Capillary Coated with Graphene Oxide as Stationary Phase for the Separation of Brucine and Strychnine by Capillary Electrophoresis

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Received 5 September 2013; revised 29 March 2014

A new capillary electrophoresis (CE) method was developed by using graphene oxide (GO) as a stationary phase for the separation of brucine and strychnine. The separation performance, reproducibility and stability of GO-coated capillary were investigated for the analysis of brucine and strychnine. After optimization of the separation conditions, a phosphate solution (40 mM, pH 7.0) containing 25% (v/v) acetonitrile was selected as the running buffer. Compared with uncoated capillary, higher separation efficiency was achieved by GO-coated capillary as a result of the increasing interactions between the analytes and the stationary phase of capillary. The linear ranges of these two alkaloids were 4.0–100.0 μg mL⁻¹ with a satisfied correlation coefficients (R > 0.9994), and this novel method provided an efficient separation of brucine and strychnine as well as a good reproducibility and stability. Finally, the developed method was successfully applied for the determination of these two alkaloids in a pharmaceutical formulation of traditional Chinese medicines.

Introduction

Brucine and strychnine are active ingredients in some traditional Chinese medicines (TCMs), such as strychnos plants, and their chemical structures are showed in Figure 1. They are usually used as anti-inflammatory drugs for central nervous system diseases, arthritic pain and traumatic pain (1). These two alkaloids are highly toxic compounds, and unintentional ingestion of plants containing them or abuse of these alkaloids drugs is dangerous. Therefore, the analysis of brucine and strychnine in strychnos plants or TCMs containing these alkaloids is essential to estimate the medicinal value.

Recently, some analytical methods have been reported for the analysis of brucine and strychnine, such as thin-layer chromatography (TLC) (2, 3), gas chromatography (GC) (4), high-performance liquid chromatography (HPLC) (5–7), ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS) (8) and HPLC-MS (9–11). The performance of these methods showed a great promise for the determination of brucine and strychnine in TCMs and biological samples. Furthermore, capillary electrophoresis (CE) as one of the practical tools for the separation and analysis of ingredients in herbal medicine was widely used because of its high separation efficiency, short analysis time, small sample requirement and low operation cost (11). Recently, some CE methods were proposed for the analysis of brucine and strychnine (12–26).

With the development of nanoscience, nanomaterials were used for the modification of capillary in a result of the improvement of separation efficiency and sensitivity for CE method. Recently, graphene and graphene-based materials were used in the field of CE analysis (27), including open-tubular capillary electrophromatography (CEC) (28–34), monolithic column (35) or chip-based open-tubular CEC (36). The development of high-resolution method about open tubular CEC with carbon nanotubes had been used for the determination of low concentration strychnine (37).

In this work, capillary coated by graphene oxide (GO) was used as a stationary phase for the separation of brucine and strychnine. Compared with the uncoated capillary, a higher resolution was obtained for these two alkaloids when GO-coated capillary was used under the same CE conditions. The chromatographic performance of GO-coated capillary was evaluated, and this novel capillary was successfully applied for the determination of brucine and strychnine in Shufengdington pills (SFDTP).

Experimental

Reagents and materials

Graphene oxide (GO) was purchased from Xianfeng Nano-materials Technology Co., Ltd (Nanjing, China). HPLC-grade methanol and acetonitrile (ACN) were obtained from Merck (Darmstadt, Germany). Sodium hydroxide, 1, 3-dicyclohexylcarbodiimide, brucine, strychnine and 3-aminopropyltriethoxysilane (APTES) were supplied by Sigma-Aldrich (Shanghai, China). Glutaraldehyde, acetone, N,N-dimethylformamide (DMF), disodium tetraborate decahydrate, sodium dihydrogen phosphate, disodium hydrogen phosphate, phosphoric acid and hydrochloric acid were obtained from Sinopharm Chemical Reagent Beijing Co. Ltd (Beijing, China). Shufengdington pills (SFDTP) were purchased from the local drugstore. Water (18.2 MΩ) was purified by a Milli-Q system (Millipore, MA, USA). All other chemicals and reagents were of analytical grade unless otherwise stated.

Apparatus

All CE experiments were performed on a Beckman P/ACE MDQ CE system (Beckman Coulter, CA, USA) equipped with a photo diode array (PDA) detector. The instrument control and data acquisition were carried out by using 32 Karat software (Version 8.0, Beckman Coulter, CA, USA). Uncoated fused silica capillary (375 μm o.d. × 75 μm i.d.) was supplied by Yongnian Optic Fiber Plant (Hebei, China). The characterization of the surface morphology of GO-coated capillary was performed by Raman spectrometer (Renishaw, Gloucestershire, UK) and S4800 field-emission scanning electron microscopy (SEM, Hitachi, Tokyo, Japan).
Preparation of the GO-coated capillary

According to the developed strategy of GO-coated capillary in our previous reports (30, 31), the GO was grafted onto the APTES-treated capillary with the effect of covalent bonding. There were four main steps in the fabrication procedures. (i) A new uncoated capillary was rinsed with NaOH solution (0.1 M) for 30 min and water for 5 min, respectively. (ii) The capillary was rinsed about 15 min by using APTES solution (2% v/v) prepared in anhydrous acetone for the introduction of an amino group, and then followed by flushing with water (5 min) and methanol (5 min) to eliminate the excess of APTES. (iii) The APTES treated capillary was rinsed for 1 h with a glutaraldehyde (10% v/v) dissolved in 50 mM borate buffer at pH 9.0. (iv) In order to introduce the GO onto the inner-wall of capillary, GO solution (5 mg mL\(^{-1}\)) dissolved in a DMF containing 0.5 mg of 1,3-dicyclohexylcarbodiimide was used for the flushing through the capillary for 1 h. After the coating procedure, the capillary was rinsed with water to remove the unimmobilized GO. The GO-coated capillary was kept at least 1 day before CE analysis.

CE conditions

The optimization of buffer conditions and the separation of brucine and strychnine were performed in a 49.0 cm total length (effective length 38.0 cm) GO-coated capillary. The optimal buffer was 40 mM phosphate buffer solution (PBS, pH 7.0) containing 25% (v/v) ACN. The samples were injected under a pressure of 0.5 psi for 5 s, and the applied voltage was at a constant voltage of 16 kV. The detection wavelength was set at 214 nm, and the capillary temperature was maintained at 25°C. Before the first use, the uncoated capillary was sequentially conditioned with methanol (10 min), water (5 min), 0.1 M NaOH (30 min), water (5 min) and running buffer (2 min). However, when the separation was carried out with the GO-coated capillary prior to injection, the GO-coated capillary was sequentially rinsed with water and running buffer for 2 min. Between two runs, the capillary was also rinsed by water and running buffer for 2 min.

Preparation of solutions

The stock standard solutions of brucine and strychnine were prepared individually in methanol/water (1:1, v/v) at a concentration of 1.0 mg mL\(^{-1}\). A series of mixed working solutions were prepared by diluting the stock solution with a mixed solution of methanol and water (1:1, v/v) to the appropriate concentration. The stock solutions and working solutions were all stored at 4°C. The pH value of the phosphate solution was adjusted to the range of 4.0–9.0 by using sodium dihydrogen phosphate, disodium hydrogen phosphate and phosphoric acid solutions.

The crude drug of SFDTP was finely ground to powder, and then about 0.2 g pulverized SFDTP was weighed accurately and extracted with 2.0 mL hydrochloric acid solution (20 mM) in an ultrasonic bath for 30 min. Subsequently, the solution was centrifuged at 10,000 rpm for 10 min, and the resulting supernatant was filtered by a 0.22 μm pore size filter (Tianjin, China). The extract was stored at 4°C.

Results

The analytical characteristics of the developed method for the analytes were investigated. A series of the standard solutions of brucine and strychnine in the range of 4.0–100.0 μg mL\(^{-1}\) were estimated for the linearity. The results are listed in Table I, and a good linear relationship with correlation coefficients (\(R > 0.9994\)) were obtained for these two alkaloids. The limits of detection (LOD, \(S/N = 3\)) and recovery are also listed in Table I.

The precisions of intra-day, inter-day and capillary-to-capillary were investigated for the GO-coated capillaries. The reproducibility of migration time and peak areas under optimal separation conditions for the intra-day precision and inter-day precision were obtained by the continuous six injections in a day and two injections for 3 days, respectively. As shown in Table II, the RSDs for brucine and strychnine of intra-day precision and inter-day precision by migration time and peak area were below 5.05 and 7.67%, respectively. Furthermore, the RSDs (\(n = 6\)) of capillary-to-capillary were listed in Table II, and
these results indicated that the developed method has potential application for the analysis of brucine and strychnine in real samples. In this experiment, the reproducibility of GO-coated capillary was investigated for the separation of brucine and strychnine under optimal separation conditions by the continuous injections for at least 20 days, and the RSDs based on migration time and peak areas were 5.54 and 8.48% for strychnine (n = 20), and 6.65 and 8.91% for brucine (n = 20), respectively. These results demonstrated that the GO-coated capillary possessed a good stability for the separation of brucine and strychnine within at least 20 days.

The developed method was applied for the determination of brucine and strychnine in SFDTP. A typical electropherogram of the extract of SFDTP is shown in Figure 2, and alkaloids were identified by spiking single analyte into the extract solution. The contents of brucine and strychnine in SFDTP are 0.16 and 0.29 mg g⁻¹, respectively. In this study, the reproducibility were also investigated for the extract of SFDTP with the GO-coated capillary, and the intra-day precision, inter-day precision as well as three GO-coated capillary precision of brucine and strychnine by migration time and peak area are listed in Table II. These results indicated that this proposed method could be a potential strategy for the evaluation of TCMs containing brucine and strychnine.

**Discussion**

**Characterization of GO-coated capillary**

In this work, the GO-coated capillary was evaluated by SEM, EDX and Raman spectra based on our previous works (30, 31). According to the characterization of SEM as well as EDX images, it can be seen clearly that a great amount of wrinkle GO were observed on the inner-surface of capillary. Moreover, compared with the EDX image of uncoated capillary, the peak of carbon element was found in the image of GO-coated capillary, but the relative peak was not detected from the uncoated capillary, and the results are listed in Table III. In the Raman spectra of GO-coated capillary, the characteristic peaks of GO could be detected in the Raman spectra of GO-coated capillary. According to these morphological characterization of GO-coated capillary, GO was combined onto the inner-surface of capillary.

**Optimization of CE conditions**

**Effect of buffer pH and organic additives on the separation of brucine and strychnine**

As shown in Figure 1, their chemical properties of brucine and strychnine are very similar, and the pKa value of brucine was 8.28, and pKa 8.26 was for strychnine (13). In order to obtain the separation of brucine and strychnine, buffer pH was considered as one of the important parameter to be investigated, and the effect of buffer pH on the resolution of brucine and strychnine was also investigated in the range of pH 4.0–8.0. Meanwhile, in order to improve the resolution of those two alkaloids, ACN was selected to reduce the peak tailing. Different percentages of ACN in buffer from 5 to 30% were investigated for the separation. The optimization of buffer pH and ACN concentration in the buffer was performed by using Matlab software. Although a high resolution was obtained when the acidic buffer (pH4.0) was used, the tailing peaks of brucine and strychnine were obtained. When the pH value was increased above pH7.0, the migration time was decreased and the peak shape of those two alkaloids was improved with the increasing of buffer pH. Furthermore, in order to obtain a good separation of those two alkaloids, ACN was used. A satisfied separation was obtained when PBS with pH7.0 [containing 25% (v/v) ACN] was used.

**Effect of applied voltage on the separation of brucine and strychnine**

The applied voltage is an important parameter for the resolution of analytes, which has a large effect on the migration time, current strength as well as EOF in CE. In order to obtain a good separation of brucine and strychnine, the applied voltage was investigated in the range of 12–18 kV. The migration time of two alkaloids were decreased when the applied voltage was increased. Considering the resolution and the migration time of brucine and strychnine, a voltage of 16 kV was selected for the separation of two alkaloids in CE analysis.

**Effect of temperature on the separation of brucine and strychnine**

The influence of temperature for the new stationary phase and the separation of brucine and strychnine were investigated in

**Table III**

<table>
<thead>
<tr>
<th>Element Uncoated capillary</th>
<th>GO-coated capillary</th>
</tr>
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<tbody>
<tr>
<td>C</td>
<td>0</td>
</tr>
<tr>
<td>Si</td>
<td>63.88</td>
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</table>

Figure 2. Typical electropherograms of brucine and strychnine in SFDTP by CE with GO-coated capillary. CE conditions: a GO-coated capillary with 38.0 cm effective length was used as the separation column. Running buffer was 40 mM PBS (pH 7.0) containing 25% (v/v) ACN. Sample was injected under a pressure of 0.5 psi for 5 s. The separation voltage was 16 kV, and the temperature was set at 25°C. The detection wavelength was set at 214 nm. 1: strychnine; 2: brucine.
the range of 15–45°C. Because the viscosity of buffer was affected by the different temperature, the migration time and resolution of two alkaloids were decreased with the temperature increasing; while the RSDs for brucine and strychnine by peak area at different temperature were below 7.15% and 6.13%, which demonstrated that the GO-coated capillary had a good stability. Considering about a good resolution and the migration time of brucine and strychnine, 25°C (ambient temperature) was selected for the separation of two alkaloids in CE analysis.

**Separation of brucine and strychnine using GO-coated capillary**

Brucine and strychnine were separated under the optimal CE conditions by using an uncoated capillary (as shown in Figure 3a) and GO-coated capillary (Figure 3b). A better resolution of brucine and strychnine was obtained with the GO-coated capillary; however, these two alkaloids were not separated from each other under the same CE conditions when the uncoated capillary was used. It may be caused by the GO on the inner face of coated capillary, which has unique characteristics. The specific properties of GO make it as a promising candidate for stationary phase to provide more sites for the desired interactions between GO and analytes, including electrostatic interaction, π–π stacking and hydrogen bonding.

**Conclusion**

In conclusion, a novel stable GO-coated capillary was prepared by a simple coating procedure with covalent bonding, and applied for the separation of brucine and strychnine in SFDTTP. The linearity, precisions and LODs were evaluated for the established method under the desired conditions with good results, which indicated that the developed method represents a useful application for the separation of brucine and strychnine in TCMs and other pharmaceutical formulations containing these alkaloids.

**Funding**

This work was supported by the Beijing Natural Science Foundation (grant number 2133061), the National Natural Science Foundation of China (grant number 21005050) and the Funding Project for Academic Human Resources Development in Institutions of Higher Learning under the Jurisdiction of Beijing Municipality (grant number PHR201108147).

**Conflict of interest statement.** The authors have declared no conflict of interest.

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