Flow injection chemiluminescence analysis for highly sensitive determination of noscapine

Yafeng Zhuang a, Xilan Cai a,b, Junsheng Yu a, Huangxian Ju a,b,∗

a Department of Chemistry, Institute of Analytical Science, State Key Laboratory of Coordination Chemistry, Nanjing University, Nanjing 210093, PR China
b Department of Technology, Jiangsu Police Officer College, Nanjing 210012, China

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Abstract

Noscapine is an antitussive drug and possesses potent antitumor activity. This work establishes a highly sensitive method for its determination by flow injection chemiluminescence (CL) technique. This method is based on its strong sensitizing effect on the weak CL reaction between sulfite and acidic permanganate. The mechanism for the sensitizing process is proposed on the basis of fluorescence and CL spectra. Under optimal experimental conditions, the CL response is proportional to the concentration of noscapine over the range of 2.0 × 10⁻⁸ to 2.0 × 10⁻⁶ mol/l with a correlation coefficient of 0.9998 and a detection limit of 8.0 × 10⁻⁹ mol/l (3σ/H9268). The relative standard deviation for 11 repetitive determinations of 5.0 × 10⁻⁷ mol/l noscapine is 1.2%. Most of metal ions and some alkaloids such as morphine, codeine and heroin do not interfere with the determination. The interference of coexisted papaverine in the opium can be eliminated by the dilution of sample solution. The method has been satisfactorily used for the determination of noscapine in synthesized samples.

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1. Introduction

Noscapine (narcotine, as shown in Scheme 1) is the second most abundant alkaloid in opium, present in concentrations of 2–8% [1] and is usually used as an antitussive drug. Unlike morphine and codeine, noscapine has no analgesic activity or abuse potential. Its major pharmaceutical action is its antitussive activity, which has been reported to be equivalent to that of codeine [2]. Recent studies indicate noscapine can cause apoptosis in many cell types and has potent antitumor activity against solid murine lymphoid tumors and human breast and bladder tumors implanted in nude mice [3,4]. These works suggest that the antitumor activity of noscapine might lie in its initiation of apoptotic pathways. Compared with other microtubule drugs, noscapine has low toxicity and wide efficacy in animal models [5]. Thus, the quantitative determination of noscapine can provide important information on its biological function.

The British Pharmacopoeia (BP) procedures [6,7] for the quantitative determination of noscapine in pharmaceutical preparations include an absorption method, which lacks specificity, and an acid-base titrimetric method in a non-aqueous medium with potentiometric end-point detection. Neither method is suitable for the determination of low levels of the alkaloid [8]. Other previously published analytical assays include high-performance liquid chromatography (HPLC) [9–13], gas chromatography [14] and spectrophotometry [8]. The spectrophotometric method developed by Suliman et al can determine noscapine down to 3.64 μmol/l (1.5 ppm) [8]. A linear range from 1.74 × 10⁻⁸ to 6.53 × 10⁻⁷ mol/l for noscapine determination has been obtained using solid-phase extraction and HPLC [11]. For the practical application, it is necessary to develop a more simple and sensitive method for noscapine determination.

Chemiluminescence (CL) is becoming a powerful analytical tool due to its high sensitivity, wide dynamic range and simple instrumentation [15]. It has been exploited with a wide range of applications in different fields, such as biotechnology, pharmacology, molecular biology, and environmental chemistry [16,17]. A variety of organic and inorganic compounds, such as bile acids [18], riboflavin [19], morphine [20–22] and ascorbic acid [23], have been determined by using KMnO₄ as a reagent to generate CL. Recently, a wide range of analytical applications of
K\textsubscript{2}MnO\textsubscript{4} in CL reactions has been reviewed [24]. The nature of those reactions has been postulated to involve the following excited state species: manganese(II) or a complex thereof [20,25–27], singlet oxygen [28,29], sulfur dioxide [18,30,31], molecular nitrogen [32,33], and fluorescent oxidation products of the analyte [34]. Although the excited state species of manganese(II) or a complex thereof in the presence of hexametaphosphate has been well characterized by Barnett et al. [27] in acidic potassium permanganate system, the CL spectrum of K\textsubscript{2}MnO\textsubscript{4}–Na\textsubscript{2}SO\textsubscript{3}–H\textsubscript{2}SO\textsubscript{4} system shows the emission of the exited sulfur dioxide between 450 and 600 nm as reported in [30]. This work studies the effect of noscapine on the CL intensity emitted from the reaction of sulfite with acidic KMnO\textsubscript{4}. A sensitizing effect of the noscapine on the CL emission is observed. Based on the sensitizing effect, a simple, rapid and sensitive flow injection CL method for noscapine detection is proposed. To our best knowledge, this is the first CL application to the determination of noscapine. It possesses a good accuracy and precision and has been used to determine noscapine in synthesized samples. In view of the facts that other alkaloids such as morphine, codeine and heroin do not interfere in the determination of noscapine and that the sensitizing effect of papaverine in a low level is unobservable, this method would be of superior selectivity for the noscapine detection. It has been reported that some illicit heroin samples contain a high content of noscapine (up to 61%) [35], therefore, this method could also be used for determination noscapine assay in these samples.

2. Experimental

2.1. Reagents

All reagents were of analytical grade. All solutions were prepared with deionized water of 18 M\textsubscript{Ω} purified from a Milli-Q purification system. Noscapine was obtained from the State Narcotic Laboratory, Beijing. K\textsubscript{2}MnO\textsubscript{4} (Jintan, China) was used as received. The stock solution of 3.0 × 10\textsuperscript{-3} mol/l KMnO\textsubscript{4} was prepared daily by diluting the stock solution of 0.01 mol/l KMnO\textsubscript{4} with 0.1 mol/l sulfuric acid. The solution of 0.01 mol/l sodium sulfite was prepared daily.

2.2. Apparatus

The FIA (flow injection analysis)-CL system is the same as that reported in [36]. Two pumps of Luminescence Analyzer (IFFM-D, Remex Electronic Instrument Limited Co., Xi’an, China) were used to deliver flow streams. Polytetrafluoroethylene (PTFE) tubing (0.8 mm i.d.) was used to connect all components in the flow system. The flow cell was a 10 cm length of spiral glass tubing (2.0 mm i.d.) and the distance between injection valve and flow cell was about 10 cm. Fluorescence spectra were recorded by a RF-5301 spectrofluorimeter (Shimadzu, Japan). The CL spectrum was obtained with a series interference filters by the static method. The filters were inserted between the sample cuvette and the photomultiplier tube (PMT).

2.3. Procedures

Noscapine and acidic K\textsubscript{2}MnO\textsubscript{4} solutions were mixed via a Y-shaped element and injected into the carrier stream (water) through a six-way injection valve. Sulfite solution was mixed with carrier stream via another Y-shaped element in front of the flow cell. The CL signal was detected by the photomultiplier tube (PMT) (CR-105, Hamamatsu Japan) placed near the flow cell and was recorded with a computer equipped with an A/D card. The wavelength of its max sensitivity of the PMT was 420 nm. The spectrum response range of the PMT was from 300 to 650 nm.

3. Results and discussion

3.1. Optimization of experimental variables

The typical CL signal of acidic KMnO\textsubscript{4} upon addition of 4.0 × 10\textsuperscript{-4} mol/l Na\textsubscript{2}SO\textsubscript{3} was shown in Fig. 1. The reaction

![Fig. 1. Typical CL signals of 3.0 × 10\textsuperscript{-3} mol/l KMnO\textsubscript{4} + 0.1 mol/l H\textsubscript{2}SO\textsubscript{4} upon addition of 4.0 × 10\textsuperscript{-4} mol/l Na\textsubscript{2}SO\textsubscript{3} in the absence (a) and presence (b) of 5.0 × 10\textsuperscript{-7} mol/l noscapine.](image-url)
between KMnO₄ and Na₂SO₃ produced a weak CL emission. When 5.0 × 10⁻⁷ mol/l noscapine was added in this system, the CL emission increased by 16.9 times. The significant increase indicated noscapine was a sensitive enhancer on the CL reaction of permanganate-sulfite. Furthermore, the emission intensity increased with an increasing concentration of noscapine. The sensitizing effect of noscapine on the weak CL emission was also related to the pH value of solution and the concentrations of KMnO₄ and sulfite. Thus, a series of experiments were performed to optimize the conditions for the production of maximum CL emission.

The effect of acid contained in the solution on the CL emission was initially examined. The CL emission intensity of noscapine-KMnO₄–Na₂SO₃ system in the presence of HCl, HNO₃, CH₃COOH, H₆P₄O₁₃, H₃PO₄ or H₂SO₄ at the same concentration was detected. The results indicated that the strongest CL emission occurred in acidic medium containing H₂SO₄. With the increasing concentration of H₂SO₄, the CL emission intensity increased and reached a maximum value at 0.1 mol/l. The intensity unchanged at higher H₂SO₄ concentrations. Therefore, 0.1 mol/l H₂SO₄ was chosen as the acidic medium for the reduction of permanganate.

Fig. 2 shows the effect of KMnO₄ concentration on the CL intensity. With an increasing concentration of KMnO₄, the CL intensity increased and then reached a maximum value at the KMnO₄ concentration of 3.0 × 10⁻⁵ mol/l. At the KMnO₄ concentrations higher than 3.0 × 10⁻⁵ mol/l, the emission intensity decreased probably owing to the permanganate absorbing the emitted light [26,37]. Therefore, 3.0 × 10⁻⁵ mol/l KMnO₄ was used for subsequent work.

The dependence of the CL intensity on the concentration of Na₂SO₃ showed a strongest emission at the concentration of 4.0 × 10⁻⁴ mol/l, which was chosen for the present work. In flow injection analysis, the flow rate of each reagent stream is generally an important parameter. The flow rates of Na₂SO₃ solution and the carrier were set at the same value and were twice those of both KMnO₄ and sample solutions.

The signal intensity increased with the increasing flow rate, as it was expected from the increased mixing rate. However, high flow rate led to much consumption of reagents and sample solutions but little gain in CL intensity and unstable CL signal. It was decided to supply the KMnO₄ and Na₂SO₃ solution at 0.9 and 1.8 ml/min, respectively.

3.2. Kinetic characteristics of the CL reaction

The kinetic behavior of the CL reaction of noscapine-MnO₄⁻–SO₃²⁻ was studied with a static method. Fig. 3 shows the typical kinetic curve. The CL reaction occurred immediately after mixing Na₂SO₃ with the solution containing KMnO₄ and noscapine and reached a maximum within 0.30 s. The CL reaction could be completed within 0.80 s after the reaction started. Thus, the CL reaction is very rapid. It is a flash-type emission and is apparently controlled by the mixing speed.

3.3. Analytical characteristics of noscapine

Under the optimum conditions mentioned above, the calibration curve was obtained for noscapine determination by plotting the CL signal versus noscapine concentration (Fig. 4), which gave a linear range from 2.0 × 10⁻⁸ to 2.0 × 10⁻⁶ mol/l with a correlation coefficient of 0.9998. The detection limit was 8.0 × 10⁻⁹ mol/l, which was calculated as the amount of noscapine required to yield a net peak three times the standard deviation of the background signal (3σ). The relative standard deviation for 11 repetitive determinations of 5.0 × 10⁻⁷ mol/l noscapine was 1.2%, showing a good reproducibility.

3.4. Interferences

The influences of different metal ions and organic compounds on the CL intensity were investigated by determining

![Fig. 2](image-url) Effect of KMnO₄ concentration on CL intensity of 0.1 mol/l H₂SO₄ + 5.0 × 10⁻⁷ mol/l noscapine + 4.0 × 10⁻⁴ mol/l Na₂SO₃.

![Fig. 3](image-url) Emission intensity vs. time profile after mixing of sulfite with acid potassium permanganate in the presence of noscapine.

Fig. 4. Plot of CL emission intensity vs. noscapine concentration.

The CL emission of the solutions containing 5.0 × 10⁻³ mol/l noscapine and foreign species with continuously increasing concentration up to 5 × 10⁻³ mol/l. When the effect of each foreign species on the peak height was less than 5.0%, it was thought not to interfere the determination of noscapine.

Table 1

<table>
<thead>
<tr>
<th>Species added</th>
<th>Maximum tolerable mole ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb²⁺, Zn²⁺, Cd²⁺, Mg²⁺, La³⁺, Cr³⁺, Cu²⁺</td>
<td>100</td>
</tr>
<tr>
<td>Mn²⁺, Sn⁴⁺, Se⁴⁺, Cr⁶⁺</td>
<td>50</td>
</tr>
<tr>
<td>Morphine, codeine, heroin</td>
<td>100</td>
</tr>
<tr>
<td>Glucose, fructose, sucrose</td>
<td>10</td>
</tr>
<tr>
<td>Papaverine</td>
<td>1.6</td>
</tr>
</tbody>
</table>

The results obtained were summarized in Table 1. Most anions had no interference because the tolerable ratios were in the case much higher than those normally encountered in real samples. No interference could be found when 100-fold concentration of morphine, codeine or heroin and 10-fold concentration of glucose, fructose or sucrose coexisted in the solution. Thus, this method could be used for the determination of noscapine and papaverine in opium, the interference of coexisted papaverine could be eliminated by the dilution of sample solution.

In order to verify the practical application of the proposed method, two simulated samples were prepared with appropriate amounts of some foreign species. Sample 1 contained 1.0 × 10⁻⁶ mol/l morphine, 2.0 × 10⁻⁶ mol/l glucose and 1.0 × 10⁻⁷ mol/l heroin, while sample 2 contained 1.0 × 10⁻³ mol/l Zn²⁺ and Ni²⁺, 1.0 × 10⁻⁸ mol/l Co²⁺, 1.0 × 10⁻⁷ mol/l morphine and 1 × 10⁻⁷ mol/l heroin. The results for the determination of noscapine were given in Table 2. The relative standard deviations less than 5.4% with an average value of 2.4% and the recoveries between 97.2 and 104.3% with an average recovery of 100.5% were highly satisfactory and illustrated the good performance of the proposed method.

3.5. Determination of noscapine in synthesized samples

In order to investigate the possible sensitizing mechanism of noscapine on the weak CL reaction of KMnO₄–H₂SO₄–Na₂SO₃, the fluorescence spectra of noscapine, noscapine–H₂SO₄, noscapine–KMnO₄–H₂SO₄, and noscapine–KMnO₄–H₂SO₄–Na₂SO₃ were recorded, respectively. The fluorescence excitation and emission spectra of noscapine were shown in Fig. 5. The excitation spectrum detected at an emission wavelength of 402 nm showed a native fluorescence excitation wavelength of 305 nm (Fig. 5a). Its fluorescence emission resulted from the oxidized noscapine. Thus, the fluorescence emission intensity increased and then tended to a maximum value. Furthermore, with an increasing reaction time the fluorescent intensity increased and then tended to a maximum value. Thus, the fluorescence emission resulted from the oxidized noscapine.
form of noscapine and it possesses stronger fluorescence emission intensity.

The addition of Na$_2$SO$_3$ to noscapine–H$_2$SO$_4$ system did not change the fluorescent intensity of noscapine, however, the fluorescent intensity of the oxidized form of noscapine increased greatly upon addition of Na$_2$SO$_3$ to noscapine–KMnO$_4$–H$_2$SO$_4$ system. The emission wavelength also remained at the same position. On the contrast, no change was observed when Na$_2$CO$_3$ was added to noscapine–KMnO$_4$–H$_2$SO$_4$ system. Thus, a reaction occurred between the oxidized form of noscapine and HSO$_3^-$ to form a strong fluorescent compound, which resulted in the increase in fluorescent intensity.

The CL spectrum of KMnO$_4$–Na$_2$SO$_3$–H$_2$SO$_4$ system showed two emission profiles extending from 490 to 620 nm and 400–500 nm, respectively (Fig. 7b). The new maximum CL emission intensity occurred at about 440 nm, coinciding with the maximum fluorescence emission wavelength of the oxidized form of noscapine. Thus SO$_2^*$ and the excited oxidized noscapine species might be the emitters in this system. The later could be produced from the oxidation of [noscapine]ox• by MnO$_4^-$.

\[
\text{nospamine + MnO}_4^- \rightarrow [\text{nospamine}]_{ox}^{*+} + \text{manganese complex}
\]
\[
[\text{nospamine}]_{ox}^{*+} + \text{HSO}_3^- \rightarrow \text{HSO}_3^{*+} + [\text{nospamine}]_{ox}^{*}
\]
\[
2\text{HSO}_3^{*} \rightarrow \text{S}_2\text{O}_6^{2-} + 2\text{H}^+
\]
\[
\text{S}_2\text{O}_6^{2-} \rightarrow \text{SO}_4^{2-} + \text{SO}_2^*
\]
\[
\text{SO}_2^* \rightarrow \text{SO}_2 + h\nu(535 \text{ nm})
\]

with maximum emission intensity at about 535 nm (Fig. 7a), which was similar to those measured with interference filters by Stauff and Jaeschke [30,31]. According to the suggestion reported in [18,30,31], the emitter was the exited sulfur dioxide. Thus, the oxidation product of HSO$_3^-$ by the oxidized form of noscapine should be HSO$_4^*$ radical. Two HSO$_4^*$ radicals then combined to produce S$_2$O$_6^{2-}$, which gave the excited intermediate product SO$_2^*$ with an emission when it returned to its ground state [18].

In noscapine–KMnO$_4$–Na$_2$SO$_3$–H$_2$SO$_4$ system, the CL spectrum showed two bands around 490–620 nm and 400–400, respectively (Fig. 7b). The new maximum CL emission intensity occurred at about 440 nm, coinciding with the maximum fluorescence emission wavelength of the oxidized form of noscapine. Thus SO$_2^*$ and the excited oxidized noscapine species might be the emitters in this system. The later could be produced from the oxidation of [noscapine]$_{ox}^{*}$ by MnO$_4^-$. The mechanism could be expressed as follows:

\[
\text{nospamine + MnO}_4^- \rightarrow [\text{nospamine}]_{ox}^{*+} + \text{manganese complex}
\]
\[
[\text{nospamine}]_{ox}^{*+} + \text{HSO}_3^- \rightarrow \text{HSO}_3^{*+} + [\text{nospamine}]_{ox}^{*}
\]
\[
2\text{HSO}_3^{*} \rightarrow \text{S}_2\text{O}_6^{2-} + 2\text{H}^+
\]
\[
\text{S}_2\text{O}_6^{2-} \rightarrow \text{SO}_4^{2-} + \text{SO}_2^*
\]
\[
\text{SO}_2^* \rightarrow \text{SO}_2 + h\nu(535 \text{ nm})
\]
4. Conclusions

The weak CL reaction of sulfite and acidic KMnO₄ can be enhanced significantly in the presence of noscapine. The sensitizing effect of noscapine on the reaction is due to the excited oxidized form of noscapine molecule and SO₂⁺, which are produced from the reaction of sulfite and the oxidation product of noscapine by KMnO₄. Based on the sensitizing effect a CL method for determination of noscapine is purposed. This method is simple, highly sensitive and selective, and can be satisfactorily used in the determination of noscapine in practical sample.

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