Flow Injection Chemiluminescence Determination of Polyhydroxy Phenols in the Presence of Rhodamine B as a Sensitiser

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A novel flow injection chemiluminescence (CL) system for determination of polyhydroxy phenols, including phloroglucinol, pyrogallol, resorcinol and pyrocatechol has been described. The system utilises CL reaction of ferricyanide with polyhydroxy phenols in sodium hydroxide media sensitised by the fluorescent dye Rhodamine B. The proposed procedure has a linear range of 0.05–5.0 μg mL⁻¹ for phloroglucinol, and 0.01–1.0 μg mL⁻¹ for pyrogallol, resorcinol and pyrocatechol. The method is sensitive, simple, and fast. The chemiluminescence reaction mechanism has been also proposed and briefly discussed.

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Polyhydroxy phenols, such as phloroglucinol, pyrogallol, resorcinol and pyrocatechol have proved their applicability in food chemistry, pharmaceutical, cosmetic, printing and dyeing industries. Thus, determination procedure for these compounds is of great interest and importance. Many analytical approaches are well known and available, i.e. spectrophotometry [1–4], gas chromatography [5], high performance liquid chromatography [6–8], and electrochemical [9] and enzymatic [10] techniques.

In recent years, flow injection chemiluminescence (CL) analysis has received much attention in various fields for its high sensitivity, wide linear working range and instrumental simplicity [11–14]. Several assays of polyhydroxy phenols estimated by flow injection chemiluminescence method have been published already in the literatures [15–16]. Du et al. [15] have described determination procedure for polyhydroxy phenols (pyrogallol, phloroglucinol, quinol, resorcinol) utilising their chemiluminescence in the reaction with luminol in alkaline media. He et al. [16] have proposed another chemiluminescence method based on the inhibition effect of polyhydroxy phenols (phlorogenic acid, catechol, resornol) on the luminol-ferricyanide system. However, these CL systems suffer from low sensitivity (the linear range for the determination of polyphenols was ranging from 0.1 to 1 µg mL⁻¹). Furthermore, they belong to the luminol CL system, so their selectivity is insufficient.

For the determination of some pharmaceuticals there are known some analytical procedures utilising chemiluminescence, which is being enhanced by fluorescent compounds (such as rhodamine B, rhodamine 6G and fluorescein). Zhang et al. [17] have investigated chemiluminescence of the rhodamine B-sensitised reaction of Ce(IV) with captopril. Huang et al. have proposed a CL system based on Ce(IV) oxidation sensitised with rhodamine 6G for the determination of analgin [18] and chlorpromazine hydrochloride [19].

It was reported that potassium ferricyanide directly oxidises thiamine [20] and tetracyclines [21] to produce weak chemiluminescence in alkaline medium. In these systems, the products of the analyte oxidation generated chemiluminescence. We have found that phloroglucinol, pyrogallol, resorcinol and pyrocatechol react with ferricyanide to produce weak chemiluminescence in alkaline medium. This CL reaction can be effectively sensitised by rhodamine B. According to these findings, a novel flow injection chemiluminescence method for the determination of polyhydroxy phenols including phloroglucinol, pyrogallol, resorcinol and pyrocatechol has been developed. One recorded chemiluminescence spectrum and confirmed rhodamine B to be a CL emitter. There is an opportunity to apply ferricyanide-rhodamine B system for the determination of polyhydroxy pharmaceuticals.
EXPERIMENTAL

Reagents

All reagents were of analytical grade, and doubly distilled water was used for the preparation of the solutions. Phloroglucinol, pyrogallol, resorcinol and pyrocatechol were obtained from the Shanghai Chemical Regent Factory. Rhodamine B and potassium ferricyanide were obtained from Acros and Chongqing Chemical Regent Factory, respectively. Stock standard solutions of phloroglucinol, pyrogallol, resorcinol and pyrocatechol (1.0 μg mL⁻¹) were prepared by dissolving 0.1 g of each in water and diluting with water to 100 mL, respectively. Standard solutions were stored in the refrigerator and protected from light. Working standard solutions were prepared by the appropriate dilutions of the stock solutions with water.

Apparatus

Flow injection chemiluminescence (FI–CL) system is shown in Figure 1. Two peristaltic pumps provided the flow rate of 1.5 mL min⁻¹. PTFE tubings (0.8 mm I.D.) were used as connecting elements. An IFFM–A Chemiluminescence Analyser (Xi’an Ruike Electronic Ltd, China) equipped with a CR–105 photomultiplier tube (PMT) (Hamamatsu, Japan) served for the intensity measurements of CL signals. Signals were recorded using an IBM-compatible computer, equipped with a data acquisition interface. Data acquisition and treatment were performed with IFFM–D software running under Windows 98.

![Figure 1](image.png)

**Figure 1.** A scheme of the flow injection chemiluminescence system for determination of polyhydroxy phenols. P – peristaltic pump; V – six-way injection valve; F – flow cell

Procedures

As shown in Figure 1, the tubes were immersed into the ferricyanide, sodium hydroxide, carrier (rhodamine B) and standard solutions, respectively. The pumps were started to wash the whole system. Then 100 μL of the standard solution was injected into the carrier stream, which was mixed with ferricyanide in the flow cell, producing CL emission. The concentration of polyhydroxy phenols was quantified measuring CL intensity.
RESULTS AND DISCUSSION

Influence of rhodamine B concentration

Preliminary experiment has shown that polyhydroxy phenols react with ferricyanide to produce a weak CL emission, and this CL reaction could be sensitised by some fluorescing compounds. So, the influence of fluorophores, including rhodamine B, rhodamine 6G, fluorescein, dichloro fluorescein and 8-hydroxy quinoline-5-sulfuric acid has been studied. According to the data in Table 1, rhodamine B occurred to be the best fluorophore for the CL reaction. Thus the effect of rhodamine B was examined in more detail. It is obvious from Figure 2 that maximum CL intensity appears for the concentration of rhodamine B of $5.0 \times 10^{-5}$ mol L$^{-1}$ and this value was finally chosen for the further study.

Table 1. The influence of some fluorophores on the relative CL intensity (results for resorcinol)

<table>
<thead>
<tr>
<th>Fluorophore</th>
<th>Optimum concentration, mmol L$^{-1}$</th>
<th>Relative CL intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>—</td>
<td>1.0</td>
</tr>
<tr>
<td>8-Hydroxy quinoline-5-sulfuric acid</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Fluorescein</td>
<td>0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Dichloro fluorescein</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Rhodamine 6G</td>
<td>0.05</td>
<td>35.2</td>
</tr>
<tr>
<td>Rhodamine B</td>
<td>0.05</td>
<td>51.1</td>
</tr>
</tbody>
</table>

Figure 2. The effect of Rhodamine B concentration on the CL intensity of the polyhydroxy phenols. Ferricyanide – $5.0 \times 10^{-4}$ mol L$^{-1}$, sodium hydroxide – 0.5 mol L$^{-1}$, resorcinol – 0.1 μg mL$^{-1}$
Influence of potassium ferricyanide concentration

The effect of potassium ferricyanide concentration on the CL intensity was examined over the concentration range of $1.0 \times 10^{-4} - 1.1 \times 10^{-3}$ mol L$^{-1}$. The results are presented in Figure 3. CL intensity increases with the ferricyanide concentration. Above its concentration of $5.0 \times 10^{-4}$ mol L$^{-1}$, CL intensity decreases. Again, the optimum concentration for ferricyanide was chosen as $5.0 \times 10^{-4}$ mol L$^{-1}$.

![Figure 3](image)

Figure 3. The effect of ferricyanide concentration on the relative CL intensity. Rhodamine B – $5.5 \times 10^{-5}$ mol L$^{-1}$, sodium hydroxide – 0.5 mol L$^{-1}$, resorcinol – 0.1 μg mL$^{-1}$

Influence of sodium hydroxide

The concentration of sodium hydroxide was being changed in the range from 0 to 2.5 mol L$^{-1}$ in order to investigate its influence on the CL intensity. Maximum CL intensity was obtained for the NaOH concentration of 1.0 mol L$^{-1}$ (Fig. 4) and was maintained at this level in the further work.

![Figure 4](image)

Figure 4. The effect of sodium hydroxide concentration on the relative CL intensity. Rhodamine B – $5.5 \times 10^{-5}$ mol L$^{-1}$, ferricyanide – 0.5 mmol L$^{-1}$, resorcinol – 0.1 μg mL$^{-1}$
Interference studies

Increasing amounts of various ionic interfering species were added to the 0.1 μg mL⁻¹ standard solution of resorcinol. Concentration ratios allowing one to maintain interferences at the 5% level were over 1000 for K⁺, Na⁺, Ca²⁺, Cl⁻, 500 for acetate, acetic ether, glucose, fructose, sucrose, 200 for Zn²⁺, Al³⁺, Mg²⁺, NO₃⁻, PO₄³⁻, SO₄²⁻, Br⁻, maltose, citrate, lactate, 10 for NO₂⁻, Fe²⁺, Fe³⁺ and 5 for ascorbic acid. These results have confirmed a satisfactory selectivity of the studied method.

Performance of the system for determination of polyhydroxy phenols

One prepared calibration plots for phloroglucinol, pyrogallol, resorcinol and pyrocatechol under optimum experimental conditions. The slopes, intercepts, as well as the range of linearity, detection limits and relative standard deviations are given in Table 2.

Possible mechanism of the CL reaction

In order to find the possible mechanism of the CL reaction, the CL spectrum of ferricyanide and polyhydroxy phenols with and without the addition of rhodamine B has been thoroughly examined. Spectra were obtained with a RF–540 fluorimeter. The wavelengths corresponding to the maximum measured signals were 440 nm, 450 nm, 460 nm and 435 nm for phloroglucinol, pyrogallol, resorcinol and pyrocatechol without rhodamine B, respectively. However, in the presence of rhodamine B only one maximum appeared at 580 nm, regardless of the compound, and it referred to the maximum emission of pure rhodamine B (Fig. 5). As predicted, this is an excited rhodamine B, which is responsible for the emission. One may propose the following mechanism of the chemiluminescence reaction:

\[
\text{ferricyanide + polyhydroxy phenols} \rightarrow \text{ferrocyanide + polyhydroxy phenols (ox)*}
\]
polyhydroxy phenols (ox)* + rhodamine B → polyhydroxy phenols (ox) + rhodamine B*

rhodamine B* → rhodamine B + hv (580 nm)

Figure 5. CL spectrum of ferricyanide + resorcinol + rhodamine B (b), ferricyanide + resorcinol (c) and the fluorescence emission spectrum of rhodamine B (a). Resorcinol – 10 μg mL⁻¹, rhodamine B – 0.1 mmol L⁻¹

CONCLUSIONS

In this work a novel method utilising chemoluminescence of polyhydroxy phenols: phloroglucinol, pyrogallol, resorcinol and pyrocatechol produced during their oxidation with ferricyanides in the presence of rhodamine B as a sensitisier has been presented. Further studies on the analytical applicability of the ferricyanide-rhodamine B chemiluminescence system to the determination of other polyhydroxy compounds have been already undertaken in our laboratory.

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REFERENCES


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