Sensitive Assay for Catecholamines in Pharmaceutical Samples and Blood Plasma Using Flow Injection Chemiluminescence Analysis

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A rapid and sensitive chemiluminescence (CL) method using flow injection analysis was described for the determination of three catecholamines: dopamine, adrenaline and dobutamine, based on their greatly enhancing effects on the CL reaction of luminol-potassium periodate in basic solutions. Under the optimized conditions, the calibration graphs relating the increase of CL intensity to the concentration of the analytes were linear. The present method allows for the determination of dopamine, adrenaline, and dobutamine over the range of $1.0 \times 10^{-10}$ - $1.0 \times 10^{-7} \text{ g/ml}$. The relative standard deviations for measurements ($n = 11$) of dopamine, adrenaline and dobutamine were 2.9, 2.3 and 1.8% when the concentrations of three catecholamines were at $1.0 \times 10^{-8} \text{ g/ml}$, respectively. The detection limits of the method were $2.0 \times 10^{-11} \text{ g/ml}$ dopamine, $1.0 \times 10^{-11} \text{ g/ml}$ adrenaline and $4.0 \times 10^{-11} \text{ g/ml}$ dobutamine. The method was successfully applied to the determination of three catecholamines in pharmaceutical samples and blood plasma.

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Introduction

Dopamine, adrenaline and dobutamine are known as catecholamines, which play important roles in the central nervous system as neurotransmitters. These catecholamines, as well as metaraminol and phenylephrine with a structure of the phenylethyl amine group, are widely used to treat hypertension, bronchial asthma, cardiac arrest, myocardial infarction, and cardiac surgery. Therefore, a highly sensitive method is necessary for the determination of the catecholamines, both in pharmaceutical samples and biological fluids.

A variety of techniques have been utilized for the determination of three catecholamines, such as chromatography,1-3 capillary electrophoresis,4-6 spectrophotometry,7 fluorometry,8-10 and electrochemical11,12 detection. However, these methods are complicated (they need derivatization or combination with various means of detection). Also, some of these methods suffer low sensitivity and specificity, such as the detection limit of the method,9 which is $1.0 \times 10^{-5}$ g/ml.

The CL methods for the determination of active compounds in pharmaceutical samples or biological fluids are widely used because of their simple, rapid, and sensitive properties. In recent years, there have been a few reports on the determination of catecholamines using the chemiluminescence (CL)13-16 technique. Among these methods, many of them determine the content of catecholamines in pharmaceutical preparations. Only Li et al.16 combined on-line microdialysis sampling with a plant tissue-based chemiluminescence flow biosensor to monitor the variation of the dopamine level in the blood of rabbit. In this method, dopamine was oxidized by oxygen under the catalysis of polyphenol oxidase in the tissue column to produce hydrogen peroxide, which reacted with luminol in the presence of the peroxidase of potato tissue, and generated a CL signal. The method allowed the determination of dopamine over the range of $1.0 \times 10^{-5}$ - $1.0 \times 10^{-7} \text{ g/ml}$ with a detection limit of $5.3 \times 10^{-8}$ g/ml.

In our work we found that catecholamines could strongly enhance the CL reaction of luminol-potassium periodate in an alkaline solution; the increase of the CL intensity was dependent on the concentration of the studied drug. Based on these observations, a simple, sensitive and rapid new assay for catecholamines has been developed using the flow-injection CL technique. The proposed method was successfully applied to the determination of dopamine, adrenaline and dobutamine, not only in pharmaceutical samples, but also in blood plasma. Moreover, the possible mechanism of the CL reaction was briefly considered.

Experimental

Apparatus

A schematic diagram of the flow-injection chemiluminescence (FI-CL) system used in this work is shown in Fig. 1. One peristaltic pump was used to deliver luminol and KIO$_4$ solutions with a flow rate of 2.7 ml/min. Another peristaltic pump was used to pump the sample solution at a flow rate of 0.8 ml/min. PTFE tubing (0.8 mm i.d.) was used to connect all components in the flow system. A six-way injection valve was used to inject the sample solutions. The streams of luminol, KIO$_4$ in the alkaline medium, and analytes were combined in a flow cell. The CL signal produced in the flow cell was collected with a photomultiplier tube. Data acquisition and treatment were handled by an IBM-compatible computer, running IFFL-DD flow-injection CL analysis system software (Xi’an Rumax Electronic Equipment Corporation, Xi’an, China).

Reagents and solutions

All chemicals used were of analytical reagent grade; doubly distilled water was used through the experiment. Dopamine

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hydrochloride, adrenaline hydrochloride and dobutamine hydrochloride were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Luminol was obtained from the Institute of Analytical Science of Shaanxi Normal University (Xi’an, China). Potassium periodate (KIO\textsubscript{4}) was obtained from Guangzhou Chemical Reagent Factory (Guangzhou, China).

A 0.01 mol/l luminol stock solution was prepared by dissolving appropriate luminol in a 0.02 mol/l sodium hydroxide solution. A luminol working solution was prepared by directly diluting this stock solution to 1.0 \times 10^{-4} \text{ mol/l} with water. Potassium periodate stock solution was prepared by dissolving 0.5750 g of potassium periodate in water and diluting to 250 ml with water. The working solution of potassium periodate was prepared by diluting this stock solution to 1.0 \times 10^{-4} \text{ mol/l} with an approximate amount of 1.0 mol/l sodium hydroxide to provide a final concentration of 0.1 mol/l sodium hydroxide in the diluted solution.

Standard solutions (1.0 \times 10^{-3} \text{ g/ml}) of dopamine, adrenaline and dobutamine were prepared freshly with water. The standard solutions were stored in a refrigerator (4°C) and protected from light before use. Before analysis, working solutions were prepared by appropriate dilution of these standard solutions with water to ensure concentrations within the linear range of the calibration graphs (Table 1).

**Preparation for the studied catecholamines injections**

Pharmaceutical injections containing dopamine hydrochloride (20 mg per ampoule), adrenaline hydrochloride (1 mg per ampoule), and dobutamine hydrochloride (20 mg per ampoule) were obtained from the First Affiliated Hospital of Xi’an Jiaotong University (Xi’an, China). The solution was diluted appropriately with water to a certain concentration, respectively, before use, and the content was calculated from the corresponding calibration graph or by using the regression equation (Table 1).

**Preparation for plasma**

Fresh whole blank blood was centrifugated at 3000 rpm for 10 min. The supernatant was transferred to a test tube and used as blank plasma. Acetonitrile (0.2 ml) was added to an aliquot of plasma (0.1 ml) in a 1.5 ml centrifuge tube, and the solutions were shaken for 5 min, and homogenized. The precipitated protein was removed by centrifugation for 10 min at 10000 rpm. The supernatant was blown dry with nitrogen gas and transferred into a 100 ml calibrated flask and completed to volume with distilled water.

**Procedure**

A series of working solutions with different concentrations were prepared by diluting respectively prepared standard solutions with water. A certain amount of catecholamines solution was injected into the flow system by a six-way injection valve and mixed with the mixed luminol-KIO\textsubscript{4} solution. Then, the mixed solution was transferred into the flow cell and an increased CL signal was obtained. The increased value of the CL intensity (\(\Delta I\), the difference of CL intensity between the catecholamine solution and the blank reagent without the catecholamine) was proportional to the concentration of the catecholamine.

**Results and Discussion**

**CL kinetic property**

In this work, it was observed that the CL reaction of lumionl-KIO\textsubscript{4} could be strongly enhanced by dopamine hydrochloride in alkaline solution. The CL signal reached the maximum intensity at 2.4 s, and was then extinguished within 5.4 s after mixing the dopamine hydrochloride solution and the lumionl solution in a sodium hydroxide with KIO\textsubscript{4} solution.

**Effect of different alkaline concentrations**

It is well known that the luminol CL reaction requires alkaline conditions. Thus, several alkaline buffer solutions (carbonate, phosphate, borate and sodium hydroxide) were tested, and we found that sodium hydroxide was the most suitable medium for the present CL reaction. In addition, we also found that the CL intensity was stronger when an appropriate amount of NaOH was added into the KIO\textsubscript{4} solution, rather than being added into the luminol solution. Based on these observations, a series of experiments were performed to select the optimum reaction conditions. Also, the effect of NaOH within the range of 0.01 – 0.30 mol/l concentration on the CL reaction was further tested when the concentrations of luminol and KIO\textsubscript{4} were both fixed at 1.0 \times 10^{-4} \text{ mol/l} (Fig. 2). The \(\Delta I\) continued to change with increasing the NaOH concentration. When 0.2 mol/l NaOH was used in the KIO\textsubscript{4} solution, the CL reaction gave the maximum \(\Delta I\). Considering the stability of CL signal, the best concentration of NaOH was 0.1 mol/l.

**Effect of the luminol concentration**

As the luminescence reagent of the CL reaction, the luminol concentration affected the CL intensity. The influence of 1.0 \times 10^{-5} to 3.0 \times 10^{-4} \text{ mol/l} luminol on the CL reaction was examined with 1.0 \times 10^{-4} \text{ g/ml} of a dopamine hydrochloride solution as the analyte when the concentration of KIO\textsubscript{4} was

\[
\begin{array}{c|c|c|c|c}
\text{Catecholamine} & \text{Con. range/} ng \text{ ml}^{-1} & \text{Limit of detection/} ng \text{ ml}^{-1} & \text{Calibration curve} & \text{Correlation coefficient} \\
\hline
\text{Dopamine} & 0.1 - 1 & \Delta I = 3.01 + 15.37C & 0.9969 \\
& 1 - 10 & \Delta I = 3.56 + 13.01C & 0.9989 \\
& 10 - 100 & \Delta I = 113.20 + 0.99C & 0.9965 \\
\text{Adrenaline} & 0.1 - 1 & \Delta I = 6.58 + 44.63C & 0.9964 \\
& 1 - 10 & \Delta I = 18.97 + 11.79C & 0.9987 \\
& 10 - 100 & \Delta I = 107.82 + 3.46C & 0.9982 \\
\text{Dobutamine} & 0.1 - 1 & \Delta I = 8.29 + 72.92C & 0.9985 \\
& 1 - 10 & \Delta I = 30.06 + 16.26C & 0.9989 \\
& 10 - 100 & \Delta I = 156.30 + 5.59C & 0.9975 \\
\end{array}
\]

\(a. \Delta I = a + bC\), where \(C\) is the concentration of catecholamines in ng/ml.
fixed at $1.0 \times 10^{-4}$ mol/l (Fig. 3). The maximum $\Delta I$ was obtained when the concentration of luminol was $1.0 \times 10^{-4}$ mol/l, and thus $1.0 \times 10^{-4}$ mol/l luminol was used in a further study.

**Effect of KIO$_4$ concentration**

KIO$_4$ was used as the oxidant in the reaction. The influence of the KIO$_4$ concentration on the CL reaction was examined in the range of $1.0 \times 10^{-5}$ - $3.0 \times 10^{-4}$ mol/l when the concentrations of NaOH and luminol were at 0.1 mol/l and 1.0 $\times 10^{-4}$ mol/l (Fig. 4). The results showed that the CL intensity increased as the concentration of KIO$_4$ was increased to $1.0 \times 10^{-4}$ mol/l. When the concentration of KIO$_4$ was above $1.0 \times 10^{-4}$ mol/l, the CL intensity decreased. Thus, the concentration of $1.0 \times 10^{-4}$ mol/l KIO$_4$ was used in the following experiments.

**Calibration curves of the studied catecholamines in pharmaceutical injections**

The working curves of the studied catecholamines were established under the optimum experimental conditions mentioned above. A $1.0 \times 10^{-5}$ g/ml stock solution of the studied catecholamines was prepared in distilled water. Serial dilutions with distilled water were made to cover the working ranges (Table 1). Table 1 lists the parameters of the calibration curves, and the relative standard deviations for the detections ($n = 11$) of dopamine, adrenaline and dobutamine were 2.9, 2.3 and 1.8% when the concentrations of the three catecholamines were at $1.0 \times 10^{-6}$ g/ml, respectively. Table 2 gives the results of this experiment.

**Calibration curves of dopamine hydrochloride in rabbit blank plasma**

Following the method described in the section “Preparation for plasma”, the relative standard deviations for the measurement ($n = 11$) of dopamine, adrenaline and dobutamine were 2.1, 2.7 and 2.3% when the concentrations of three catecholamines were at $5.0 \times 10^{-9}$ g/ml, respectively. Table 2 lists the parameters of the calibration curves, and the relative standard deviations for the detections ($n = 11$) of dopamine, adrenaline and dobutamine were 2.9, 2.3 and 1.8% when the concentrations of the three catecholamines were at $1.0 \times 10^{-9}$ g/ml, respectively. Table 2 lists the parameters of the calibration curves, and the relative standard deviations for the detections ($n = 11$) of dopamine, adrenaline and dobutamine were 2.9, 2.3 and 1.8% when the concentrations of the three catecholamines were at $5.0 \times 10^{-9}$ g/ml, respectively. Table 2 gives the results of this experiment.

**Interference studies**

The effects of various substances (excipients and common ions) that often accompany injections of the three catecholamines were studied by analyzing a standard solution of dopamine hydrochloride ($1.0 \times 10^{-6}$ g/ml) into which increasing amounts of interfering analyte were added. It was considered not to interfere if a foreign species caused a relative error of less than $\pm 5\%$ during the determination of $1.0 \times 10^{-9}$ g/ml of dopamine hydrochloride. No interference was found when including up to 1000-fold K$^+$, Na$^+$, Cl$^-$, SO$_4^{2-}$, NO$_3^-$, SO$_3^{2-}$, PO$_4^{3-}$; 100-fold NH$_4^+$, Ba$^{2+}$, Mg$^{2+}$; glucose, lactose; 50-fold sodium pyrosulfate; and 1-fold Ca$^{2+}$, Al$^{3+}$, ascorbic acid.

**Determination of the studied catecholamines injections**

Following the method described in the section “Preparation for the studied catecholamines injections”, the results are listed in Table 3, and agreed well with those obtained by the method recommended by the Pharmacopoeia of People’s Republic of China. 17

**Determination of catecholamines in rabbit plasma**

The injection of 0.1 ml catecholamine ($4.0 \times 10^{-6}$ g/ml) was added to 0.1 ml of blank plasma; following the method described in section “Preparation for plasma”, the results are listed in Table 4. As can be seen, the proposed method proved to be satisfactory for determining the studied catecholamines in rabbit plasma.
Under the experimental conditions described above, the CL spectra of the luminol-KIO₄ reaction in both the absence and presence of the studied catecholamines were obtained using a refitted RF-540 spectrofluorometer. It was observed that the CL spectra of the luminol-KIO₄ reaction had the maximum emission wavelength at 425 nm, which is the characteristic spectrum of the luminol reaction. These results indicated that the potential CL emitter is the excited state of 3-aminophthalate ion + hydroxy and/or superoxide radicals + the other products; hv → excited 3-aminophthalate ions; and/or superoxide radicals + the other products; 3-aminophthalate ion + KIO₄ → excited 3-aminophthalate ions; 3-aminophthalate ions → 3-aminophthalate ion + hv (λₑₓᵣᵣ = 425 nm).

### Table 3 Determination of the catecholamine injections

<table>
<thead>
<tr>
<th>Substance</th>
<th>Claimed/ Found/ Added/ Recovered/ Rec., %</th>
<th>Official method/ mg ml⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine</td>
<td>10 9.87 2.00 3.04 3.93</td>
<td>98.3</td>
</tr>
<tr>
<td></td>
<td>3.00 1.95 1.01 3.93</td>
<td>97.5</td>
</tr>
<tr>
<td></td>
<td>4.00 1.95 1.01 3.93</td>
<td>97.5</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>1 0.98 2.00 2.09 3.00</td>
<td>95.7</td>
</tr>
<tr>
<td></td>
<td>3.00 2.87 1.01 3.93</td>
<td>95.7</td>
</tr>
<tr>
<td></td>
<td>4.00 3.78 1.01 3.93</td>
<td>95.7</td>
</tr>
<tr>
<td>Dobutamine</td>
<td>10 9.65 2.00 1.89</td>
<td>94.5</td>
</tr>
<tr>
<td></td>
<td>3.00 2.90 1.01 3.93</td>
<td>96.7</td>
</tr>
<tr>
<td></td>
<td>4.00 4.17 1.01 3.93</td>
<td>104.3</td>
</tr>
</tbody>
</table>

a. Mean value of five measurements.

### Possible CL reaction mechanism

Based on the enhancement effects of catecholamines on the CL from the reaction of luminol with KIO₄ in an alkaline solution, a new flow injection CL method was developed for the determination of dopamine, adrenaline and dobutamine. The present method was applied to the analysis of three catecholamines in pharmaceutical samples and blood plasma with satisfactory results.

### Conclusion

Based on the enhancement effects of catecholamines on the CL from the reaction of luminol with KIO₄ in an alkaline solution, a new flow injection CL method was developed for the determination of dopamine, adrenaline and dobutamine. The present method was applied to the analysis of three catecholamines in pharmaceutical samples and blood plasma with satisfactory results.

### Acknowledgements

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