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Water-dispersible molecularly imprinted nanohybrids via co-assembly of carbon nanotubes with amphiphilic copolymer and photocrosslinking for highly sensitive and selective paracetamol detection

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ABSTRACT

A novel kind of water-dispersible molecularly imprinted nanohybrids containing multi-walled carbon nanotubes (MWCNTs) was prepared via the combination of co-assembly and photocrosslinking, and employed to construct an electrochemical sensor. A photo-crosslinkable amphiphilic copolymer (Poly(AA-*co*-VMc-*co*-EHA), PDHES) was firstly synthesized, which could co-assemble with MWCNTs in the presence of template molecule (paracetamol, PCM) in aqueous solution, generating photo-crosslinkable molecularly imprinted nanohybrids (MIP-MWCNTs). A robust MIP-MWCNTs film was formed on the electrode surface via the deposition of MIP-MWCNTs nanohybrids and the subsequent photo-crosslinking. After extracting paracetamol molecules, an electrochemical MIP-MWCNTs sensor was successfully developed. The as-prepared MIP-MWCNTs sensor showed a significantly wide linear detection range (0.1–2500 μM) and low detection limit (0.02 μM), owing to the large surface area of MIP-MWCNTs nanohybrids and superior electrical conductivity of MWCNTs, which affords a direct channel for the electron transfer from the recognition cavities to the electrode surface and thus enhances the response signal. In addition, good selectivity toward paracetamol (the imprinting factor α is 7.5) and ultrafast response time (less than 180 s) of MIP-MWCNTs sensor have also been demonstrated. The MIP-MWCNTs sensor also exhibited satisfactory repeatability and stability, which has been successfully utilized to measure paracetamol in urine samples with good results, demonstrating a promising feature for applications in medical diagnostics.

1. Introduction

Molecularly imprinted technology has proven to be a crucial approach for preparing receptors with tailor-made recognition sites complementary to the template molecules in shape, size and functional groups. Owing to their advantages of easy preparation, good stability, and low prices, the resulting molecularly imprinted polymers (MIPs) with affinity and specificity towards templates, have been widely applied in sample pretreatment, chromatographic separation and sensing of active molecules, pharmaceuticals, environmental pollutants and many other fields (Hu et al., 2013; Martin-Esteban, 2013; Tiwari and Prasad, 2015; Uzun and Turner, 2016; Wackerlig and Lieberzeit, 2015).

In the last decade, MIPs are preferred to be prepared into nanosized particles or surface imprinting materials to overcome the drawbacks of traditional monolith MIPs (incomplete template removal, poor site accessibility, and small binding capacity). Nanosized MIPs possess high surface-to-volume ratio and large surface area. As a result, most template imprinting sites are located at or close to the materials surface, which leads to more complete templates removal, faster binding kinetic, and larger binding capacity (Alizadeh and Allahyari, 2013; Bazrafshan et al., 2017; Gao et al., 2007; Li et al., 2006; Wackerlig and Schirhagl, 2016). Although great progress has been achieved in nanosized MIPs, there are still some challenges remaining to be resolved. For example, previously prepared nanosized MIPs are normally synthesized in organic solvents, whereas most of the practical applications of MIPs are related with aqueous environment such as environmental monitoring, food safety control, and clinical diagnostics. It is well-known that the specific recognition performances of MIPs prepared in organic solvents is not so good when applied in aqueous environments, which puts significant limit in their practical applications (Arabi et al., 2017; Ostovan et al., 2018; Yang et al., 2009; Zhang, 2014; Zhang and Li, 2013).

To address this issue, various water-compatible synthesis strategies have been developed to prepare water-compatible nano-sized MIPs,

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such as reversible addition-fragmentation chain transfer (RAFT) precipitation polymerization employing hydrophilic macromolecular chain-transfer agents (Arabi et al., 2017; Ma et al., 2013; Zhao et al., 2014), surface-core cross-linking of a surfactant micelle (Awino and Zhao, 2015), stimuli-responsive MIPs (Wu et al., 2016), and imprinting in metal–organic gels such as porogen (Ma et al., 2015). In recent years, our group has developed an efficient and versatile strategy combining the self-assembly of amphiphilic copolymers and molecular imprinting technology to prepare a series of nano-sized MIPs in aqueous solution. The easy availability and versatility of the amphiphilic copolymer and the one-pot assembly process in aqueous solution makes this strategy highly efficient and could be applicable for various small organic molecules as well as proteins (Fan et al., 2013; Luo et al., 2013; Yang et al., 2011; R.L. Zhang et al., 2015; Q.L. Zhang et al., 2015; Zhang et al., 2016). And the as-prepared water-compatible nano-sized MIPs have been successfully employed in constructing MIP electrochemical sensors, which showed specific recognition toward the template molecules and wide detection range in water. However, the sensitivity of the developed MIP sensors was far from satisfactory, which should be attributed to poor electrical conductivity of the nano-sized MIPs composed of the electrically insulating amphiphilic copolymers, generating a serious barrier for the electron transfer and greatly limiting the signal transduction process (Mazzotta et al., 2016). In order to solve this problem, electroactive components such as conducting polymer and Au NPs have been successfully incorporated in the macromolecular assembly system to construct conductive or electroactive molecular imprinted nanohybrids very recently. The electrical response and sensitivity of the prepared MIP electrochemical sensor from these conductive or electroactive molecular imprinted nanohybrids have been greatly enhanced (Luo et al., 2017, 2016a, 2016b; Zhao et al., 2018).

In addition to conducting polymer and metal materials, carbon nanomaterials possess outstanding electrical conductivity and have been frequently employed as sensing elements. In particular, carbon nanotubes, cylindrical carbon materials with well-ordered arrangement of carbon atoms linked via sp^2 bonds, have been widely used in biosensing platforms due to their outstanding electrical conductivity, large surface area, robust mechanical strength and chemical stability (Anirudhan et al., 2014; Liu et al., 2016a, 2014a; Yang and Zhao, 2015). So it is highly envisioned that if carbon nanotubes could be incorporated into the assembly system, a novel water-compatible MIP nanohybrids with high electrical conductivity can be produced.

Herein, a novel kind of water-dispersible molecularly imprinted nanohybrids with good electrical conductivity was prepared via the co-assembly of multi-walled carbon nanotubes (MWCNTs) with amphiphilic photo-crosslinkable copolymer in the presence of template molecules. As a proof of concept, paracetamol (PCM), widely employed as analgesic and antipyretic drug, was chosen as the template molecule. A robust MIP-MWCNTs film was formed on the electrode surface via the deposition of MIP-MWCNTs nanohybrids and the subsequent photo-crosslinking. After extracting paracetamol molecules, an electrochemical MIP-MWCNTs sensor was successfully developed for the detection of PCM. The whole fabrication strategy of MIP-MWCNTs nanohybrids and MIP-MWCNTs sensor was shown in Scheme 1. The resulted MIP-MWCNTs nanohybrids have been characterized. The sensing capability of the MIP-MWCNTs sensor such as selectivity, detection range, detection limit, and stability has been investigated.

2. Experimental section

2.1. Chemicals and reagents

MWCNTs (> 95%) were purchased from Chengdu Organic Chemicals Institute, Chinese Academy of Sciences, China. Diphenylketone, paracetamol (PCM), ascorbic acid (AbA), uric acid (UA), 4-aminophenol (4-AP), 4-nitrophenol (4-NP), and lactic acid were bought from Sinopharm Group Chemical Reagent. The deionized water ($18.2\,M\Omega\,cm^{-1}$) was obtained from a Millipore Milli-Q water purification system. All the other chemicals and reagents were of analytical grade and used as received.

2.2. Preparation of MIP-MWCNTs nanohybrids

The photo-crosslinkable amphiphilic copolymer PDHES was prepared according to Yang's et al. (2011) work with slight modifications. Its chemical structure and synthetic route are shown in Scheme 1A and Fig. S1, respectively. The NMR and FT-IR spectra are provided in the supporting information (Fig. S2), convincing the structure of the PDHES copolymer. Its molecular weight was determined by GPC analysis and calculated to be $Mn = 5.6 \times 10^4$ g/mol.

To prepare the molecularly imprinted nanohybrids, 20 mg of PDHES copolymer, 20 mg of PCM, 10 mg of diphenylketone (photoinitiator) and 1.0 mg of MWCNTs were co-dissolved in10 mL of DMF to give a 2 mg/mL copolymer solution containing 0.1 mg/mL MWCNTs. Meanwhile, 1 vol% lactic acid was added to keep the solution pH below 4.5 to ensure the protonation of dimethylamino group. The mixture solution was stirred for 12 h to ensure the complexation of PDHES copolymers with MWCNTs. A predetermined amount of ultrapure water as poor solvent was dropwise added into the mixtures to induce the co-assembly of PDHES copolymer, PCM, and MWCNTs to generate imprinted nanohybrids. The imprinted photocrosslinkable MIP-MWCNTs nanohybrids was obtained after dialyzing against water for 36 h to remove DMF and free PCM molecules.

As control samples, nonimprinted photosensitive nanohybrids (NIP-MWCNTs) and MIP nanoparticles were prepared with the same method but in the absence of the template molecules and MWCNTs, respectively.

2.3. Preparation of the MIP-MWCNTs sensor

Prior to preparation procedure, a bare glass carbon electrode (GCE, Φ 3 mm) was polished with 0.1 and 0.05 µm alumina slurries to produce a mirror-like surface, sonicated in ultrapure water, anhydrous alcohol, ultrapure water, respectively, and finally dried with nitrogen stream. A certain volume of MIP-MWCNTs water dispersion was cast on GCE surface and dried at room temperature. Then the MIP-MWCNTs modified electrode was exposed under UV irradiation for 30 min (UV light generated by a spot-curing system with a wavelength of 365 nm and a power of $16\,mW/cm^2$) to induce the photo-crosslinking and lock the recognition sites, leading to a robust MIP-MWCNTs film. Finally, the PCM template molecules were extracted from the MIP-MWCNTs film by treating the modified electrode with a mixture of acetic acid and methanol (1:4, v/v), generating the MIP-MWCNTs sensor.

As control samples, a nonimprinted sensor (NIP-MWCNTs sensor) was prepared following the same procedure for the MIP-MWCNTs sensor, but using nonimprinted photosensitive nanohybrids (NIP-MWCNTs). In addition, an imprinted control sensor (MIP sensor) was prepared using MIP nanoparticles instead of MIP-MWCNTs.

2.4. Characterization and measurement

^1H NMR spectrum of the amphiphilic photo-crosslinkable copolymer was recorded by a Bruker (400 MHz) instrument. Fourier transmission infrared (FTIR) spectrum is recorded with a FTIR spectrophotometer (FTLA 2000-104). The morphology of nanohybrids was observed by the transmission electron microscope (TEM) using JEOL JEM-2100 at 120 kV and a field emission scanning electron microscope (FE-SEM, Hitachi S-4800) operating at 2.0 kV. Dynamic light scattering (DLS) was carried out using an ALV-5000 laser light scattering spectrometer.

All electrochemical experiments were performed using a CHI660E electrochemical workstation with a three-electrode cell, which includes a saturated calomel electrode as reference electrode, GCE as the