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Towards Ultralow Detection Limits of Aromatic Toxicants in Water using Pluronic Nanoemulsions and Single-Entity Electrochemistry

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Abstract: We demonstrate a new electroanalytical technique using nanoemulsions (NEs) as a nanoextractor combined with single entity electrochemistry (SEE) to separate, preconcentrate analytes from bulk media, and even detect them in situ, enabling ultratrace level analysis. This approach is based on our hypothesis that the custom-designed NEs would enable to effectively scavenge compounds from bulk media. Herein, we use Pluronic F-127 functionalized NEs to extract, preconcentrate target analytes e.g., ferrocene derivatives as a model aromatic toxicant dissolved in the water, and employ SEE to in situ detect and quantitatively estimate analytes extracted in individual NEs. Extraction was markedly efficient to reach ~8 orders of magnitude of preconcentration factor under the true equilibrium, thereby enabling ultratrace level analysis with a detection limit of ~0.2 ppb. The key step to attain high sensitivity in our measurements was to modulate the total amount of added NEs respect to the total volume of bulk solution, thereby controlling the extracted amount of analytes in each NE. Our approach is readily applicable to investigate other aromatic toxicants dissolved in the water, thus detecting hazardous carcinogen, 2-aminobiphenyl in the water up to ~0.1 ppb level. Given the excellent detection performance as well as the broad applicability for ubiquitous aromatic contaminants, the combination of NEs with SEE offers great prospects as a sensor for environmental applications.
Introduction

The global concern about the water quality and its contamination in the environment has been drastically growing as industrialized nations and developing countries have exploded with abundance in recent years. Particularly, water contamination by aromatic toxicants (or polycyclic aromatic hydrocarbons, PAH) is critical, since they are ubiquitous as byproducts of combustion process as well as a natural component of fossil fuels. Despite low concentration in water due to the low solubility, aromatic toxicants accumulate in sediment, soil and aquatic organisms, thus leaching out and finally leading to bioaccumulate / biomagnify in the food chain. Indeed, PAH have been found at the level of 0.17 µM in the breast milk of nursing mothers living in oil spill affected regions. The potential health effects of aromatic toxicants include the disruption of pulmonary, gastrointestinal, renal, and dermatologic systems, ending to a cancer. Currently, the U.S. Environmental Protection Agency (EPA) declares 0.2 – 0.4 ppb PAH in drinking water.

Due to the carcinogenicity, the ultratrace level analysis of aromatic toxicants in water is significant for a public health perspective. Gas chromatography combined with either mass spectrometry (MS) or fluorescence (or UV-Vis) spectroscopy is commonly used techniques with high sensitivity, whereas they require high purchase/running costs, and a skilled operator with laborious and time consuming work. Considering the importance of high throughput assays with an easy access, the advancement in analytical system offering high sensitivity, rapidity, miniaturizability (or portability), and accessibility within reasonable costs is ultimately demanded.

For the advancement in these analytical processes, we introduce nanoemulsions (NEs) as a new turning point. NEs are oil-in-water (o/w) emulsions with mean droplet diameters ranging from 50 to 500 nm. Owing to the small size and the hydrophobic environment inside NEs, NEs can be a suitable strategy for an efficient extractor of lipophilic analytes. We recently studied NEs functionalized with triblock copolymer, Pluronic F-127 having c.a. 40 nm diameter with a high monodispersity, and measured a partition coefficient at intact NEs in situ, which is remarkably large as ~1.9 x 10^10 as a result of both thermodynamic distribution and intermolecular interaction of extracted compounds in the partitioned system. The resultant extraction was markedly efficient to reach ~8 orders of magnitude for the preconcentration factor. Herein, we utilize these key physicochemical properties to make an attractive attempt that NEs are used for both separation of analytes from environments and preconcentration in situ. Additionally, the fast mass transport
of analytes to NEs is anticipated due to a small size of NEs, leading to true equilibrium in the given system. In fact, the ability of nanoparticles to remove or separate toxic compounds from subsurface or environments in situ, rapidly, and efficiently at a reasonable price has been extensively explored.\textsuperscript{15} Such attempts have been only focused on solid nanoparticles with high adsorption capacity as such or chemically functionalized.\textsuperscript{16} And yet, these nanoparticles are combined with conventional analytical methods, e.g. inductively coupled plasma atomic absorption (or emission) spectrometry (ICP-AAS, or ICP-AES), ICP-MS, or high performance liquid chromatography (HPLC), while effective, very often costly and time consuming.\textsuperscript{15}

Innovatively, we combine Pluronic F-127 functionalized NEs with single entity electrochemistry (SEE) to in situ separate, preconcentrate, and even detect analytes at ultratrace level. This unique combination provides distinct analytical merits. First, NEs as an efficient nanoextractor enable ultrasensitive analysis with ultralow detection limit via extraction and preconcentration in situ without additional sample treatment. Second, the application of SEE allows for in situ detection of discrete NEs preconcentrating analytes using a simple instrumentation. Thereby, we can attain ultra-high sensitivity, rapidity, miniaturizability, and easy accessibility in this analysis.

Herein, we systematically vary the concentrations of ferrocenemethanol (FcMeOH) as a model aromatic toxicant and NEs in aqueous bulk solution, perform SEE measurements to construct a calibration curve, thus quantitatively establishing a new analytical method. Throughout blind sample tests, the analytical capability of this method is further validated. Also, our ultrasensitive analysis is readily applicable to other aromatic toxicants such as 2-aminobiphenyl (2-ABP), a typical carcinogen.\textsuperscript{17} Notably, this demonstrated application of NEs and SEE opens up a new route to ultratrace level analysis for environmental pollutants in water, whereas SEE has been widely applied to study single events, and discretely characterize size distribution of polydisperse soft particles including NEs as opposed to ensemble measurements.\textsuperscript{18}

**Experimental Section**

**Chemicals.** Pluronic F-127 (F-127), bis(2-ethylhexyl)- sebacate (DOS, 97.0%), tetrahydrofuran (THF, 99.9%), ferrocenemethanol (FcMeOH, 97.0%), potassium tetrakis (pentafluorophenyl) borate (KTFPB, 97.0%), ammonium hexafluorophosphate (NH₄PF₆, 99.98%), ammonium
chloride (NH₄Cl, 99.5%) and potassium nitrate (KNO₃, 99.0%) were purchased from Sigma-Aldrich used as obtained. Uranless negative stain, and glass vials were purchased from Fisher Scientifics. Nanopure water (18.2 MΩ·cm, TOC 2 ppb; Milli-Q Integral 5 system, Millipore) was used to prepare all the aqueous electrolyte solutions as well as in NE synthesis.

**Synthesis of Nanoemulsions.** NEs were synthesized by dissolving 1.8 mg of KT_FP_B and 250.0 mg of F127 in 3.0 mL of THF to form a homogeneous solution. Then, 8.8 μL of DOS was added to this solution. Resulting solution was vortexed for 1 hour using a vortex mixer (Fisher Scientific, Pittsburgh, PA) at a spinning speed of 3000 rpm. After mixing, 0.1 ml of the solution was forcefully injected into 4.0 mL of deionized water on a vortex with a spinning speed of 4000 rpm for 2 minutes. This process was repeated for another batch. The resulting 8 mL solution was then combined, and further homogenized for 2 min at a rate of 4900 rpm using homogenizer (Kinematica AG, Polytron system PT 10-35 GT, Switzerland). Finally, the resulting solution was purged with N₂ gas to fully evaporate THF for 1 hour under a flow rate of 40 psi. After evaporation, the final solution was used as a NE stock solution.

**Measuring the Size and Zeta Potential of Nanoemulsions.** NEs were characterized by the dynamic light scattering (DLS) to measure the size distribution and ζ-potential (Malvern Zetasizer Nano ZS, Malvern Instruments Inc., MA). Measurements were taken at 90° angle for NEs. For the DLS sample preparation, 1.0 mL of NE stock solution was diluted with 3.0 mL of nanopure water, and a 1.0 mL aliquot of this diluted solution was taken to fill a DLS cuvette (Malvern DTS 1070, Malvern Instruments Inc., MA). DLS measurements were performed at 25 °C.

**Fabrication of a Pt UME.** Pt UME (5 μm diameter) was fabricated using CO₂−laser capillary puller (model P-2000, Sutter Instrument). First, 25 μm dia. Pt wire (Goodfellow, annealed) was inserted in a borosilicate capillary (I.D. 0.2 mm, O.D. 1mm, item No 9-000-2000, Drummond scientific company, Broomall, PA) and was pulled together with CO₂−laser puller. An as-pulled Pt UME was milled by a homemade polisher to expose Pt disk resulting in an inlaid disk-shaped electrode. Then, the Pt UME was cleaned in piranha solution for 10s, followed by through rinsing in water. Prepared Pt UMEs were also used under 30 % or higher relative humidity at 22–23 °C. Herein, the size of 5 μm diameter Pt UMEs is chosen to observe well resolved individual collisions of each NE as well as reasonable collisional frequency where current spikes are expected in tens of second range.18
**SEE Measurements.** SEE was performed in a two-electrode cell using a bipotentiostat (CHI 760E or CHM8022D, CH Instrument, Austin, TX) at ambient temperature (20 °C). Pt UME (5 μm diameter) was immersed in the aqueous solution containing 10 mM NH₄PF₆ as the supporting electrolyte. Prior to SEE measurements, aqueous solution with added NEs was vortexed for 15 min at 1000 rpm, and left on the benchtop for 2 hrs for homogeneous mixing of picomolar ~ femtomolar level of NEs and micromolar ~ nanomolar level of analytes (after rigorous mixing with vortex for 15 min, no discernible difference was observed between with and without 2 hr delay). Amperometric current was measured over time under a constantly applied potential of 0.40 V or 0.85 V vs a Pt quasi-reference electrode (Pt QRE) to oxidize FcMeOH or 2-ABP extracted inside NEs from the bulk media, respectively. The time interval for data acquisition was 5–10 ms.

**Results and Discussions**

**Nanoemulsions with High Monodispersity and a Large Partition Coefficient.** We utilized NEs functionalized with amphiphilic block copolymer (Pluronic F-127) and castor oil plasticizer (Figure 1A). In earlier works, we elucidated important physicochemical properties of these NEs such as size, electrochemical activity relevant to the intrinsic inner structure, and the partition coefficient. Here, we selected NEs with the optimized composition showing 40 ± 5 nm diameter with high monodispersity, high electroactivity, and high partition coefficient. As shown in Figure 1B, the high monodispersity was observed by TEM measurements consistent with dynamic light scattering (DLS) measurements with 38 nm diameter and 0.17 ± 0.05 polydispersity index (PDI) (Figure S1).

Previously, a partition coefficient at intact NEs could be determined as $P = 1.9 \times 10^{10}$ by in situ SEE measurements. The large $P$ for aromatic compounds promises a potential application of NEs as efficient nanoextractors, thereby enabling in situ separation of analytes from environments, and preconcentration inside NEs. In combination with SEE, all three steps of separation by extraction, preconcentration, and even detection of analytes can be studied in situ with a simple instrumentation. Herein, we explore the ability of NEs to remove aromatic toxicants from water, preconcentrate them, thus enabling ultratrace level analysis. FcMeOH is studied as a model.
aromatic toxicant dissolved in the water, which undergoes extraction, preconcentration, and the electrolysis inside NEs during the electrochemical detection.

Figure 1. (A) A schematic illustration of NE composed of triblock polymer (F-127), ion exchanger (TFPB−), and dioctyl sebacate oil (DOS). (B) TEM image of monodispersed NEs with 40 nm diameter. (C) Diffusion-limited loading of a NE with analytes, e.g. FcMeOH.

Efficient and Rapid Extraction by Nanoemulsions. The feasibility of new applications of NEs in preconcentration and ultratrace level analysis from the environmental sources relies on how efficiently and rapidly NEs can extract the target compounds. Owing to the large partition coefficient at NEs, FcMeOH can be effectively partitioned and extracted into a NE from the aqueous bulk solution. The mass transfer of FcMeOH followed by extraction into the NEs readily occurs due to the small dimension of NEs, thereby the extremely short loading time is expected from the high diffusional flux of FcMeOH to a NE (Figure 1C). At a steady state, the diffusional flux of FcMeOH to the surface of a spherical NE, J, can be estimated as,

\[ J = \frac{D_{FcMeOH} C_{FcMeOH}}{r_0} \]

(1)

where \( D_{FcMeOH} \) and \( C_{FcMeOH} \) are the diffusion coefficient and concentration of FcMeOH in the aqueous sample solution and \( r_0 \) is radius of a NE. Assuming that FcMeOH at the NE/solution interface is instantaneously partitioned and extracted, the time required for the diffusion-limited loading of NEs, \( \tau \), is given by,

\[ \tau = \frac{C_{fill} r_0^2}{3D_{FcMeOH} C_{FcMeOH,aq}} \]

(2)
where \( C_{\text{fill}} \) is the filling capacity in a NE with varying from 0.0 to 15.7 M, \( C_{\text{FcMeOH,aq}} \) is the concentration of FcMeOH in aqueous solution. The filling capacity varies depending on the concentration of FcMeOH in aqueous phase and the amount of NEs added in the aqueous bulk solution.

Under the equilibrium, the filling capacity is determined by,

\[
C_{\text{fill}} = \left[ \frac{V_{\text{total}}}{V_{\text{aq}} + V_{\text{NE}}} \right] \cdot C_{\text{FcMeOH}}^{\text{tot}} \approx \left[ \frac{V_{\text{total}}}{V_{\text{NE}}} \right] \cdot C_{\text{FcMeOH}}^{\text{tot}}
\]  

(3)

where \( V_{\text{aq}} \), \( V_{\text{NE}} \), and \( V_{\text{total}} \) are the volume of aqueous phase, NE phase, and their sum, respectively (Table S1 in SI), and \( P \) is the partition coefficient at NEs. In our earlier work, the maximum filling capacity of an individual NE was empirically estimated as 15.7 M.\(^{13}\) Also, our recent work evaluated \( P (=1.9 \times 10^{10}) \) of intact NEs for aromatic compounds such as 2-ABP and FcMeOH.\(^{14}\) Due to the large \( P \), the \( C_{\text{fill}} \) is mainly determined by the volume ratio between \( V_{\text{total}} \) and \( V_{\text{NE}} \), and the total concentration of FcMeOH present in the total solution, \( C_{\text{FcMeOH}}^{\text{tot}} \) under \( V_{\text{aq}} / P \ll V_{\text{NE}} \). Hence, this implicit eq (3) linearly relates \( C_{\text{fill}} \) with \( C_{\text{FcMeOH}}^{\text{tot}} \). Overall, based on the eq (2) and (3), with a diffusion coefficient of FcMeOH as \( 6 \times 10^{-6} \text{ cm}^2/\text{s} \), 0.1 to 3.0 \( \mu \text{M} \) FcMeOH in the presence of 8 pM NEs in the aqueous solution can be accumulated into each NE to yield \( \sim 0.30 \) to 15.7 M in 600 to 1000 ms, respectively. The expected loading times would be fast enough to perform SEE measurements under the true equilibrium, thereby supporting the feasibility of in situ separation, preconcentration, and detection of analytes (e.g. FcMeOH) from the aqueous bulk solution.

**Single Entity Electrochemistry with Individual Nanoemulsions.** In Figure 2A, SEE measurement is schematically illustrated, where a Pt UME applied with a constant potential of 0.40 V vs. Pt QRE is immersed in the aqueous solution containing both FcMeOH at a concentration lower than 10 \( \mu \text{M} \) and freshly prepared NEs. This aqueous solution was vortexed for 15 min once NEs were added, and left on the benchtop for 2 hrs prior to SEE measurements to ensure the system under the equilibrium. FcMeOH concentration in the aqueous bulk solution is too low to give any substantial background current in the current-time \((i-t)\) response during SEE measurements. NEs extracting/preconcentrating FcMeOH spontaneously diffuse and collide onto a Pt UME, where an oxidative potential is applied. Upon a collision of an individual NE to a Pt UME, FcMeOH preconcentrated in a NE is immediately oxidized leading to anodic currents, which decay over
time due to the depletion of FcMeOH in a NE, thereby showing a current spike upon an individual collision of a NE. The integration of a current spike over time gives charges needed for electrolysis of FcMeOH, thus an amount of FcMeOH extracted/preconcentrated in a NE.

Herein, we performed three sets of SEE measurements in the presence of three different concentrations of NEs, such as 8.0 pM, 0.8 pM, or 80 fM. In each set of SEE measurements, the concentration of FcMeOH in aqueous solution varied about three orders of magnitudes. The first set of SEE was performed in the presence of 8 pM of NEs with the concentration of FcMeOH at from 10.0 μM to 0.1 μM. For example, with 8 pM of NEs and 10.0 μM FcMeOH in aqueous solution, i-t curve was obtained with a series of current spikes showing nearly uniform current magnitudes (Figure 2B). Each current spike showed a characteristic decay with time. This collisional response, i-t decay was fitted with bulk electrolysis model, assuming that FcMeOH is extracted, diffuses in the castor oil inside a NE rather than adsorption on NE surface, and undergoes an electrolytic reaction at a contact point between a NE and a Pt UME (See SI and Figure S2 for more details). In Figure 2B insets, a good agreement between the experimental i-t curve (black solid lines) and the simulation (red open circles) is obtained, which evidently indicates that extracted (and preconcentrated) FcMeOH resides in a NE filled with castor oil, not on the surface of a NE.

Continuously, charges from integrated current spikes were collected to construct a size distribution curve of NEs preconcentrating FcMeOH. Current spikes at least 3 times larger than the background noise signal were counted for this analysis. Using the concentration of FcMeOH extracted in a NE determined by eq (3), 15.7 M, and the integrated charge (Q) from i-t curve, diameter of a NE, d is calculated by

$$d = 2^{\frac{3}{4}} \sqrt[3]{\frac{3Q}{4\pi F C_{fill}}}$$

(4)

where F is Faraday constant (96485 C/mol).

The discrete size distribution estimated from current spikes in Figure 2B, is illustrated in Figure 2C. The resultant d ranges 30 ~ 60 nm with a peak at 40 nm, which is consistent with the DLS data. This consistency validates $C_{fill}$ determined by eq (3). Notably, a narrow distribution of d could be attributed to the fully equilibrated system as well as intrinsically monodisperse NEs. In addition,
this equilibrated system along with the unique combination of SEE and the highly monodisperse NEs, is advantageous to attain the high accuracy and precision in our analysis, since repetitive measurements of similar NEs can be made during SEE.

Further, FcMeOH concentration in aqueous solution was sequentially decreased up to 1.0 μM in the presence of 8 pM NEs, and the subsequent SEE was performed (Figure 2D). The respective $C_{\text{fill}}$ value, 6.0 M from eq (3) was used to estimate diameter of NEs from each $i$-$t$ curve result and eq (4), and the corresponding size distribution curve was constructed (Figure 2E). The resultant $d$ ranging 30 ~ 50 nm with a peak at 40 nm showed good consistency with the DLS data, which validates $C_{\text{fill}}$ determined by eq (3) as well.

Likewise, another SEE was performed in the presence of 8 pM of NEs with FcMeOH concentration at 0.1 μM. In Figure 2F, a typical $i$-$t$ curve with 8 pM NEs and 0.1 μM FcMeOH is illustrated. Current spikes fitted well with bulk electrolysis model indicating an extraction of FcMeOH inside a NE (insets in Figure 2F). Based on the charges integrated from each current spike and eq (4) as well as the $C_{\text{fill}}$ as 0.60 M using eq (3), the size distribution curve was constructed, narrowly spanning between 40 and 70 nm with a peak at 50 nm (Figure 2G). Since the current spikes under this condition are close to the limit of detection in our measurements, charges integrated from $i$-$t$ curve tend to be slightly overestimated, thus leading to the larger mean diameter of NEs, 50 nm. Overall, as FcMeOH concentration in aqueous solution decreases from 10.0 μM to 0.1 μM in the presence of 8 pM NEs, the $C_{\text{fill}}$ validated by SEE measurements linearly decreases from 15.7 M to 0.6 M as expected under the fully equilibrated system.

Two more sets of SEE measurements were performed under 1 μM ~ 10 nM FcMeOH with 0.8 pM NEs, and 0.1 μM ~ 1.0 nM FcMeOH with 80 fM of NEs, respectively. We could observe the same trends of SEE results as the case with 8 pM NEs aforementioned. Hence, a linear decrease in $C_{\text{fill}}$ from 15.7 M to 0.6 M is also seen, as FcMeOH concentration in aqueous solution sequentially decreases under each 0.8 pM and 80 fM NEs, respectively. A characteristic $i$-$t$ curve and the corresponding size distribution curve of NEs under 1.0 nM FcMeOH with 80 fM of NEs are shown in SI (Figure S3).
Figure 2. (A) A schematic illustration of SEE measurements using NEs as nanoextractor to effectively scavnage target compounds, A from water to NEs, and electrochemically sense them by oxidation ($E_{\text{tip}} = 0.4$ V vs Pt QRE) upon the collision of NE. (B) $I-t$ curves in the presence of 10 μM FcMeOH with 8 pM NEs. The insets magnified typical current spikes fitted with bulk electrolysis model (red open circles), (also see Figure S2). (D), (F) $I-t$ curves in the presence of 1 μM FcMeOH with 8 pM NEs, and 0.1 μM FcMeOH with 8 pM NEs, respectively. The insets show magnified current spikes fitted with bulk electrolysis model (red open circles) as well. (C), (E) and (G) Size distribution curves of NEs based on the electrochemical measurements (orange bars) from (B), (D) and (F), and compared with DLS measurements (black solid lines), respectively.
Construction of Calibration Curves and the Analytical Validation with a Blind Sample. We estimated a charge density at individual NEs using the integrated charges and the electrochemically determined diameter of NEs from three sets of SEE measurements, and finally constructed a plot of charge density vs. total concentration of FcMeOH (Figure 3A). In this plot, three curves for each NE concentration are depicted with showing sigmoidal trends. This plot is analytically useful, since it can be utilized as a calibration curve for the quantitative analysis of unknown samples. Once excluding the saturated region, each three curve was fitted with exponential function (Figure 3B). The respective exponential equations and $R^2$ correlation coefficients ($\geq 0.99$) are given in Figure 3B. Note that three curves are almost identical, only laterally shifted, indicating that the amount of FcMeOH in individual NEs extracted from aqueous bulk solution can be modulated by varying the volume ratio between total NEs and the total solution. Every an order of magnitude decrease in the volume ratio lowers the limit of detection (LOD) with an order of magnitude as well. Hence, 0.2 ppb LOD could be achieved in 1 nM FcMeOH solution in the presence 80 fM NEs.

The analytical merit of this charge density plot is validated by testing a blind sample. A blind sample of FcMeOH solution was provided to the researchers without any information in advance. Three sets of SEE measurements were performed in the presence of 80 fM 0.8 pM, and 8 pM NEs, separately (Figure 4). Each $i-t$ curve shows characteristic current spikes, where a current decay is fitted with bulk electrolysis model implying the extraction/preconcentration of FcMeOH inside NEs.

In Figure 4, with 80 fM NEs, our observed collision frequency was 0.03 Hz (6 peaks in 200 s), approximately 5 fold higher than the theoretically expected frequency of 0.0056 Hz with assuming a diffusion-governed collision. With 0.8 pM NEs, the observed frequency of 0.020 Hz (10 peaks within 500 s) is ~3 fold lower than the theoretical value of 0.056 Hz, which is still reasonable range. With 8 pM, however, the observed frequency was 0.020 Hz (4 peaks in 200 s), 28 fold lower than the theoretical value of 0.56 Hz. Due to the stochastic feature in SEE measurements, the observed frequencies at 0.8 pM and 80 fM NEs are still within reasonable ranges. Only the case with 8 pM NEs shows significantly lower collisional frequency. Notably, under 8 pM NEs, the measured current spikes and the corresponding charge densities were very close to the limits of detection (LOD). Also, current spikes smaller than 3 times of background noise level were not counted in
our estimation. Resultantly, our observable number of current spikes at 8 pM NEs was limited, thereby the resulting collisional frequency was largely deviating from the theoretical prediction.

We repeated each set of SEE measurements three times (data not shown). From each set of SEE data, average current densities were determined as 12.5 (± 0.5) × 10⁻⁴, 2.5 (± 0.2) × 10⁻⁴, and 3.0 (± 0.2) × 10⁻⁴ fC/nm³ for 80 fM, 0.8 pM, and 8 pM NEs respectively. Using a goal-seek function in Excel and the exponential equations obtained from each NE concentration, the corresponding concentrations of FcMeOH in the blind sample were estimated as 22 (± 2) nM, 34 (± 3) nM, and 430 (± 50) nM FcMeOH for 80 fM, 0.8 pM, and 8 pM NEs, respectively.

In the charge density plot (Figure 3B), we defined an accuracy range as 30 – 95 % of maximum charge density (15.5 × 10⁻⁴ fC/nm³), where the slope of a curve is steep enough to sensitively correlate the total analyte concentration with the charge density in NEs. If the estimated charge density is outside the accuracy region, i.e., less than 30 % or higher than 95 %, it would give only upper limit or lower limit of analyte concentration, respectively. Based on this definition of accuracy range, we could accept the estimated FcMeOH concentration, 22 (± 2) nM determined by the curve with 80 fM NEs, which is close to the true value of the blind sample concentration, 20 nM. So, unknown sample concentration was accurately determined within 10 % of error range.
This successful blind sample test validates our quantitative analysis using SEE combined with NEs as a nanoextractor via separation, preconcentration, and detection in situ.

Figure 3. (A) Charge density curves vs. logarithm of total FcMeOH concentration in the presence of 8 pM (blue circles), 0.8 pM (red circles), and 80 fM NEs (grey circles), respectively. The charge density varies from $1.0 \times 10^{-4}$ to $15.5 \times 10^{-4}$ fC/nm$^3$ within $\sim 2$ orders of magnitude of FcMeOH concentration, showing a sigmoidal trend. The LODs under each 8 pM, 0.8 pM, and 80 fM NEs are 100 nM, 10 nM, and 1 nM FcMeOH (i.e. 20, 2, and 0.2 ppb), respectively. (B) Calibration curves for corresponding three concentrations of NEs. Calibration curves are plotted within the dynamic range in (A), where calibration equations are obtained by fitting with exponential functions.
Ultra trace Level Detection of Ubiquitous Aromatic Toxictants in Water. Our ultrasensitive analysis is readily applicable to other aromatic toxicants. As typical carcinogen, 2-Aminobiphenyl (2-ABP) was studied with SEE combined with NEs. As aforementioned, three sets of SEE measurements under a constant potential ($E_{tip} = 0.85$ V vs Pt QRE) were performed in the presence of 8 pM, 0.8 pM, and 80 fM NEs, separately. We could observe similar trends in SEE data to the case of FcMeOH. For example, a typical $i$-$t$ curve is illustrated with 1 nM 2-ABP in the aqueous bulk solution in the presence of 80 fM NEs (Figure 5A). Current spikes were well fitted with the bulk electrolysis model, implying the extraction/preconcentration of 2-ABP inside each NE. Based
on the integrated charges from this \(i-t\) curve, eq (3) (i.e. \(C_{\text{fill}} = 0.6 \text{ M}\)) and eq (4), the corresponding size distribution curve of NEs was constructed as shown in Figure 5B. A narrow range within 40 nm – 70 nm with a peak at 60 nm diameter was obtained, which is fairly consistent with DLS measurement. Since the current spikes under this condition are close to the limit of detection in our measurements, charges integrated from \(i-t\) curve could be slightly overestimated, thus leading to the larger mean diameter of NEs, 60 nm than DLS or TEM data.

Finally, using the integrated charges and the electrochemically estimated size of NEs from all sets of SEE data, the charge density plot vs. total concentration of 2-ABP could be constructed (Figure 5C, a numerical fitting with exponential function is depicted in SI). The resultant plot shows not only the LOD up to 1 nM, i.e., 0.17 ppb of 2-ABP, but also nearly identical trends to those for FcMeOH. Notably, this trend indicates that the extraction/preconcentration behavior of NEs as a nanoextractor can be universal for ubiquitous aromatic compounds, which are common as environmental pollutants. Given the excellent detection performance as well as the broad applicability for ubiquitous aromatic toxicants, the combination of NEs with SEE offers great prospects as a sensor for environmental applications. Further, this remarkable sensitivity will be synergized by incorporating selectivity factors such as a chelator inside NEs.
Conclusions

In conclusions, we could successfully employ NEs as nanoextractors, and explored the ultra-trace level sensing of aromatic toxicants from water using the stochastic electrochemistry, SEE. The direct and in-situ measurements of analytes extracted from water into NEs were enabled via electrochemical oxidation upon the collision of each individual NE to Pt UME. This new analytical approach exhibited a detection limit of ~0.1 ppb level for aromatic compounds dissolved in water owing to the unprecedentedly high partition coefficient at intact NEs. The extraction efficiency of

Figure 5. (A) I-t curves in the presence of 1 nM 2-ABP with 80 fM NEs ($E_{tip} = 0.85$ V vs Pt QRE). The insets magnified typical current spikes fitted with bulk electrolysis model (red open circles). (B) Size distribution curves of NEs based on the electrochemical measurements (orange bars) from (A) and DLS measurements (black solid lines). (C) Charge density curves vs. logarithm of 2-ABP concentration in aqueous bulk solution in the presence of 8 pM (blue circles), 0.8 pM (red circles), and 80 fM NEs (grey circles), respectively.
NEs was remarkable to reach the preconcentration factor up to ~8 orders of magnitude, which is considered superior to previously reported analytical sensing. Notably, any prolonged preconcentration step is not required in the present work owing to the fast mass transport of analytes to NEs, whereas traditional stripping voltammetry techniques with subnanomolar level of LOD needs hour-long preconcentration steps.\textsuperscript{20} In addition, our demonstrated approach is transformative to other NE systems. If differently sized NEs are utilized, the overall analytical processes should undergo three steps: (1) Characterizing the size distribution of given NE system, (2) Constructing calibration curves (as shown in Figure 3) from SEE measurements, (3) Quantify the unknown sample using calibration curves. Given the excellent detection performance as well as the broad applicability for ubiquitous aromatic contaminants, the combination of NEs with SEE offers great prospects as a sensor for environmental applications.

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**Author Contributions**

All authors have given approval to the final version of the manuscript

**Notes**

The authors declare no competing financial interest.

**Supporting Information**

Characterization of NEs by Dynamic Light Scattering, Additional Experimental Data, Fitting \(i-t\) Decay with Bulk Electrolysis Model, Additional SEE Data with FcMeOH, Electrochemistry of 2-ABP in THF Cocktail Solution, Calibration curves for 2-ABP with 8 pM, 0.8 pM, 80 fM NEs, \(i-t\) Curves with NEs added to the Blank Aqueous Solution Containing no FcMeOH.

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