Flow-injection chemiluminescent determination of nitrite in water based on the formation of peroxynitrite from the reaction of nitrite and hydrogen peroxide

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Abstract

In the present work, a new flow-injection chemiluminescent (CL) method for the determination of nitrite has been proposed. In acidic medium, nitrite reacts with hydrogen peroxide to form peroxynitrous acid, which is an unstable compound in acidic solution and can be quenched into peroxynitrite in basic solution. During the decomposition of peroxynitrite a weak CL was observed. With the presence of ethyldimethylcetylammonium bromide (EDAB) and uranine, the CL intensity was enhanced significantly. This CL system was developed as a flow-injection method for the determination of nitrite. The CL intensity is proportional to the concentration of nitrite in the range from $1.0 \times 10^{-7}$ to $5.0 \times 10^{-5}$ M. The analytical frequency is 30 samples/h. The detection limit (S/N = 3) is $5.0 \times 10^{-8}$ M and the relative standard deviation for nine repeated measurements of $1.0 \times 10^{-6}$ M nitrite was 4.3%. The interferences of some cationic and anionic ions on the CL were studied. The anionic ions have almost no effect on the nitrite determination. After using a cation-exchange column as pre-treatment, the interferences of the cationic ions can be removed well. This method has been satisfactorily applied to determine nitrite in tap and well waters with recoveries of 94–106%.

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1. Introduction

Nitrite is a common and toxic pollutant in environment [1,2]. When nitrite is present in water, it can react with secondary amines to form carcinogenic N-nitrosoamines which are harmful to human health [3]. Furthermore, nitrite interferes with oxygen transport in the blood stream because it can convert oxyhemoglobin into methemoglobin. Therefore, the determinations of nitrite in environment and human bodies have attracted much attention, and a simple, sensitive and specific determination of nitrite is highly desirable.

Due to the importance of nitrite to nature and human bodies, a lot of analytical methods [4] have been developed for the determination of nitrite, e.g. spectrophotometric [5–8], electrochemical [9–12] and fluorometric [13–15] methods. The commonly spectrophotometric determinations of nitrite are based on the diazo coupling reaction. Some of them give good sensitivity and
selectivity but they require close control of pH and temperature during the diazotization step and a relatively long coupling time. The electrochemical detection of nitrite almost suffers from the complicated preparation process of the electrodes and low sensitivity. The fluorometric method for nitrite detection is often based on the reactions of nitrite with fluorescent dyes and the limit of its detection is relatively low. However, the fluorescent dyes are difficult to meet the requirement for the analysis of nitrite in real samples because of potential strong background fluorescence resulting from the dissolved organic substances in real samples.

CL analysis is becoming increasingly important in various fields for its high sensitivity, simple instrumentation, wide dynamic range and good reproducibility. In the last 20 years, the assay of nitrite by CL methods has also been described. To our knowledge, the majority of the CL assays for nitrite are based on the conversion of nitrite to nitric oxide (NO), which is detected in the gas phase by the CL reaction with ozone [16,17]. However, transformation of the NO in the phase need usually strip NO from solution with an inert gas. Thus, such techniques suffer from tedious procedure and low sensitivity. Moreover, Kumamaru and co-workers [18] described a CL post-column determination of nitrite, based on its ability to suppress the CL reaction of nitric acid and potassium hydroxide using iron(III) as catalyst. This method is simple in system and can be used to determine not only nitrite but also the other inorganic anions in water samples with ion-exchange chromatography, but the detection limit of nitrite is only $1.5 \times 10^{-6}$ M, which is not good enough for the trace analysis of nitrite.

Mikuska et al. [19] reported the determination of nitrite, based on the reactions of nitrite with acidified hydrogen peroxide to form peroxynitrous acid [20–25], with subsequent reaction with alkaline luminol, which is the light emitter. In the present work, without any special CL reagent, e.g. luminol and lucigenin, based on the decomposition of the peroxynitrile to form NO$^-$ ion and singlet oxygen ($^1$O$_2$) which is the light emitting species, a flow-injection CL method for the determination of nitrite is described. Although the detection limit of the present work is 50 times higher than that of the work of Mikuska et al., the selectivity of the present work is good because of the selective reaction of peroxynitrile. The method is adequate for the levels of nitrite allowable in tap and well waters. When a CL enhancer, e.g. uranine is present in the reaction system, a part of the energy of $^1$O$_2$ is transferred to uranine, which results in increased CL emission. A suitable micellar solution often enhances CL quantum or energy transfer efficiency during the CL reactions [26–28]. The target of this work is to find a simple and sensitive method to determine nitrite in natural waters directly in the liquid phase.

2. Experimental

2.1. Reagents

All chemicals were of analytical grade and used without further purification. Water was obtained from Milli-Q purification system (Japan Millipore, Tokyo). A 0.01 M nitrite solution was prepared by dissolving 0.069 g NaNO$_2$ (Beijing Chemical Reagent Company, Beijing, China), pre-dried at 110°C for 4 h, in 100 ml of water. A small amount of sodium hydroxide (NaOH) was added to the above solution to prevent its decomposition and 1.0 ml of chloroform to inhibit bacterial growth. Working standard solution was freshly prepared by diluting the stock solution with water. A working solution of hydrogen peroxide (H$_2$O$_2$) was freshly prepared by volumetric dilution of 30% (v/v) H$_2$O$_2$. A 0.01 M stock solution of ethyldimethylcetylammonium bromide (EDAB) (Tokyo Kasei Kogyo, Tokyo, Japan) was prepared with water and diluted to low concentration before use.

2.2. Apparatus

A flow system is illustrated in Fig. 1. A BPCL ultra-weak luminescence analyzer (Institute of Biophysics, Chinese Academy of Sciences, Beijing, China) was used. The three flow lines $R_1$, $R_2$ and $R_3$ are for delivering the carrier (water), H$_2$O$_2$–HCl solution and NaOH–EDAB–uranine solution, respectively. Sample solution is injected by means of a 50 μl loop valve injector. A column (3.0 cm length, 0.5 cm i.d.) packed with Dowex 50W X4 resin (Dow Chemical, Midland, USA) assembled behind the injection valve was used to remove the cationic metal ions from the samples. Black Teflon tube (1 mm i.d.) is used for the
Fig. 1. Schematic diagram of the flow-injection chemiluminescence system. P1 and P2, peristaltic pumps; M, mixing coil (20 cm long); S, 50 ml sample injector; C, 5.0 mm i.d. × 3.0 cm length column packed with Dowex 50W X4 resin; F, flow cell; R1, carrier (water) at 1.8 ml/min; R2, 0.01 M H2O2–0.06 M HCl solution at 1.8 ml/min; R3, 0.006 M EDAB–0.001 M uranine in 0.15 M NaOH solution at 2.5 ml/min.

flow lines. The peak height of the signal recorded was measured as CL intensity. The batch CL method was carried out with a batch style BPCL luminescence analyzer (Institute of Biophysics, Beijing).

2.3. Procedure

As shown in Fig. 1, a 50 μl of nitrite standard solution or sample solution was injected into the carrier stream through the injector and mixed with H2O2–HCl solution through a three-way piece. A 20 cm mixing coil was used for efficient mixing of sample and H2O2–HCl solution. A cation-exchange column located after the loop valve was used for pre-treatment of sample. Finally, the sample–H2O2–HCl solution mixed with NaOH–EDAB–uranine solution in a spiral flow CL cell which was placed in front of the photomultiplier tube. The CL signal was recorded with a portable recorder (Sichuan Fourth Instrument Factory, Chongqing, China). The stocking time of the tap and well waters should be controlled as short as possible.

3. Results and discussion

3.1. Mixing order of reagents and timing for nitrite injection

CL is defined as the emission of light from an electronically excited state species, which are produced during the chemical reaction. Therefore, the proper mixing order of reagent solution is extraordinarily crucial in CL reaction. In our former works [29,30], we often found that no or weakly light emission may be observed with unsuitable mixing orders. In order to obtain the highest CL signal, various mixing orders of reagents were measured by the batch method. The results indicated that there was no CL generation by the injection of 100 μl of NaOH solution into H2O2–HCl–NO2– solution. The replacement of H2O by 0.06 M HCl solution gave a weak CL emission. This phenomenon indicated that the reaction NO2– with H2O2 does not only need the presence of an acid catalyst, but also NaOH solution must be added subsequently in order to generate CL signal. If the NaOH solution was added before HCl solution, there was also no CL emission recorded. Therefore, the mixing order of NaOH solution and HCl solution to NO2––H2O2 system is important. The NaOH must be lastly added to the system, which has been considered in the flow system.

In order to improve CL sensitivity, the effects of surfactant and enhancer were also examined. EDAB and uranine were mixed with NaOH by three different orders. We can find that without the addition of EDAB and uranine together to the basic solution, the CL intensity has not enhanced significantly. The injection of NaOH–EDAB–uranine mixing solution into H2O2–HCl–NO2– solution provided the strongest CL emission.
Finally, the effect of the mixing time of H₂O₂ and HCl solutions on the CL intensity was studied. It is known that H₂O₂ is relatively stable in acidic medium. Our experimental results showed that a mixing solution of 0.01 M H₂O₂ and 0.06 M HCl was stable at least 3 h. The CL intensity was almost the same for different H₂O₂ and HCl mixing time from 10 s to 180 min. Therefore, in order to make the flow system as simple as possible, the H₂O₂ solution and HCl solution were mixed in advance and flow from line R₂ (Fig. 1). The surfactant, uranine can be prepared in NaOH solution before using. Based on the batch experiments, a flow system was assembled as in Fig. 1, where, R₂ flow line was used for uranine, surfactant and NaOH mixing solution.

3.2. Optimization of acids

The formation of peroxynitrous acid through the reaction of nitrite and hydrogen peroxide needs the presence of acid as catalyst. Therefore, no CL phenomenon was observed in alkaline or neutral solution. Four different acids HCl, H₂SO₄, H₃PO₄ and HNO₃ were compared. The effects of their concentrations on the CL intensity were shown in Fig. 2a. All these acids can be used as catalyst for the NO₂⁻–H₂O₂ reaction. However, among them HCl gave the highest CL signal. This is presumably due to the fact that Cl⁻ ion takes part in the reaction of H₂O₂ and HNO₂ (reactions (1)–(3)) [21].

\[
\begin{align*}
\text{H}^+ + \text{HNO}_2 & \rightarrow \text{H}_2\text{NO}_2^+ \\
\text{H}_2\text{NO}_2^+ + \text{Cl}^- & \rightarrow \text{NOCl} + \text{H}_2\text{O} \\
\text{NOCl} + \text{H}_2\text{O}_2 & \rightarrow \text{HOONO} + \text{H}^+ + \text{Cl}^-
\end{align*}
\]

The strongest CL intensity was obtained at the concentration of HCl 0.06 M which was chosen as the optimum concentration in further experiments.

3.3. Concentration of sodium hydroxide

Peroxynitrous acid is easily to be decomposed into NO₃⁻ and H⁺, it is often quenched into peroxynitrite with alkaline solution [24,25]. In the absence of NaOH, no CL signal was obtained with our CL apparatus. Therefore, the concentration of NaOH is a key parameter in this work. The CL intensity (Fig. 2b) was increased with the increase of the concentration of NaOH from 0 to 0.12 M, and then kept at a constant in the range of 0.12–0.16 M NaOH. The raising of NaOH concentration over 0.16 M caused the decrease of CL intensity. In the concentration range of 0.12–0.16 M NaOH the CL intensity was the strongest and remained on a plateau. A concentration of 0.15 M NaOH was chosen as the most convenient one for the following experiments.

3.4. Concentration of hydrogen peroxide

The effect of the concentration of hydrogen peroxide on the CL intensity was investigated in the range of 0.002–0.05 M H₂O₂. Without H₂O₂ in the reaction, there was no CL emission observed. As shown in Fig. 2c, when the concentration of H₂O₂ is 0.01 M, the most suitable CL signals can be recorded and the CL emission is the strongest. The concentrations of hydrogen peroxide higher than 0.01 M caused low CL intensity and many gaseous bubbles appeared in the waste solution. These phenomena can be explained by the H₂O₂ decomposing in stronger alkaline solution and the inhibiting the formation of peroxynitrite [31]. Therefore, the system was operated at 0.01 M H₂O₂ throughout this study.

3.5. Effect of the surfactant

It is known that the organized surfactant micelle as reaction medium can enhance CL intensity. Upon reaching a certain minimum concentration called the critical micelle concentration (CMC), amphiphilic surfactant molecules tend to associate dynamically in aqueous solution to form micelles. The local microenvironment in micellar media is drastically different from that in homogeneous media. In the present system, the CL was proposed as a result of the generation of singlet oxygen. Therefore, the yield of singlet oxygen was crucial for the CL efficiency. Various surfactants were added to the nitrite–H₂O₂ system in order to investigate their influences on the CL intensity. As shown in Table 1, the concentration of each surfactant is optimum. Anionic and non-ionic surfactants have no effect on the CL intensity. The results showed that EDAB was the most promising one in eight cationic surfactants examined, giving a 14-fold enhanced CL signal. The optimum concentration of EDAB was
Fig. 2. Effect of various conditions on the CL intensity: (a) concentration of HCl (●), HNO₃ (●), H₂SO₄ (●) and H₃PO₄ (●); (b) concentration of NaOH on the CL intensity; (c) concentration of H₂O₂; (d) concentration of EDAB; (e) concentration of uranine.

also examined. The CL intensity is increased near linearly with the increasing of EDAB concentration from 0.001 to 0.006 M (Fig. 2d). A concentration of 0.006 M EDAB was chosen as the optimum condition on account of the solubility of EDAB in water.

3.6. Choice of CL enhancer

CL enhancing method is often used to increase the light emission by an energy transfer from an excited intermediate that is a weak emitter in itself to
Table 1
Effect of surfactants on the CL intensity

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Concentration (mM)</th>
<th>Relative intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Cetyltrimethylammonium bromide (CTAB)</td>
<td>5.0 (0.9)</td>
<td>12.4</td>
</tr>
<tr>
<td>Myristyltrimethylammonium bromide (MTAB)</td>
<td>7.0 (3.5)</td>
<td>11.0</td>
</tr>
<tr>
<td>Didodecyldimethylammonium bromide (DDAB)</td>
<td>4.0 (0.18)</td>
<td>1.0</td>
</tr>
<tr>
<td>Octadecyltrimethylammonium chloride (OTAC)</td>
<td>5.0</td>
<td>12.6</td>
</tr>
<tr>
<td>Ethylidimethylcetylammonium bromide (EDAB)</td>
<td>6.0</td>
<td>14.0</td>
</tr>
<tr>
<td>Dodecyltrimethylammonium bromide (DTAB)</td>
<td>18.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Dioctadecyldimethylammonium chloride (DODAC)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Didodecyldimethylammonium bromide (DODAB)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Sodium dodecyl sulfate (SDS)</td>
<td>10.0 (8.0)</td>
<td>1.0</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>0.1% (v/v)</td>
<td>1.0</td>
</tr>
<tr>
<td>Tween-85</td>
<td>0.1% (v/v)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

The values in parentheses are the critical micelle concentration (CMC).

a Normalized with respect to the signal in the absence of surfactant.

Table 2
Effect of sensitizer on the relative intensity

<table>
<thead>
<tr>
<th>Fluorescent compound (1.0 mM)</th>
<th>Relative CL intensity a</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.0</td>
</tr>
<tr>
<td>Uranine</td>
<td>9.0</td>
</tr>
<tr>
<td>Eosin Y</td>
<td>6.2</td>
</tr>
<tr>
<td>Fluorescein</td>
<td>4.4</td>
</tr>
<tr>
<td>Rhodamine</td>
<td>1.0</td>
</tr>
<tr>
<td>8-Hydroxyquinoline</td>
<td>1.0</td>
</tr>
<tr>
<td>Rhodamine B</td>
<td>1.0</td>
</tr>
<tr>
<td>Acridine</td>
<td>1.0</td>
</tr>
<tr>
<td>Brilliant sulfolavine</td>
<td>1.0</td>
</tr>
</tbody>
</table>

The values in parentheses are the critical micelle concentration (CMC).

a Normalized with respect to the signal in the absence of sensitizer.

A fluorescent compound added purposely. In this work, eight fluorescent compounds, which are often used as enhancer in CL analysis, were compared. The results were shown in Table 2. We can find that fluorescein, eosin Y and uranine have evidence CL enhancing ability for the nitrite determination. Especially, uranine can increase the CL efficiency of the system about nine-fold, and CL signal is very stable as a result that the energy transfer is high enough for uranine, while is deficient for the other sensitizers tested. Therefore, uranine was selected as an enhancer in the system to enhance the weak CL. Fig. 2e showed the effect of the uranine concentration on the CL intensity. The maximum CL intensity was obtained for 0.001 M uranine. Concentrations exceeding 0.001 M uranine caused a sharp decrease of the CL signal, which might be attributed to the absorption of the emitted light for the yellow color of the excess of uranine, hence 0.001 M uranine was chosen for the analytical procedure.

3.7. Standard curve and detection limit

Under the above optimum conditions, a standard curve, \( Y = 7.0 \times 10^6 X + 2.77 \), was obtained for nitrite determination. \( Y \) is the relative CL intensity (the ratio of signal to noise, SN) and \( X \) the concentration of nitrite. A good calibration graph was linear in the concentration range from \( 1.0 \times 10^{-7} \) to \( 5.0 \times 10^{-5} \) M nitrite. The detection limit (SN = 3) was \( 5.0 \times 10^{-8} \) M nitrite. The correlation coefficient was 0.9995. The relative standard deviation for nine repeated measurements of \( 1.0 \times 10^{-6} \) M nitrite was 4.3%.

Table 3
Tolerance limit of various coexistent substances on the determination of \( 1.0 \times 10^{-6} \) M nitrite

<table>
<thead>
<tr>
<th>Coexistent substances</th>
<th>Tolerance (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( 5.0 \times 10^{-4} )</td>
</tr>
<tr>
<td>Ca (^{2+}), Br(^-), Cl(^-), F(^-), SO(_4^{2-}), CO(_3^{2-}), HCO(_3^-), HPO(_4^{2-}), PO(_4^{3-}), Pb(^{2+}), Mg(^{2+}), Cu(^{2+}), CH(_3)COO(^-), OCT(^-)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( 5.0 \times 10^{-5} )</td>
</tr>
<tr>
<td>Zn(^{2+}), Cd(^{2+}), Mo(^{6+}), Fe(^{3+}), Cu(^{2+})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( 1.0 \times 10^{-6} )</td>
</tr>
<tr>
<td>1(^+), Hg(^{2+}), Al(^{3+}), Nb(^{5+}), Cr(VI)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( 1.0 \times 10^{-12} )</td>
</tr>
<tr>
<td>Cu(^{2+}), Cr(^{3+})</td>
<td></td>
</tr>
</tbody>
</table>

* Normalized with respect to the signal in the absence of enhancer.
Table 4
Determination of nitrite in tap and well water samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Proposed method (10^{-6} M)</th>
<th>Spectrophotometric method</th>
<th>Added (10^{-6} M)</th>
<th>Found (10^{-6} M)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water</td>
<td>0.67 ± 0.02</td>
<td>0.72 ± 0.04</td>
<td>1.0</td>
<td>1.06 ± 0.02</td>
<td>106 ± 2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.0</td>
<td>1.96 ± 0.05</td>
<td>98.0 ± 2.5</td>
</tr>
<tr>
<td>Well water</td>
<td>0.42 ± 0.04</td>
<td>0.27 ± 0.02</td>
<td>1.0</td>
<td>0.94 ± 0.04</td>
<td>94.0 ± 4.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.0</td>
<td>2.08 ± 0.03</td>
<td>104 ± 1.5</td>
</tr>
</tbody>
</table>

All the results are the mean of three determinations ± S.D.

3.8. Interferences

The effect of various interferences concurrently presented in water for the determination of 1.0 × 10^{-6} M nitrite using the proposed method was investigated. The tolerance limit was taken as the amount which caused an error of ±5% in peak height. The results are summarized in Table 3. The influences of anionic ions, except I\(^-\) ion, on the CL intensity were slightly. They can have a high concentration coexisting with nitrite. However, without use of a cation-exchange column, many cationic ions, especially cobalt(II) and chromium(III), interfered seriously. Instead of nitrite solution, injection of 1 × 10^{-8} M cobalt(II) or chromium(III) into the flow system, a strong CL signal was also appeared. This phenomenon may be due to the CL emission of the decomposition of H\(_2\)O\(_2\) catalyzed by transition metals [32]. Therefore, this reaction system may also be developed as a CL method for the trace analysis of cobalt(II) and chromium(III). In order to eliminate the interferences from cationic ions, a cation-exchange column of Dowex 50W X4 resin was assembled behind the injector. The interferences of the cationic ions can be removed well. The concentrations of these cationic ions lower than 10^{-5} M have no influence on the determination of 10^{-6} M nitrite solution.

3.9. Analysis of real samples

The concentrations of nitrite in tap and well waters were determined by the proposed method. Well water comes from deep-groundwater, which is relatively clean and can be drunk directly without pre-treatment. Freshly collected samples were filtered through a membrane filter of 0.45 µm pore size, kept in a refrigerator at about 4 °C and analyzed by the recommended procedure within 4 h of collection. The interferences of the cationic ions were eliminated with the cation-exchange column that was furnished with glass wool at both ends. The results in Table 4 agreed well with those obtained by a standard spectrophotometric method [33], and the recoveries for the samples were 94–106%. The nitrite concentration of tap water is higher than that well water in Haidian district of Beijing.

4. Conclusion

A new flow-injection CL method for the determination of nitrite was developed. It is a simple, rapid and convenient method compared with other reported CL methods based on the conversion of nitrite to nitric oxide and detection using the gas phase CL reaction system. After using a cation-exchange column as pre-treatment, the interferences of the cationic ions can be removed well. The present method demonstrated that it can be used successfully for the determination of nitrite in tap and well waters.

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