Sensitive determination of heroin based on electrogenerated chemiluminescence of tris(2,2′-bipyridyl)ruthenium(II) immobilized in zeolite Y modified carbon paste electrode

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A novel method for rapid, inexpensive, sensitive and selective determination of heroin was proposed by flow injection electrogenerated chemiluminescence (ECL). Zeolite Y sieves were used for the preparation of an ECL sensor by immobilizing tris(2,2′-bipyridyl)ruthenium(II) (Ru(bpy)) in their supercages, which was achieved through the ion exchange properties of the sieves. The electrochemical and ECL behaviors of Ru(bpy) immobilized in zeolite Y modified carbon paste electrode was investigated. The immobilized Ru(bpy) displayed a pair of surface-controlled redox peaks with an electron transfer rate constant of 1.2 ± 0.1 s⁻¹ in 0.1 mol dm⁻³ pH 6.3 phosphate buffer. The modified electrode showed an electrocatalytic response to the oxidation of heroin, producing a sensitized ECL signal. The ECL sensor showed a linear response to flow injection of heroin in the range of 2.0–80 μmol dm⁻³ with a detection limit of 1.1 μmol dm⁻³. This method for heroin determination possessed good sensitivity and reproducibility with a coefficient of variation of 1.99% (n = 15) at 50.0 μmol dm⁻³. The ECL sensor showed good selectivity and long-term stability. Its surface could be renewed quickly and reproducibly by a simple polish step.

Introduction

Drug abuse has become a serious and growing social problem. The determination of heroin is of great importance for fighting its abuse and investigating the metabolism and toxicology of heroin in the body. Various methods for the analysis of heroin have been described, which mainly encompassed chromatographic techniques such as gas chromatography with flame ionization detection, gas chromatography-mass spectrometry (GC-MS), reversed-phase HPLC using UV detection and capillary electrophoresis. Although the capillary electrophoresis separation can detect heroin with a high sensitivity, it is necessary to develop a rapid, convenient and inexpensive method for sensitive and selective determination of heroin. Electrogenerated chemiluminescence (ECL), also known as electrochemiluminescence and a production of light from electrochemically generated reagents, has become an important and sensitive detection method in recent years. It usually uses tris(2,2′-bipyridyl)ruthenium(II) (Ru(bpy)) as the ECL compound because of its stability, regenerability, excellent luminescence properties and compatibility with a wide range of analyses. Previous studies have shown Ru(bpy) can react with a range of tertiary amino groups such as trialkylamines, pyrrolidines and piperidines to produce electrogenerated chemiluminescence for their detections. This method has been extended to the determination of codeine and related alkaloids, including heroin. However, these methods need to add continuously Ru(bpy) to the flow stream to produce the ECL response of amines. Thus, their applications are limited due to the consumption of a large amount of the ruthenium complex. The electrochemical regeneration and recycled utilization of Ru(bpy) can be achieved by immobilizing it on an electrode surface.

The immobilization of Ru(bpy) on an electrode surface has offered a number of advantages. When Ru(bpy) is immobilized permanently or at least for a prolonged time without significant loss of activity, the ECL analysis can be designed without the need of Ru(bpy) delivery to the electrode. This not only cuts the consumption of Ru(bpy), but also allows simpler instrumentation. Additional tubing, mixing chamber and pump to deliver the reagent to the electrochemical cell may also be removed compared to other systems using Ru(bpy) in solution. So far various methods have been carried out for the immobilization of Ru(bpy) to develop a cost-effective, regenerable chemical sensor. Ru(bpy) can be immobilized onto the electrode surface as a monolayer or by forming a self-assembled monolayer with the derivatives of Ru(bpy). However, these films are unstable because of the highly applied potentials needed. The incorporation of Ru(bpy) within the cation exchange polymer such as Nafion by the electrostatic attachment is another popular method. Nafion film has drawbacks because of the slow mass transfer through the film and the partition of Ru(bpy) into the more hydrophobic regions of Nafion, though these properties have been improved by incorporation of silica nanocomposites or sol–gel-derived titania into Nafion. The modified films exhibit faster response and higher sensitivity for ECL detection. However, the long-term stability is still limited by the high hydrophobicity of Nafion.

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Although the immobilization of Ru(bpy)$_3^{2+}$ in chitosan shows an improved selectivity, its stability is also not improved. The immobilization of Ru(bpy)$_3^{2+}$ in Eastman-AQ55-silica composite thin-films, silica sol–gels, and the combination of sol–gels and polyHEMA membranes for preparation of electrochemiluminescence sensors for oxalate, tripropylamine and chlorpromazine have been reported. These sensors show good sensitivity and stability. This work used zeolite Y as a matrix to immobilize Ru(bpy)$_3^{2+}$ for ECL detection.

Zeolite Y, one of the zeolites with the general formulation (C$^{m+}$)$_x$[(AlO$_2$)$_y$(SiO$_2$)$_z$]$_m$(H$_2$O) which are widely used adsorbents, ion exchangers, or catalysts, is a hydrated crystalline aluminosilicate mineral, natural or synthetic. Based on ion-exchange properties of zeolite Y, Ru(bpy)$_3^{2+}$ have been entrapped in the supercages of zeolite Y to study the oxidizing properties of immobilized Ru(bpy)$_3^{2+}$. To the best of our knowledge, however, the ECL behavior and application of immobilized Ru(bpy)$_3^{2+}$ in zeolites have not been reported yet. This work immobilized Ru(bpy)$_3^{2+}$ in the supercages of zeolite Y by ion exchange. Combining the advantages of carbon paste electrode (CPE) a novel ECL sensor as well as a new method for heroin detection was developed. The sensor showed good reproducibility, selectivity and long-term stability for flow injection analysis. The surface of the sensor could be renewed by simple polishing. This method could be used for rapid and sensitive ECL quantification of heroin. These results indicated that zeolite Y was a promising material in electrogenerated chemiluminescence sensing applications.

**Experimental**

**Chemicals and reagents**

Sodium zeolite Y was obtained from Nanjing inorganic chemistry factory (China). This powdered zeolite had an average particle size less than 300 mesh. Tris(2,2’-bipyridyl) ruthenium(II) chloride hexahydrate was obtained from Aldrich and used without further purification. Heroin was obtained from the State Narcotic Laboratory (Beijing, China). Carbon graphite powder (<325 mesh, Johnson Matthey) and paraffin oil were from Fluke. All other reagents were of analytical reagent grade. Solutions were prepared with twice-distilled water.

**Ion exchange**

The ion-exchanged zeolite Y particles were prepared by stirring a suspension of 0.5 g of Na–Y in 10 ml of water containing approximate 0.1 mol dm$^{-3}$ Ru(bpy)$_3^{2+}$ for 24 h. The obtained mixture was filtered, washed several times with distilled water, and air-dried at 40 °C to produce Ru(bpy)$_3^{2+}$-zeolite Y particles. The concentration of Ru(bpy)$_3^{2+}$ in the zeolite was determined to be (2–3) × 10$^{-6}$ mol g$^{-1}$ of zeolite by comparing UV-visible spectra of the aqueous solutions before and after the exchange. This value was consistent with that reported previously, indicating the Ru(bpy)$_3^{2+}$ molecules were exchanged into the supercage of zeolite Y.

**Sensor preparation**

The electrodes were prepared as described previously. Prior to use, the graphite powder was treated at 700 °C for 30 s in a muffle furnace and then cooled to room temperature in a desiccator in the presence of activated silica gel. 100 mg Ru(bpy)$_3^{2+}$-zeolite Y particles were mixed with 200 mg carbon graphite. 70 mg of the mixture and 25.2 µl paraffin oil were then mulled together by hand-mixing to obtain a uniformly wetted paste. The zeolite Y-CPE or ordinary CPE used for comparison was prepared in the same way by using zeolite Y instead of Ru(bpy)$_3^{2+}$-zeolite Y or omitting the Ru(bpy)$_3^{2+}$-zeolite Y addition step. A portion of the modified carbon paste was placed into the end of a Teflon tube (3 mm id). Electrical contact to the paste was established by inserting a copper wire down the tube and into the back of the mixture. Prior to use the electrode tip was gently rubbed on a fine piece of paper to produce a flat surface.

**Apparatus**

Electrochemical experiments were performed on CHI 812 (Shanghai, China) with a three-electrode system. The CPE or modified CPE, platinum electrode, and Ag/AgCl electrode with saturation KCl solution were used as working, counter and reference electrodes. All potentials were measured and reported with respect to Ag/AgCl reference electrode. The ECL emission was recorded with CHI 812 (Shanghai, China) and Luminescence Analyzer (IFFM-D, Remex Electronic Instrument Limited Co., Xi’an, China) at room temperature. They were controlled by a personal computer. Fig. 1 shows a schematic diagram of the flow injection analysis apparatus used in this study. Two pumps of Luminescence Analyzer (IFFM-D) were used to deliver flow streams. Polytetrafluoroethylene (PTFE) tubing (0.8 mm id) was used to connect all components in the flow system. Sample solutions were injected into the carrier stream (water) through a six-way injection valve. The flow rate was optimized at 1.20 ml min$^{-1}$. The flow-through electrolytic cell was placed directly in front of the photomultiplier tube (PMT) window. Unless noted, the PMT was biased at 800 V. As shown in Fig. 2, the flow cell was assembled with a Ru(bpy)$_3^{2+}$ modified electrode (3 mm) placed against a transparent Plexiglas window. A Teflon spacer was inserted between the electrode and the Plexiglas to create a volume of 50 µl flow.
cell. Visible-region diffuse-reflectance spectra were recorded using a UV-2401 PC spectrophotometer (Shimadzu, Japan). The ECL spectrum was obtained with series interference filters by the static method. The filters were inserted between the sample cuvette and the PMT.

**Results and discussion**

**Visible-region diffuse-reflectance spectra**

The visible-region diffuse-reflectance spectra of zeolite Y and Ru(bpy)$_3^{2+}$-zeolite Y are shown in Fig. 3. The spectrum of zeolite Y did not show any absorption band, while Ru(bpy)$_3^{2+}$-zeolite Y exhibited an absorption band at 450 nm, which was the characteristic absorption of orange Ru(bpy)$_3^{2+}$ complexes, and thus demonstrating that Ru(bpy)$_3^{2+}$ had been immobilized in zeolite Y sieves. The diameter of Ru(bpy)$_3^{2+}$ was about 1.08 nm, which was small enough to fit the 1.3 nm internal diameter of zeolite supercage for stable immobilization of Ru(bpy)$_3^{2+}$ in the large cavities of zeolite Y through ion exchange.

**Electrochemistry of Ru(bpy)$_3^{2+}$-zeolite Y-CPE**

Fig. 4 shows the cyclic voltammograms of CPE, zeolite Y-CPE and Ru(bpy)$_3^{2+}$-zeolite Y-CPE in 0.1 M pH 6.3 phosphate buffer at 100 mV s$^{-1}$. The zeolite Y-CPE showed a larger background current than CPE, while no peak was observed at either CPE or zeolite Y-CPE. Ru(bpy)$_3^{2+}$-zeolite Y-CPE exhibited a couple of redox peaks at $+1.047$ V and $+0.985$ V and a smaller background current than zeolite Y-CPE. Obviously, the response was attributed to the oxidation and reduction of the electroactive center of Ru(bpy)$_3^{2+}$ immobilized on an electrode surface. With an increasing scan rate in the range from 50 to 400 mV s$^{-1}$ both the oxidation and reduction currents increased linearly, their peak potentials shifted in positive and negative directions, respectively (Fig. 5), and the peak-to-peak separation increased, indicating a surface-controlled quasi-reversible electrode process. From the peak-to-peak separations of cyclic voltammograms of the immobilized Ru(bpy)$_3^{2+}$ at different scan rates, an average electron transfer rate constant of 1.2$\pm$0.1 s$^{-1}$ was obtained using Laviron’s model and the electron transfer number of 1 with an electron transfer coefficient, $x$, of 0.77 that was obtained from the slope of plot $E_{pc}$ vs. $\ln v$. The average surface concentration of Ru(bpy)$_3^{2+}$ was calculated from the peak areas of cyclic voltammograms and the real area of the CPE to be $(8.6\pm1.5) \times 10^{-10}$ mol cm$^{-2}$.
The real area was determined by the slope of plot of the anodic peak current of 1.0 mmol dm$^{-3}$ K$_3[Fe(CN)_6]$ in 0.1 mol dm$^{-3}$ KCl $\text{vs.}$ the square root of scan rate to be $6.6 \times 10^{-2}$ cm$^2$. The value of coverage obtained was much larger than the theoretical value of $1.81 \times 10^{-10}$ mol cm$^{-2}$ calculated from the 1.08 nm diameter of Ru(bpy)$_3^{2+}$ for monolayer coverage. Thus, the Ru(bpy)$_3^{2+}$ molecules were immobilized in the supercages without the surface of zeolite Y. The porous structure resulted in a high coverage. The inhabitancy of Ru(bpy)$_3^{2+}$ molecules in part of the pores of zeolite Y resulted in a smaller background current of Ru(bpy)$_3^{2+}$-zeolite Y-CPE than zeolite Y-CPE.

**Electrocatalytic response of immobilized Ru(bpy)$_3^{2+}$ to oxidation of heroin**

Heroin is one typical compound of tertiary amino groups that can be oxidized with one electron transfer at an enough positive potential.$^{11,42}$ The cyclic voltammogram of heroin at zeolite Y-CPE in 0.1 mol dm$^{-3}$ pH 6.3 phosphate buffer is shown in Fig. 6. One oxidation peak of heroin could be observed at +0.891 V at 100 mV s$^{-1}$. The oxidation peak potential was more negative than +1.047 V of the immobilized Ru(bpy)$_3^{2+}$. Thus, it was possible thermodynamically that the oxidation state of Ru(bpy)$_3^{2+}$ oxidized heroin to produce an electrocatalytic response. Fig. 7A shows the change of electrochemical response of Ru(bpy)$_3^{2+}$-zeolite Y-CPE upon addition of 40.0 μmol dm$^{-3}$ heroin into the pH 6.3 phosphate buffer. The anodic peak current increased by 19.2 μA, while the cathodic peak current decreased. The increased peak current value was much larger than 3.6 μA of the peak current of heroin at zeolite Y-CPE at the same concentration, showing an obviously electrocatalytic action of the immobilized Ru(bpy)$_3^{2+}$ to the oxidation of heroin. The electrocatalytic process resulted in the formation of an emitting species to produce the ECL signal.

**Electrogenerated chemiluminescence**

Fig. 7B shows the ECL signal of Ru(bpy)$_3^{2+}$-zeolite Y-CPE in the presence of heroin upon the oxidation process. At potentials more negative than the oxidation peak potential of the immobilized Ru(bpy)$_3^{2+}$ no ECL signal was observed. The onset of luminescence occurred near +0.98 – +1.02 V. The ECL signal then arose steeply and reached the maximum at +1.16 V. These results were consistent with the oxidation of the immobilized Ru(bpy)$_3^{2+}$. With a decreasing scan rate from 400 to 50 mV s$^{-1}$ the ECL curve became wider and the peak intensity of ECL stayed relatively constant. ECL experiment
conducted with Ru(bpy)$_3^{2+}$-zeolite Y-CPE in phosphate buffer without the presence of heroin showed only a weak background ECL emission, while no ECL emission was observed at zeolite Y-CPE in heroin solution, indicating the ECL emission of the system presumably arose from the energetic electron-transfer reaction between electrogenerated Ru(bpy)$_3^{3+}$ and the reducing intermediate, the deprotonated form of oxidized heroin ion free radical, to produce the excited state Ru(bpy)$_3^{2+}$, an emitting species. The ECL spectrum of this system showed an emission peak at nearly 616 nm (Fig. 8), close to the emission peaks at 610 or 620 nm for Ru(bpy)$_3^{2+}$ dissolved in aqueous solution, and about 616 nm for Ru(bpy)$_3^{2+}$ in aqueous solution, indicating a similar emission process to that reported in the literature.

**Flow injection analysis of heroin**

Using flow injection technique and water as the carrier stream, samples containing heroin in 0.1 mol dm$^{-3}$ pH 6.3 phosphate buffer were detected at a constant potential of 1.2 V (versus Ag/AgCl). With a decreasing flow rate the ECL intensity increased, however, significant peak broadening was observed and a prolonged analysis time was needed at lower flow rates. Therefore, a value of 1.2 ml min$^{-1}$ was selected as a compromise between these two opposing factors. According to the electrochemical and ECL responses 0.1 mol dm$^{-3}$ phosphate buffer was suitable for preparation of sample solutions. At buffer concentrations greater than 0.2 mol dm$^{-3}$, the ECL signal gradually decreases and then diminished. The ECL response of 40.0 µmol dm$^{-3}$ heroin showed a dependence on the pH value of 0.1 mol dm$^{-3}$ phosphate buffer ranging from 3 to 8. With an increasing of pH value the ECL emission of heroin increased, suggesting that the deprotonation of oxidized heroin was required for efficient ECL. When the pH value was more than 6.5 the emission decreased. Thus, pH 6.3 phosphate buffer was used for all experiments.

The ECL intensity increased linearly with the increasing heroin concentration over the range from 2.0 to 80.0 µmol dm$^{-3}$. The linear regression equation was $I_{ECL} = 0.0256 + 0.258c$ (c: µmol dm$^{-3}$) with a correlation coefficient of 0.9973. The detection limit was 1.10 µmol dm$^{-3}$ at a signal-to-noise ratio of 3. Although this sensitivity was lower than those of 45 nmol dm$^{-3}$ based on continuous addition of Ru(bpy)$_3^{2+}$ to the flow stream to produce the ECL response and 27 nmol dm$^{-3}$ using capillary electrophoresis analysis, it corresponded to those of 1 µmol dm$^{-3}$ for oxalate and tripropylamine based on immobilized Ru(bpy)$_3^{2+}$ ECL sensors, and was better than those of 184 and 13 µmol dm$^{-3}$ for heroin by HPLC and 1.35 µmol dm$^{-3}$ for heroin by GC-MS. It was also enough for determination of heroin in seized heroin products for fighting its abuse, in which the contents of heroin were more than 20%.

When samples injections were made every 30 s, the ECL peak intensity showed good reproducibility (Fig. 9), with a coefficient of variation of 1.99% for 15 repetitive determinations at 50 µmol dm$^{-3}$ heroin. The injection interval was half of that for tripropylamine determination at Ru(bpy)$_3^{2+}$/Eastman-AQ55D-silica thin-film and quarter of that for oxalate determination at Ru(bpy)$_3^{2+}$/Nafion modified electrode, indicating that the sensor based on the immobilization of Ru(bpy)$_3^{2+}$ in zeolite Y particles had a fast ECL response.

**Selectivity and interference studies**

Heroin is known to be an abuse drug. The components of heroin products include heroin, monomethylcodeine, morphine derivatives and other substances such as metal ions. Some biomolecules such as urea, glucose, and amino acid also coexist with heroin in biological samples for the investigation of metabolism and toxicology of heroin in the body. Thus, it is of interest to find out the selectivity of the present ECL sensor for assessing its analytical applicability. The presence of organic solvents such as methyl alcohol, acetonitrile in phosphate buffer (30 : 70 v/v) did not affect the determination of heroin. Table 1 lists the relative ECL signals of other interferents to the signal of heroin at 1.0 mmol dm$^{-3}$. All compounds were tested for ECL emission in 0.1 mol dm$^{-3}$ phosphate buffer at pH 6.3. The results showed most compounds present in biological samples and papaverine, one main component of opium, did not affect the determination of heroin.
Table 1  Selectivities for various compounds

<table>
<thead>
<tr>
<th>Test compound</th>
<th>ECL intensity (1.0 mM)</th>
<th>Test compound</th>
<th>ECL intensity (1.0 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid</td>
<td>0</td>
<td>Papaverine</td>
<td>0</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>0</td>
<td>Glucose</td>
<td>0</td>
</tr>
<tr>
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<td>Oxalate</td>
<td>0.0115</td>
</tr>
<tr>
<td>Cystine</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Codeine</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ The ratio of the ECL intensity of 1.0 mM test compound to that of 1.0 mM heroin. $^b$ The ratio was obtained at the morphine and heroin concentrations of 20 μM.

Stability of Ru(bpy)$_3^{2+}$-zeolite Y-CPE

When the sensor was not in use, it was stored dryly at room temperature. The sensor could retain the initial ECL signal of 20.0 μmol dm$^{-3}$ heroin upon storage for 15 days. When the sensor was immersed in phosphate buffer for a potential scan between +0.6 and +1.4 V, no obvious decrease in the peak current was observed until 100 cycles. A longer scan showed a slight decrease in peak current. Thus, the electrochemical stability of Ru(bpy)$_3^{2+}$ immobilized in zeolite Y and the sensor was good. After the ECL or electrochemical response displayed an obvious decrease, the sensor surface could be renewed by a gently rubbing step on a fine paper. The ECL response of the renewed surface examined at a heroin concentration of 20.0 μM gave a relative standard deviation of 2.0% for six successive renewals. Over an examining period of six months the repetitively renewed surface did not show obvious differences in an ECL signal of 20.0 μmol dm$^{-3}$ heroin. Thus the method was rapid, easy, and more importantly, reproducible to renew the sensor surface.

Conclusions

Immobilization of Ru(bpy)$_3^{2+}$ in zeolite Y is successfully achieved for preparation of a novel ECL sensor. Both visible-region diffuse-reflectance spectroscopic and electrochemical studies indicate that Ru(bpy)$_3^{2+}$ molecules are immobilized in the large cavities (supercages) of zeolite Y through ion exchange. The immobilized Ru(bpy)$_3^{2+}$ shows a surface-controlled quasi-reversible electrode process and has an obviously electrocatalytic action to the oxidation of heroin, which results in the formation of an emitting species to produce ECL signal for sensitive determination of heroin using a flow injection ECL analysis system. The sensor displays an excellent selectivity for heroin over a wide range of potential interferences including oxygen, urea, iron cation and glucose. The method presented has a good reproducibility and sensitivity. It possesses a good selectivity and could be used for flow injection analysis of heroin products by combining with separation methods to eliminate the interference of codeine. The sensor shows good stability, fast response and ease of preparation, and can be regenerated by renewing the sensor surface with a simply and gently rubbing step on a fine paper. This immobilized method is promising in electrogenerated chemiluminescent sensing applications.

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