Talanta 119 (2014) 440–446

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Ion-exchange chromatography combined with direct current amperometric detection at CuNPs/reduced graphene oxide–chitosan composite film modified electrode for determination of monosaccharide composition of polysaccharides from *Phellinus igniarius* ☆

Lingling Xi *, Fengli Wang, Zuoyi Zhu, Zhongping Huang, Yan Zhu

Department of Chemistry, Xixi Campus, Zhejiang University, Hangzhou 310028, China

ARTICLE INFO

Article history:
Received 24 July 2013
Received in revised form
5 November 2013
Accepted 7 November 2013
Available online 19 November 2013

Keywords:
Nano-copper particles
Graphene
Ion chromatography
Direct current amperometric detection
Monosaccharide composition
Phellinus igniarius

ABSTRACT

A novel Cu nanoparticles/reduced graphene oxide–chitosan (CuNPs/r-GO–chitosan) composite film modified glassy carbon electrode (GCE) was fabricated by dispersing CuNPs uniformly on a stable r-GO–chitosan thin film through electrodeposition process. The modified electrode was characterized by cyclic voltammetry, scanning electron microscopy (SEM) and electrochemical impedance spectroscopy (EIS), and exhibited efficiently electrocatalytic oxidation toward monosaccharides with high stability. The good electrocatalytic activity of this modified electrode might be attributed to the synergistic effect of r-GO and CuNPs, and the stability might be attributed to the r-GO and chitosan thin matrix film. When the CuNPs/r-GO–chitosan/GCE was used as an electrochemical sensor in high performance anion exchange chromatography-direct current amperometric detection (HPAEC-DC) flowing system for the determination of monosaccharides under constant working potential of +0.55 V, the detection limits ($S/N=3$) ranged from 0.006 to 0.02 mg L^{-1} for the analyzed sugars, and the dynamic linear ranges spanned from 0.02 to 500 mg L^{-1}. The proposed method has been applied for the determination of monosaccharide composition of crude polysaccharides from *phellinus igniarius* real samples, and the results were satisfactory.

1. Introduction

Carbohydrates can be readily separated by high performance liquid chromatography (HPLC), high performance anion-exchange chromatography (HPAEC) and capillary electrophoresis [1–4], but their detection with conventional spectrophotometric methods such as UV–vis absorption or fluorescence approach is difficult to be realized because of the absence of strong chromophores. For this reason, the analysis of carbohydrates had been performed using pre-column or post-column derivatization methods [5–7]. Non-derivatizing detection methods include refractive index [8–10], evaporative light scattering [11–13] and mass spectrometry [4,5]. Refractive index and evaporative light scattering are unable to detect possibly low concentrations of analytes in some biologically real samples due to their lower detection sensitivity. Mass spectrometry is more complicated and expensive. Another non-derivatizing detection method is electrochemical detection (ECD), and pulsed amperometric detection (PAD) is often combined with HPAEC for the sugars determination [14–17]. The hydroxyl groups in carbohydrates can be partially deprotonated in basic media, therefore, HPAEC directly using strong alkaline eluents can simultaneously meet the requirements of electrochemical detection and separation. Unfortunately, an electrocatalytically active noble metal working electrode such as Au electrode must be used in PAD mode owing to the scarce electroactivity of sugars, and a multistep potential waveform must be required in order to restore the electrocatalytic activity of Au electrode. Additionally, it must raise the pH of mobile phase to approximate 13 because of the detection sensitivity. To resolve these problems, the effective approach seems to be the application of chemically modified electrodes (CMEs). Characterized by either surface-confined or three-dimensional dispersions of catalytic sites, CMEs have been proved to be competitive over conventional bulk metal electrodes and permit direct current amperometric operation mode (DC) in HPAEC analysis of carbohydrates and amino acids in alkaline media [18,19]. Recently considerable efforts have been devoted to the development of CMEs based on the electrocatalytic activity of specific redox mediators for the detection and quantitation of non-absorbing UV–visible and scarcely electroactive compounds.

* Corresponding author. Tel.: +86 571 88273637; fax: +86 571 88273637.
E-mail address: XLL98731@zju.edu.cn (L. Xi).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.11.024

Transition metals and their oxides, such as nickel [20,21], cobalt [22,23] and copper [24,25] have attracted much interest as electrode modifying materials because of their good electrocatalytic property in alkaline solutions towards the oxidation of carbohydrates. Graphene, a single layer of carbon atoms in a closely packed honeycomb two-dimensional lattice, shows many advantages such as exceptional thermal and mechanical properties, excellent electrical conductivity and fast electron transportation ability, and has attracted considerable attention in recent years [26–30]. Due to its unique properties, graphene has been considered as an attractive new material in the design of novel electrochemical sensors [31–34]. Luo et al. has developed a non-enzymatic glucose sensor based on graphene and CuO nanocubes [35]. Chen et al. has also prepared a graphene-copper nanoparticles composite sensor and applied it for the amperometric detection of carbohydrates in capillary electrophoresis–ECD system [36]. But the modifying methods which are reproducible and can make the modifiers electrocatalytic activity not deteriorate during operation in flowing systems such as HPAEC are still needed. Chitosan (CTS) with abundant amino groups exhibits good compatibility with metal nanoparticles and excellent film-forming ability [37–39]. So it is a very suitable matrix for immobilizing metal nanoparticles and constructing electrochemical sensors [40–42]. However, as far as we know, studies on using chitosan as the matrix for hosting the nanocomposite of graphene and Cu nanoparticles (CuNPs) to construct an electrochemically amperometric sensor, and employ it in HPAEC-DC flowing system for stable and sensitive determination of carbohydrates have not been reported.

Phellinus igniarius (*P. igniarius*, PIE), a kind of well-known chinese traditional medicinal mushroom containing many bioactive compounds, has been well-known for many centuries as Sanghuang (yellow polyporus) [43]. The main compounds polysaccharides isolated from *P. igniarius* have attracted great attention for many years due to their various biological functions such as anti-inflammatory, antioxidative activities, inhibiting tumor growth and metastasis with low toxicity [44–46]. The knowledge and analysis of the monosaccharide composition of *P. igniarius* polysaccharides are essential because of its important effect to above functions of polysaccharides [44,47]. Thus, it is important to establish a rapid and sensitive analytical method for the accurate determination of monosaccharide composition of polysaccharides from *P. igniarius* [44,47,48].

In the present work, combining the above-mentioned benefits of CuNPs, graphene and chitosan, a novel nanocomposite modified electrode CuNPs/reduced-graphene oxide-chitosan/GCE (CuNPs/r-GO–chitosan/GCE) was prepared by electrochemical reduction of Cu ion and oxidative graphene. Using the CuNPs/r-GO–chitosan modified GCE as an amperometric sensor, a new method was established for the monosaccharides determination combining the good selectivity of HPAEC with the high sensitivity of ECD. The CuNPs/r-GO nanocomposite showed good electrocatalytic activity toward the studied monosaccharides (glucose, fucose, arabinose, galactose and mannose). Moreover, the resulting CuNPs/r-GO–chitosan modified electrode demonstrated favorable reproducibility and stability when utilized as amperometric sensor in HPAEC-DC flowing system for the determination of carbohydrates. The application of the established new method using CuNPs/r-GO–chitosan modified electrode was tested by analyzing the monosaccharide composition of polysaccharides from *P. igniarius*, and this application has not been reported by now.

2. Experimental

2.1. Materials

Chemicals copper sulfate and sodium hydroxide were of analytical-reagent grade, and they were used without further purification. Distilled deionized water of 18.2 MΩ cm was used throughout. Graphite powder, glucose, fucose, arabinose, galactose and mannose were purchased from Shanghai Aladdin Chemical Reagent Company (Shanghai, China). Chitosan was purchased from Sigma. Stock solutions of glucose, fucose, arabinose, galactose and mannose were prepared with deionized water at a concentration of 1000 mg L^{-1}, stored in refrigerator (4 °C) and diluted before use.

Graphene oxide was synthesized from graphite according to the method [49,50]. Briefly, natural flake graphite was reacted with concentrated sulfuric acid, nitric acid and potassium chlorate for 96 h. After oxidation of graphite, the mixture was added to excess water, washed with a 5% solution of HCl, and then repeatedly washed with water until the pH of filtrate was neutral. Then through extremely rapid heating and successful splitting of graphite oxide, wrinkled graphene sheets functionalized with hydroxyl and carboxylic groups were obtained. The prepared GO powder was exfoliated by ultrasonication to form a 1.0 mg mL^{-1} GO colloidal dispersion.

2.2. Instrumentation

Electrochemical measurements were performed on a CHI 660A electrochemical workstation (CH Instrument Co., Austin, TX, USA). A conventional three-electrode system was employed, involving a 3.0 mm diameter bare or modified GCE as the working electrode, a platinum wire as the counter electrode and an Ag/AgCl electrode as the reference electrode. A Hitachi Scientific Instruments (London, U.K.) model S-3000H scanning electron microscope at an accelerating voltage of 15 kV was used for surface image measurements. Electrochemical impedance spectroscopy (EIS) measurements of the bare GCE and modified electrodes were carried out by a Model VWP2 (Park Corporation, Canada) in the frequency range of 0.01 Hz–100 kHz at the open circuit potential versus Ag/AgCl with potential amplitude of 10 mV.

Chromatographic analysis was performed on an ICS-2000 (Dionex, Sunnyvale, CA, USA) chromatograph, which was equipped with a gradient pump, a 6-port valve with a 25 μL loop and an electrochemical detector (model ED40). A flow-through thin-layer electrochemical detection cell composed of a 1.0 mm diameter bare or modified GCE, a pH-Ag/AgCl combination reference electrode and a titanium body counter electrode was used. Separations were accomplished on a Dionex anion exchange column, CarboPac PA10 (250 × 4 mm i.d.) coupled with a guard column (50 × 4 mm i.d.). 15 mM NaOH was chosen as eluent. The flow rate was 1.0 mL min^{-1}.

2.3. Preparation of modified electrodes

After being soaked for few minutes in 37% w/w perchloric acid to remove any trace of metal from the electrode surface, a GCE was polished with 0.5 μm Al_2O_3 powder, and cleaned in an ultrasonic bath with ethanol and deionized water respectively. The prepared electrode was dried under a nitrogen stream and used for modification immediately. To fabricate the CuNPs/r-GO–chitosan modified electrode, firstly a mixture containing same volume of 0.25 mg mL^{-1} GO and 0.5% (wt) chitosan aqueous solutions was sonicated for 1 h, 2 μL of the mixture dispersion was dropped on the surface of above-prepared GCE and air-dried at room temperature to obtain the GO–chitosan modified electrode. Then CuNPs (mostly 20–100 nm) were electrochemically deposited on this GO–chitosan modified electrode by the CV method scanning from 0.0 to −1.5 V for 3 cycles at 20 mV s^{-1} scan rate in 5 mM $CuSO_4$, during this process the GO was in-situ reduced electrochemically. After the modification with Cu-NPs, the electrode was thoroughly washed to remove unbound materials from the electrode surface and dried in air for 30 min. The controlled r-GO–chitosan/GCE was prepared by dropping 2 μL of the mixture

dispersion of 0.25 mg mL^{-1} GO and 0.5% chitosan aqueous solutions on the surface of GCE and then scanned from 0.0 to −1.5 V for 3 cycles at 20 mV s^{-1} scan rate in 5 mM Na_2SO_4 without Cu ions. The controlled CuNPs/chitosan/GCE and CuNPs/r-GO/GCE were prepared by depositing CuNPs by CV method scanning from 0.0 to −1.5 V for 3 cycles at 20 mV s^{-1} scan rate in 5 mM $CuSO_4$ on chitosan/GCE and GO/GCE, which were fabricated by dropping 2 μL of 0.25% (wt) chitosan solution and 2 μL of 0.125 mg mL^{-1} graphene oxide solution on the surface of GCE respectively.

2.4. Sample preparation

2.4.1. Extraction of crude polysaccharide from P. igniarus

The dried fruiting bodies of *P. igniarius* were cut into small pieces and reflux extracted with ethanol for 12 h to remove lipids. The residue was air dried and extracted 3 times each for 2 h with six times volume of boiling water. The water-insoluble material was removed by filtration, and the supernatant was concentrated into one-tenth of the original volume. Then 95% ethanol was added into the concentrated solution slowly until the final alcohol concentration reached 65%. The resulting precipitate was collected and then air dried to become the crude polysaccharide.

2.4.2. Hydrolysis of crude polysaccharide from Phellinus igniarus

The obtained crude polysaccharide (2.0 mg) from *P. igniarius* above was hydrolyzed with 2 M H_2SO_4 at 110 °C for 2 h. The hydrolyzed liquid was diluted by deionized water, and then filtered through a 0.2 μm nylon membrane (Nylaflo Aldrich) to obtain the mixture of monosaccharides.

3. Results and discussion

3.1. Characteristics of CuNPs/r-GO–chitosan modified electrode

Cu-NPs were deposited electrochemically onto GO–chitosan modified GCE in 5 mM $CuSO_4$ under scanning potentials from 0 to −1.5 V (vs. AgCl/AgCl) at 20 mV s^{-1} scan rate. The loading mass of Cu-NPs on electrode was controlled by varying the deposition cycle. The CVs were recorded in 0.10 M NaOH solution containing 100 mg L^{-1} glucose after the deposition of Cu-NPs on GO–chitosan/GCE by changing the scanning cycle. It was found that the response current of Cu-NPs/r-GO–chitosan/GCE towards glucose oxidation increased during the first five deposition cycles. With further increase of the deposition cycle, the response current of Cu-NPs/r-GO–chitosan/GCE decreased. This was probably because that the size of Cu-NPs clusters became increased with the increase of sweeping cycle, and the larger sized particles decreased the electrocatalytic activity and stability of the Cu-NPs/r-GO–chitosan/GCE. On these accounts, we selected three sweeping cycles for the deposition of Cu-NPs on GO–chitosan/GCE throughout our experiments. Besides, different concentrations from 0.10 to 1.0 mg mL^{-1} of graphene oxide aqueous solutions when they were mixed with chitosan solution for preparing GO–chitosan/GCE were also investigated. Results showed that a highly sensitive and stable response of CuNPs/r-GO–chitosan/GCE towards glucose oxidation was obtained when 0.25 mg mL^{-1} graphene oxide solution was chosen. The graphene oxide was well dispersed in chitosan solution, forming a stable and dark suspension. The edge plane of graphene sheets yielded chemical functional groups, such as –OH and –COOH, in the thermal exfoliation process, which let graphene oxide sheets be more hydrophilic and easier to interact with chitosan, facilitating the preparation of GO–chitosan composite film as a uniform and stable matrix for depositing CuNPs. The existence of a certain amount of r-GO could enhance the conductivity, electrocatalytic activity of CuNPs/r-GO–chitosan/GCE and the existence of chitosan could enhance the stability of the nanocomposite modified electrode.

In order to investigate the morphology of Cu-NPs/r-GO–chitosan composite film, different parts of the CuNPs/r-GO–chitosan/GCE surface were observed by scanning electron microscopy (SEM). Fig. 1 shows a typical image of the surface morphology of CuNPs/r-GO-chitosan film on GCE. Near-perfect Cu-NPs are relatively dispersed on the whole surface of r-GO–chitosan matrix. This may be attributed to the uniformity and stability of the matrix graphene–chitosan thin film in the copper deposition process since both graphene and chitosan have excellent film-forming ability. Moreover, the electrostatic interaction between the graphene–COO^- anions and amino cations of chitosan led to form a stable GO–chitosan composite thin film on GCE. Additionally, chitosan, which could adhere strongly to the surface of GCE, was modified on GCE to eliminate the possible fouling, and enhance the compatibility between CuNPs and GCE, correspondingly prevent the leaching of CuNPs from the surface of GC electrode.

The modified process of GCE surface was also monitored by EIS, whose semicircle portion corresponded to the electron transfer limited process. Fig. 2 shows the results of EIS on bare GCE (a) and different modified GC electrodes (inset, b and c) respectively in the presence of redox probe $Fe(CN)_6^{3-/4-}$. After chitosan was modified on GC electrode, a very large electron transfer resistance (R_{et}) was observed in curve inset, which proved that chitosan was effectively modified on GCE surface. Compared to chitosan/GCE, it could be observed that the diameter of the semicircle plot was decreased with the presence of r-GO (curve b). It is demonstrated that r-GO present on chitosan/GCE facilitated the electron-transfer

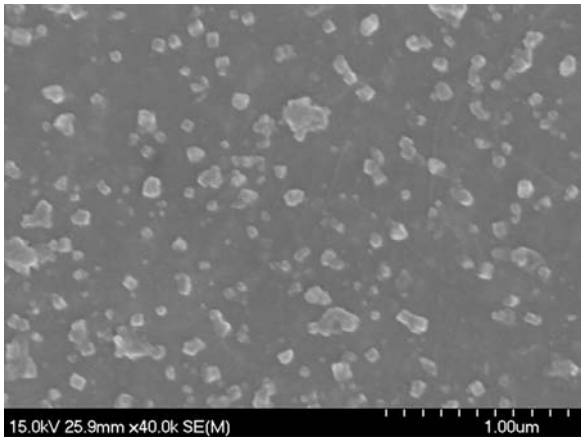

Fig. 1. SEM image of CuNPs/r-GO–chitosan/GCE.

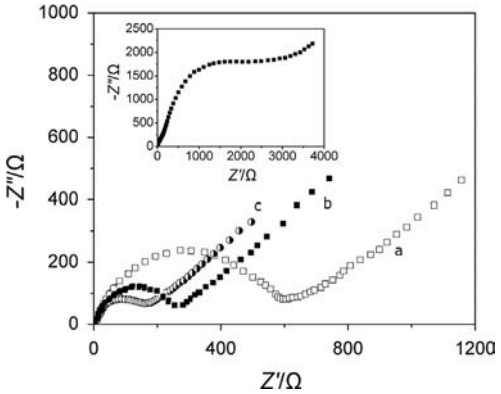

Fig. 2. Electrochemical impedance spectroscopy of GCE (a), chitosan/GCE (inset), r-GO–chitosan/GCE (b), CuNPs/r-GO–chitosan/GCE (c) in 0.1 mol L^{-1} KCl containing 1.0 mmol L^{-1} $Fe(CN)_6^{3-/4-}$.

process on the electrode surface due to its smaller electron transfer resistance. It could be observed in curve c that the diameter of the semicircle plot was decreased largely. This is demonstrated that CuNPs present on r-GO–chitosan/GCE further facilitated the electron-transfer process on the electrode surface due to its smaller electron transfer resistance.

3.2. Electrocatalytic oxidation of monosaccharides at CuNPs/r-GO-chitosan/GCE

The electrocatalytic activity of CuNPs/r-GO–chitosan/GCE toward monosaccharides oxidation was studied. Fig. 3 shows the CVs of r-GO–chitosan/GCE, CuNPs/chitosan/GCE and CuNPs/r-GO–chitosan/GCE in the absence (curve a) and presence (curve b) of 100 mg L^{-1} glucose in 0.1 M NaOH. There was no significant oxidation peak observed at r-GO–chitosan/GCE (Fig. 3A) in the presence of glucose in the potential range from −0.2 to 0.8 V, suggesting that this electrode is relatively inactive toward the oxidation of glucose. The results shown in Fig. 3B demonstrated that CuNPs/chitosan could electrocatalyze the oxidation of glucose. This was due to the electrocatalytic activity of CuNPs film. However, at the CuNPs/r-GO–chitosan/GCE (Fig. 3C), when 100 mg L^{-1} glucose was added to 0.1 M NaOH solution, an obvious increase of anodic current signal resulting from the oxidation of glucose and the more negative peak potential, were observed in the potential range from −0.20 to 0.80 V. The electrocatalytic activity of CuNPs modified electrode toward the oxidation of glucose in alkaline medium may be mainly attributed to the involvement of Cu (II) and Cu (III) species on surface of electrode. The enhanced electrocatalytic performance of CuNPs/r-GO–chitosan/GCE might be attributed to the synergistic effect of the electrocatalytic CuNPs film and the large surface-to-volume ratio r-GO film with high conductivity and fast electron transportation ability.

In addition, the CV experiments of other four monosaccharides (fucose, arabinose, galactose and mannose) oxidation were also investigated under the same conditions. The typical voltammetric curves of these four monosaccharides at CuNPs/r-GO–chitosan/GCE are shown in Fig. 4. Similarly, the addition of each monosaccharide to the blank NaOH caused a notable enhancement of the anodic current in the potential range from −0.20 to 0.80 V. Therefore, the CuNPs/r-GO–chitosan/GCE can be applied as an electrochemical sensor in HPAEC-DC system for the simultaneous and sensitive determination of these monosaccharides. Moreover, the anodic oxidation peak was considerably reproducible in the presence of monosaccharide after fifty consecutive scan times, showing almost no surface passivation, likely because the thin-layer of electroactive species is less affected by fouling and wastage. These observations illustrated that direct current amperometric detection of monosaccharides in HPAEC flow system at CuNPs/r-GO–chitosan/GCE would seem to be feasible.

3.3. Selection of chromatographic separation and DC amperometric detection conditions

Using CuNPs/r-GO–chitosan/GCE as the amperometric sensor, the effect of applied detection potentials on the current responses of fucose, arabinose, galactose, glucose and mannose in HPAEC-DC was examined in the range from 0.35 to 0.65 V. Fig. 5 shows the relationship of current responses of analytes with applied working potentials. It can be seen that the current responses of analytes increased as the potential increased from 0.35 to 0.55 V. When the potential increased above 0.60 V, the baseline noise and background current increased, which was disadvantageous for the sensitive and stable detection of analytes. Therefore, an applied potential of 0.55 V (vs. Ag/AgCl) was chosen as the optimal detection potential in our HPAEC-DC system.

The effect of eluent concentrations on retention times and separation resolutions of monosaccharides was also investigated. To get the ionization of sugar hydroxyl groups, the alkaline medium NaOH, which can simultaneously meet the requirement of direct current amperometric detection, was chosen as the eluent for anion-exchange chromatographic separation of monosaccharides. With the increasing of NaOH concentration, retention times of monosaccharides were accordingly shortened and the current responses became greater because sugars are more easily oxidized at higher pH. In order to obtain a reasonable separation resolution of chromatographic peaks and the maximum signal to noise ratios, 15 mM moderate NaOH solution was chosen as the eluent. Under the optimum conditions, it needed only 30 min to attain the equilibrium of HPAEC-DC system. Fig. 6 shows a typical chromatogram of a mixture of 5.0 mg L^{-1} fucose, arabinose, galactose, glucose and mannose standard solutions detected at the CuNPs/r-GO–chitosan/GCE. Good chromatographic separation of the five sugars with symmetric peaks was obtained within a short time.

3.4. Analyses of monosaccharides by HPAEC-DC

Under the optimum chromatographic and DC detection conditions described above, a series of five monosaccharides (fucose, arabinose, galactose, glucose and mannose) mixed standard solutions ranged from 0.02 to 500 mg L^{-1} respectively were analyzed. The linearities were evaluated by analyzing standard solutions concentrations (X, mg L^{-1}) with respect to peak areas (Y, nA min). The analytical results in terms of detection limits (LOD), linear ranges and correlation coefficients of each monosaccharide were summarized in Table 1. Linear responses were generally obtained over a range of approximately four orders of magnitude for these five monosaccharides. Limits of detection determined from the lowest concentration solutions according to $S/N=3$, ranged from 0.006 to 0.02 mg L^{-1} for the five monosaccharides. The relative standard deviations (RSDs) of peak areas for a set of six

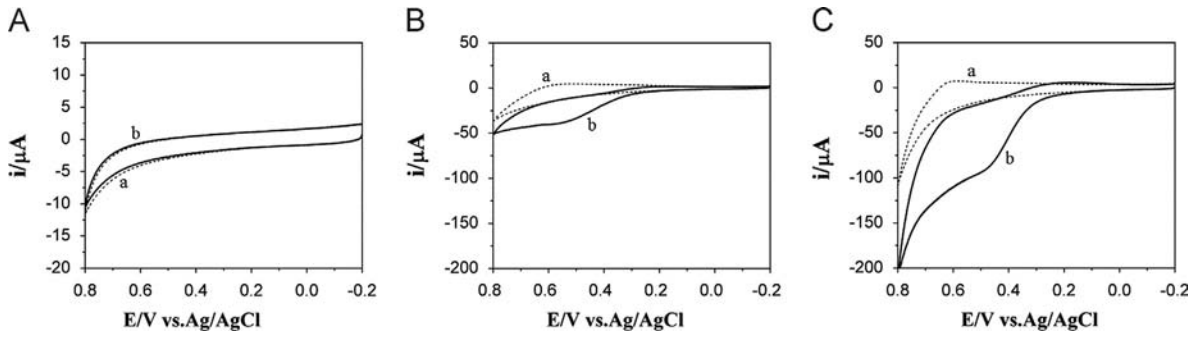

Fig. 3. Cyclic voltammograms of 0.1 M NaOH at r-GO–chitosan/GCE (A), CuNPs/chitosan/GCE (B) and CuNPs/r-GO–chitosan/GCE (C) without (a) and with (b) glucose (100.0 mg L^{-1}). Scan rate: 100 mV s^{-1}.

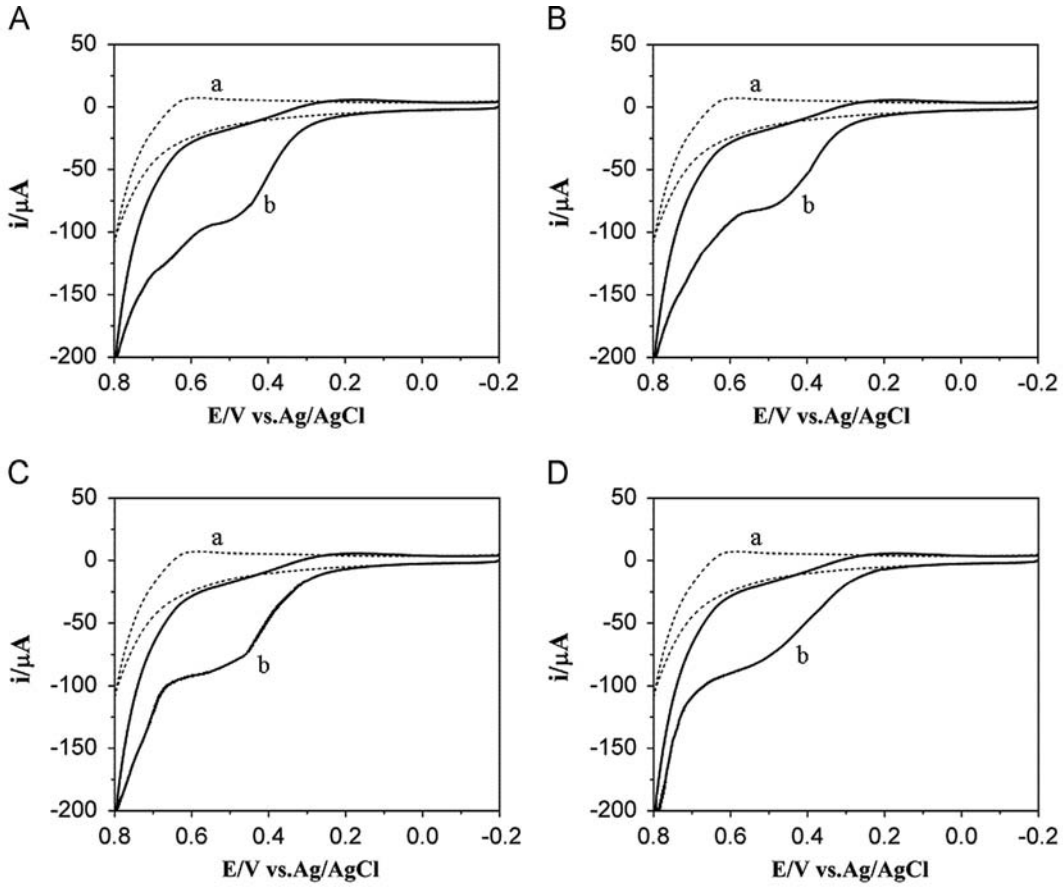

Fig. 4. Cyclic voltammograms of CuNPs/r-GO–chitosan/GCE in 0.1 M NaOH before (curve a) and after (curve b) the addition of 100.0 mg L^{-1} fucose (A), arabinose (B), galactose (C) and mannose (D). Scan rate: 100 mV s^{-1}.

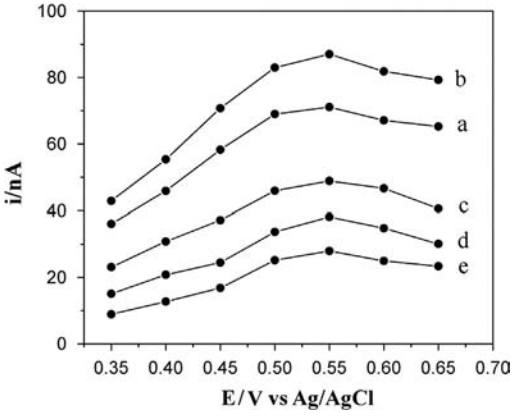

Fig. 5. The relationship of current responses at CuNPs/r-GO-chitosan/GCE and applied potentials of 2.0 mg L^{-1} fucose (a), arabinose (b), galactose (c), glucose (d), and mannose (e) respectively in HPAEC-DC system. Column: CarboPac PA10 (250×4 mm^2) coupled with a guard column (50×4 mm^2). Eluent: 15 mM NaOH. Flow rate: 1.0 mL min^{-1}.

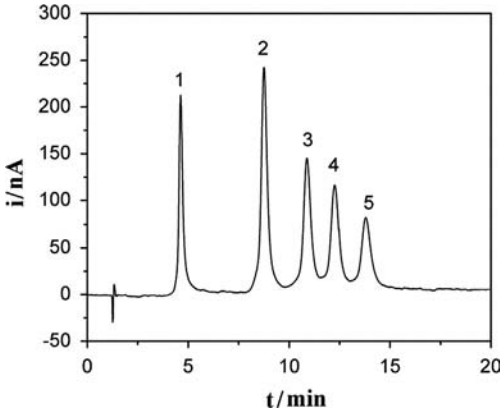

Fig. 6. Chromatogram of a mixture of 5 mg L^{-1} fucose (1), arabinose (2), galactose (3), glucose (4) and mannose (5) using CuNPs/r-GO–chitosan/GCE as amperometric sensor. Working potential: +0.55 V (vs. Ag/AgCl). Chromatographic conditions were the same as in Fig. 5.

consecutive chromatographic injections of a standard mixture of 5.0 mg L^{-1} of each monosaccharide were investigated and ranged from 1.9% to 2.8%. It is interesting to observe that the obtained analytical parameters such as LODs and linear ranges of this work are generally comparable with those obtained by utilizing other amperometric sensors in HPAEC analysis of sugars [18,19]. In addition, this CuNPs/r-GO–chitosan modified electrode has better reproducibility.

The goal of this work was to develop a sensitive and stable amperometric sensor in HPAEC-DC system for the detection of

Table 1
Analytical data of five monosaccharides obtained by HPAEC-DC.

Compounds	Linear range (mg L^{-1})	Regression equation	Correlation coefficient	RSD ($n=6$) (%)	LOD (mg L^{-1})
Fucose	0.02–500	$Y=41.35X+0.1556$	0.9997	2.3	0.006
Arabinose	0.02–500	$Y=45.82X+0.2765$	0.9992	1.9	0.006
Galactose	0.03–200	$Y=29.66X-0.1417$	0.9998	2.4	0.01
Glucose	0.03–200	$Y=28.33X-0.2882$	0.9999	2.8	0.01
Mannose	0.05–200	$Y=20.64X-0.3318$	0.9993	2.0	0.02

monosaccharides. The stability of CuNPs/r-GO–chitosan/GCE was tested in HPAEC-DC flowing system by measuring the change of signals upon injections of 5 mg L^{-1} fucose, arabinose, galactose, glucose and mannose mixture periodically performed during 16 h of continuous analyses. In this part, two modified electrodes, CuNPs/r-GO/GCE and CuNPs/r-GO–chitosan/GCE were investigated. As a result, the amperometric responses of the investigated five sugars detected at CuNPs/r-GO/GCE presented gradual decrease up to 17–24%. However, using CuNPs/r-GO-chitosan/GCE as the working electrode, the amperometric responses showed variation of only 9–11% over about 16 h of operation for these five sugars, demonstrating that the adding of chitosan to the CuNPs/r-GO nanocomposite film enhanced the stability of the amperometric sensor CuNPs/r-GO–chitosan/GCE in HPAEC flowing system. Additionally, the day-to-day stability was also investigated for 6 days by analyzing six injections of 5 mg L^{-1} glucose each day using a same CuNPs/r-GO-chitosan/GCE. The results showed that the amperometric signals were relatively stable, and the RSD was 6.5%.

3.5. Determination of monosaccharide composition of P. igniarius polysaccharides

To demonstrate the applicability of the CuNPs/r-GO–chitosan/GCE as an amperometric sensor in HPAEC-DC, the determination of carbohydrates in hydrolyzed samples of crude polysaccharides from *P. igniarius* in different regions of china was performed. Representative chromatograms of hydrolyzed polysaccharides samples of *P. igniarius* from different regions are shown in Fig. 7. The peaks identification of the analyzed molecules was based on the retention times in Fig. 6 and was further confirmed by adding authentic standard solutions to diluted samples. Quantitative analyses of the considered analytes were performed using the corresponding regression equations in Table 1. And then the relevant concentrations were expressed as the monosaccharide compositions (mole ratios) of *P. igniarius* polysaccharides. Based on the moles of mannose, the mole ratios of monosaccharides of *P. igniarius* polysaccharides from different regions were listed in Table 2. As can be seen, *P. igniarius* polysaccharide samples from different regions showed similar monosaccharide composition (including fucose, arabinose, galactose, glucose and mannose), and glucose occupied the highest level in all analyzed samples. However, the results in our work were different from some previous reports. For example, the polysaccharide isolated from *P. igniarius* mycelium reported by Wu et al. was composed only of glucose, galactose and mannose [44]. And Chen et al. had reported monosaccharide composition of *P. igniarius* polysaccharide was *n* (xylose):*n* (mannose):*n* (fucose):*n*

Table 2
Monosaccharide composition (moral ratio) of *P. igniarius* polysaccharides from different regions.

P. igniarius oringin	Monosaccharide composition *n* (Fuc):*n* (Ara):*n* (Gal):*n* (Glu):*n* (Man)				
	Fucose	Arabinose	Galactose	Glucose	Mannose
Jilin	0.32	0.04	0.80	2.47	1
Guizhou	0.08	0.04	0.44	3.78	1
Shandong	0.37	0.01	0.86	2.11	1
Yunnan	0.40	0.04	0.80	2.69	1

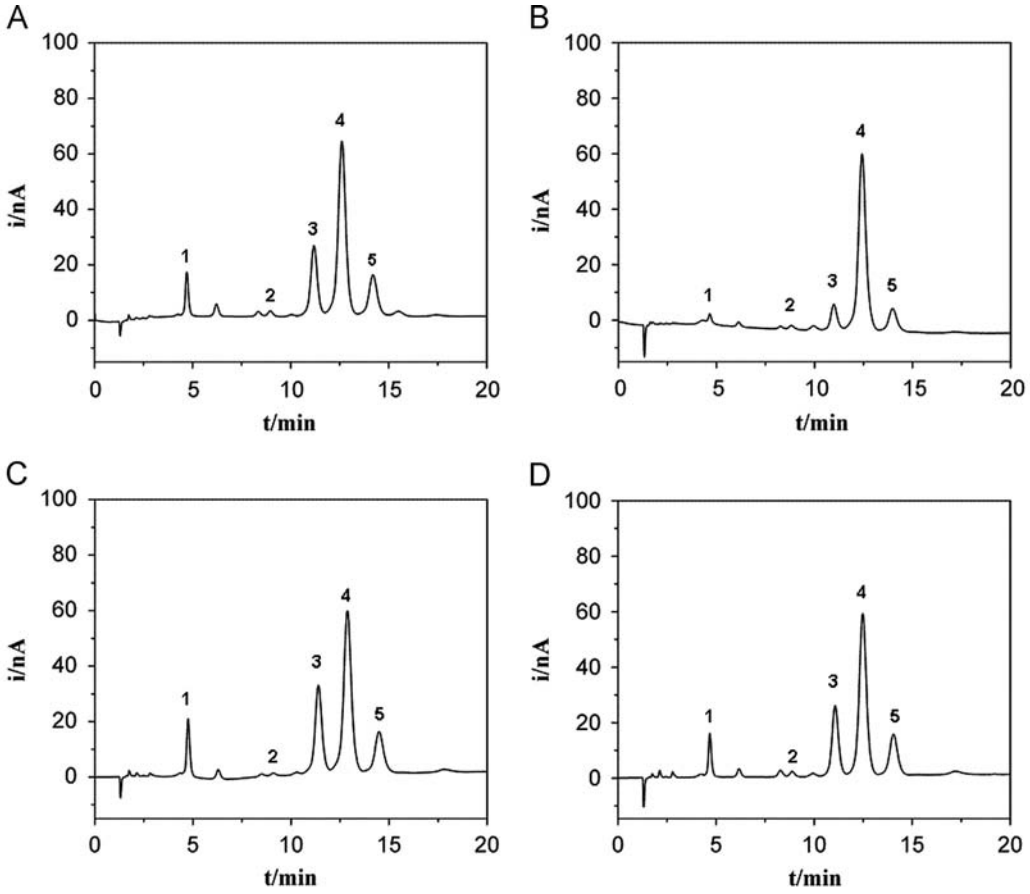

Fig. 7. Chromatograms of four real hydrolyzed samples of *P. igniarius* polysaccharides containing fucose (1), arabinose (2), galactose (3), glucose (4) and mannose (5) from different regions in China. (A) From Jilin Province. (B) From Guizhou Province. (C) From Shandong Province. (D) From Yunnan Province. Experimental conditions were the same as in Fig. 6.

Table 3
Recoveries and RSDs of monosaccharides in *P. igniarius* polysaccharide hydrolyzed liquid samples.

Compounds	Found ($mg\ L^{-1}$)	Original ($mg\ L^{-1}$)	Added ($mg\ L^{-1}$)	Recovery (%)	RSD ($n=3$) (%)
Fucose	0.959	0.464	0.50	99.00	3.9
Arabinose	0.136	0.038	0.10	98.00	4.3
Galactose	2.017	1.012	1.00	100.5	3.7
Glucose	5.235	2.676	2.50	102.4	2.3
Mannose	2.176	1.180	1.00	99.60	2.7

(glucose):*n* (galactose)=2.3:1:6.4:22.1:19.83 by GC [46]. Another study revealed that monosaccharides of a new heteropolysaccharide isolated from fruit bodies of *P. igniarius* consisted only of galactose, mannose and fucose by HPAEC-PAD [47]. But Yang et al. reported a novel heteropolysaccharide isolated from fruiting bodies of *P. igniarius* was composed of fucose, glucose, mannose, galactose and 3–O–Me–galactose in a ratio of 1:1:1:2:1 [48]. These indicated that the sugars compositions were different between mycelia and fruiting bodies. The relevant recoveries were evaluated for each constituent by spiking the hydrolyzed samples with stock solutions of monosaccharides at an approximate level of the measured content, and the results were reported in Table 3. The recoveries of the relevant monosaccharides were between 98% and 102%, and the RSDs, evaluated from three chromatographic injections, ranged from 2.3% to 4.3% for all determined compounds.

4. Conclusion

A novel modified electrode CuNPs/r-GO–chitosan/GCE was constructed and showed highly electrocatalytic activity toward carbohydrates oxidation with reproducibility and stability. It was successfully applied as an amperometric sensor for the determination of carbohydrates in HPAEC combined with ECD system under a constant applied potential. The CuNPs/r-GO–chitosan/GCE exhibited synergistic combination of the advantages of CuNPs, graphene and chitosan toward the electrocatalytic oxidation of carbohydrates. The developed HPAEC-DC method has been successfully applied to determine the monosaccharide composition of crude polysaccharides from *P. igniarius* with acceptable level of sensitivity and recoveries. As a result, the proposed method appears to be useful and effective for the routine practical determination of common carbohydrates in complex real matrices without any derivatization procedure. Ease of preparation and good stability confirmed the potential interest of this modified electrode as amperometric sensor in carbohydrates analytical applications. In addition, utilization of the modified electrode eliminated the need for time-consuming polishing and reequilibrating of working electrode which was required using bulk bare gold electrode in HPAEC-PAD flowing system.

Acknowledgments

This work was supported by Scientific Instruments Major Project of the Ministry of Science and Technology of China (No. 2012YQ09022903) and by Zhejiang Provincial Natural Science Foundation of China (No. Y407153, Y4100190), and sponsored by Zhejiang Provincial Assay Foundation of China (No. 2012C37003).

References

[1] J.D. Oliver, M. Gaborieau, E.F. Hilder, P. Castignolles, J. Chromatogr. A 1291 (2013) 179–186.
[2] Q.Y. Sheng, Y.X. Ke, K.Y. Li, D.P. Yu, X.M. Liang, J. Chromatogr. A 1291 (2013) 56–63.
[3] T. Wang, X.B. Yang, D.Y. Wang, Y.D. Jiao, Y. Wang, Y. Zhao, Carbohydr. Polym. 88 (2012) 754–762.
[4] M. Raessler, TrAC-Trend Anal. Chem. 30 (2011) 1833–1843.
[5] S. Rakete, M.A. Glomb, J. Agric. Food Chem. 61 (2013) 3828–3833.
[6] V.M. Rizelio, L. Tenfen, R. da Silveira, L.V. Gonzaga, A.C.O. Costa, R. Fett, Talanta 93 (2012) 62–66.
[7] A. Harazono, T. Kobayashi, N. Kawasaki, S. Itoh, M. Tada, N. Hashii, A. Ishii, T. Arato, S. Yanagihara, Y. Yagi, A. Koga, Y. Tsuda, M. Kimura, M. Sakita, S. Kitamura, H. Yamaguchi, H. Mimura, Y. Murata, Y. Hamazume, T. Sato, S. Natsuka, K. Kakehi, M. Kinoshita, S. Watanabe, T. Yamaguchi, Biologicals 39 (2011) 171–180.
[8] E. Muntean, Chromatographia 71 (2010) S69–S74.
[9] D.W. Togami, B.J. Poulsen, C.W. Batalao, W.A. Rolls, Biotechniques 10 (1991) 650–655.
[10] R.G. Bell, K.L. Newman, J. Chromatogr. 632 (1993) 87–90.
[11] P.B. Filson, B.E. Dawson-Andoh, Bioresour. Technol. 100 (2009) 6661–6664.
[12] L. La Pera, G. Di Bella, R. Magnisi, V. Lo Turco, G. Dugo, Ital. J. Food Sci. 19 (2007) 319–328.
[13] R. Slimestad, I.M. Vagen, J. Chromatogr. A 1118 (2006) 281–284.
[14] D. Kotnik, A. Smidovnik, P. Jazbec-Krizman, M. Krizman, M. Prosek, J. Chromatogr. B 879 (2011) 3700–3706.
[15] A.P. Sullivan, N. Frank, D.M. Kenski, J.L. Collett, J. Geophys. Res.-Atmos. 116 (2011).
[16] A. Steinbach, A. Wille, Lc Gc Eur. (2008) 15–16.
[17] T.R.I. Cataldi, M. Angelotti, G. Bianco, Anal. Chim. Acta 485 (2003) 43–49.
[18] I.G. Casella, M. Contursi, Anal. Bioanal. Chem. 376 (2003) 673–679.
[19] M. Vidotti, C.D. Cerri, R.F. Carvalhal, J.C. Dias, R.K. Mendes, S.I.C. de Torresi, L. T. Kubota, J. Electroanal. Chem. 636 (2009) 18–23.
[20] F. Chekin, S. Bagheri, A. Arof, S.B. Abd Hamid, J. Solid State Electrochem. 16 (2012) 3245–3251.
[21] R. Ojani, J.B. Raoof, S. Fathi, Electroanalysis 20 (2008) 1825–1830.
[22] A. Arvinte, A.M. Sesay, V. Virtanen, Talanta 84 (2011) 180–186.
[23] S. Buratti, B. Brunetti, S. Mannino, Talanta 76 (2008) 454–457.
[24] F. D'Eramo, J.M. Marioli, A.H. Arevalo, L.E. Sereno, Talanta 61 (2003) 341–352.
[25] L. Hua, L.S. Chia, N.K. Goh, S.N. Tan, Electroanalysis 12 (2000) 287–291.
[26] H.C. Chen, Y.H. Chen, S.L. Chen, Y.T. Chern, R.Y. Tsai, M.Y. Hua, Biosens. Bioelectron. 46 (2013) 84–90.
[27] K. Radhapyari, P. Kotoky, M.R. Das, R. Khan, Talanta 111 (2013) 47–53.
[28] T.Y. Zhang, X.Q. Li, S.Z. Kang, L.X. Qin, W.F. Yan, J. Mu, J. Colloid Interf. Sci. 402 (2013) 279–283.
[29] X. Chen, Y.C. Dai, Z.B. Zheng, K.Z. Wang, J. Colloid Interf. Sci. 402 (2013) 107–113.
[30] X.Z. Shi, H. Gong, Y.J. Li, C. Wang, L. Cheng, Z. Liu, Biomaterials 34 (2013) 4786–4793.
[31] S.G. Ge, M. Yan, J.J. Lu, M. Zhang, F. Yu, J.H. Yu, X.R. Song, S.L. Yu, Biosens. Bioelectron. 31 (2012) 49–54.
[32] X.L. Sun, J. Ji, D.L. Jiang, X.W. Li, Y.Z. Zhang, Z.J. Li, Y.N. Wu, Biosens. Bioelectron. 44 (2013) 122–126.
[33] W.J. Lian, S. Liu, J.H. Yu, J. Li, M. Cui, W. Xu, J.D. Huang, Biosens. Bioelectron. 44 (2013) 70–76.
[34] R.Y. Li, Q.F. Xia, Z.J. Li, X.L. Sun, J.K. Liu, Biosens. Bioelectron. 44 (2013) 235–240.
[35] L.Q. Luo, L.M. Zhu, Z.X. Wang, Bioelectrochemistry 88 (2012) 156–163.
[36] Q.W. Chen, L.Y. Zhang, G. Chen, Anal. Chem. 84 (2012) 171–178.
[37] N. Kocak, M. Sahin, S. Kucukkolbasi, Z.O. Erdogan, Int. J. Biol. Macromol. 51 (2012) 1159–1166.
[38] L. Carson, K. Hibbert, F. Akindoju, C. Johnson, M. Stewart, C. Kelly-Brown, G. Beharie, T. Fisher, J. Stone, D. Stoddart, A. Oki, G.M. Neelgund, G. Regisford, P. Traisawatwong, J.R. Zhou, Z.P. Luo, Spectrochim. Acta A 96 (2012) 380–386.
[39] F. Chekin, J.B. Raoof, S. Bagheri, S.B.Abd Hamid, Anal. Methods-Uk 4 (2012) 2977–2981.
[40] W. Sun, X.Z. Wang, X.H. Sun, Y. Deng, J. Liu, B.X. Lei, Z.F. Sun, Biosens. Bioelectron. 44 (2013) 146–151.
[41] M.K. Patel, M.A. Ali, M. Zafaryab, V.V. Agrawal, M.M.A. Rizvi, Z.A. Ansari, S.G. Ansari, B.D. Malhotra, Biosens. Bioelectron. 45 (2013) 181–188.
[42] Y. Pan, Y.Z. Zhang, Y. Li, J. Appl. Polym. Sci. 128 (2013) 647–652.
[43] X.D. Shang, Q. Tan, R.N. Liu, K.Y. Yu, P.Z. Li, G.P. Zhao, Int. J. Med. Mushrooms 15 (2013) 165–174.
[44] M.J. Wu, D.Z. Jiang, T.M. Liu, L.P. Zhang, Chem. Res. Chin. Univ. 22 (2006) 708–711.
[45] Y.H. Shon, K.S. Nam, Biotechnol. Lett. 24 (2002) 1005–1010.
[46] L. Chen, J.Z. Pan, X. Li, Y. Zhou, Q.L. Meng, Q. Wang, Int. Immunopharmacol. 11 (2011) 255–259.
[47] Y. Yang, L.B. Ye, J.S. Zhang, Y.F. Liu, Q.J. Tang, Biosci. Biotechnol. Biochem. 73 (2009) 134–139.
[48] Y. Yang, J.S. Zhang, Y.F. Liu, Q.J. Tang, Z.G. Zhao, W.S. Xia, Carbohydr. Res. 342 (2007) 1063–1070.
[49] H.C. Schniepp, J.L. Li, M.J. McAllister, H. Sai, M. Herrera-Alonso, D.H. Adamson, R.K. Prud'homme, R. Car, D.A. Saville, I.A. Aksay, J. Phys. Chem. B 110 (2006) 8535–8539.
[50] M.J. McAllister, J.L. Li, D.H. Adamson, H.C. Schniepp, A.A. Abdala, J. Liu, M. Herrera-Alonso, D.L. Milius, R. Car, R.K. Prud'homme, I.A. Aksay, Chem. Mater. 19 (2007) 4396–4404.