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Preparation and characterisation of core–shell CNTs@MIPs nanocomposites and selective removal of estrone from water samples

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ABSTRACT

This paper reports the preparation of carbon nanotubes (CNTs) functionalized with molecularly imprinted polymers (MIPs) for advanced removal of estrone. CNTs@Est-MIPs nanocomposites with a well-defined core–shell structure were obtained using a semi-covalent imprinting strategy, which employed a thermally reversible covalent bond at the surface of silica-coated CNTs for a large-scale production. The morphology and structure of the products were characterised by transmission electron microscopy and Fourier transform infrared spectroscopy. The adsorption properties were demonstrated by equilibrium rebinding experiments and Scatchard analysis. The results demonstrate that the imprinted nanocomposites possess favourable selectivity, high capacity and fast kinetics for template molecule uptake, yielding an adsorption capacity of 113.5 μmol/g. The synthetic process is quite simple, and the different batches of synthesized CNTs@Est-MIPs nanocomposites showed good reproducibility in template binding. The feasibility of removing estrogenic compounds from environmental water using the CNTs@Est-MIPs nanocomposites was demonstrated using water samples spiked with estrone.

1. Introduction

During recent years, emerging contaminants such as steroid sex hormones, which have been referred to as endocrine disruptors or endocrine disrupting compounds (EDCs), are of particular concern because of the volume of these substances that are used and because of their activity as endocrine disruptors. Both natural and synthetic steroid hormones can be found in the environment, and they are believed to enter the water system via human or animal farming. Hormones, such as estradiol, estrone and ethinylestradiol, have been found in water at ng/L levels [1–3], but even at these low concentrations, some of these hormones may induce estrogenic responses and cause adverse effects in aquatic and terrestrial organisms and in humans [4]. Estrone is one of the naturally estrogenic hormones, which can be toxic and carcinogenic even at low levels. Estrone has been reported to influence the normal development and maturation of females; in addition to estrone's suspected carcinogenic activity, it has been identified as a major contributor to the endocrine-disrupting activity observed from environmental water samples [5,6]. To prevent these uncontrolled and deleterious effects on human health and the aquatic environment, the development of new adsorbents for the separation of estrogenic compounds is of significant interest. Currently, there is considerable interest in developing new, selective and sensitive methods for extracting and isolating components from complex environmental matrices. As a result, solid phase extraction and clean-up methods based on molecularly imprinted polymers (MIPs) have been developed to increase selectivity in the extraction of target analytes from very complex samples [7–10].

Molecular imprinting has become an attractive technique that allows the construction of tailor-made binding sites for a given target or group of target molecules [11]. MIPs are usually prepared by polymerizing a mixture of template, functional monomer and an excess of crosslinker; the template is then removed from the crosslinked polymer network to leave functional recognition sites with a shape that is complementary to the template. These specific sites provide the capacity for specific rebinding with the template. Due to their mechanical and chemical stability, easy preparation and low cost, MIPs have been used in a variety of application, such as for separation media [12], mimicking antibodies [13] and chemical and biomimetic sensors [14].

Recently, core–shell nano-structured MIPs composites based on magnetic nanoparticles [15–18] and carbon nanotubes (CNTs) [19,20], which combine the advantageous properties of both materials, are actively being pursued due to their unique physicochemical properties and great potential in magnetic, biomedical,

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electronic, catalytic and biosensor applications. Theophylline-imprinted polymers were immobilized on CNTs and grafted onto iniferter-modified CNTs by Lee et al. [19,20]. An electrochemical sensor fabricated by modifying MWCNTs-MIPs on a glassy carbon electrode surface to recognize dopamine has been reported by Kan et al. [21], and Yu and Lai [22] have prepared MIPs by electrochemical polymerization of pyrrole onto a stainless steel frit, using ochratoxin A as the template and CNTs as nano-structured fibres to construct a micro-solid phase pre-concentration device. Choong et al. [23] have demonstrated a surface imprinting technique via electropolymerisation of a conducting polypyrrole thin film onto an array of vertically aligned CNTs. Despite the extraordinary properties of CNTs, however, most of their applications are suppressed by their lack of solubility in any solvents. The aforementioned studies did not address this problem; in their methods, carrier CNTs must be used in an organic solvent. In this study, we focused on the development of a new methodology for using CNTs as carriers in both organic and aqueous solvents. Coating CNTs with SiO_2 was investigated as a potential synthetic route because SiO_2 has well-known insulating properties and biocompatibility [24]. Furthermore, the fabrication of a CNT-SiO_2 core@shell structure could eliminate the undesirable attractive interactions between the nanotubes and improve interfacial bonding with the matrix [25–27].

The objective of our work is to synthesize a novel imprinted material based on CNTs use of a "semi-covalent" imprinting technique that forms a reversible covalent bond; this material was applied as a sorbent for the removal of estrone at low concentration levels in water samples. In addition, the "semi-covalent" imprinting strategy, which was first developed by Whitcombe and co-workers [28,29] can be viewed as a hybrid approach that combines the advantages of both covalent and non-covalent imprinting methods, in which the imprinting is covalent but the rebinding is non-covalent in nature. Recently, a new imprinted film on the silver, magnetic nanoparticles or silica spheres, which used a "semi-covalent" imprinting strategy for imprinting estrone and testosterone, were reported [18,30–32]. Due to the formation of big bundles held strongly together, CNTs are difficult to disperse homogeneously in different solvents, which greatly limits their application. The physical and chemical properties of CNTs can be significantly altered through chemical surface modification, doping and coating. Herein, silica was selected to encapsulate the CNTs, since silica coatings can screen the CNTs and favor the dispersion of CNTs in liquid media. Furthermore, functionalizing CNTs with SiO_2 will not only alter the exposed surface of CNTs to silica without disruption of the CNTs' structure, but also probably make them biocompatible and further achieve applications in cell imaging, and delivery of proteins and drugs [33,34]. In this study, core@shell nano-structured estrone-imprinted CNTs@Est-MIPs composites were prepared via sol–gel procedure using a semi-covalent imprinting strategy that formed thermally reversible covalent bonds at the surface of CNTs coated with silica. The functionalization of the surface of CNTs with MIPs possessing tailor-made binding sites permits the fabrication of novel one-dimensional hybrid materials. The CNTs@MIPs composites with distinctive outer surfaces can be an ideal candidate of multifunctional nanomaterial for recognition and preconcentration of a given target. The resulting CNTs@Est-MIPs nanocomposite were characterized using transmission electron microscope (TEM) and Fourier transform infrared (FT-IR) spectrometer. The adsorption properties were demonstrated by equilibrium rebinding experiments and Scatchard analysis. The selectivity of the obtained CNTs@Est-MIPs was elucidated by the different rebinding capabilities of estrone and two other estrogenic compounds. The synthetic process is quite simple, and the different batches of imprinted materials demonstrated good reproducibility as a sorbent for estrone. The resulting CNTs@Est-MIPs was successfully applied to remove estrone from spiked environmental and drinking water samples.

2. Experimental

2.1. Materials

Tetraethoxysilane (TEOS), 3-(triethoxysilyl)propyl isocyanate and 3-aminopropyltriethoxysilane (APTES) were purchased from Alfa Aesar Chemical Company. Multi-walled carbon nanotubes (MWCNTs, diameter: 60–100 nm, length: 5–15 μm) were provided by Shenzhen Nanotech Port Co. Ltd., China. Estrone, estriol and diethylstilbestrol were obtained from Sigma. HNO_3, cetyltrimethylammonium bromide (CTAB), *N*,*N*-dimethylformamide, triethylamine, ammonium hydroxide (25%), methanol, ethanol, acetone and chloroform were purchased from Tianjin Chemicals Ltd. Dimethyl sulfoxide (DMSO) was purified by standard methods prior to use. Other chemicals were used as received without further purification.

2.2. SiO_2 coating process on MWCNTs (CNTs@SiO_2)

Impurities such as amorphous carbon and metallic catalyst in the CNTs sample were removed using an HNO_3 solution (2.6 M) in a three-neck flask with vigorous stirring (500 rpm); the mixture was refluxed for 48 h. The suspension was then filtered through a 0.22 μm filter to recover the CNTs, followed by washing repeatedly with highly purified water until the pH reached 7.0, and dried under vacuum at 80 °C for further use. The SiO_2 coating on the CNTs was performed according to the method of Zhu et al. [35]. In a typical synthesis, 250 mg of pristine CNTs, 0.5 mL of APTES, 100 mg of cetyltrimethylammonium bromide (CTAB) and 48 mL of H_2O were sonicated in a conical flask for 20 min at 40 °C. After stirring for another 3 h, mixture A was obtained. When 5 mL of TEOS, 3 mL of H_2O and 50 mL of ethanol were treated exactly as mixture A, mixture B was obtained. Subsequently, mixture A and B were combined and then sonicated for 60 min at 40 °C. After stirring for another 10 min, ammonium hydroxide (25%) was added dropwise until the pH value reached 9.5. After the coating progress was performed, the product was washed with distilled water and ethanol several times. Finally, the product was dried under vacuum for use in further studies.

2.3. Preparation of estrone-imprinted polymers (CNTs@Est-MIPs) and non-imprinted polymers (CNTs@NIPs)

The template (estrone)–silica monomer complex (Est–Si) was synthesized according to the method of He et al. [32]. The synthesis of estrone-imprinted polymer CNTs@Est-MIPs using a semi-covalent imprinting strategy via a thermally reversible bond [30] is shown in Fig. 1. A mass of 0.5 g of CNTs@SiO_2 was dissolved into 30 mL of 0.1 M acetate buffer (pH 5.2), and 0.12 g of Est–Si was added to the solution. The mixture was reacted for 12 h with mechanical stirring (500 rpm) at room temperature. The product was centrifuged and then washed several times with acetone; the washed product was dried for use in further studies. The imprinting performances of products under different mass ratios of CNTs@SiO_2 to Est–Si in the present study are summarized in Table 1.

A mass of 0.3 g of the above product was added to a solution of dimethyl sulfoxide (10 mL) and water (2 mL). The mixture was stirred for 3 h at 180 °C. Finally, the estrone-imprinted CNTs@Est-MIPs nanocomposite was centrifuged and then washed several times with acetone; the washed product was dried under vacuum at 40 °C for use in further studies.

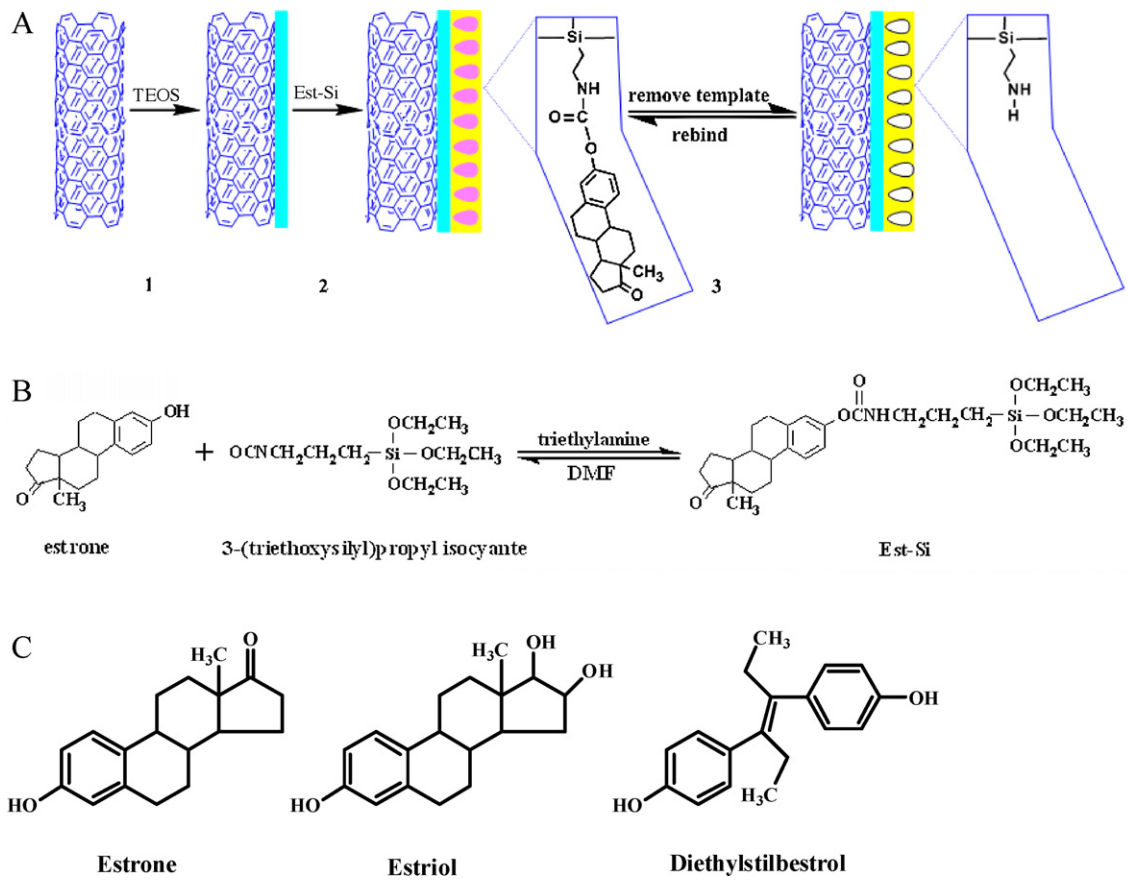

Fig. 1. Scheme for the synthesis of (A) CNTs@Est-MIPs and (B) template (estrone)–silica monomer complex (Est–Si); the chemical structures of estrone, estriol and diethylstilbestrol are presented in (C). The surface of purified CNTs was converted to a silica shell by a sol–gel process using TEOS and APTES in the presence of CTAB to give core@shell CNTs@SiO_2 (1). The CNTs@SiO_2 reacted with Est–Si to produce a silica surface functionalized with estrone-imprinted polymer (2). After removing estrone by a simple thermal reaction, estrone-imprinted polymer coated CNTs were obtained (3).

Non-imprinted polymers CNTs@NIPs were synthesized using the above procedure, except APTES was used in place of the template–monomer complex (Est–Si).

2.4. Binding experiment

2.4.1. Binding isotherm for estrone

A mass of 20 mg of CNTs@Est-MIPs and CNTs@NIPs were added separately to solutions of estrone in 10 mL of chloroform at various concentrations. After incubation for 1 h at room temperature, the CNTs@Est-MIPs and CNTs@NIPs were isolated by centrifugation. The filtrate was concentrated to dryness by evaporation of the solvent and then dissolved in methanol before HPLC analysis. The amount of estrone bound to the CNTs@Est-MIPs and CNTs@NIPs was determined by measuring the residual estrone in the filtrate using HPLC.

Table 1
Effect of polymerization conditions on imprinting performance of CNTs@Est-MIPs and CNTs@NIPs ($n = 3$).[a]

CNTs@SiO_2:Est–Si[b]	Q_{MIPs}[c] (μmol/g)	Q_{NIPs}[c] (μmol/g)	IF[d]
1:1	59.86 ± 0.34	18.96 ± 0.17	3.16 ± 0.05
2:1	72.65 ± 0.51	19.72 ± 0.21	3.68 ± 0.06
3:1	81.34 ± 0.42	19.96 ± 0.14	4.08 ± 0.05
4:1	89.58 ± 0.29	19.24 ± 0.26	4.66 ± 0.08
5:1	80.43 ± 0.67	19.39 ± 0.37	4.15 ± 0.11
6:1	64.71 ± 0.24	19.03 ± 0.25	3.40 ± 0.06
7:1	38.15 ± 0.89	19.54 ± 0.42	1.95 ± 0.08

[a] In this experiment, 20 mg of CNTs@Est-MIPs and CNTs@NIPs were incubated in the solution of estrone at a concentrations of 60 μg/mL for 1 h at 25 °C ($n = 3$).
[b] CNTs@SiO_2:Est–Si is defined as the mass ratio.
[c] Q_{MIPs} and Q_{NIPs} were the amount of analytes bound to CNTs@Est-MIPs and CNTs@NIPs at equilibrium, respectively.
[d] $IF = Q_{MIPs}/Q_{NIPs}$, which represents imprinting factor.

2.4.2. Repeatability of the CNTs@Est-MIPs

Six batches of 20 mg of CNTs@Est-MIPs that were prepared on different days were added to solutions of estrone with a concentration of 60 μg/mL in 10 mL of chloroform. After incubation for 1 h at room temperature, the binding amount of estrone was measured using HPLC.

2.4.3. Specific recognition of CNTs@Est-MIPs and CNTs@NIPs

A mass of 20 mg of CNTs@Est-MIPs and CNTs@NIPs were added separately to mixed solutions of estrone, estriol and diethylstilbestrol in 10 mL of chloroform at various concentrations. After incubation for 1 h at room temperature, the CNTs@Est-MIPs and CNTs@NIPs were isolated by centrifugation. The filtrate was concentrated to dryness by evaporation of the solvent and then dissolved in methanol before HPLC analysis. The amount of estrone, estriol and diethylstilbestrol bound to the CNTs@Est-MIPs was determined using HPLC. The mobile phase was methanol–H_2O (68/32, v/v) at a flow rate of 1.0 mL/min. The injection volume was 20 μL, and the column effluent was monitored at 280 nm.

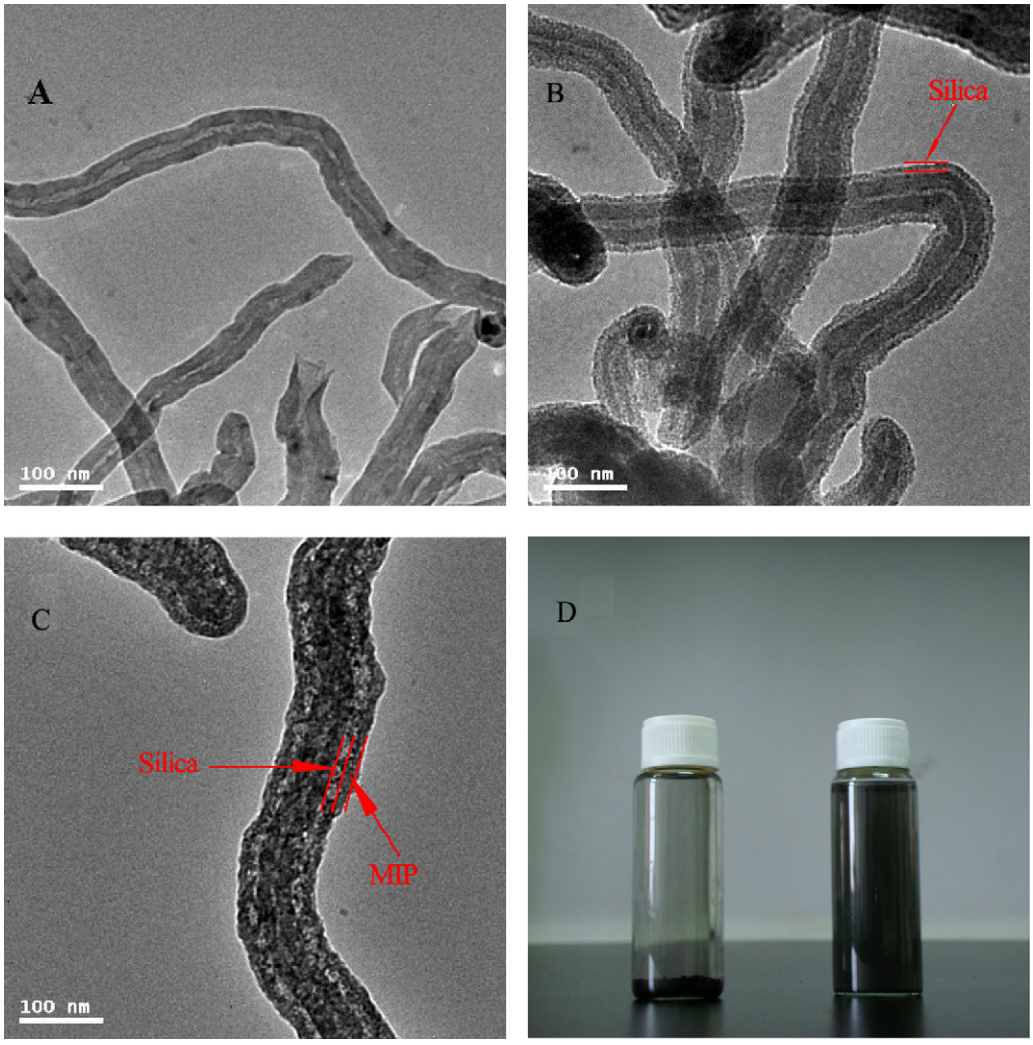

Fig. 2. TEM images of purified CNTs (A), silica film deposited on the surface of CNTs via a sol–gel process (B) and CNTs@Est-MIPs (C). Photos of CNTs (left) and CNTs@SiO_2 (right) dispersed in the aqueous phase (D).

2.5. Removal of estrone in environmental and drinking water samples

The river, lake and tap water samples collected from the Xiaoyin River, Mati Lake and the laboratory (Tianjin, China) were spiked with estrone at a concentration of 5 μg/mL and then stored in closed amber glass containers at room temperature in the dark until they were analysed, which was within 24 h after sampling. 50 mg of CNTs@Est-MIPs was added into 10 mL of the river, lake and tap water containing the spiked estrone. After incubation for 1 h at room temperature, the CNTs@Est-MIPs was isolated by centrifugation, and the supernatant was concentrated for analysis using HPLC. The mobile phase was methanol–H_2O (80/20, v/v) at a flow rate of 1.0 mL/min. The injection volume was 20 μL, and the column effluent was monitored at 280 nm. In order to assure the accuracy and repeatability of removal efficiency of CNTs@Est-MIPs to estrone in environmental and drinking water samples, we did the above experimental procedure for five runs on each samples.

2.6. Instruments

A Tecnai G2 T2 S-TWIN microscope was used to obtain transmission electron microscopy (TEM) images of CNTs, CNTs@SiO_2 and CNTs@Est-MIPs. Fourier transform infrared (FT-IR) spectra were recorded on an AVATAR 360 (Nicolet Corp., USA), and samples were dried at 80 °C in a vacuum oven for at least 12 h prior to fabrication of the KBr pellet. In this context, 2 mg of CNTs, estrone, CNTs@SiO_2, imprinted polymer that was not washed and CNTs@Est-MIPs samples were thoroughly crushed and mixed with 100 mg of KBr, and the mixtures were used for sample pellet fabrication. Fifty scans of the region between 400 and 4000 cm^{-1} were collected for each FT-IR spectrum that was recorded. ^1H NMR spectra of the Est–Si complex were investigated using a Varian Mercury Vx-300. The amount of estrone was analysed using HPLC, and HPLC analyses were performed on a Shimadzu LC-20A HPLC system that included a binary pump and a variable wavelength UV detector (Shimadzu, Kyoto, Japan). The instrument control and data processing were carried out by the LC solution software. A Shimadzu VP-ODS C18 (5 μm particle size, 150 mm × 4.6 mm) analytical column was used for the separation of analytes.

3. Results and discussion

3.1. Synthesis of imprinted materials

The synthesis of the CNTs@Est-MIPs via a multi-step procedure is illustrated in Fig. 1A; this process involves silica-shell deposition on the surface of CNTs, MIPs functionalized onto the silica surface, and final extraction of estrone by thermal reaction and generation of the recognition site. The surface of purified CNTs was converted

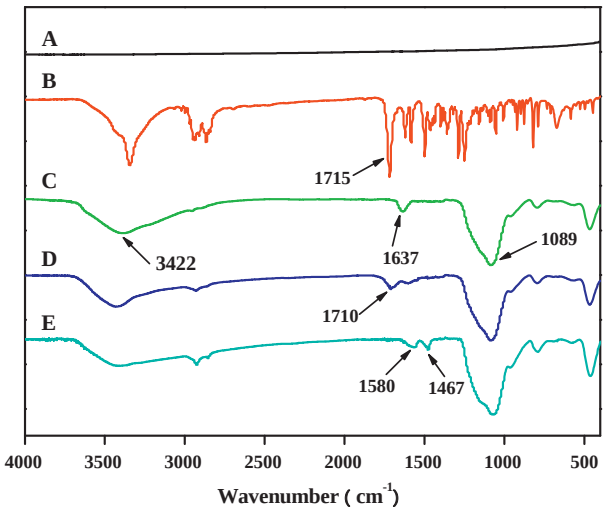

Fig. 3. FT-IR spectra of pure CNTs (A), estrone (B), CNTs@SiO_2 (C), CNTs@Est-MIPs before (D) and after (E) extraction of estrone.

to a silica shell by a sol–gel process using TEOS and APTES in the presence of the cationic surfactant CTAB to yield the CNTs@SiO_2 core–shell structure. The presence of a silica shell provides good biocompatibility, a hydrophilic surface, high dispersion in all solvents and a non-toxic coating. Furthermore, the silanol group on the silica coating can be easily modified to link bioconjugators with interesting biofunctionalities using the sol–gel method [33,34]. In this study, we used a thermally reversible bond for the preparation of the template (estrone)–silica monomer complex (Est–Si), which allowed us to remove the template by simple thermal reaction and simultaneously introduce functional groups into the cavities on the surface of the CNTs. The CNTs@SiO_2 reacted with Est–Si to produce a silica surface functionalized with MIPs.

The Est–Si complex (Fig. 1B) was synthesized by reaction of 3-(triethoxysilyl)propyl isocyanate with estrone in the presence of triethylamine and DMF under nitrogen according to the method by He et al. [32]. The reaction occurs between the isocyanate group of 3-(triethoxysilyl)propyl isocyanate and the phenol moiety of estrone, forming a thermally cleavable urethane bond. The thermal cleavage of the urethane bond was investigated by ^{1}H NMR. Est–Si was dissolved in DMSO-d_6 and its ^{1}H NMR spectra at room temperature were investigated. The aromatic ring proton peaks appeared at 7.270, 6.835 and 6.777 ppm, and the NH peak appeared at 7.675 ppm, which is consistent with formation of the urethane. The thermally cleavable urethane bond is stable at room temperature, but this bond undergoes reversible cleavage at elevated temperature. Once this bond is broken, the MIP coating was prepared by a sol–gel reaction of the Est–Si. To extract the imprinted estrone molecules from the core–shell CNTs@Est-MIPs, the estrone-imprinted CNTs@Est-MIPs were heated at 180 °C in a mixture of DMSO and water, and the dissociated isocyanto group in the silica shell was converted to an amino group via reaction with H_2O. The control CNTs@NIPs nanocomposites were prepared with 3-aminopropyltriethoxysilane and TEOS in the absence of a template molecule.

One of the important factors for successful molecular imprinting is the presence of functional monomers in the polymer matrix. To investigate the effect of the mass ratio of CNTs@SiO_2 to Est–Si on the imprinting performance of CNTs@Est-MIPs, the CNTs@Est-MIPs and the control CNTs@NIPs were prepared in the presence of the mass ratio of CNTs@SiO_2 to Est–Si from 1:1 to 7:1. The results of imprinting performance of CNTs@Est-MIPs were shown in Table 1. When the mass ratio of CNTs@SiO_2 to Est–Si increased from 1:1 to 7:1, the CNTs@Est-MIPs showed the maximal adsorption capacity and imprinting factor under the mass ratio of CNTs@SiO_2 to Est–Si of 4:1, but CNTs@NIPs showed nearly the same adsorption capacity to estrone. In this work, the mass ratio of CNTs@SiO_2 to Est–Si of 4:1 was used to prepare CNTs@Est-MIPs.

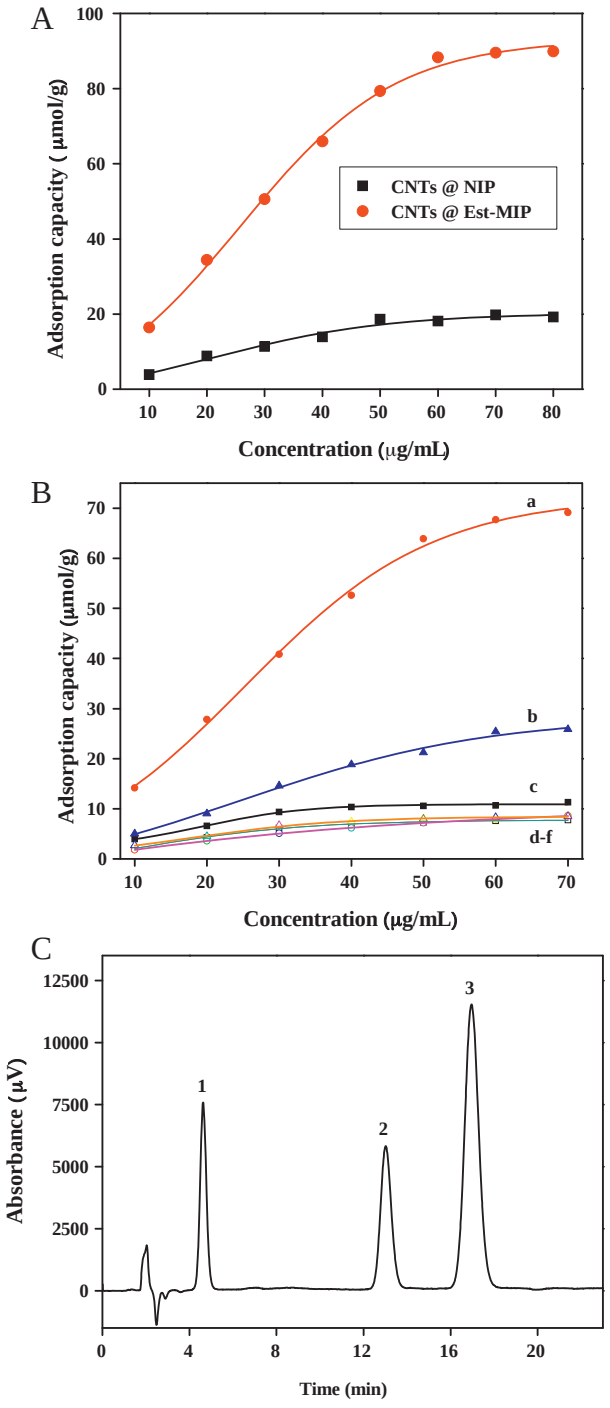

Fig. 4. Adsorption isotherm of estrone onto CNTs@Est-MIPs and CNTs@NIPs (A). Adsorption isotherms of CNTs@Est-MIPs and CNTs@NIPs onto estrone (a, d), estriol (b, e) and diethylstilbestrol (c, f) (B). A mass of 20 mg of CNTs@Est-MIPs and CNTs@NIPs were incubated in the solution of estrone (A) and the solutions of estrone, estriol and diethylstilbestrol (B) at different concentrations for 1 h at 25 °C. (C) Chromatogram of the three estrogenic analytes on C18 analytical column. Samples: 20 μL of 10 μg/mL estriol (1), estrone (2) and diethylstilbestrol (3) mixture solution; detection wavelength: 280 nm.

Table 2
Regression analysis of calibration curves and detection limits of three estrogenic compounds.

Analytes	Regression equation	r	Linear range (μg/mL)	Detection limit (μg/L)
Estrone	$Y=(7.71\times10^3)X+1.04\times10^3$	0.9999	0.050–100.0	13.7
Estriol	$Y=(9.71\times10^3)X+9.14\times10^2$	0.9999	0.050–100.0	16.1
Diethylstilbestrol	$Y=(2.57\times10^4)X+3.91\times10^3$	0.9999	0.050–100.0	10.2

3.2. Characterisation of the imprinted materials

TEM images of the CNTs, CNTs coated with silica and CNTs@Est-MIPs are shown in Fig. 2. The average diameter of the CNTs is observed at 60–80 nm (Fig. 2A) before coating; after coating with the SiO_2, the diameter of the CNTs@SiO_2 increases to about 80–100 nm, corresponding to a 10–20 nm thick SiO_2 layer covering the CNTs (Fig. 2B). The SiO_2 layers were uniformly coated with a thickness of 10–20 nm on the CNTs for all of the samples, and there were hardly any free SiO_2 particles found in the TEM images. Silica coating can mitigate the difficulty of dispersing CNTs homogeneously in different solvents, and this coating favours the dispersion of CNTs in liquid media. The same amount of un-coated CNTs and CNTs@SiO_2 were added separately to an aqueous solution and sonicated for 10 min to disperse them adequately. The un-coated CNTs were difficult to dissolve, but CNTs@SiO_2 dissolved quite well (Fig. 2D). The solubility of CNTs can be meliorated considerably in the aqueous phase after the coating process with SiO_2. The TEM image of CNTs@Est-MIPs shows the formation of the CNTs@Est-MIPs core/shell-structured nanocomposites (Fig. 2C) after Est–Si was reacted on the surface of the CNTs@SiO_2 via sol–gel reaction. The thickness of the silica film was observed to increase by repeating the sol–gel reaction. The diameter of the CNTs@Est-MIPs increased to 100–120 nm after the estrone-imprinting process, which corresponds to a 15–20 nm thick imprinted SiO_2 layer covering on the CNTs (Fig. 2C). The thickness of this imprinted polymer layer will be effective for mass transport between the solution and the surface of the CNTs@Est-MIPs.

The FT-IR spectra of the CNTs, estrone, CNTs@SiO_2, and the CNTs@Est-MIPs before and after the extraction of estrone are shown in Fig. 3. There was no apparent peak in the spectrum of the blank CNTs (curve A). The strong peaks at about 1089 cm^{-1} (curve C), 1092 cm^{-1} (curve D) and 1091 cm^{-1} (curve E) are attributed to the stretching frequencies of Si–O–Si; the –OH vibration was detected at 3422 and 1637 cm^{-1}, and these peaks indicated the formation of the silica film [36]. The peaks at 1710 and 1715 cm^{-1} represent the typical stretching vibrations of carbonyl groups in the urethane bond and estrone, respectively [37]. Before extraction of estrone, the observed peak at 1710 cm^{-1} indicated the stretching vibration of carbonyl groups (curve D), and this peak is absent in the CNT@Est-MIPs spectrum after the extraction of the template molecules (curve E). The infrared data for the samples demonstrated the successful formation of estrone-imprinted polymer on the surface of the CNTs@SiO_2. The characteristic amido peak at 1467 cm^{-1} and 1580 cm^{-1} [36–38] also verified the removal of template molecules. These results are consistent with the conclusion that the CNTs@Est-MIPs were obtained with binding sites complementary to the template in size, shape and position of the functional groups.

3.3. Binding properties of CNTs@Est–Si MIPs

3.3.1. Isothermal binding properties of CNTs@Est-MIPs and CNTs@NIPs

The adsorption kinetics of estrone solution of 60 μg/mL onto CNTs@Est-MIPs was investigated. The estrone molecules approached the surface imprinting cavities of CNTs@Est-MIPs only need 1 h to reach adsorption saturation, but it took about 8 h [39], 12 h [40] and 24 h [41] to reach adsorption saturation by the traditional bulk polymers. In comparison to the traditional bulk polymers, the nanosized and uniform imprinted sites of CNTs@Est-MIPs allowed efficient mass transport, thus overcoming some drawbacks such as mass transfer, low integrity of the polymer structure, and the production of heterogeneous binding sites of traditionally bulk imprinted materials.

The estrone recognition ability of the CNTs@Est-MIPs and control CNTs@NIPs was investigated by a steady-state binding method. As can be seen in Fig. 4A, the amount of estrone bound in the CNTs@Est-MIPs is higher than that in the CNTs@NIPs. When the initial concentration of estrone is 60 μg/mL, the amounts bound estrone in the CNTs@Est-MIPs and CNTs@NIPs are 89.58 and 19.24 μmol/g, respectively.

The saturation binding data were further processed with a Scatchard equation to estimate the binding properties of the CNTs@Est-MIPs. The Scatchard equation is:

$$\frac{Q}{[\mathrm{Est}]} = \frac{(Q_{\max} - Q)}{K_D}$$

where Q was the amount of estrone bound to the CNTs@Est-MIPs at equilibrium, Q_{max} was the apparent maximum number of binding sites, [Est] was the free analytical concentration of estrone at equilibrium and K_D was the dissociation constant. The values of K_D and Q_{max} can be calculated from the slope and intercept of the Scatchard plot.

The Scatchard analysis for the CNTs@Est-MIPs was performed, and the Scatchard plot was a single straight line and the linear regression equation for the linear region was $Q/[\mathrm{Est}] = 130.3 - 1.148Q$ ($R^2 = 0.9987$). From the slope and the intercept of the straight line obtained, the values of K_D and Q_{max} were 0.8711 μmol/L and 113.5 μmol/g, respectively.

In comparison with previous work, the binding capacity of CNTs@Est-MIPs in the present work is 6 [38,41] and 10 [42,43]

Table 3
The adsorption capacity, imprinting factors and selectivity coefficients of estrone, estriol and diethylstilbestrol onto CNTs@Est-MIPs and CNTs@NIPs ($n = 3$).[a]

Analytes	Q_{MIPs} (μmol/g)[b]	Q_{NIPs} (μmol/g)[b]	IF[c]	SC[d]
Estrone	63.92 ± 0.87	7.21 ± 0.13	8.86 ± 0.04	–
Estriol	10.55 ± 0.53	7.37 ± 0.49	1.43 ± 0.02	6.19 ± 0.07
Diethylstilbestrol	21.26 ± 0.71	8.08 ± 0.26	2.63 ± 0.00	3.07 ± 0.29

[a] In this experiment, 20 mg of CNTs@Est-MIPs and CNTs@NIPs were incubated in the mixing solution of estrone, estriol and diethylstilbestrol at a concentrations of 60 μg/mL for 1 h at 25 °C ($n = 3$).
[b] Q_{MIPs} and Q_{NIPs} were the amount of analytes bound to CNTs@Est-MIPs and CNTs@NIPs at equilibrium, respectively.
[c] $IF = Q_{MIPs}/Q_{NIPs}$, which represents imprinting factor.
[d] $SC = IF_{estrone}/IF_i$, which stands for selectivity coefficient. $IF_{estrone}$ and IF_i are the imprinting factors for estrone and two other estrogenic compounds, respectively.

times more than the estrone-imprinted polymers based on bulk polymerization. This is because of the process of producing MIPs-functionalized onto the CNTs@SiO_2 by "semi-covalent" imprinting strategy, after imprinting polymerization, a high density of effective imprinted sites was obtained on the imprinted polymer. Therefore, the high surface/volume ratios of nanosized imprinted CNTs@Est-MIPs with attractive tubular structure were expected not only to improve the binding capacity, but also to provide an excellent accessibility to target molecules.

3.3.2. Repeatability of the CNTs@Est-MIPs

The repeatability of the CNTs@Est-MIPs was also investigated using six batches of the CNTs@Est-MIPs prepared on different days. For each batch, three independent replicates of the estrone-binding amount were measured. The average binding amount of the six batches of product CNTs@Est-MIPs was 77.58 μmol/g. Results showed that the reproducibility of the six batches of imprinted materials was satisfactory, exhibiting a relative standard deviation of less than 8.0% (2.4%, 4.6%, 6.4%, 8.0%, 3.4% and 0.53% for individual batches, respectively).

3.3.3. Specific recognition of CNTs@Est-MIPs and CNTs@NIPs

We investigated the specific recognition ability of the CNTs@Est-MIPs and CNTs@NIPs through a steady-state binding experiment in a mixed solution of estrone with estriol and diethylstilbestrol, which are two structural analogues of estrone (Fig. 1C) at different concentrations.

In order to quantitatively determine three estrogenic analytes, the calibration curves were drawn. The linear equation, linear range and detection limit of estrone, estriol and diethylstilbestrol were shown in Table 2. The linearity for three estrogenic compounds was performed by injecting of 20 μL standard solutions of three analytes. The calibration curves showed a range of linearity between 0.050 and 100 μg/mL for three estrogenic analytes. The higher linear regression coefficients ($r > 0.9999$) were obtained for all tested analytes. The detection limits (S/N = 3) are 13.7, 16.1 and 10.2 μg/L for estrone, estriol and diethylstilbestrol, respectively (Table 2). The typical chromatogram of separation of the three estrogenic compounds was shown in Fig. 4C. The retention time of estrone, estriol and diethylstilbestrol was 12.98, 4.62 and 16.97 min, respectively.

The adsorption isotherms of the CNTs@Est-MIPs and CNTs@NIPs with estrone, estriol and diethylstilbestrol are shown in Fig. 4B. The CNTs@Est-MIPs showed significantly higher specific recognition ability for estrone over both estriol and diethylstilbestrol in the mixture solution; in contrast, the binding capacity of estrone, estriol and diethylstilbestrol were nearly the same on the CNTs@NIPs (Table 3). These results indicated that the imprinted materials are suitable as a recognition element for template molecules.

The amount of estrone, estriol and diethylstilbestrol adsorbed onto the CNTs@Est-MIPs and CNTs@NIPs were measured according to the following formula:

$$Q = \frac{(C_I - C_F)V}{m}$$

where Q is the amount of estrone, estriol and diethylstilbestrol bound to the CNTs@Est-MIPs or CNTs@NIPs, C_I is the initial molecule concentration, C_F is the final molecule concentration, V is the total volume of the solution and m is the mass of the polymer in each solution. Additionally, the imprinting factor (IF) and selectivity coefficient (SC) were generally used to evaluate the selective-binding properties of the CNTs@Est-MIPs and CNTs@NIPs toward estrone and the structurally related compounds estriol and diethylstilbestrol. The IF and SC were calculated by the following formulas:

$$\text{Imprinting factor (IF)} = \frac{Q_{MIPs}}{Q_{NIPs}}$$

$$\text{Selectivity coefficient (SC)} = \frac{IF_{estrone}}{IF_i}$$

where Q_{MIPs} is the amount of bound molecules on the CNTs@Est-MIPs, and Q_{NIPs} is the amount of bound molecules on the

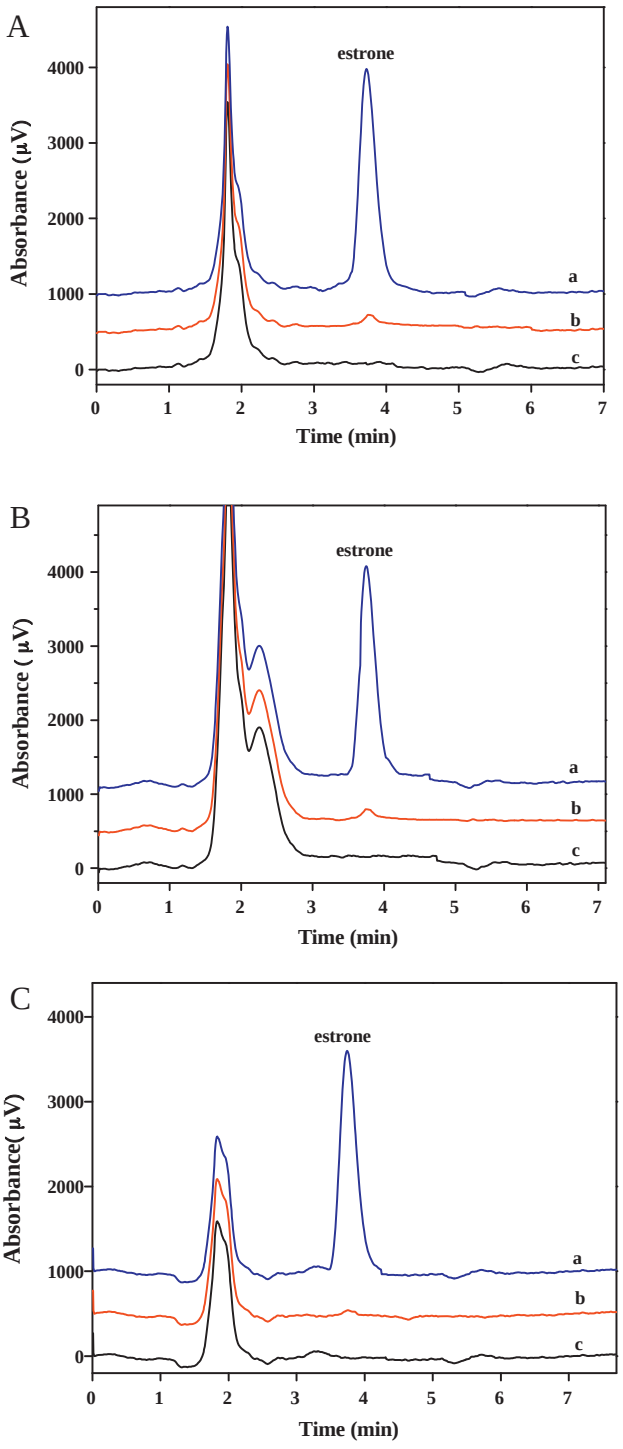

Fig. 5. Chromatograms of estrone in the river (A), lake (B) and tap (C) water samples. Samples spiked with estrone at a concentration of 5 μg mL^{-1} (a), spiked samples after adsorption by CNTs@Est-MIPs (b) and blank water samples (c). The retention time of estrone was 3.74 min.

CNTs@NIPs. $IF_{estrone}$ and IF_i are the imprinting factors for estrone and the two other estrogenic compounds, respectively.

Table 3 lists the adsorption capacity, IF and SC values of different molecules on CNTs@Est-MIPs and CNTs@NIPs. The binding capacity of estrone on the CNTs@Est-MIPs is about six- and three-fold higher than that of estriol and diethylstilbestrol, respectively. The imprinting factors for binding estrone, estriol and diethylstilbestrol are 8.86, 1.43 and 2.63, respectively. These results indicate that the template molecule had a relatively higher affinity for the imprinted polymer than its analogues. The selectivity of CNTs@Est-MIPs is about three-fold at least when compared with the results obtained by Zhang and Hu [44], which hardly has selectivity for analogue. This could be due to the different polymerization methods to prepare MIPs. During the preparation of the imprinted material via the semi-covalent method, estrone was imprinted on the silica layers; after removal of estrone, the complementarity of the cavities in the imprinted polymers to the template in size and shape was established. These results demonstrate satisfactory imprinting efficiency of the present method.

3.4. Removal of estrone from environmental and drinking water

The feasibility of removing estrogenic compounds from environmental and drinking water by the CNTs@MIPs nanocomposites was demonstrated using water samples spiked with estrone. The water samples, including river, lake and tap water, were spiked at a concentration of 5 μg/mL, respectively. To evaluate the accuracy and the removal efficiency of the CNTs@Est-MIPs, after treating water samples spiked with estrone with CNTs@Est-MIPs, the residue levels of estrone were analysed. For each sample, five measurements were performed. The chromatograms of the spiked water samples, the spiked samples after adsorption by CNTs@Est-MIPs and blank water samples without spiked estrone are displayed in Fig. 5. It can be seen that the estrone was almost completely removed after adsorption by the CNTs@Est-MIPs. The removal efficiency of the CNTs@Est-MIPs for estrone ranged from 96.14% to 98.03%. These results indicate that the CNTs@Est-MIPs were an excellent material for removing estrone from the water samples.

4. Conclusion

In this study, a low-cost and reproducible method was adopted for the first time to prepare core–shell structured CNTs@Est-MIPs nanocomposites using a semi-covalent imprinting strategy, which formed a thermally reversible covalent bond at the surface of CNTs coated with silica. The thickness of the silica film can be controlled by repeating the sol–gel reaction. The results of binding experiments demonstrated that the imprinted nanocomposites possessed favourable selectivity, high capacity and fast kinetics for uptake of the template molecule. The synthetic process is quite simple, and different batches of the imprinted materials showed good reproducibility in template binding experiments. Additionally, the prepared CNTs@Est-MIPs nanocomposites showed high removal efficiency as a sorbent for estrone in water samples. The core–shell CNTs@MIPs nanocomposites developed in this work can also be applied as a selective coating for electrochemical or quartz crystal microbalance sensors to monitor for estrone residue in environmental water.

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