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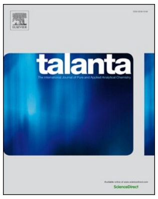

Development of a novel amine- and carboxyl-bifunctionalized hybrid monolithic column for non-invasive speciation analysis of chromium

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ABSTRACT

A novel amine- and carboxyl-bifunctionalized organic-inorganic hybrid monolithic column (A&C-HMC) was synthesized *via* one-pot co-condensation of N-(2-aminoethyl)-3-aminopropyltriethoxysilane, carboxyethylsilanetriol sodium salt and tetramethoxysilane with cetyltrimethylammonium bromide and polyethylene glycol 6000 as binary porogens in this work. The introduction of the binary porogens controllably improved the morphology and pore structure of A&C-hybrid monolith (HM) and made the active sites of amine and carboxyl groups more prolific, compared with the monolith prepared with either of porogens. It is found that Cr(VI) and Cr(III) can be selectively adsorbed on A&C-HM under different pH ranges, and eluted by aqueous nitric acid solution completely. The A&C-HMC was used as needle-solid phase microextraction (SPME) matrix for direct separation and enrichment of inorganic chromium species coupled with inductively coupled plasma mass spectrometer without any oxidation/reduction treatment. Various parameters of SPME operation and analytical performance were investigated systematically, and the adsorption mechanism was also discussed and explained in depth. In view of the advantages of facile preparation, low cost, excellent speciation selectivity and high adsorption capacity to Cr(VI) and Cr(III), the A&C-HMC based SPME protocol is a promising alternative for non-disturbed speciation analysis of inorganic chromium in real environmental water samples.

1. Introduction

Chromium (Cr) is extensively used for various industrial purposes, and the unjustified utilization of Cr may cause serious Cr contamination in the environment, which has been of great concern [1–3]. In natural water, Cr is found mainly in its oxidation states, hexavalent chromium (Cr(VI)) and trivalent chromium (Cr(III)) [4]. Cr(VI) is highly toxic and recognized as a human carcinogen. In contrast, Cr(III) is considered to be an essential trace element for glucose metabolism and maintenance of normal human function, while high doses of Cr(III) may be toxic to organisms [5,6]. Different Cr species exhibit wide toxicity differences and state lability in the environment. Therefore, it is particularly important to develop novel species separation materials and analytical methods for highly sensitive, selective and non-invasive speciation of chromium [7].

Many functional groups have been widely applied for the speciation analysis of trace elements including chromium based on various interactions. For instance, amine group is used for selectively adsorbing Cr (VI), Se(VI) and As(V) [7], thiol group for Cr(VI) [8], As(III) and Sb(III) [9], and carboxyl group for Cr(III) [10], Sb(III) [11] and rare earth elements [12]. Besides these common groups, some chelating ligands are also employed for specifically adsorbing some element species, for example, pyrrole for Cr(VI) [13,14], and zincon for Cr(VI) and Cr(III) [15], etc.

Due to the different physical and chemical properties of Cr(VI) and Cr(III), it is difficult to achieve the selective extraction and evaluation of both two chromium species using the absorbents modified with single functional groups. The common solutions are as follows. Cr(VI)/Cr(III) is selectively separated and enriched with single-functionalized material first, and the other species is just calculated by subtraction from the total chromium in original samples [10]. Or all the remanent chromium in supernatant/effluent is regarded as other chromium

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species and detected directly without enrichment because no interaction happens between them and the material [3]. These two methods might be inaccurate for the samples with very low chromium level and bring higher results for Cr(III)/Cr(VI), since other chromium species besides Cr(VI) and Cr(III) likely exist in the samples. The rest inorganic chromium species could also be transferred into Cr(VI)/Cr(III) by introducing oxidant/reductant in the supernatant/effluent and enriched again under the same conditions [7], which may cause labile species to change and introduce interferences. Another solution is to enrich total inorganic chromium and one of them under different solution pH respectively, and then calculate other species by subtraction [15,16], which is relatively reliable, but operation-complex and time-consuming. Even though amine-functionalized materials have been reported not only to capture Cr(VI), but also to adsorb a bit of Cr(III) under different conditions [3,7,17], there are more or less cross interferences between Cr(VI) and Cr(III) on the adsorbents modified with single group. In addition, a combination strategy of two single-functionalized materials is also suggested for the separation and enrichment of element species [18,19]. However, these proposals need at least two absorbents of different function groups, multiple-steps operation and more time consumption.

During the past decade, in order to overcome the shortages of single-functionalized material, multifunctionalized material has aroused concern gradually to be used for separating and enriching different element species with many merits, such as cost-saving and simple operation, comparing with the strategies of combining multiple single-functionalized materials. Besides these, the synergistic or antagonistic effects may also present between multiple groups [19–21], which could further improve the performance of these materials. However, the reports of multifunctionalized materials for elemental speciation analysis are relatively few [19,20,22–24]. Among these, only one work is for chromium speciation analysis. Shevchenko et al. proposed a thiol- and sulfonic acid-bifunctionalized mesoporous silica, on which thiol group reduces Cr(VI) into Cr(III) and sulfonic acid group adsorbs the reduced Cr(III) [24].

Based on existing reports, many kinds of separation methods have been developed for the determination of inorganic chromium species, including packed column SPE [7], disperse solid phase extraction (d-SPE) [10], magnetic solid phase extraction [15,17], stir bar sorptive extraction [25], capillary monolithic column solid phase microextraction (SPME) [3] and so on. Compared with other methods, SPME based on capillary monolithic columns can not only pretreat liquid sample on site as needle-SPME [20], but also couple with detectors for on-line operation [12], which have received extensive attention due to the series of merits of the monolithic columns, like the uniform structure, controllable morphology, convective mass transfer and good adsorption capacity [26]. Organic-inorganic hybrid monolithic columns (HMC) prepared *via* sol-gel process combines the advantages of traditional organic polymer and inorganic silica monolithic columns with good biocompatibility, large specific surface area and high mechanical stability [27,28]. The preparation method to directly introduce function groups into the skeleton of monolith during sol-gel process, called "one-pot", can make the monolith more uniform and reproducible than the post-modified ones [29,30]. Li et al. from our research group proposed a strategy by using a centrifugal microfluidic platform integrating amine-functionalized monolithic capillary columns for selectively extracting Cr(VI) with high throughput [3]. We also developed a carboxyl-functionalized HMC as needle-SPME matrix to selectively adsorb Cr(III) [11]. As far as we know, there have been no reports of the multifunctional monolithic column applied for chromium speciation analysis. In our recent work, a thiol- and amine-bifunctionalized HMC was prepared for the separation and enrichment of two arsenic species [20]. In fact, there are significant differences between amine and carboxyl groups, especially when affected by the solution pH, they often have diametrically opposite charges [12,18,27,31]. Not only that, based on our findings, the target element species could be eluted from carboxyl-functionalized monolith much more easily than from thiol functionalized one owing to weaker chelation interaction of carboxyl group to metal ions [32]. Therefore, it is very meaningful to develop HMCs modified with amine and carboxyl groups simultaneously for non-invasive speciation analysis of chromium, which is expected to adsorb Cr(VI) and Cr(III) at different pH that eliminates the interference of non-target species.

2. Materials and methods

2.1. Reagents and materials

Carboxyethylsilanetriol sodium salt (CES), 25 wt% solution in H_2O and tetramethoxysilane (TMOS) were purchased from J&K (Shanghai, China) and Alfa Aesar (Tianjin, China), respectively. N-(2-aminoethyl)-3-aminopropyltriethoxysilane (AEAPTES), polyethylene glycol 6000 (PEG6000) and acetic acid (HAc) were all purchased from Ourchem (Shanghai, China). Cetyltrimethylammonium bromide (CTAB) was obtained from TCI (Tokyo, Japan). HNO_3 was of guaranteed reagent grade and all other chemicals were at least of analytical grade and used without further purification. Deionized water used throughout the experiment (18.25 MΩ·cm) was prepared from a Milli-Q water system (Millipore, Bedford, MA, USA). The fused-silica capillary with 530 μm i.d. and 690 μm o.d., used for preparing monolithic columns, was provided by Reafine Chromatography Ltd. (Hebei, China).

The stock standard solutions of Cr(VI) and Cr(III) (1000.0 mg L^{-1}) were GSB04-1723-2004(a) and GBW08614 respectively supplied by China National Measuring Science Research Institute (Beijing, China). Lower concentration standard solutions were prepared daily by proper dilutions from their stock solutions.

Yangtze River and Qinhuai River waters were respectively taken from the Nanjing section of Yangtze River and Qinhuai River in Gulou District of Nanjing in Jiangsu Province, China. Tap water was collected in our Xianlin campus of Nanjing University in Qixia District, Nanjing. All water samples were stored at 4 °C refrigerator and filtered through a 0.45 μm cellulose acetate membrane prior to use.

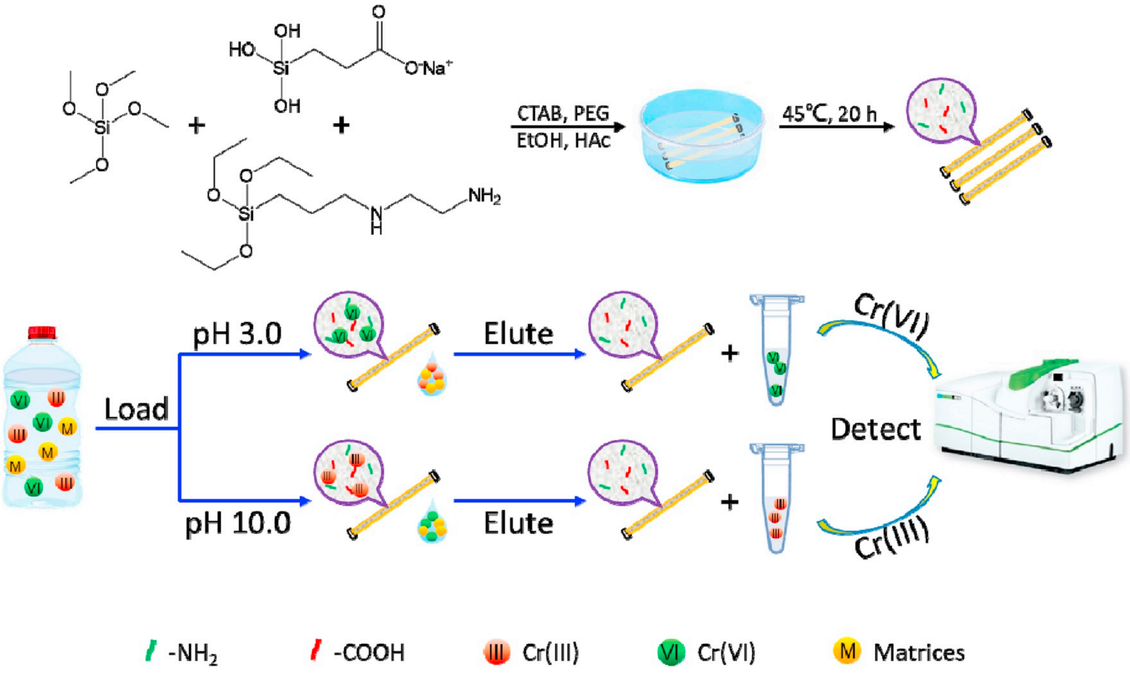
2.2. Instrumentation

A PerkinElmer NexION 350D ICP-MS (PerkinElmer SCIEX, Concord, Canada) with kinetic energy discrimination (KED) mode is used for the determination of different chromium species, and the optimum operation conditions are summarized in Table S1. The pH of solutions was adjusted with diluted HNO_3 or $NH_3{\cdot}H_2O$, and controlled by a FiveEasy Plus pH meter (Mettler-Toledo, Shanghai, China). For introducing the solution through the monolithic column during the SPME process, a syringe infusion pump (LSP04-1A, LongerPump, Hebei, China) was applied.

The scanning electron microscopy (SEM) and element mapping images of the monolithic materials were obtained using a Hitachi S-3400 II SEM (Hitachi, Tokyo, Japan). The transmission electron microscopy (TEM) images of the monoliths were obtained from a JEOL JEM-2100 TEM (JEOL, Japan). Fourier-transform infrared (FT-IR) spectra in KBr were collected on a NEXUS 870 FT-IR spectrometer (Nicolet, USA). An Elementar Vario EL II elemental analyzer (Elementar, German) was used to obtain the elemental analysis (EA) result of the monolith with oxygen as the combustion gas. X-ray photoelectron spectroscopy (XPS) analyses were carried out on a PHI5000 Versa Probe photoelectron spectrometer (ULVAC-PHI, Kanagawa, Japan). N_2 adsorption-desorption measurement was recorded with a Micromeritics ASAP 2020 BET surface analyzer system (Micromeritics, Shanghai, China) to calculate the surface areas and pore sizes of materials with the Brunauer-Emmett-Teller (BET) and Barrett-Joyner-Halenda (BJH) methods, respectively.

2.3. Preparation of A&C-HMC

The fused-silica capillary was activated before used referencing our



Scheme 1. Schematic illustration of the preparation of A&C-HMC (upper) and workflow of Cr speciation analysis procedure (lower).

previous work [20]. The A&C-hybrid monolith (HM) is mainly prepared by using sol-gel method from a solution containing TMOS, CES and AEAPTES as silane reagents, CTAB and PEG6000 as binary porogens, ethanol and HAc solution as cosolvent, which is illustrated in Scheme 1. In detail, 16.7 mg CTAB and 22.5 mg PEG6000 were weighed in a 1.5 mL Eppendorf vial and completely dissolved in a mixture of 242 μL ethanol and 186 μL 1.0 mol L^{-1} HAc. Subsequently, 120 μL TMOS, 60 μL CES and 15 μL AEAPTES were added into the above mixture. After vortexed at room temperature for 30 s and ultrasonicated at 0 °C for 30 s, the resulting sol was quickly introduced into the activated capillary by a syringe. The capillary sealed with silicone rubber at both ends was further reacted at 45 °C for 24 h. The hybrid monolith formed within the capillary was rinsed with methanol and water to remove porogens and unreacted compounds. For comparison, the columns with only CTAB or PEG6000 as porogen were also prepared under the same conditions, named as A&C-HMC-CTAB and A&C-HMC-PEG column, respectively.

2.4. Speciation analysis procedure

As a needle-SPME material, A&C-HMC can selectively adsorb Cr(VI) and Cr(III) in different pH ranges, which can be used for separation and enrichment of inorganic chromium species, as shown in Scheme 1. To realize separation and enrichment of Cr(VI), 3.0 mL aqueous sample solution (pH 3.0) was pumped through 3.5 cm-length A&C-HMC at a flow rate of 150 μL min^{-1}. Then 150 μL of 10% HNO_3 (v/v) as eluent was allowed to flow through the column at the same flow rate to desorb the uptaken Cr(VI) and the effluents were collected for Cr(VI) determination by ICP-MS. For Cr(III), the SPME procedure was nearly the same as that for Cr(VI) except the pH of sample solution was adjusted to 10.0 prior to extraction.

3. Results and discussion

3.1. Preparation and characterization of A&C-HMC

3.1.1. Preparation of A&C-HMC

The A&C-HMC was synthesized with TMOS, AEAPTES and CES as the precursors at low temperature. The amine group from AEAPTES in the bifunctional monolithic materials could capture Cr(VI), however, hardly adsorbed Cr(III). When the volume rates of AEAPTES and CES were higher than 1:2, because CES used in this work is 25 wt% aqueous solution and the amine groups in the prepared materials were much more than carboxyl groups under these ratios of organic silica precursors. In order to make the two functional groups in the material as equal as possible, the volume ratio of AEAPTES to CES was controlled as 1:4. What was more, considering that the skeleton structure of this bifunctional monolith is more fragile than single amine- or carboxyl-functionalized monolith, high dose TMOS is required to increase the stability of the skeleton. The final ratio of precursors was determined as TMOS : CES : AEAPTES = 8:4:1 (v/v/v) after repeated attempts.

The synthesis of this HMC was based on typical sol-gel process. Due to the certain weak alkalinity of CES and AEAPTES and the quick hydrolysis of TMOS, the gelling speed of the mixture is too fast to fit the pre-polymerization mixture into the capillary column. HAc solution with different concentrations from 0.1 to 1.5 mol L^{-1} was introduced to slow down the reaction. It was found that the gel was difficult to form when the concentration of HAc was above 1.2 mol L^{-1}, while still too quickly below 0.9 mol L^{-1}. Thus, the concentration of HAc was set to be 1.0 mol L^{-1}. Moreover, we found that it was benefit to reduce the backpressure of the monolithic column by properly increasing the dose of ethanol in the mixture, but too much ethanol would make the monolith become fragile. In the end, the ratio of 1.0 mol L^{-1} HAc and ethanol was selected as 186:242 (v/v).

Porogen is an important component of prepolymerization mixture to improve the specific surface area and permeability of the resultant monolithic columns. The suitable porogen can not only adjust the macroporous size of the monolithic column, but also create a certain size of mesoporous structure in the material skeleton. Usually, one porogen was introduced in the preparation of monolithic column [33], or just using the solvent as porogen [34,35], while only a few monoliths produced with multiple porogens [36]. The use of multiple porogens may result in a graded pore structure in the obtained monolithic skeleton, which may make the monolith not only porous owing the abundant active sites of functional groups, but also possible for size exclusion chromatography [36,37]. Here, the influence of porogens was investigated. The A&C-HMC was prepared in presence of CTAB and PEG6000 as binary porogens. For comparison, A&C-HMC-CTAB and A&

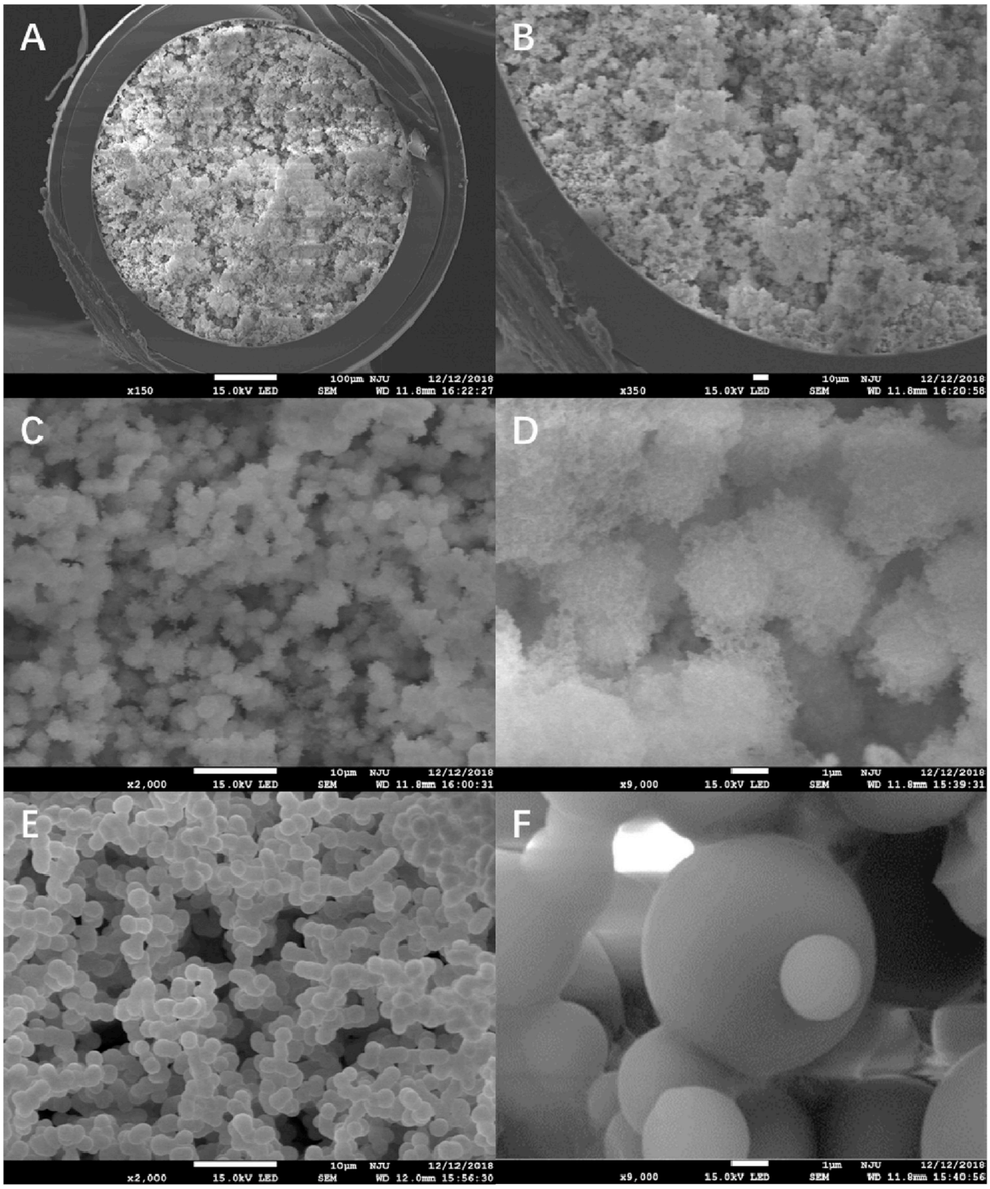


Fig. 1. SEM images of amine- and carboxyl-bifunctionalized monolithic columns. A~D: A&C-HMC (A. × 150; B. × 350; C. × 2000; D. × 9000); E and F: A&C-HMC-CTAB (E. × 2000; F. × 9000).

C-HMC-PEG were also prepared. As shown in Fig. S1, wet A&C-HM-PEG gel was semitransparent obviously, and the other two monolithic gels (A&C-HM-CTAB and A&C-HM) were opaque, which meant that it was not very suitable to prepare amine- and carboxyl-bifunctionalized monolith only using PEG6000 as porogen under this preparing condition. The influence of porogens on the morphology of the bifunctionalized monolith was further discussed. It can be seen clearly from the SEM images (Fig. 1(C) to (F)) that both A&C-HM and A&C-HM-CTAB were formed by continuous and homogeneous interconnecting spheres. The difference was that the skeleton surface of A&C-HM-CTAB was smooth, while that of A&C-HM was rambutan-shaped. The extremely rough surface of A&C-HM facilitates the exposure of functional groups, more active sites and more efficient mass transfer. Furthermore, the average BET surface areas of the A&C-HM and A&C-HM-CTAB were 536.27 and 672.87 m^2 g^{-1} respectively, which were both larger than most reported monolithic columns [33,35,38–40]. The high surface areas can result in high adsorption capacities of target element species. Moreover, the N_2 adsorption-desorption isotherms and pore size distributions of monoliths are shown in Fig. S2. It was clearly seen that hysteresis loops were existed in the N_2 adsorption-desorption isotherms for both two monoliths (Fig. S2(A) and (B)), which illustrated that both A&C-HM and A&C-HM-CTAB owned pores in their skeletons. The difference was that A&C-HM-CTAB showed a H4 hysteresis loop, which meant that the pores of A&C-HM-CTAB had uniform shape and size. This result was consistent with the relatively narrow peak in the corresponding pore size distributions. Comparatively, two sizes of mesopores ($\sim$3.5 and $\sim$30 nm) were existed in the skeleton of A&C-HM, which matched H3 hysteresis loop shown in Fig. S2(A) and the SEM images. TEM was also characterized to visualize the mesoporous structures of the monoliths. As shown in Fig. S3, both A&C-HM-CTAB and A&C-HM were porous. Moreover, compared with A&C-HM, the mesoporous in A&C-HM-CTAB were more crowded and denser, which was consistent with the results of BET surface area. The presence of multi-size pores caused by binary porogens also endues A&C-HM with the ability to separate and enrich other targets basing on size exclusion. Therefore, A&C-HMC was chosen for further application in view of the unique porous morphology of its skeleton.

3.1.2. Characterization of A&C-HMC

As shown in the cross-sectional SEM images of A&C-HMC (Fig. 1(A) and (B)), the A&C-HM was tightly bound on the inner wall of capillary and had well-controlled continuous macroporous structure, which ensures the low backpressure, high permeability and fast homogeneous mass transfer during sample analysis procedure. Furthermore, the column backpressure of A&C-HMC was just slightly higher than the background pressure of the instrument (Labtech P600 HPLC pump (Beijing, China)) and linearly related to the flow rate in the whole tested range (10–500 μL min^{-1}), which meant that the A&C-HMC had good mechanical strength (Fig. S4).

Many characterization analyses, such as EA, element mapping, FT-IR and XPS spectra, were used to examine the existence of amine and carboxyl groups on the prepared monoliths. Because of the overlap between the absorption peaks in FT-IR spectrum of COO$^-$M$^+$ and N–H bonds at around 1600 cm^{-1}, A&C-HM was washed by aqueous HNO$_3$ solution. As can be seen from Fig. 2, not only C═O stretching vibration peak at 1715 cm^{-1}, but also C–N stretching vibration at 1455 cm^{-1} and N–H stretching vibrations at 1620 cm^{-1} existed in FT-IR spectrum of A&C-HM and matched with these of single carboxyl- or amine-functionalized monoliths, respectively. Compared with single amine-functionalized material, the N–H vibration absorption peak appeared slightly red shift due to the presence of carboxyl groups in the A&C-HMC.

XPS was employed for further evaluating the bifunctionalized material (Fig. 3). The XPS survey scan result showed that C 1s, O 1s, N 1s and Si 2p core-levels existed distinctly, and detail C 1s spectrum of the material was drawn in the right inset in Fig. 3. Among the seven deconvoluted peaks of C 1s, 286.1 and 287.1 eV were respectively assigned to the binding energies of C–O and C═O, and 287.9 eV belonged to the binding energy of carbon bonding with nitrogen (C–N). In the meanwhile, the EA results also showed significant existence of nitrogen element (Table S2). All above demonstrated that amine and carboxyl groups had been successfully decorated onto the A&C-HM material. What was more, as shown in the element mapping images (Fig. S5), the uniform distribution of Si, O, C and N elements indicated that the synthesized A&C-HM material had good uniformity.

3.2. Optimization of speciation analysis procedure

3.2.1. Effect of pH on adsorption

As a key factor to affect the surface property of A&C-HM and the distribution of inorganic chromium species, the solution pH has great impact on the adsorption behavior of Cr(VI) and Cr(III) onto the adsorbent. To investigate the effect of solution pH, 1.0 mL 20 μg L^{-1} Cr(VI)/Cr(III) solutions in the different pH (1.0–10.5) were pumped through the A&C-HMC under the flow rate of 20 μL min^{-1}. The results are presented in Fig. 4. Cr(VI) and Cr(III) can be selectively adsorbed by A&C-HM, respectively, from pH 2.5–3.5 and from pH 9.5–10.2. This phenomenon could be explained by the electrostatic force resulted from amine and carboxyl groups together [10,15]. The acid dissociation constant (pK_a) values of amine groups of AEAPTES are 6.8 and 9.3, respectively. When pH is below 6.8, AEAPTES is mainly protonated as positive divalent ion, marked as AH$_2^{2+}$ (-NH$_2^+$-CH$_2$-CH$_2$-NH$_3^+$), and gradually deprotonated to AH$^+$ (–NH–CH$_2$-CH$_2$-NH$_3^+$ or –NH$_2^+$-CH$_2$-CH$_2$-NH$_2$) and A (–NH–CH$_2$-CH$_2$-NH$_2$) as the solution pH increasing. Contrarily, the carboxyl groups of CES are predominantly presented as neutral COOH under strong acidic condition, and deprotonated as anions COO$^-$ in weakly acidic and alkaline solutions based on its pK_a of 4.4. Cr(VI) always existed as anions, HCrO$_4^-$, CrO$_4^{2-}$ and/or Cr$_2$O$_7^{2-}$, during the tested pH range, while Cr(III) were mainly cations (Cr^{3+}, Cr(OH)$^{2+}$ or Cr(OH)$_2^+$) [41]. As seen apparently from Fig. 4, during pH from 4.4 to 9.3, there might be some antagonism between amine and carboxyl groups due to their opposite charges, which may weaken the strong electrostatic attractions between Cr(VI) and amine groups or that between Cr(III) and carboxyl groups in this pH range, thereby reducing mutual interference between the two species. Not only that, carboxyl groups also can form strong complexation towards Cr(III) [10].

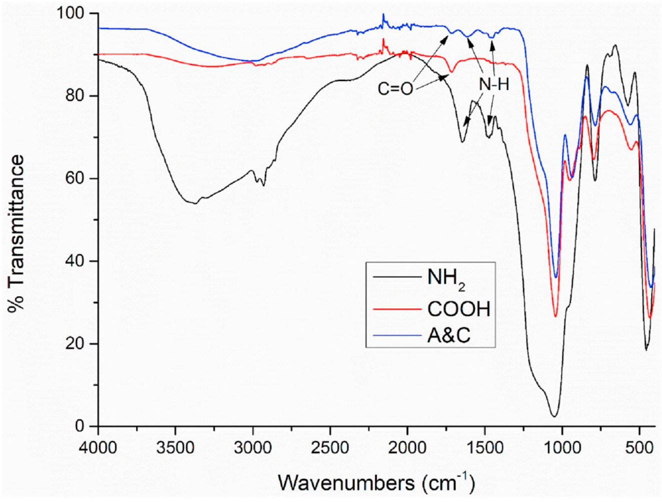
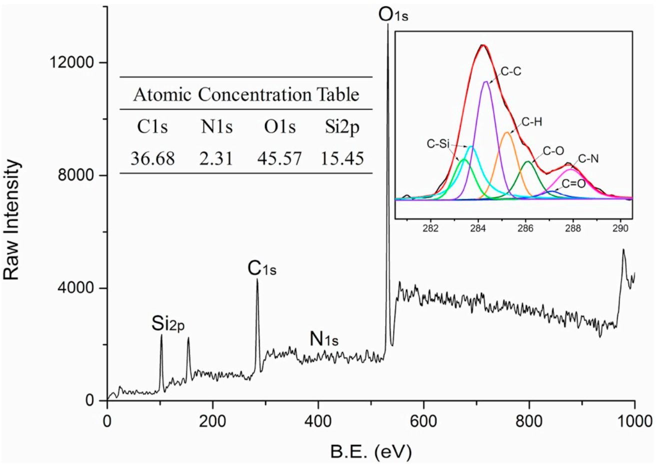


Fig. 2. FT-IR spectra of A&C-HMC, amine- and carboxyl-functionalized HMC.



Atomic Concentration Table

C1s	N1s	O1s	Si2p
36.68	2.31	45.57	15.45

Fig. 3. XPS spectra of A&C-HMC. The right inset is the detail C 1s spectrum of A&C-HM material.

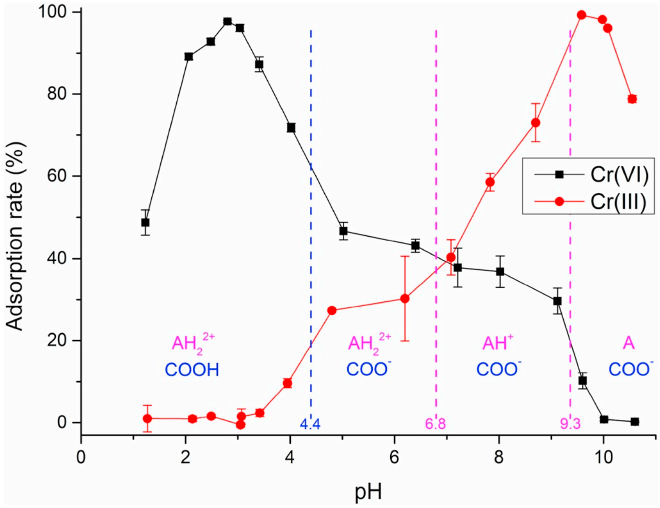


Fig. 4. Adsorption rates of inorganic chromium on A&C-HMC under different pH. Concentration of each species: 20 μg L^{-1}. Flow rate: 20 μL min^{-1} "AH$_2^{2+}$", "AH$^+$" and "A" in the figure means amine-containing groups in AEAPTES with different charges.

Therefore, in the subsequent experiments, the sample pH of 3.0 and 10.0 were selected for adsorbing Cr(VI) and Cr(III), respectively, with A&C-HMC.

To further validate the adsorption mechanism of chromium species on A&C-HMC, the A&C-HM materials adsorbing Cr(VI) and Cr(III) (A&C-HM-VI and A&C-HM-III) were respectively prepared for XPS measurement to characterize the attraction of the monolith to Cr(VI)/Cr(III). The results, listed in Table S3, indicated that the element Cr existed in both A&C-HM-VI and A&C-HM-III. In Jiang's work [15], Cr(III) peak existed in the both spectra of materials absorbing Cr(VI)/Cr(III), which was explained as that the adsorbed Cr(VI) was reduced into Cr(III) by phenolic group of zincon-immobilized magnetic nanoparticles. The similar phenomenon that the adsorbed Cr(VI) would be reduced to Cr(III) by absorbents also existed in other reports [24,42]. Differently, as shown in Fig. S6, only Cr(VI) or Cr(III) peak was observed in the spectrum of corresponding monolith, A&C-HM-VI or III, respectively [15], which proved that two chromium species were not denatured significantly at their respective pH during the adsorption process. The detail Cr 2p spectrum of A&C-HM-VI can be deconvoluted into four peaks as Fig. 5(A), in which, 579.6, 578.9 and 578.6 eV were assigned to the binding energies of CrO_4^{2-}, 580.1 eV to $Cr_2O_7^{2-}$, and no peak belonging to other coordinate bonds. This is consistent with the mechanism explanation of Cr(VI) adsorbed due to the electrostatic attraction. In the five peaks deconvoluted from the detail Cr 2p spectrum of A&C-HM-III (Fig. 5(B)), two distinct peaks of coordinate bonds belonged to the binging energies of Cr(III) complexed by carbonyl (575.7 eV) and carboxyl groups (574.5 eV), while no peak was ascribed to the binging energy of Cr(III) complexed by amine group, which revealed that not only electrostatic attraction, but also complexation interaction have taken place between Cr(III) and carboxyl groups on the A&C-HM at solution pH of 10.0.

3.2.2. Effect of flow rate

To investigate the effect of sample loading flow rate on Cr(VI)/Cr(III) retention, 1.0 mL sample solution (pH 10.0 for Cr(III) and pH 3.0 for Cr(VI)) containing 20 μg L^{-1} Cr(VI)/Cr(III) was passed through the A&C-HMC under different flow rates varying from 20 to 250 μL min^{-1}. According to the results shown in Fig. S7, the adsorption rates did not significantly reduce with the increasing loading flow rate, and were still above 95% in the whole tested range, which meant that the developed monolithic column had good mechanical strength. Subsequently, the influence of elution flow rate on the recovery rates of Cr(VI)/Cr(III) was also evaluated in detail. As illustrated in Fig. S8, the recoveries had basically no change with different elution flow rates, but the errors were slightly increased at 250 μL min^{-1}. In order to ensure the proposed SPME method time-saving and safe enough for the skeleton of A&C-HMC, 150 μL min^{-1} was much more suitable and adopted in both the sample loading and elution steps.

3.2.3. Effect of sample volume

The effect of sample volume on the adsorption rates of A&C-HMC to Cr(VI) and Cr(III) was measured by continuing pumping sample solutions containing 20 μg L^{-1} Cr(VI)/Cr(III) under the optimum pH and flow rate, and the effluents were collected and detected each 1.0 mL. As can be seen from Fig. S9, the adsorption rates of Cr(VI) and Cr(III) had no apparent variation with the sample volume increase. To balance the higher enrichment factor against the longer experiment time using larger sample volume, 3.0 mL sample solution was chosen for subsequent experiments after comprehensive consideration.

3.2.4. Eluent

It can be seen from the results of the sample pH experiment that both Cr(VI) and Cr(III) were hardly adsorbed on A&C-HMC under strong acid condition. Based on this, nitric acid was selected as eluent to release the adsorbed Cr(VI) and Cr(III) from the monolithic column, and the concentration of HNO_3 and eluent volume were optimized (Fig. 6). The recoveries of Cr(VI) and Cr(III) increased with the increasing HNO_3 concentration, and closely reached 100% when HNO_3 concentration exceeded 7% (v/v). To obtain better precision, 10% HNO_3 was used as eluent for further experiments. The eluent volume was investigated referencing our previous works [11,20]. It was obvious from Fig. 6(B) that 150 μL 10% HNO_3, the minimum injection volume of ICP-MS in our lab, was able to elute Cr(VI) and Cr(III) completely with recoveries of ~100%. Therefore, the elution volume was set to be 150 μL for getting a high enrichment factor.

3.3. Interference study

The interference of other main coexisting ions in environmental waters, such as Na^+, K^+, Ca^{2+}, Zn^{2+}, Mg^{2+}, NO_3^-, SO_4^{2-}, etc., on the determination of Cr(VI) and Cr(III) was examined using 1.0 mL solution containing 10 μg L^{-1} each target species and a certain concentration of interfering ions as sample under the optimized conditions described above. The results in Table S4 indicated that in the presence of 100 mg L^{-1} Na^+, K^+ and Ca^{2+}, 5.0 mg L^{-1} Mg^{2+}, Zn^{2+} and Al^{3+}, 1.0 mg L^{-1} Ni^{2+}, Cu^{2+}, Pb^{2+} and Fe^{3+}, 34.5 mg L^{-1} NO_3^-, 10 mg L^{-1} PO_4^{3-}, 20 mg L^{-1} SO_4^{2-} and 177.5 mg L^{-1} Cl^-, the recoveries of two chromium species, Cr(VI) and Cr(III), were still above 90%. Therefore, the proposed method could be applied for the speciation analysis of inorganic chromium in the real water samples with good tolerance of interference.

3.4. Adsorption capacity and reusability of A&C-HMC

To evaluate the performance of A&C-HMC, its adsorption capacity was examined by the breakthrough curve experiment of 2.0 mg L^{-1} Cr

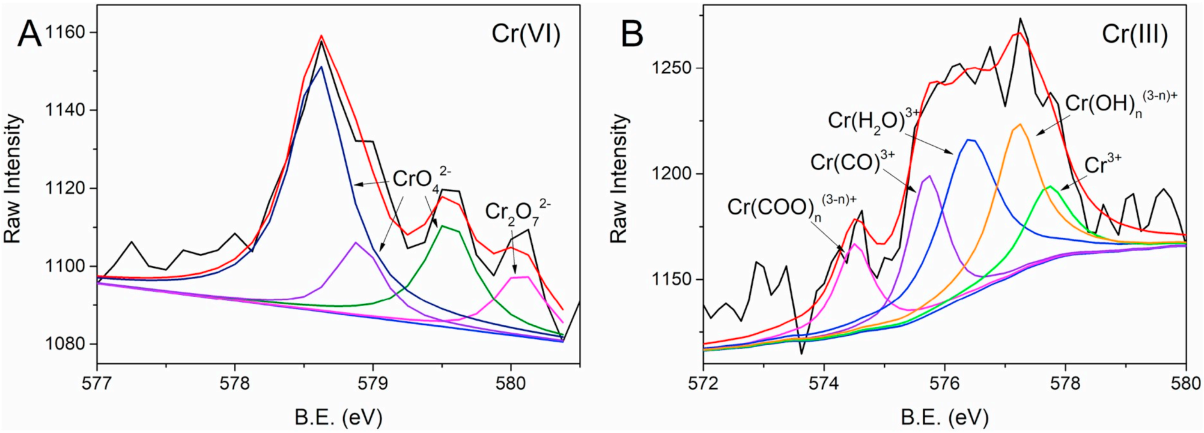


Fig. 5. Detail Cr 2p spectra of A&C-HMs adsorbing Cr(VI) and Cr(III). (A) A&C-HM-VI; (B) A&C-HM-III.

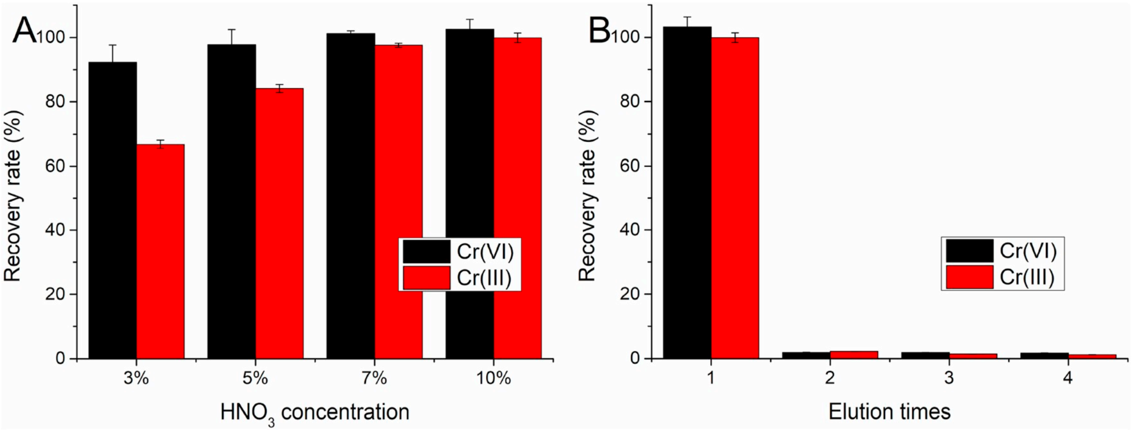


Fig. 6. Effect of eluent. A. Effect of HNO_3 concentration in eluents for Cr(VI) and Cr(III) (the eluent volumes are 300 μL); B. Effect of volume in eluents for Cr(VI) and Cr(III): 150 μL each time. Sample volume: 3.0 mL. Concentration of each species: 20 μg L^{-1}. Solution pH: 3.0 for Cr(VI) and 10.0 for Cr(III). Flow rate: 150 μL min^{-1}.

Table 1
Analytical results of inorganic Cr in standard solutions (ST1 and ST2) and water samples (mean ± sd, *n* = 3).

Sample	Spiked (μg L^{-1})		Found (μg L^{-1})		Recovery (%)	
	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)
ST1	5.0	0.5	4.916 ± 0.253	0.499 ± 0.038	98.3	99.9
ST2	0.5	5.0	0.501 ± 0.026	4.799 ± 0.079	100	96.0
Tap	0	0	0.258 ± 0.015	0.165 ± 0.041	N/A	N/A
	0.3	0.3	0.573 ± 0.047	0.481 ± 0.037	103	103
	0.6	0.6	0.838 ± 0.028	0.765 ± 0.009	97.7	100
Yangtze River	0	0	0.270 ± 0.012	0.143 ± 0.013	N/A	N/A
	0.3	0.3	0.576 ± 0.024	0.444 ± 0.028	101	100
	0.6	0.6	0.887 ± 0.051	0.737 ± 0.049	102	99.2
Qinhuai River	0	0	0.316 ± 0.055	0.293 ± 0.016	N/A	N/A
	0.3	0.3	0.603 ± 0.025	0.578 ± 0.026	97.9	97.5
	0.6	0.6	0.929 ± 0.010	0.857 ± 0.034	101	96.0

(VI)/Cr(III). The results shown in Fig. S10 demonstrated that the adsorption capacities of A&C-HMC were 1.83 μg cm^{-1} (~5.38 mg g^{-1}) for Cr(VI) and 2.51 μg cm^{-1} (~7.39 mg g^{-1}) for Cr(III) at 5% breakthrough volume, which were much higher than our actual application needs.

The reusability was another key factor in evaluating the performance of monolithic column, and investigated by measuring the recoveries of Cr(VI)/Cr(III) on the A&C-HMC after several adsorption/elution steps. As shown in Fig. S11, the A&C-HMC could enable at least four loading and elution cycles without losing analytical performance or causing any identifying bleeding of the monoliths, which indicated that the developed A&C-HMC had good stability and reusability.

3.5. Analytical performance and sample analysis

The needle-SPME method by taking A&C-HMC as adsorbent was proposed and the analytical performance under the optimized conditions in this work were as follows: the theoretical enrichment factor was 20-fold and the calibration curves were 0.05–100 μg L^{-1} ($r^2 > 0.997$ for Cr(VI) and $r^2 > 0.994$ for Cr(III)). The limits of detection (LODs, defined as 3-fold signal-to-noise ratio) were both 0.007 μg L^{-1} for Cr (VI) and Cr(III), which were considerably lower than the allowed limits of Cr in environmental waters [3].

Furthermore, two standard solutions (ST1 and ST2) and three kinds of real water samples were applied for assessing the feasibility of protocol in this work. As listed in Table 1, Cr(VI) and Cr(III), in standard solutions, original and spiked water samples, could be well selectively extracted by A&C-HMC under their respective optimal conditions, with the recoveries of 97.7–103% and 96.0–103% for two inorganic chromium species, respectively. The experiment results indicated that this proposed method could be applied for analyzing the speciation of inorganic chromium in environmental waters.

4. Conclusions

Bifunctionalized A&C-HMC was developed with binary porogens *via* one-pot co-condensation and used as needle-SPME matrix for the species speciation of inorganic chromium in environmental waters. The A&C-HM possesses an extremely rough rambutan-shaped surface compared with the same monolithic material prepared with single porogen, which could significantly increase the surface exposure of functional groups gaining abundant amine and carboxyl active sites. Very interestingly, the proposed A&C-HM can selectively capture Cr(VI) and Cr (III) in different pH ranges, respectively, without any species variation. This is because electrostatic attraction happens between Cr(VI) and amine group on A&C-HM, while both electrostatic attraction and complexation interaction do between Cr(III) and carboxyl group. To the best of our knowledges, this work is the first time to synthesize a multifunctionalized HMC by facile “one-pot” aiming at the non-invasive separation of inorganic chromium species. Moreover, the good mechanical strength endowed the A&C-HMC with the potentials for not only on-site pretreatment of collected water samples, but also on-line capillary SPME coupled with detectors.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

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