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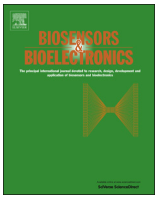

Calix[8]arene functionalized single-walled carbon nanohorns for dual-signalling electrochemical sensing of aconitine based on competitive host-guest recognition

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ABSTRACT

A dual-signalling electrochemical approach has been developed towards aconitine based on competitive host–guest interaction by selecting methylene blue (MB) and p-sulfonated calix[8]arene functionalized single-walled carbon nanohorns (SCX8–SWCNHs) as the “reporter pair”. Upon the presence of aconitine to the performed SCX8–SWCNHs · MB complex, the MB molecules are displaced by aconitine. This results in a decreased oxidation peak current of MB and the appearance of an oxidation peak of aconitine, and the changes of these signals correlate linearly with the concentration of aconitine. A linear response range of 1.00–10.00 μM for aconitine with a low detection limit of 0.18 μM (S/N=3) was obtained by using the proposed method. This method could be successfully utilized to detect aconitine in serum samples. This dual-signalling sensor can provide more sensitive target recognition and will have important applications in the sensitive and selective electrochemical detection of aconitine.

1. Introduction

Aconitine is a diester-diterpene alkaloid derived from some Chinese medicinal herbs of genus *Aconitum* in the family of Ranunculaceae such as *Aconitum carmichaeli* Debx. and *Aconitum Kusnezoffii* Reichb. These medicinal herbs are widely used in clinics in China and other East Asian countries because of their effects against rheumatoid arthritis and some other inflammations (Li and Cai, 2013). However, the toxicity resulting from these alkaloids in these plants, such as aconitine, talatisamine, and Yunaconitine cannot be neglected. The tubers and roots of aconites, which have been proved to be neurotoxic and cardiotoxic, are applied only after cautious processing (usually boiling) in order to reduce their toxicity. Nevertheless, unexpected poisoning incidents caused by these toxic alkaloids remained in the herbal medicines have occurred from time to time because of improper processing (Yang et al., 2010). Thus, the development of a valid and sensitive method to determine the *Aconitum* alkaloids is of great importance. So far, several methods have been reported for the determination of *Aconitum* alkaloids, including HPLC (Wang et al., 2006), GC–MS (Wada et al., 2006), LC-MS (Beyer et al., 2007), etc. However, most of these methods need complex sample pretreatments, such as liquid-phase extraction, solid phase extraction, and pre-column derivation, which are time-consuming, tedious, or need large amounts of toxic organic solvents (Yang et al., 2010). Therefore, designing and developing an effective, rapid, and simple analytical method for the determination of *Aconitum* alkaloids is highly desirable. Electrochemical detection is an attractive alternative because it features high sensitivity, instrument simplicity, fast response, low cost, and feasibility of miniaturization (Gupta et al., 2007a, 2007b, 2012, 2015a, 2015b; Jain et al., 2010; Goyal et al., 2010; Karimi-Maleh et al., 2014a, 2014b; Ghaedi et al., 2015).

The host–guest interaction is extensively used to construct nanoscale electrochemical devices in the field of molecular recognition because its recognition motifs are specific and bioorthogonal and can be used without an additional catalyst (Uhlenheuer et al., 2010; Chinai et al., 2011). However, the use of synthetic receptors with competitive binding assays has gained popularity within the past two decades, and the so-called “indicator displacement assay” or IDA, has become a standard strategy for molecular sensing, complementary to the approach of direct sensing (You et al., 2015). The sensing principle of IDA relies on the competition between a test substance and an indicator for the same binding site on the host. When an analyte is added to a solution containing a host · indicator complex, the analyte displaces the indicator from the binding site. Upon displacement of the indicator, a change in signal is observed (Ghale and Nau, 2014).

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Although IDA has been widely applied in the optical sensing field (Praetorius et al., 2008; Ghale et al., 2011; Mao et al., 2012; Biedermann and Nau, 2014; Li et al., 2015; Yang et al., 2015), it has not been extensively investigated for electrochemical sensing applications except for a few researchers that contributed to this area (Agnihotri et al., 2015; Yang et al., 2016).

Calixarenes, recognised as the third generation of macrocyclic host molecule after crown ethers and cyclodextrins, which show high supramolecular recognition and enrichment capability with various guest molecules (Dsouza et al., 2011; Gupta et al., 2013). Some water-soluble calixarenes, particularly p-sulfonated derivatives, have been widely investigated to develop different electrochemical sensing platforms due to their biocompatibility and simplicity of synthesis (Zhou et al., 2013; Yang et al., 2016). Single-walled carbon nanohorns (SWCNHs), not only have advantages of conventional carbon nanomaterials but also possess excellent catalytic properties, high purity and low toxicities, which could be explored as a replacement of nanotubes to be used in the electrochemical sensing or biosensing field (Yang et al., 2014). Recently, Ojeda et al. reported an electrochemical biosensing platform using CNHs as a scaffold and their results indicated that the CNHs-based sensing platform showed response superior to that of CNTs-based (Ojeda et al., 2014). It has been reported that the composites of calixarenes and carbon materials could be formed by π–π interactions and hydrogen interactions (Zhou et al., 2013; Chen et al., 2015). If SWCNHs are modified with water-soluble calixarenes, it is possible to obtain new functionalized materials that simultaneously possess the unique properties of SWCNHs and calixarenes. Therefore, the integration of SWCNHs and p-sulfonated calixarenes can be potentially applied in the electrochemical sensing or biosensing field, and thus arouses extensive research interest.

Herein, to the best of our knowledge, this is the first time a dual-signalling electrochemical approach for aconitine sensing based on a competitive host–guest recognition between p-sulfonated calix[8]arene (SCX8) and signal probe/target molecules using a SCX8–SWCNHs modified electrode was developed. As illustrated in Scheme 1, methylene blue (MB) and aconitine were selected as the probe and target molecules, respectively. The MB molecules can enter into the hydrophobic inner cavity of SCX8 due to the host–guest interaction, and the SCX8–SWCNHs modified electrode displays a remarkable anodic peak. In the presence of aconitine, a competitive interaction to SCX8 occurs and the MB molecules are displaced by aconitine. This results in a decreased oxidation peak current of MB and the appearance of an oxidation peak of aconitine. Thus, the aconitine can be easily detected by such a dual-signalling electrochemical sensing strategy.

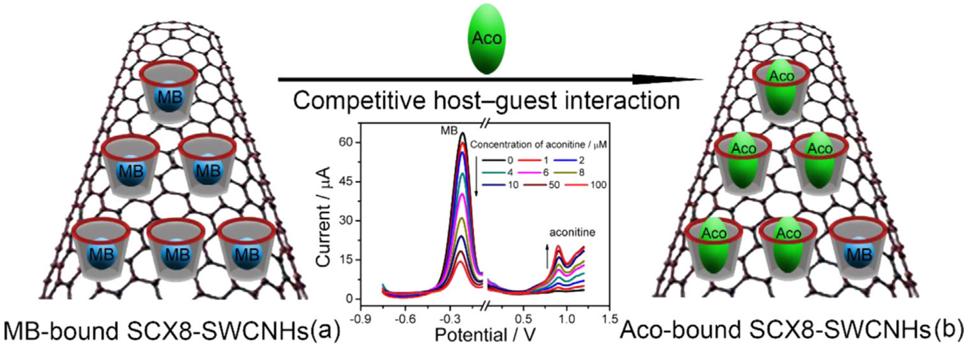
2. Materials and methods

The experimental details are provided in **ESI**.

3. Results and discussion

3.1. Characterization of the SCX8-SWCNHs composite

A green and clean approach was adopted to prepare the SCX8–SWCNHs with excellent dispersibility in water, which was carried out by sonicating the SWCNHs in SCX8 aqueous solution. Firstly, the dispersibility of SWCNHs and SCX8–SWCNHs was evaluated as shown in Fig. 1**A**. It can be seen that the dispersion ability of the pristine SWCNHs in aqueous solution is rather poor due to the strong π–π interaction. However, the obtained SCX8–SWCNHs could be stably dispersed in water even after removing free SCX8 via high-speed centrifugation (16,000 rpm) and no obvious precipitates are observed after being stored for more than 6 months, which is caused by the hydrophilic property of the SCX8. This implies that the SCX8 is important for efficiently inhibiting the aggregation of the SWCNHs. As shown in Fig. 1**B**, the TEM image of SWCNHs exhibited the typical morphology of dahlia bundles with a diameter of approximately 40 nm (Ojeda et al., 2014). A TEM image of SCX8–SWCNHs (Fig. S2) was also obtained compare with SWCHs. Since it was difficult to distinguish the SCX8 molecules on the TEM images, the SCX8–SWCNHs was further characterized by FTIR, TGA, and zeta potential analysis. The synthesized SCX8–SWCNHs was characterized by FTIR as shown in Fig. 1**C**, by comparing FTIR spectra of SCX8, SCX8–SWCNHs, and SWCNHs, significant features can be observed: Firstly, the peaks for $-SO_3^-$ at 1076 and 1045 cm^{-1}, as seen in the spectra of pure SCX8, also appeared in the spectra of SCX8–SWCNHs, indicating that SCX8 was attached to the surface of SWCNHs (Zhou et al., 2013). Secondly, the alteration of the peak value at 3440 cm^{-1} of –OH in SCX8 stretching vibrations shifting to 3433 cm^{-1} in SCX8–SWCNHs was identified as a result of hydrogen interactions between the oxygen-containing groups of SWCNHs and hydroxyl groups of SCX8 (Mao et al., 2012). In addition, it has been reported that the composites of calixarenes and carbon materials could be formed by π–π interactions and hydrogen interactions (Zhou et al., 2013; Chen et al., 2015). These results demonstrated that SCX8 had successfully self-assembled to SWCNHs and formed SCX8–SWCNHs nanohybrid. TGA was used to determine the amount of SCX8 on the SWCNHs, as shown in Fig. 1**D**. For the pristine SWCNHs, the minor loss in mass (4.0%) at a temperature of approximately 600 °C was due to the pyrolysis of a very small amount of the oxygen-containing functional groups. The pure SCX8 exhibited an abrupt mass loss at approximately 300 °C. The mass loss of the SCX8–SWCNHs reached about 22 wt% when the temperature was 600 °C. To deduct the mass loss of SWCNHs, the



Scheme 1. Protocol of the dual-signalling electrochemical sensing platform towards aconitine (Aco) by using SCX8–SWCNHs against MB.