraction and quantification.

[a] Mean ± S.D., $n = 4$.

[b] RSD (%) = S.D./mean × 100%.

chemistry may have been changed due to the surface coating by both peat and clay. A smaller fraction of pristine sand in the mixture would lower recovery due to stronger sorption by the peat or clay in the matrix. Moreover, our data suggest different extraction efficiency can be expected for sediments or soils with different compositions. In application of this method to a higher organic content solid matrix, more extraction cycles could be applied to increase the extraction efficiency. Following worst case scenario of 100% clay, 72% recovery was reached. In order to obtain a higher recovery up to 80%, 3 extraction cycles is suggested following the residual analyte rules of $(1-x)^n$. Conversely, increasing temperature may not be ideal to increase the extraction efficiency due to its negative enthalpy of solubility maxima. In contrast to the conventional wisdom of increasing temperature for higher recovery, fullerenes behave in the opposite way. [31,34,35]

We extracted and analyzed sediment samples that were collected at the termination of a 10 d benthic exposure study. Quantification of C60 in the sediment ($n = 4$) containing nominal concentration of 0.05–11.33 mg/kg indicated means of 0.03 ± 0.02, 0.08 ± 0.04, 0.47 ± 0.07, 1.27 ± 0.15, 2.53 ± 0.39, and 9.36 ± 1.25 mg/kg, respectively (Table 5). Precision (RSD%) of the extraction (11–71%) is higher than was observed for the method validation (4–6%). Results show a higher RSD% of 71.4% and 50.6% in groups of lower concentration 0.05 and 0.13 mg/kg C60, respectively, while producing RSD% of 14.7%, 11.8%, 15.4% and 13.4% in groups of higher concentration 0.44, 1.51, 3.67, and 11.3 mg/kg C60, respectively.

The function describing the linear relationship between C60 concentration in sediment and reproducibility (RSD%) (Fig. 2) in concentration range of 0.05–0.13 mg/kg is:

$$y = 84.4 - 260x$$

The function describing the linear relationship between C60 concentration in sediment and reproducibility (RSD%) in concentration range of 0.44–11.33 mg/kg is:

$$y = 13.9 - 0.0236x$$

where x is the C60 concentration in dry sediment and y is the recovery reproducibility (RSD%).

These two recovery functions intersect at the point (0.27 mg/kg dw, 14.4%). These results suggest a threshold concentration of 0.27 mg/kg dw C60 in sediment is required to allow a quantifiable concentrations (RSD% < 15%) using this method. Concentrations lower than 0.27 mg/kg dw, produced poorer precision (RSD% > 50%). Limit of quantification (LOQ) is defined as S/N = 6, which implies a threshold of 16.6% (RSD%). Therefore, the 15% (RSD%), as the LOQ in the sediment experiments, is comparable to the estimated LOQ of 0.15 mg/kg determined in the method validation. Moreover, the higher RSD% observed for extraction of trial samples rather than the method validation samples could be due to the different spiking volume of the sample, as well. In the method optimization, we spiked each 10 g sample, which was totally extracted afterwards. However, in the exposure experiment, we spiked a larger mass (about 100 g) of sediment for each replicate, and only a 10 g portion of was collected for extraction. Fullerene extraction from sediment samples after toxicity test has a lower extraction precision compared to the method validation. It could be due to the matrix heterogeneity, aging, and/or complexity. Bioturbation of sediment by *L. varigatus* could increase the inhomogeneity of the sediment in comparison with the matrix prior to the toxicity test.

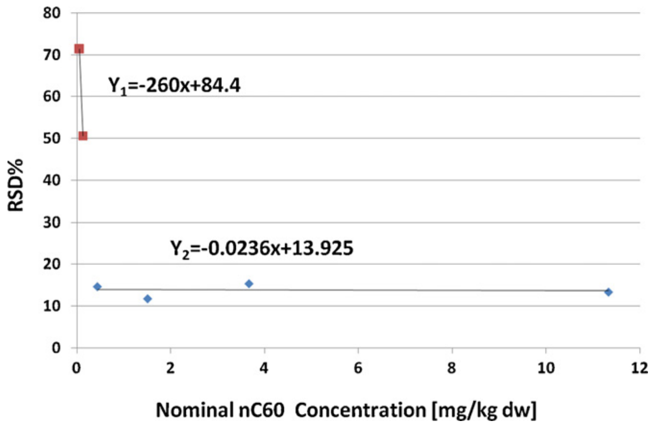

Fig. 2. Function of precision (RSD%) of nominal C60 concentration [mg/kg dw] in range of 0.05–11.33 mg/kg dw.

4. Conclusion

This study describes a simple, inexpensive and reproducible method to quantify fullerenes (C60) in artificial sediment samples. The procedure demonstrated high recoveries (83.9–102%) at all three tested concentrations (1.62, 0.65, and 0.32 μg/g dry sediment) for the analyte. Using less expensive (silanized glass) vessels allowed high recovery as (83.9–102%) compared to Teflon™ tubes (88.0–108%). This method has been applied in further experiments for C60 determination from sediment.

References

[1] V.L. Colvin, Nat. Biotechnol. 21 (10) (2003) 1166–1170.
[2] R.J. Aitken, et al., Occup. Med. (Lond.) 56 (5) (2006) 300–306.
[3] R.D. Handy, R. Owen, E. Valsami-Jones, Ecotoxicology 17 (5) (2008) 315–325.
[4] R.A. Freitas, Nanomed. Nanotechnol. Biol. Med. 1 (1) (2005) 2–9.
[5] R. Owen, M. Depledge, Mar. Pollut. Bull. 50 (6) (2005) 609–612.
[6] EPA, Resource Conservation and Recovery Act, EPA, 1976.
[7] EPA, Toxic Substances Control Act, EPA, 1976.
[8] OSHA, Occupational Safety & Health Act, OSHA, 2004.
[9] A. Baun, et al., Ecotoxicology 17 (5) (2008) 387–395.
[10] B.M. Simonet, M. Valcarcel, Anal. Bioanal. Chem. 393 (1) (2009) 17–21.
[11] L.K. Duncan, J.R. Jinschek, P.J. Vikesland, Environ. Sci. Technol. 42 (1) (2008) 173–178.
[12] J.D. Fortner, et al., Environ. Sci. Technol. 39 (11) (2005) 4307–4316.
[13] X. Ma, D. Bouchard, Environ. Sci. Technol. 43 (2) (2009) 330–336.
[14] C.M. Sayes, et al., Biomaterials 26 (36) (2005) 7587–7595.
[15] S.B. Lovern, R. Klaper, Environ. Toxicol. Chem. 25 (4) (2006) 1132–1137.
[16] E. Oberdöster, Environ. Health Perspect. 112 (10) (2004).
[17] E. Oberdöster, et al., Carbon 44 (6) (2006) 1112–1120.
[18] X. Zhu, et al., J. Nanopart. Res. 11 (1) (2009) 67–75.
[19] T.B. Henry, et al., Environ. Health Perspect. 115 (7) (2007) 1059–1065.
[20] Q. Li, et al., Environ. Sci. Technol. 43 (10) (2009) 3574–3579.
[21] D. Li, et al., Environ. Toxicol. Chem. 27 (9) (2008) 1888–1894.
[22] Y. Wang, Y. Li, K.D. Pennell, Environ. Toxicol. Chem. 27 (9) (2008) 1860–1867.
[23] A. Baun, et al., Aquat. Toxicol. 86 (3) (2008) 379–387.
[24] B. Espinasse, E.M. Hotze, M.R. Wiesner, Environ. Sci. Technol. 41 (21) (2007) 7396–7402.
[25] J. Gao, et al., Environ. Sci. Technol. 43 (9) (2009) 3322–3328.

[26] B. Xie, et al., Environ. Sci. Technol. 42 (8) (2008) 2853–2859.
[27] C.Y. Chen, C.T. Jafvert, Environ. Sci. Technol. 43 (19) (2009) 7370–7375.
[28] A. Shareef, G. Li, R.S. Kookana, Environ. Chem. 7 (3) (2010) 292–297.
[29] d. Heymann, et al., Cretaceous Res. 17 (1996) 367–380.
[30] J. Jehlicka, et al., Carbon 43 (9) (2005) 1909–1917.
[31] C.W. Isaacson, M. Kleber, J.A. Field, Environ. Sci. Technol. 43 (17) (2009) 6463–6474.
[32] P. Grathwohl, Environ. Sci. Technol. 24 (11) (1990) 1687–1693.
[33] OECD, Test No. 225: Sediment-Water Lumbriculus Toxicity Test Using Spiked Sediment, OECD Guildlines for the Testing of Chemicals, Section 2: Effects on Biotic Systems, OECD, 2007.
[34] K.M. Kadish, R.S. Ruoff, Fullerenes: Chemistry, Physics and Technology, Wiley-Interscience, New York, 2000.
[35] R.S. Ruoff, et al., Nature 362 (6416) (1993) 140–141.