Electrochemistry Hot Paper

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Electrochemical Sizing of Organic Nanoparticles**

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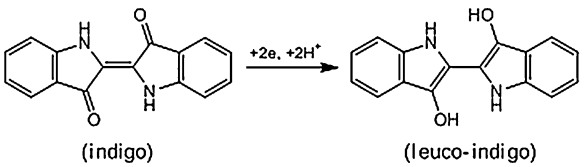
Size plays a crucial role in the applications of organic nanoparticles across many fields such as biomedicine, optoelectronics, and environmental science.[1–4] So far, microscopy and spectroscopy techniques have played central roles in organic nanoparticle detection; however, unlike metal nanoparticles, the widespread use of microscopy such as TEM or SEM is limited as the organic particle size may be compromised after drying when imaging is conducted. In comparison to metal and inorganic nanoparticles, the real-time in situ sizing of organic nanoparticle challenges existing techniques because of their presence in complex aqueous media which may affect their aggregation/agglomeration state and stability over time.[5] Light-scattering techniques such as DLS provide a powerful sizing technique for organic nanoparticles in complex solution; however, they measure the overall signal from the whole bulk of nanoparticles which makes it very sensitive to the presence of large particles. It can thus be influenced by the presence of possible dust particles or small amounts of large aggregates which will influence the size determination. More recently, alternative advanced optical[6,7] and ultracentrifugation methods[8] have been developed for sizing of nanoparticles, but they generally require expensive instrumentation and long processing times.

Electrochemistry as an alternative method to optical methods provides a more efficient and cost-effective approach for characterization of nanoparticles (NPs).[9–14] Our group has developed the nanoimpact method based on the detection of solution-phase NPs upon their random collision by virtue to their Brownian motion with the imposed electrode. This method has been successfully applied for determining the size,[9] concentration,[10] the presence of molecular tags,[11] and aggregation[12] of silver and other metal nanoparticles.[13] However, no studies have reported the electrochemical characterization, sizing and detection of organic NPs, although Bard and co-workers have investigated luminescent organic nanoparticles.[15a,b,c]

Here, we demonstrate for the first time the direct electrochemical sizing of organic nanoparticles using indigo nanoparticles as a model, based on Faradaic charge transfer when nanoparticles strike an electrode. This strategy is shown to be both feasible and quantitative, and could be used for size monitoring of a wide range of organic nanoparticles in real environments.

Indigo nanoparticles of various sizes were synthesized by the re-precipitation method, as described in Ref. [16]. Further information can be found in the Experimental Section. First, to determine the reduction potential of indigo NPs for impacting experiments, cyclic voltammetry (CV) was performed by using an indigo-modified carbon macroelectrode. A known amount of the suspension of Indigo NPs was drop-casted on the carbon macroelectrode to modify the electrode, and left to dry under a N_2 atmosphere. Cyclic voltammograms were recorded with the modified carbon macroelectrode immersed in phosphate-buffered saline (PBS) buffer, yielding a reduction feature of indigo NPs associated with the following known reaction [Eq. (1)].[17] The CV scan of a significant reduction peak of indigo NPs at −0.650 V is shown in the Supporting Information. The potential used for reduction of indigo NPs for impact experiments was −0.70 V.



(1)

Next, a carbon microelectrode was placed in 0.1M PBS buffer solution (pH 7.0) and a known concentration of predispersed indigo NPs added. The electrode potential was held at −0.70 V. Under potentiostatted conditions (between −0.7 V and −0.9 V), reductive (Faradaic) spikes from individual indigo NPs were observed (Figure 1 a), showing for the first time that direct reduction of individual organic nanoparticles during random collision events can be observed and quantified. As expected, the onset of these Faradaic spikes was found dependent on the reduction potential. By reducing the reduction potential (for example, to −0.3 V), no spikes were observed (see the Supporting Information), showing those spikes result from the Faradaic reduction of indigo NPs. Increasing the amount of added indigo NPs increased the frequency of spikes (see the Supporting Information). This further confirms that the spikes correspond to Faradaic reduction of indigo NPs. To further quantify the size of indigo NPs, the equation between diameter and charge of single indigo NPs is established based assuming that indigo NPs are spherical (diameter, D_{np}), and the charge Q passed as a result of complete reduction of single indigo NPs is given by Equation (2),

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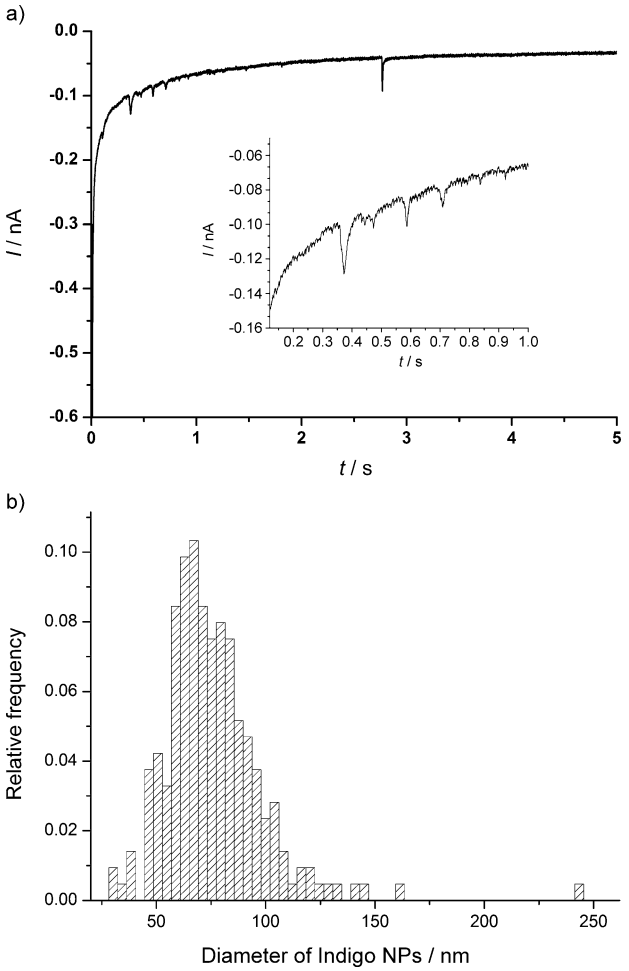

Figure 1. a) Chronoamperometric profiles showing reductive Faradaic spikes of indigo NPs in PBS buffer (pH 7) at −0.70 V versus saturated calomel electrode. The inset shows the detailed impact spikes. b) Distribution of the NP diameter derived from a charge per current spike according to Equation (2).

$$D_{np} = 2^3\sqrt{\frac{3A_rQ}{4nF\pi\rho}} \qquad (2)$$

where ρ is the NP density,[18] and A_r is the relative atomic mass. The spherical geometry of indigo nanoparticles was visualized using a dynamic light scattering microscopy (see the Supporting Information). The parameter n is the number of electrons transferred in the reduction of indigo NPs; $n = 2$ according to,[17] and has confirmed by plotting the charge obtained from the integration of the peak area of CV (see the Supporting Information) versus the total amount of indigo deposited on the electrode. Multiple repeats were performed such that total number of recorded impact events was greater than 300. By analyzing those impact spikes, the size distribution of the indigo NPs was derived, as shown in Figure 1 b. The mean diameter and standard deviation of indigo NPs were determined to be 68 ± 23 nm. The results demonstrated for the first time the electrochemical sizing of organic nanoparticles can be quantified. To verify our results from the electrochemical sizing, the size of indigo NPs from the same batch was characterized by dynamic light scattering (DLS; see the Experimental Section and Supporting Information for more details). There was an excellent agreement of the resulting sizing distribution obtained from our electrochemical sizing and the DLS analysis, as shown in Figure 2.

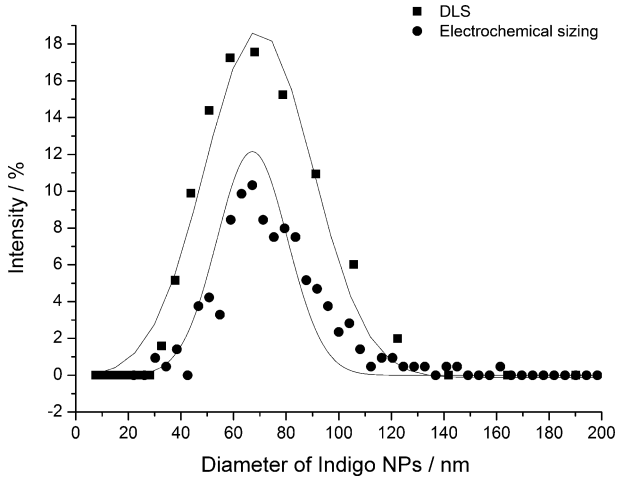

Figure 2. Indigo NP size distribution obtained from electrochemical sizing experiment (dots) and DLS analysis (squares) with small nanoparticles.

To further confirm our electrochemical sizing results of indigo NPs, analogous experiments were carried out to size the other batch of indigo NPs of larger size. As expected, by increasing the amount of indigo for re-precipitation, the size of indigo NPs was increased, as characterized by DLS, giving a mean size distribution of 107 nm. The size of indigo NPs was then electrochemically characterized by adding the indigo NPs into the buffer and the reductive spikes of indigo NPs of the batch were observed and subsequently analyzed. The size distribution from indigo NPs were analyzed from the charges of those individual reductive spikes with a mean diameter and standard deviation determined to be 107 ± 35 nm (Figure 3),

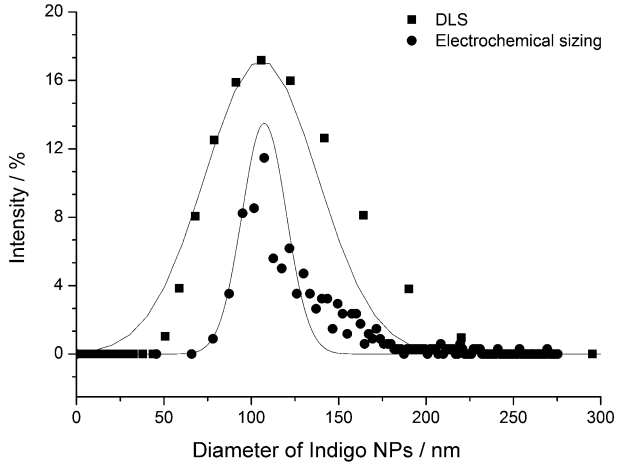

Figure 3. Indigo NP size distribution obtained from electrochemical sizing experiment (dots) and DLS analysis (squares) with large nanoparticles.

again showing an excellent agreement with pervious DLS sizing results.[19]

Herein we have shown for the first time, that direct electrochemical sizing of organic NPs is feasible and quantitative. As the electrochemical sizing enables the simultaneously sizing of individual organic NPs, we expect that this sizing strategy can be applied to determine the size range of organic NPs in drug delivery and in biological environments, which are impossible to characterize by other sizing methods currently available. This methodology is currently applied to characterize redox organic nanoparticles, however, this may further open a new strategy to electrochemically characterize a wide range of organic nanoparticles including antibodies and proteins through surface modified redox active groups. Research is underway to extend this phenomenon to characterization of other types of organic NPs as well as the detection of organic nanoparticles in authentic environments.

Experimental Section

Indigo (synthetic, 95%), dimethyl sulfoxide (DMSO, 99.9%, anhydrous) were purchased from Aldrich. Indigo was dissolved in 25 mL DMSO, giving an indigo concentration of 0.5 mM. An Indigo–DMSO solution (200 μL) was added to deionized water (20 mL) in a glass round-bottom flask and thoroughly mixed by magnetic vortexing stirring at 800 rpm for 5 minutes. The resulting mixture was a light blue, transparent dispersion with the appearance of a solution. To prepare indigo nanoparticles of larger size, the indigo–DMSO solution (800 μL; the volumetric ratio of the organic solution to water was increased four times) of was added. The mixture presented a blue transparent dispersion. The size distributions of synthesized indigo nanoparticles were measured by dynamic light scattering (Malvern Instruments Ltd, UK).

Unless stated otherwise, all chemicals in this work were acquired from Sigma–Aldrich. All solutions were made up using ultrapure water of resistivity 18.2 MΩ cm (Millipore) at 25 °C and degassed thoroughly with N_2 (oxygen-free, BOC Gases plc) before use. The electrochemical experiments were performed at room temperature in a conventional three electrode setup using an Autolab II potentiostat (Metrohm-Autolab BV, Netherlands) within a double Faraday cage. For drop-casting and voltammetric measurements experiment, a carbon macroelectrode (3 mm diameter) was used as working electrodes, a saturated calomel electrode (SCE) as reference (Radiometer, Copenhagen), and a graphite rod as the counter electrode. Electrochemical sizing and chronoamperometric recording were performed with the same reference and counter electrodes used for the voltammetric measurements above but the working electrode used was a carbon microelectrode (radius 5.5 μm). Signal Counter was developed by Dr. D Omanović at Center for Marine and Environmental Research Zagreb, Croatia and used for analysis of the impact spikes observed and OriginPro 8.5.1 for statistical analysis on nanoparticle size.

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[19] Note: the event that the two nanoparticle strike on the electrode surface simultaneously is rarely observed in the experiments, but if it did, the doubled charge will be seen.

