Quantification of fullerene aggregate $nC_{60}$ in wastewater by high-performance liquid chromatography with UV–vis spectroscopic and mass spectrometric detection

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\section{1. Introduction}

Fullerenes, a class of carbonaceous nanomaterials existing as hollow spheres, ellipsoids or tubes, possess several promising features such as high strength, special electrical and optical properties, radical scavenging capability and others. These features are expected to lead to their wide use in new high-strength materials, electronics, optics, biomedical and cosmetic applications (Jensen et al., 1996; Innocenzi and Brusatin, 2001; Sherigara et al., 2003; Tenne and Seifert, 2009). The expected industrial-scale production is expected to lead to their wide use in new high-strength materials, while SPE required filtration or reclaimed wastewater and secondary effluent of less suspended solids. Calibration curves plotted as peak areas of UV absorbance at 332 nm against spiked $nC_{60}$ concentrations showed good linearity over a range of 20–200 µg L\textsuperscript{-1} after 10-fold concentration by LLE, but only over the range of 0.8–2 µg L\textsuperscript{-1} for reclaimed wastewater and 0.8–4 µg L\textsuperscript{-1} for secondary effluent after 1000-fold concentration by SPE. Recoveries of $nC_{60}$ by LLE were in the range of 89–94% with a standard deviation (SD) not more than 2% and recoveries of $nC_{60}$ by SPE were much lower, only 18% for reclaimed wastewater and 9% for secondary effluent. The method detection limits (MDLs) of LLE with UV–vis spectroscopy were 3–4 ng L\textsuperscript{-1} for six water matrices and the MDLs of SPE with UV–vis spectroscopy were 0.42 µg L\textsuperscript{-1} for reclaimed wastewater and 0.64 µg L\textsuperscript{-1} for secondary effluent. UV–vis spectroscopy and mass spectrometry gave similar sensitivity. With LLE, mass spectrometry offered a small linear range of 20–60 µg L\textsuperscript{-1}, but it provided specificity based on the mass-to-charge ratios (m/z) of the molecular ions. This paper demonstrates the feasibility of the combination of different extraction and detection methods to quantify $nC_{60}$ in engineered wastewater matrices.

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This paper evaluates the performance of liquid–liquid extraction (LLE) and solid phase extraction (SPE) in separating and concentrating aqueous fullerene ($nC_{60}$) from wastewater and compares UV–vis spectroscopy and mass spectrometry for the quantification of $nC_{60}$, LLE was suitable for multiple wastewater matrices, while SPE required filtration or reclaimed wastewater and secondary effluent of less suspended solids. Calibration curves plotted as peak areas of UV absorbance at 332 nm against spiked $nC_{60}$ concentrations showed good linearity over a range of 20–200 µg L\textsuperscript{-1} after 10-fold concentration by LLE, but only over the range of 0.8–2 µg L\textsuperscript{-1} for reclaimed wastewater and 0.8–4 µg L\textsuperscript{-1} for secondary effluent after 1000-fold concentration by SPE. Recoveries of $nC_{60}$ by LLE were in the range of 89–94% with a standard deviation (SD) not more than 2% and recoveries of $nC_{60}$ by SPE were much lower, only 18% for reclaimed wastewater and 9% for secondary effluent. The method detection limits (MDLs) of LLE with UV–vis spectroscopy were 3–4 ng L\textsuperscript{-1} for six water matrices and the MDLs of SPE with UV–vis spectroscopy were 0.42 µg L\textsuperscript{-1} for reclaimed wastewater and 0.64 µg L\textsuperscript{-1} for secondary effluent. UV–vis spectroscopy and mass spectrometry gave similar sensitivity. With LLE, mass spectrometry offered a small linear range of 20–60 µg L\textsuperscript{-1}, but it provided specificity based on the mass-to-charge ratios (m/z) of the molecular ions. This paper demonstrates the feasibility of the combination of different extraction and detection methods to quantify $nC_{60}$ in engineered wastewater matrices.

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et al., 2006; Isaacson et al., 2007; Bouchard and Ma, 2008; Chen et al., 2008; Hyung and Kim, 2009) and solid phase extraction (SPE) (Santa et al., 1995; Bouchard and Ma, 2008; Chen et al., 2008) are applied to extract and concentrate \( n_{C60} \) from these matrices into toluene. Extraction also partially excludes potential interfering materials. High-performance liquid chromatography (HPLC) coupled with UV–vis spectroscopy at 300–400 nm (Santa et al., 1995; Moussa et al., 1997; Xia et al., 2006; Bouchard and Ma, 2008; Hyung and Kim, 2009) and/or mass spectrometry at negatively charged m/z of 720 (Santa et al., 1995; Moussa et al., 1997; Isaacson et al., 2007; Chen et al., 2008) are used to quantify \( C_{60} \)–toluene extracts. A review on \( C_{60} \) analysis is available (Isaacson et al., 2009). Only one occurrence study of wastewater treatment plant (WWTP) effluents has found measurable levels of \( n_{C60} \), reporting levels up to nearly 20 \( \mu g L^{-1} \), although the sources for this high concentration were poorly justified (Farré et al., 2010). The main challenge for the quantification of \( n_{C60} \) in wastewater is the extraction and separation of \( n_{C60} \) from wastewater, because components in wastewater are more complicated and in higher quantities than those in matrices as mentioned above, which create difficulties in the pretreatment and analysis of the samples (Shon and Vigneswaran, 2006).

The objective of this study was to evaluate the abilities of LLE and SPE to extract, separate and concentrate \( n_{C60} \) from wastewater and to quantify and compare the linear response ranges, the MDLs and the recoveries of \( n_{C60} \) by both HPLC–UV–vis and LC/MS techniques. The obtained results were also compared with the results obtained from the literature, with rather simple aqueous matrices. Finally, a demonstration case was presented.

2. Materials and methods

2.1. Materials and chemicals

\( C_{60} \) (purity >99.9\%) was purchased from the MER Corporation (Tucson, AZ, USA). Toluene and methanol (HPLC grade) were obtained from Mallinckrodt Baker (Phillipsburg, NJ, USA). All chemicals used in this study were of reagent grade or higher. All solutions used were prepared with ultrapure water (18.2 MΩ cm\(^{-1}\)) purified by a NANOpure system (Barnstead, IA, USA) with an organic free cartridge. Five wastewater samples, including raw wastewater, primary effluent, chemically-enhanced primary treatment (CEPT) effluent, secondary effluent and reclaimed wastewater, were collected to represent the domestic wastewater at different stages of treatment. The raw wastewater samples, primary effluent samples, and CEPT effluent samples were collected from the Shatin Sewage Treatment Works, Hong Kong. The reclaimed wastewater samples were obtained by filtering the secondary effluent samples using 20–25 \( \mu \)m membranes (Whatman, UK) to simulate sand filtration (Tchobanoglous et al., 2004). All wastewater samples were stored at 4 °C for less than two weeks. The characteristics of the wastewater samples are shown in Table S1 in Supplementary Information.

2.2. Aqueous \( n_{C60} \) suspension preparation and standardization

An aqueous \( n_{C60} \) stock suspension was prepared by following a solvent exchange procedure using toluene. Details of the protocol can be found in Chen et al. (2008). The resulting yellow/brown suspension was filtered through a 0.45–\( \mu \)m membrane (Advantec, Japan) and then stored in an amber glass container at 4 °C for use. The size of the aqueous \( n_{C60} \) in the stock solution was 73–191 nm and its zeta potential was approximately -40 mV. The concentration of \( n_{C60} \) in the stock solution was standardized according to Deguchi et al. (2001). In brief, 5 mL of the aqueous \( n_{C60} \) stock solution and 5 mL of a 2% NaCl solution were mixed and 5 mL of toluene was added. The mixture was vigorously agitated continuously for 30 min and then was settled until the complete separation of the aqueous and toluene phases. The upper toluene phase was withdrawn carefully and the 1-cm UV–vis absorbance of \( C_{60} \) at a wavelength of 332 nm was measured with a UV–vis spectrophotometer (Lambda 25, PerkinElmer Ltd., USA). The corresponding \( C_{60} \) concentration was calculated from the calibration curve established from a series of known concentrations of \( C_{60} \) in toluene.

2.3. Pretreatment methods

2.3.1. Liquid–liquid extraction (LLE)

A 40 mL \( n_{C60} \)-containing aqueous sample was placed in a capped bottle containing 1% NaCl by weight prior to the addition of 4 mL of toluene. The presence of NaCl avoided the emulsion problem, destabilized the aqueous \( n_{C60} \) and facilitated the transfer of \( n_{C60} \) into the toluene phase (Hyung and Kim, 2009). It should be noted that the 1% NaCl in the samples was high enough to extract \( n_{C60} \) from the aqueous phase to the toluene phase. If the ionic strengths of wastewater samples are high enough (e.g. equivalent to 1% NaCl or higher), there is no need to add the salt. After shaking the bottle on a shaker for 4 h, the aqueous phase and toluene phase were allowed to separate. Then the toluene phase was collected, filtered through a 0.45–\( \mu \)m filter (Advantec, Japan) and subjected to analysis. This method resulted in a 10 times concentration factor.

2.3.2. Solid phase extraction (SPE)

Extractions were carried out on a vacuum manifold for 24 samples (Alltech, USA) using 500 mg/6 mL Strata SDB-L cartridges from Phenomenex (Torrance, CA, USA). The SPE cartridges were sequentially preconditioned with 10 mL of methanol and 10 mL of ultrapure water. Then 1 L of an \( n_{C60} \)-containing aqueous sample was loaded onto the cartridge at a flowrate of about 5–6 mL min\(^{-1}\). The SPE cartridges were rinsed with 10 mL of ultrapure water and 10 mL of methanol. Thereafter, the cartridges were dried under vacuum and eluted using 10 mL of toluene. The resulting extracts were concentrated with a gentle stream of nitrogen to about 0.5 mL and diluted with toluene to a final volume of 1 mL, which was then filtered through a 0.45–\( \mu \)m filter (Advantec, Japan) and subjected to analysis. This method resulted in a 1000 times concentration factor.

2.4. Detection methods

2.4.1. HPLC with UV–vis spectroscopic detection

Analysis was performed on a HPLC (VP series, Shimadzu, Japan) equipped with a photodiode array UV–vis spectrophotometer (SPD-M10A VP series, Shimadzu, Japan). The analytical column was a 250 × 4.6 mm I.D. COSMOSIL Buckyprep column packed with 5 \( \mu \)m particles (Phenomenex, Torrance, CA, USA). The mobile phase used was toluene at a flowrate of 1.0 mL min\(^{-1}\). The injection volume was 100 \( \mu \)L in all cases. A wavelength at 332 nm was selected for the quantification of \( C_{60} \).

2.4.2. HPLC with mass spectrometric detection

The detection was performed on a HPLC (1200 series, Agilent, USA) equipped with a single quadrupole mass spectrometer (6120 series, Agilent, USA). The analytical column, mobile phase and injection volume were identical to those mentioned above. The mass spectrometer was operated in the negative ion mode using atmospheric pressure chemical ionization (APCI). The opti-
mal MS conditions for the APCI analysis were as follows: drying gas flow, 2.0 L min⁻¹; nebulizer pressure, 206.7 kPa; drying gas temperature, 200 °C; vaporizer temperature, 200 °C. Molecular ion [C₆₀]⁺ at negatively charged m/z of 720 was selected and monitored for the quantification of C₆₀.

2.5. The simulated CEPT process for demonstration

Chemically-enhanced primary treatment (CEPT) is used to treat most wastewater in Hong Kong. The CEPT process, including coagulation, flocculation and sedimentation steps, was simulated by jar tests using a standard jar test device (Stuart Scientific, UK). The jar test procedure was initiated by dosing alum of 50 mg L⁻¹ to raw wastewater spiked with 200 µg L⁻¹ nC₆₀ with rapid mixing at 130 rpm for 1 min, followed by slow mixing at 30 rpm for 10 min and at 20 rpm for another 10 min, consecutively, and the solution was finally allowed to settle for 30 min. The supernatant was collected for analysis of remaining nC₆₀ concentrations in duplicate by LLE followed by HPLC coupled with UV–vis spectroscopy.

3. Results and discussion

3.1. Evaluation of LLE and SPE

To evaluate the performance of LLE and SPE in separating and concentrating nC₆₀ from wastewater in terms of the linear range, the MDL and the recovery of nC₆₀, all nC₆₀-containing aqueous samples were pretreated by LLE or SPE, and then analyzed by HPLC coupled with UV–vis spectroscopy.

3.1.1. LLE

Based on some trial experiments (data not shown), LLE with 1% NaCl addition by weight achieved equilibrium within 4 h. The addition of NaCl not only destabilized nC₆₀ (Deguchi et al., 2001; Chen et al., 2008), but also avoided the emulsion problem caused by the presence of surfactants, proteins and lipids in wastewater. These impurities were suggested to cause emulsion problems to hinder the interphase transportation of C₆₀ in biological samples and natural water (Xia et al., 2006; Hyung and Kim, 2009).

A series of six nC₆₀-containing aqueous samples from 20 to 200 µg L⁻¹ were prepared by spiking the standardized nC₆₀ stock solution into ultrapure water, reclaimed wastewater, secondary effluent, CEPT effluent, primary effluent and raw wastewater. With LLE and HPLC coupled with UV–vis spectroscopy, linear calibration curves were obtained with good regression coefficients (R² ≥ 0.99) based on the peak areas of UV absorbance at 332 nm of the aqueous samples versus the spiked nC₆₀ concentrations (Fig. 1A). The slopes of these calibration curves were close, with a less than 5% difference.

The recovery of nC₆₀ was defined as the ratio of the amount extracted to the amount spiked. As shown in Table 1A, recoveries of nC₆₀ in all water matrices tested were between 88% and 97% with a standard deviation (SD) not more than 2%. Recoveries of nC₆₀ in primary effluent and raw wastewater were a little lower than those in ultrapure water, reclaimed wastewater and secondary effluent, although there existed great differences in their TSS and DOC concentrations (Table S1 in Supplementary Information). The results indicate minor matrix effects from particles, organic substances or other impurities in wastewater. The lowest nC₆₀ recovery in the CEPT effluent may be attributed to the amorphous Fe(OH)₃, on which nC₆₀ was easily adsorbed and was excluded by the 0.45-µm filter.

The method detection limits (MDLs) by LLE with UV–vis spectroscopy in different water matrices were determined to be 3–

4 µg L⁻¹ (Table 1B) and were not affected by the different water qualities, based on the calculation of 3.143 times of the standard deviation of seven replicate samples spiked with 30 µg L⁻¹ of nC₆₀.

3.1.2. SPE

The reclaimed wastewater samples can be loaded onto the SPE cartridges directly. The high quantities of suspended solids in the raw wastewater, primary effluent, CEPT effluent and secondary effluent samples may, however, block the SPE cartridges, which makes passage of water through the cartridges difficult or even impossible. Filtration through a 1-µm pore size membrane was suggested by USEPA Method 3535A (USEPA, 2007) before loading samples with high quantities of suspended solids. This filtration step, however, may exclude particle-associated nC₆₀ and naturally formed large fullerene aggregates to cause low recoveries of nC₆₀. Experiments were carried out to examine the exclusion of nC₆₀ by the 1-µm pore size membrane (Whatman GMF 150, UK) and nC₆₀ concentrations were determined using LLE followed by HPLC/UV–vis measurement. Fig. S1 in Supplementary Information shows that approximately 80%, 58%, 59% and 15% of nC₆₀ in raw wastewater, primary effluent, CEPT effluent and secondary effluent, respectively, were excluded by the membrane. The large exclusion of nC₆₀ in raw wastewater, primary effluent, CEPT effluent and secondary effluent by the membrane led to very low recoveries of nC₆₀ from these wastewater matrices. Therefore, SPE was applicable to
wastewater matrices with low quantities of particles, such as secondary effluent and reclaimed wastewater only.

Fig. 1B shows calibration curves obtained by SPE plotted as peak areas of UV absorbance at 332 nm against spiked nC60 concentrations. Linear relationships were only obtained from 0.8 to 2 l g L−1 for reclaimed wastewater and from 0.8 to 4 l g L−1 for secondary effluent.

As shown in Table 2A, recoveries of nC60 by SPE were much lower than those by LLE, only 9% with a SD of 1% for secondary effluent and 18% with a SD of 1% for reclaimed wastewater. The different nC60 recoveries can be associated with the different concentrations of TSS and COD in these two samples, which affect the exclusion of particle-associated nC60 during the prefiltration step and interfere with the adsorption/extraction of nC60 by SPE.

The MDLs were determined to be 0.42 l g L−1 for reclaimed wastewater and 0.64 l g L−1 for secondary effluent by SPE with UV–vis spectroscopy (Table 2B), which were about one order of magnitude less than those achieved by LLE with UV–vis spectrometry (Table 1B), because of the much higher enrichment ratio and the lower recoveries.

### Table 2A
**Recovery of nC60 in different water matrices by SPE.**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Percentage recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spiked concentration (µg L−1)</td>
</tr>
<tr>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
</tr>
<tr>
<td>4</td>
<td>1.8</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Average</td>
<td>–</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>–</td>
</tr>
</tbody>
</table>

3.2. Comparison of HPLC with UV–vis spectroscopic and mass spectrometric detection

To compare the two detection methods (UV–vis spectroscopy and mass spectrometry) in terms of the linear range and the MDL, all nC60-containing aqueous samples were pretreated with LLE and then separately analyzed by these two detection methods. The HPLC chromatographs with UV–vis spectroscopic detection (Figs. S2A–S2C), the peak at 3.5 min belongs to the solvent peak with wastewater impurities co-eluted and the elution time of C60 was found to be approximately 7.6 min. In the HPLC chromatographs with mass spectrometric detection (Figs. S2D–S2E), the two small peaks at 3.5 min and 5 min are the eluted impurities and the elution time of C60 was found to be approximately 9.1 min. The difference of the C60 elution times was attributed to the different HPLC systems used for the UV–vis spectroscopic and mass spectrometric detection. As shown in Figs. S2A and S2D, wastewater impurities did not produce interfering peaks at the elution times of C60 in the

### Table 2B
**MDLs in different water matrices by SPE with UV–vis spectroscopy.**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Spiked concentration (µg L−1)</th>
<th>Measured concentration (µg L−1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reclaimed wastewater</td>
<td>Secondary effluent</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>0.72</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0.89</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>0.73</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>0.99</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>0.94</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>1.02</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>0.72</td>
</tr>
<tr>
<td>MDL (µg L−1)</td>
<td>0.42</td>
<td>0.64</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.13</td>
<td>0.20</td>
</tr>
</tbody>
</table>
HPLC chromatographs of wastewater samples without $n$C$_{60}$ with UV–vis spectroscopic and mass spectrometric detection. Comparing Figs. S2B and S2C of the chromatographs of the C$_{60}$-toluene extract and the C$_{60}$-toluene standard solution, the elution times of C$_{60}$ were the same and thus were not affected by the presence of wastewater impurities. In conclusion, the UV–vis spectroscopic and mass spectrometric detections are able to measure nC$_{60}$ in the wastewater matrices tested.

Fig. 2 shows that, by mass spectrometric detection, the areas of negative ion with $m/z$ of 720 of C$_{60}$ and the spiked nC$_{60}$ concentrations show good linear response ($R^2=0.99$) in the concentration range up to 60 µg L$^{-1}$, which is lower than that by UV–vis spectroscopic detection (up to 200 µg L$^{-1}$). Santa et al. (1995) also reported that the calibration curve plotted as peak areas versus nC$_{60}$ concentrations in plasma with UV–vis spectroscopy showed good linearity over a wider concentration range than mass spectrometry. The MDLs by LLE with mass spectrometric detection (up to 200 µg L$^{-1}$) and mass spectrometric detections are able to measure nC$_{60}$ in wastewater matrices tested.

Table 3 lists and compares the quantification of nC$_{60}$ in different aqueous matrices with different sample pretreatment and detection methods, in terms of the linear range, the analyte recovery, and the MDL. The data include results from the current study and those available in the literatures, which deal with less complex matrices including biological samples, freshwater salt solution, surface water, ground water, tap water and NOM-containing solutions. Among these data, three pretreatment methods including evaporation, LLE and SPE and two detectors including UV–vis spectroscopy and mass spectrometry were used. Evaporation can concentrate nC$_{60}$ from ultrapure water only, because in more complex samples, the small quantity of nC$_{60}$ in the dried samples tends to be embedded in or covered by co-concentrated inorganic and nonvolatile components so that the resolubilization of C$_{60}$ into toluene is hindered, leading to a low recovery of nC$_{60}$ (Chen et al., 2008). LLE and SPE can extract and concentrate nC$_{60}$ from biological and environmental samples with less salt incorporation. LLE is applicable to all water and wastewater matrices and SPE is only applicable to water and wastewater matrices with less particles. Recoveries of nC$_{60}$ by LLE generally approached 90% or greater except those reported in Chen et al. (2008), which might be owing to the short extraction time (only 10 min). Recoveries of nC$_{60}$ by SPE were generally much lower than those by LLE and were easily affected by the aqueous matrices. The cleaner the water, the better nC$_{60}$ recoveries by SPE. It is also the case in our study where the nC$_{60}$ recovery from reclaimed wastewater is much higher than that from secondary effluent and both of which are much lower than those from ultrapure water and tap water reported in Chen et al. (2008). The high recovery of nC$_{60}$ (74–80 ± 10%) by SPE reported in Bouchard and Ma (2008) might be owing to the small volume of samples pretreated (only 30 mL). Generally, SPE gave lower MDL than LLE, owing to the higher enrichment ratio (1000:1) used in this study and Chen et al. (2008). The relatively low method quantification limit with SPE achieved by Isaacson et al. (2007) was due to the large-volume injection (500 µL). The low detection limit of 0.34 µg L$^{-1}$ reported by Xia et al. (2006) is lower than the MDL (the enrichment ratio was 8.9:1). A lower MDL may be achievable in the current study if the enrichment ratio of LLE is increased or large-volume injection is used.

### Table 3

<table>
<thead>
<tr>
<th>Sample pretreatment</th>
<th>Detector</th>
<th>Linear range (µg L$^{-1}$)</th>
<th>Recovery (%)</th>
<th>MDL (µg L$^{-1}$)</th>
<th>Matrix</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLE</td>
<td>UV–vis</td>
<td>–</td>
<td>94–100</td>
<td>0.34$^a$</td>
<td>Biological samples</td>
<td>Xia et al. (2006)</td>
</tr>
<tr>
<td>LLE</td>
<td>Mass</td>
<td>0.1–150</td>
<td>93 ± 7.0</td>
<td>0.40$^b$</td>
<td>Freshwater salt solution</td>
<td>Isaacson et al. (2007)</td>
</tr>
<tr>
<td>LLE</td>
<td>UV–vis</td>
<td></td>
<td>78.2 ± 3.2</td>
<td>2.48</td>
<td>Surface and ground water</td>
<td>Bouchard and Ma (2008)</td>
</tr>
<tr>
<td>SPE</td>
<td>–</td>
<td>(74–80) ± 10</td>
<td>–</td>
<td>–</td>
<td>Ultrapure water</td>
<td>Chen et al. (2008)</td>
</tr>
<tr>
<td>Evaporation</td>
<td>Mass</td>
<td>5–25</td>
<td>35 ± 4.9</td>
<td>2.78</td>
<td>Tap water</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5–25</td>
<td>2.1 ± 0.4</td>
<td>–</td>
<td>Ultrapure water</td>
<td>–</td>
</tr>
<tr>
<td>LLE</td>
<td>Mass</td>
<td>10–40</td>
<td>39 ± 3.2</td>
<td>3.33</td>
<td>Tap water</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5–25</td>
<td>32 ± 7.0</td>
<td>–</td>
<td>Ultrapure water</td>
<td>–</td>
</tr>
<tr>
<td>SPE</td>
<td>Mass</td>
<td>0.5–5</td>
<td>42 ± 2.9</td>
<td>0.30</td>
<td>Tap water</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5–5</td>
<td>33 ± 2.6</td>
<td>–</td>
<td>NOM-containing solution</td>
<td>Hyung and Kim (2009)</td>
</tr>
<tr>
<td>LLE</td>
<td>UV–vis</td>
<td>–</td>
<td>Almost 100</td>
<td>–</td>
<td>Ultrapure water, wastewater$^c$</td>
<td>This study</td>
</tr>
<tr>
<td>LLE</td>
<td>UV–vis</td>
<td>20–200</td>
<td>(89–94) ± 2</td>
<td>3–4</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mass</td>
<td>20–60</td>
<td>4–11</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>SPE</td>
<td>UV–vis</td>
<td>0.8–4</td>
<td>9 ± 1</td>
<td>0.64</td>
<td>Secondary effluent</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.8–4</td>
<td>18 ± 1</td>
<td>0.42</td>
<td>Reclaimed wastewater</td>
<td>–</td>
</tr>
</tbody>
</table>

$^a$ The detection limit.

$^b$ The concentration that gave an S/N of 10:1.

$^c$ Five wastewater matrices, including raw wastewater, primary effluent, CEPT effluent, secondary effluent and reclaimed wastewater.
3.4. \( nC_{60} \) removal by the demonstrated CEPT process

To demonstrate the applicability of the quantification method of LLE followed by HPLC coupled with UV–vis spectroscopy to environmental study, the removal of \( nC_{60} \) by the simulated CEPT process was conducted. Considering the MDLs of the quantification method, a higher concentration of \( nC_{60} \) (200 \( \mu g \) L\(^{-1}\)) than those reported in WWTP effluent (Farré et al., 2010) was spiked into raw wastewater and the alum dosage was 50 mg L\(^{-1}\). After the jar test simulating coagulation, flocculation and sedimentation, the remaining \( nC_{60} \) concentration in the supernatant was determined to be 13.8 ± 1.2 \( \mu g \) L\(^{-1}\) and the removal efficiency of \( nC_{60} \) by the simulated CEPT process under the specific condition was 93%. The result demonstrates that the reported method can be used to study the treatability of \( nC_{60} \) in engineered wastewater treatment systems or units.

4. Conclusions

This paper evaluated the performance of liquid–liquid extraction (LLE) and solid phase extraction (SPE) in separating and concentrating \( nC_{60} \) from wastewater and compared HPLC coupled with UV–vis spectroscopy and mass spectrometry for the quantification of \( nC_{60} \). LLE was suitable for multiple wastewater matrices, while SPE was only applicable to reclaimed wastewater and secondary effluent of less suspended solids. The linear ranges of the calibration curves covered the concentration range of 20–200 \( \mu g \) L\(^{-1}\) for six water matrices by LLE, but covered the range of 0.8–2 \( \mu g \) L\(^{-1}\) for reclaimed wastewater and 0.8–4 \( \mu g \) L\(^{-1}\) for secondary effluent by SPE. Recoveries of \( nC_{60} \) by LLE were in the range of 88–97% with a SD not more than 2% while recoveries of \( nC_{60} \) by SPE were much lower, only 18% for reclaimed wastewater and 9% for secondary effluent. The MDLs of LLE with UV–vis spectroscopy were 3–4 \( \mu g \) L\(^{-1}\) for six water matrices and the MDLs of SPE with UV–vis spectroscopy were 0.42 \( \mu g \) L\(^{-1}\) for reclaimed wastewater and 0.64 \( \mu g \) L\(^{-1}\) for secondary effluent. Sensitivity of UV–vis spectroscopy and mass spectrometry was similar, while a linear relationship could be obtained by LLE with UV–vis spectroscopy in a range of 20–200 \( \mu g \) L\(^{-1}\) which is wider than that obtained by LLE with mass spectrometry (20–60 \( \mu g \) L\(^{-1}\)). However, mass spectrometry provides specificity to differentiate different order fullerenes based on the m/z of charged molecules. This paper demonstrated the feasibility of the combination of different extraction and detection methods to quantify \( nC_{60} \) in engineered wastewater matrices. It should be noted that the assessment covers typical municipal wastewater after different stages of treatment. Whether these MDLs and ranges of linearity are applicable to other industrial wastewaters with high strengths of pollutants requires further confirmation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2010.03.052.

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