Chemiluminescent flow-through sensor for automated dissolution testing of analgin tablets using manganese dioxide as oxidate

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Abstract

A chemiluminescence (CL) flow-through sensor for the determination of analgin was described. It was based on the direct oxidation of analgin by manganese dioxide to produce a weak CL in the absence of luminescence reagent. The CL intensity in acidic medium was enhanced by the addition of Rhodamine B (RhB). The solid-phase manganese dioxide was immobilized on the sponge rubber inside the CL flow cell by a very simple means. The calibration graph is linear in the range $4 \times 10^{-5}$ to $1 \times 10^{-3}$ g/ml with a detection limit of $2.7 \times 10^{-5}$ g/ml ($S/N = 3$). The sensor was successfully used for automated dissolution testing of analgin tablets and sampling frequency was 120 times/h. The dissolution profiles of analgin tablets from three different plants were obtained, which demonstrate the stability, sensitivity, large dynamic measuring range and robustness of the system.

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1. Introduction

Analgin (dipyrone, novagin, metamizol), the sodium salt of \{2,3-dihydro-1,5-dimethyl-3-oxo-2-phenyl-1H-pyrazol-4-yl\}methyamino\}methanesulfonic acid, is a therapeutic agent commonly used as an analgesic, antipyretic, and antispasmodic. It forms the active constituent of other drugs. Quality control of analgin dosage and its monitoring in body fluids by quick automated techniques are important analytical tasks. Some analytical methods have been reported for the determination of analgin including spectrophotometry \cite{1,2}, fluorometry \cite{3}, amperometry \cite{4}, titrimey \cite{5}, chromatography \cite{6}, as well as spot test \cite{7}.

Chemiluminescence (CL) analysis offers high sensitivity, wide linear range and simple instrument. When coupled with flow-injection analysis (FIA), the CL-based FIA method provides cheap, rapid, simple and reproducible means of detection and, therefore, has been successfully applied to many drugs detection \cite{8-12}. The advantages of CL-FIA make it an important analytical method. However, in the CL flow system, the continuous delivery of reactants into the reaction zone is required, and the continuous flow of the reagents causes reagent waste and environmental pollution, which limits the widespread application of CL.

In recent years, CL flow sensor systems with immobilized reagents have received much attention and many analytical applications have appeared in the literature \cite{13-20}. Compared with the use of continuously delivered reagents in the conventional CL flow systems, these CL flow-through sensors are advantageous not only for operational convenience and instrumental simplification but also for cost, environment, and resource considerations. Solid manganese dioxide has a high redox potential ($+1.23$ V) in mineral acid solution \cite{21}. As a strong oxidant, manganese dioxide can oxidize a large variety of organic and inorganic substances and is used in the spectrophotometrical analysis \cite{22,23}. Alapont et al. \cite{24} has also used the MnO$_2$ as oxidant in the CL reaction, but it was based on the inhibitory effect of the Mn(II) (released from MnO$_2$) on the CL reaction of luminol–H$_2$O$_2$–K$_3$Fe(CN)$_6$ system. It was well known that the sensitivity of the inhibitory CL system was low and the selectivity involving luminol CL reaction was poor. In this paper, we found, in the absence of CL reagents, manganese dioxide can directly oxidize analgin in H$_2$SO$_4$ medium to produce weak CL, which could be sensitized by Rhodamine B (RhB). By a very simple means, manganese dioxide was immobilized inside of the CL flow cell as solid-phase CL.
oxidant. Compared to the report with polyester resin beads as an immobilized support [23], this flow sensor, which was easy to fabrication and replacing, could be used continuously 400 times. This method has been successfully applied to the determination of analgin and automated dissolution testing of analgin tablets.

2. Experimental

2.1. Reagents and solutions

All reagents were of analytical grade and the water used was deionized and doubly distilled. A $1 \times 10^{-3}$ g/ml stock solution of analgin (Xi’an Pharmaceutical Plant) was stored in the refrigerator (4 °C). Working standard solutions were prepared daily immediately before use. Manganese dioxide was obtained from Xi’an Chemical Reagents Plant (Xi’an, China). Sulfuric acid and RhB were from Xi’an Reagents Plant, Xi’an, China. Analgin tablets from three different Plants were purchased from the local market.

2.2. Apparatus

The schematic diagram of the FI–CL system for dissolution testing of analgin tablets is shown in Fig. 1. A peristaltic pump (Model DDB, Zhejiang Xiangshan Shipu Haitian Electronic Instrument Plant, China) was used to deliver all flow streams. PTFE tubing (0.8 mm i.d.) was used as connection material in the flow system. Sample solution was injected by a six-way injection valve into the carrier stream (H2O) and merged with the RhB and H2SO4 solution and then reached the solid-phase reactor, producing CL signal. The CL signal produced in the solid-phase reactor was detected and recorded with a computerized ultra-weak luminescence analyzer (type BPCL, manufactured at the Institute of Biophysics, Chinese Academy of Sciences, Beijing, China). The solid-phase reactor was located directly facing the window of the CR-105 photomultiplier tube (operated at $-800$ V, Hamamatsu, Tokyo, Japan). Data acquisition and treatment were performed with BPCL software running under Windows 98.

In the process of dissolution testing of tablet, there would be some undissolved suspended particles flowing into cell, so an on-line filter was needed in the FI–CL drug-dissolution testing system. The on-line filter was produced by modifying a plastic syringe filter equipped with a 10 mm diameter micropore membrane filter (0.45 μm) sandwiched between an O-ring and a perforated plastic support in a 300 μl dead volume filter chamber. The filtering area was about 75 mm². The filter was connected with PTFE tubing to the FI-CL system.

The FI–CL system was coupled with a paddle dissolution tester equipped with a dissolution vessel in a thermostatic water bath (Model 501, Shanghai Precision Instrument Plant, China).

2.3. Procedures

2.3.1. Preparation of the solid-phase reactor

About 1.5 g clean and CR-1211 sponge rubber was cut into small pieces (about 2 mm x 2 mm x 2 mm), this then was stirred with 1.5 g powder of manganese dioxide for 30 min. The sponge absorbing MnO2 was packed into a glass column (70 mm length, 5.5 mm i.d.) and some glass wool was inserted at both the ends to prevent loss of sponge. Each packed reactor had to be conditioned for at least 30 min before use. Conditioning involved pumping water through the reactor for 15 min, followed by pumping the carrier (H2SO4) for another 15 min.

2.3.2. Drug-dissolution conditions

The dissolving conditions for dissolution studies of analgin tablets were prescribed by the Chinese Pharmacopoeia.

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![Schematic diagram of the FI-CL system for dissolution testing of analgin tablets](image)

Fig. 1. Schematic diagram of the FI-CL system for dissolution testing of analgin tablets. a: RhB + H2SO4; b: H2O; E: electronic stirrer; P: peristaltic pump; V: injection valve; F: flow cell; D: detector; PC: personal computer.
using the paddle method. One liter of water was used as dissolution medium filled into dissolution vessel. The temperature (37°C) was maintained in the whole process of dissolution test. The rotation speed of the paddle was 100 rpm.

2.3.3 Drug-dissolution studies
A analgin tablet was dropped in the dissolution fluid thermostated 37 ± 0.5°C. The sample solution in the dissolution fluid at different times was injected into FI–CL system at a sampling frequency of 120 times/h, as shown in Fig. 1, 120 l sample solution was injected into the carrier through an on-line filter, then the stream was merged with RhB and H2SO4 into the solid reactor, producing the light. The concentration of sample was quantified by the CL intensity.

3. Results and discussion
3.1. Mechanism of the MnO2 chemiluminescence system
The structure of analgin was shown in Fig. 2, it included a sulfite anion group (–SO₃²⁻). While, the oxidation of –SO₃²⁻ in acid solutions was a well known CL reaction and the analytical properties of the reaction have been thoroughly studied with MnO₄⁻ [26–28], Ce(IV) [29,30], Ag(II) [31] and Mn(III) [32]. According to the different authors, the radical mechanism has been proposed for the CL oxidation of sulfite [26–33], which attributes the weak CL emitted by the reaction between sulfite and oxidant to the formation of the excited sulfur dioxide (SO₂∗). Moreover, some fluorescent compounds could sensitize the CL intensity because the energy of the SO₂∗ can easily be transferred to a fluorophore intentionally added to the system [27,31,33].

In the present work, MnO₂ can oxidize analgin to produce a weak CL which can be sensitized by RhB. We found the rate of the CL reaction in the solution was very fast: from the reagents mixing to the peak maximum, only 0.5 s was needed and it took about 5 s for the signal to reach the zero again. We also examined the CL spectra by a modified RF-540 spectrofluorophotometer, which showed only one peak at about 430 nm (same as the maximum in the emission spectrum of RhB). Therefore, the possible CL mechanism of the reaction may be attributed to the following reactions in the simple form:

\[
\text{MnO}_2 + 2\text{HSO}_3^- + 4H^+ \rightarrow \text{Mn}^{2+} + 2\text{HSO}_3^* + 2H_2O
\]  

(1)

\[
2\text{HSO}_3^* \rightarrow \text{S}_2\text{O}_6^{2-} + 2H^+
\]

(2)

\[
\text{S}_2\text{O}_6^{2-} \rightarrow \text{SO}_4^{2-} + \text{SO}_2^*
\]

(3)

\[
\text{SO}_2^* + \text{RhB} \rightarrow \text{SO}_2^+ + \text{RhB}^*
\]

(4)

\[
\text{RhB}^* \rightarrow \text{RhB} + h\nu
\]

(5)

3.2. Effect of RhB on the CL system
The effect of RhB concentration on the CL intensity is shown in Fig. 3. The CL intensity increased as the RhB concentration increased from 0 to 5 × 10⁻⁵ g/ml, after which the CL intensity started decreasing. The optimum RhB concentration was chosen to be 5 × 10⁻⁵ g/ml.

3.3. Effect of acidic media on the CL intensity
Because MnO₂ has a high redox potential only in acidic medium, the acidic solution was used as the CL reaction media. The influences of different acidic media on the CL intensity were studied (Table 1). The results showed the sulfuric acid was the optimum medium, the same with the CL system involving Ce(IV) or MnO₄⁻ [26,27,34–36]. Furthermore, the effect of H₂SO₄ concentration was studied (Fig. 4). The CL intensity increased with the increasing of H₂SO₄ concentration in the range of 0.0–0.01 mol/l, probably because the oxidation potential of MnO₂ increased. However, above the concentration of 0.01 mol/l, the CL intensity declined probably because the fluorescent quantum of RhB

<table>
<thead>
<tr>
<th>Acid (0.05 mol/l)</th>
<th>Relative CL intensity a</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl</td>
<td>25</td>
</tr>
<tr>
<td>CH₃COOH</td>
<td>42</td>
</tr>
<tr>
<td>HNO₃</td>
<td>30</td>
</tr>
<tr>
<td>H₃PO₄</td>
<td>51</td>
</tr>
<tr>
<td>H₃PO₄O₃</td>
<td>53</td>
</tr>
<tr>
<td>H₂SO₄</td>
<td>100</td>
</tr>
</tbody>
</table>

a Corresponding to the normalized maximum light intensity.

Fig. 3. Effect of RhB concentration on the CL intensity. Analgin concentration: 1 × 10⁻⁴ g/ml; H₂SO₄ concentration: 0.01 M.
decreased at higher [H\(^{+}\)]. (The experiment showed that the fluorescent intensity of RhB at the same concentration decreased with increasing H\(_2\)SO\(_4\) concentration in the range of 0.01–0.1 mol/l.) So, we selected the 0.01 mol/l H\(_2\)SO\(_4\) as the optimum medium.

3.4. Effect of flow rate

In the flow injection analysis, the flow rate is an important factor. In this work, we investigated the effect of flow rate on the CL intensity in the range of 0–6 ml/min. The results showed the CL intensity increased with the increasing rate in the range of 0.5–3.0 ml/min. Flow rate >3.0 ml/min led to greater consumption of reagent, unacceptable reproducibility and higher pressure on the solid reactor. Therefore, a flow rate of 3.0 ml/min was chosen for further study.

3.5. The lifetime of the solid-phase reactor

The lifetime of each other was established by comparing the CL intensity of the same analgin concentration in the different times. When the CL intensity started to decrease systematically and significantly, the reactor had to be replaced. In addition, we use the white sponge to immobilize the black MnO\(_2\). So, another indication that the reactor was losing its oxidation capacity was the color of the packaging itself. The color of the packaging of a new reactor was black. After pumping solution into the reactor, the color of the packing at the front-end of the reactor started to become pale yellow, and then white. When the one-third of the reactor became white, it usually had to be replaced. The reactor may be continuously used about 400 times during a period of 50 h. Moreover, it was easy to prepare and change the solid-phase reactor.

3.6. Performance of the system for analgin measurements

Under the optimum conditions described above, the calibration graph of emission intensity (I, mV) versus analgin concentration was linear in the range of 4 \(\times\) 10\(^{-5}\) to 1 \(\times\) 10\(^{-3}\) g/ml. The detection limit was 2.7 \(\times\) 10\(^{-5}\) g/ml. The regression equation was \(I = 8.00 \times 10^{3} C + 19.79\) (C being analgin concentration, g/ml) with a correlation coefficient of 0.9975. The relative standard deviation for seven replication injection of 1 \(\times\) 10\(^{-4}\) g/ml is 2.5%.

3.7. Interference studies

The interference of common ions and excipients in the pharmaceutical dosage was investigated in the determination of 1 \(\times\) 10\(^{-4}\) g/ml analgin. The tolerable concentration ratios for interference at the 5% level were 1000 for K\(^{+}\), Na\(^{+}\), Ca\(^{2+}\), Cl\(^{-}\) and NO\(_3\)\(^{-}\), 100 for glucose, lactose, 10 for Fe\(^{2+}\), Co\(^{2+}\) and NH\(_4\)\(^{+}\), 1 for Fe\(^{3+}\), Mn\(^{2+}\), carbamide, respectively. The results showed that the proposed method has good selectivity.

3.8. Performance of the chemiluminescence flow-through sensor for analgin dissolution testing

The analgin tablets was dissolved in 1000 ml water at 37 \(\pm\) 0.5\(^{\circ}\)C and 100 rpm. The system studied one analgin tablet per dissolution. After the undissoved parts were separated by an on-line filter, the dissolution fluid was injected into the flow-cell through valve at different times at a sampling frequency of 120 times/h. The dissolution profiles of three different commercial products of analgin tablets were studied using the method described above. The data points represent the average values of three parallel determinations, as shown in Fig. 5. The different analgin tablets from three different plants have different dissolution rates. On the whole, the analgin tablets have rapid release rate.

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References


Biographies

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