Determination of Epinephrine Using Sodium Iodate in Chemiluminescence

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Epinephrine was determined using a lab-made chemiluminescence (CL) system with air pump. Luminol-sodium IO_4^- chemiluminescence system was employed to produce the luminescence of epinephrine. In the reaction, epinephrine was oxidized to produce superoxide or singlet oxygen by periodate in alkaline solution, which enhanced CL of luminol. For optimization, various buffers, such as phosphate, borate, and tris, were studied in this experiment. Compared to NaOH, the phosphate and borate buffer showed better reproducibility with similar sensitivity. Small amount of sample, 22 μL, was required for a measurement. The limit of quantification for epinephrine was obtained to be ~10^{-9} g/mL after optimization.

Key Words : Chemiluminescence, Epinephrine, Luminol, Sodium iodate

Introduction

Epinephrine is known as catecholamine, which is the major component of adrenal medullar, and it has been used as an indicator for pheochromocytoma and neuroblastoma. For clinical purpose, high sensitivity and small sample consumption are required to determine epinephrine quantitatively because of limited sample volume and low concentration range of a few ng/mL in biological and clinical samples. Electrochemical detection (ED),[1-7] fluorescence detection,[8-11] mass spectrometry,[12,13] and chemiluminescence[14-17] are often used in chromatographic separations to determine catecholamine and related compounds, such as epinephrine (E), norepinephrine (NE), and dopamine (DOPA). For the biological application of ED, the release of the catecholamine, DOPA, E and NE from single vesicles can be detected electrochemically using a carbon-fiber electrode placed adjacent to a cell.[18-22] Since the native fluorescence of catecholamines with phenolic functional group shows a short Stokes shift, low sensitivity, and selectivity, the fluorescence detection with pre- and post column derivatization is more substantial and therefore it is applied more widely in present days. Though the derivatization method provides high sensitivity for DOPA and NE, it can't be used for epinephrine and other metabolites that have no primary amino groups.[6]

For the determination of epinephrine, chemiluminescence (CL) has been employed in which the excited analyte species were produced by chemical reactions with oxidation agent, and the resulting emission was measured.[23-25] Furthermore, in chromatographic application, luminescence of terbium ion was applied to determine catecholamines, and E, NE, DOPA, etc. in urine samples after the compounds of interest were separated by capillary electrophoresis.[6]

An attractive feature of CL technique is the simplicity of instrumentation. Luminescence detection for those compounds, compared with ED and fluorescence, can provide higher sensitivity and robustness due to the almost zero background emission, non-contact transducer, and direct reaction. Chemiluminescence (CL) combined with flow injection analysis (FIA) system even shows excellent sensitivity, rapidity, continuous and real time monitoring analysis; these advantages of CL have been applied to industrial, environmental and clinical fields.[26-28] Most of those CL reaction has been done by luminol/H_2O_2 system with the aid of catalytic reaction in the presence of trace metal ions, such as Fe, Cu, Co, etc. However, the hydrogen peroxide which influences the emission stability is known as relatively unstable.

In this work, the luminol/IO_4^-/OH^- CL system is used to determine trace amount of epinephrine for chemiluminescence reaction using a lab-built CL system with an air pump for the sample injection. The CL system equipped with the air pump has a function of high sensitivity, on-line analysis, minimization of sample, and reagents volume, and it makes possible to apply the system not only to environmental and semiconductor but also to clinical or biological sample analysis.[26] Use of iodate instead of hydrogen peroxide with luminol immobilized on anion exchange resin greatly enhanced the CL emission when epinephrine was determined.[29] However, sensitivity was ruined due to its poor stability on the surface of resin although it showed advantage of simplifying flow injection CL system. In this experiment, iodate was prepared in alkaline solution for better stability and mixed with luminol prior to be used. Optimization of the system for this method was performed by changing various factors, such as buffer, pH, and trace metal interferences.

Experimental

Instrument. Chemiluminescence (CL) flow injection system was described in the previous paper,[26] except sample injection system. The sample was injected through the air pump developed in our laboratory, and the reaction reagents, luminol and IO_4^-, were delivered into a reaction cell using a peristaltic pump (Instech OEM, USA) from a reservoir through Teflon tubing (1 mm i.d.). Since the flow rate of the

syringe pump was very low (~μL min^{-1}), a sample droplet slowly formed at the tip of the capillary and then fell into the cell by gravity for injection. The amount of sample injected for one measurement was about 22 μL. A Y-shaped element was used to mix luminol and IO_4^-, which positioned at the inlet of the reaction cell. The cell was made of quartz, so the emission can be detected through a bottom window. It had a cylindrical body (10 mm i.d., 8 mm in height, 2 mm in thickness) and a flat, transparent quartz window (1.0 mm in thickness) at the bottom. A mini peristaltic pump (APT instrument, USA) was used to drain the reacted reagent from the cell. The top of the reaction cell was open to inject sample. The luminol-IO_4^- reagent was changed after each measurement for reproducible quantitative analysis throughout this experiment.

The luminescence emission was detected by a PMT and the output signal was transferred to a data-acquisition board including A/D converter and displayed using lab-made graphics software.

Reagents. Luminol (5-amino-2,3-dihydrophtaazine-1,4-dione, Aldrich Chem. Co., USA) of 0.05 M was prepared in a buffer. IO_4^- solution was prepared by dissolving Na IO_4^- (Aldrich Chem. Co., USA) and mixed with luminol in a 1:1 volume ratio, and then added to the reaction cell. 1×10^{-3} g/mL of epinephrine was prepared by dissolving 0.001 g in 100 mL water. Stock solution of 1,000 μg mL^{-1} for Fe was prepared from chloride salts in 1% HCl solution. All buffers and standard working solutions were prepared using 18.3 MΩ doubly distilled deionized water (Milli-Q, Millipore, USA). Standard addition method was employed throughout this work.

Results and Discussion

Optimization

Concentration of sodium iodate: Major factors influencing emission intensity in chemiluminescence (CL) reaction were luminol, pH, oxidant, and catalyst. Optimum concentration of luminol was obtained to be 0.01 M which is the same as that for luminol-H_2O_2 CL system. At this condition, the concentration of iodate was optimized at 0.01 M by changing the concentration from 0.005 to 0.05 M, as shown in Figure 1. Since the iodate, organic oxidant, was colorless and showed no absorption in high concentration, it shouldn't make any interference for transmitting CL emission, not as other oxidants, such as potassium permanganate or Ce(IV) solution. From the figure, the CL emission was stable with relative standard deviation of ±8.0% in the luminal-iodate system. The improvement of stability to apply this CL system to biological samples was very important because small amount of sample can make the measurement unstable.

Selection of buffer. Use of iodate for CL reaction of epinephrine could require different chemical environment for alkaline condition, so various kinds of buffers, such as tris buffer, boric acid, and phosphoric acid, were tested and the results of five measurements for each buffer are shown in Figure 2. If only NaOH was used for the determination of 1 × 10^{-5} g/mL of epinephrine, the CL emission was unstable, resulted in relatively poor reproducibility of 4.92% RSD (relative standard deviation). Whereas tris produced very poor signal intensity with RSD of 13.9%, both phosphoric acid and boric acid generated strong intensity with excellent reproducibility of 0.76% and 3.69% RSD, respectively. Since the tris buffer produced poor sensitivity probably due to quenching luminescence, poor reproducibility was observed. Considerably, inorganic buffers produced stable signals, compared to NaOH. In this experiment, phosphoric acid was used for the detection of epinephrine because of its good reproducibility with enough sensitivity as well as biocompatibility.

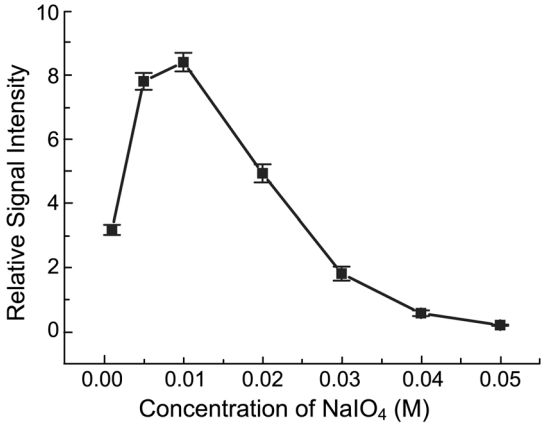

Figure 1. Optimization of the concentration of sodium iodate in chemiluminescence; PMT 500 V, op amp 10^5, 0.01 M luminol (in borate buffer), Epinephrine 1×10^{-5} g/mL.

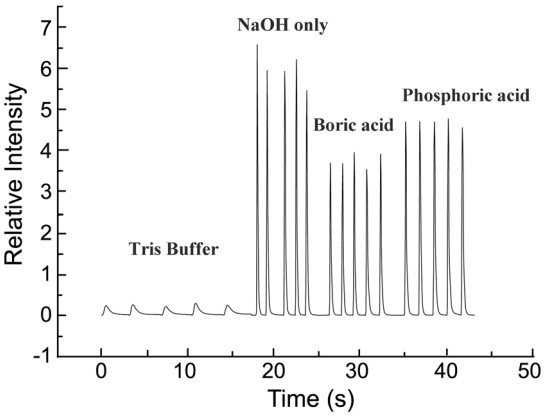

Figure 2. Effect of buffer on chemiluminescence reaction; PMT 450 V, op amp 10^5, 0.01 M luminol (in 0.1 M buffers), 0.01 M $NaIO_4$, Epinephrine 1×10^{-5} g/mL.

At this condition, pH of the phosphate buffer was optimized at 13, as shown in Figure 3, which is higher than the optimized condition of NaOH, pH 10. Interestingly abrupt signal enhancement, almost hundred times, was observed when the pH was shifted from 10 to 13. Therefore, the optimization of pH in phosphate buffer was very crucial to sensitivity, when iodate was used.

Application to determine epinephrine. At the optimized condition, detection of epinephrine was performed. Although

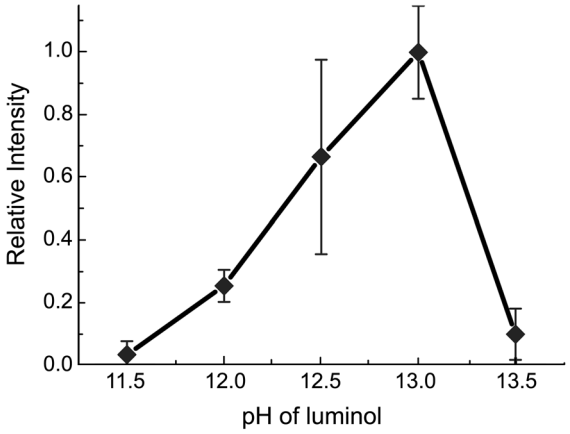

Figure 3. Optimization of pH in chemiluminescence reaction using luminal-iodate.

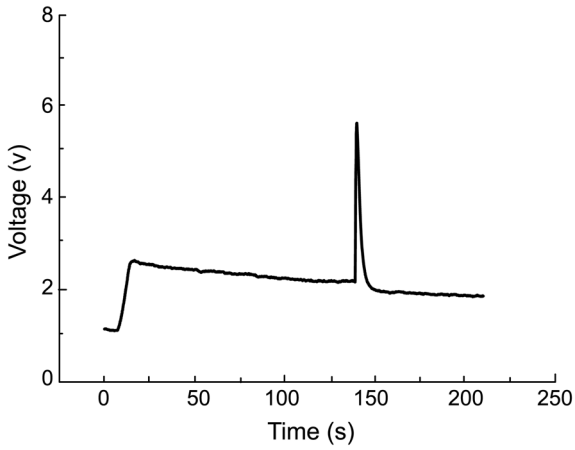

Figure 4. Time-dependent chemiluminescence of epinephrine (5×10^{-5} g/mL) in luminol-iodate system.

no analyte was injected, background was high and decreased with time. The cause of background was unclear at this moment. Noticeably, the luminol and iodate were mixed before epinephrine was injected. Fortunately, the rate of background decrease was steady and slow, which made it possible to do subtract background. Therefore, the net signal intensity of analyte was determined by background subtraction at a certain fixed time after injection, as shown in Figure 4. The reaction rate of epinephrine with luminol-iodate was fast enough to measure the peak height of injected sample.

The calibration curve for epinephrine in the concentration range of $\sim 10^{-9}$ g was shown in Figure 5. The minimum detectable concentration for epinephrine was about 5×10^{-10} g/mL with reproducibility of ±8.3% when 22 μL of sample was injected, which satisfied the requirements in sensitivity for clinical and biological application.

Interference of Fe ions. Since the lumion-iodate can react with trace amount of metallic catalysts, such as Fe, Cu, Co, etc., the interference of those metal ions should be considered when applied to biological sample. In this experiment, Fe^{2+} and Fe^{3+} ions of 0, 1, 2.5, 5, 10, and 20 μg/mL were added to epinephrine solution. Figure 6 is showing the

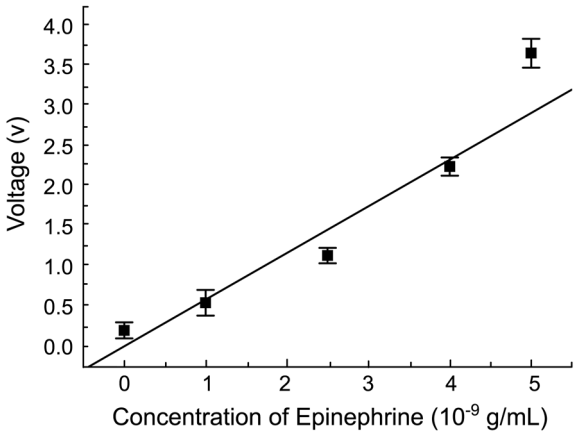

Figure 5. Response curve for epinephrine in chemiluminescence.

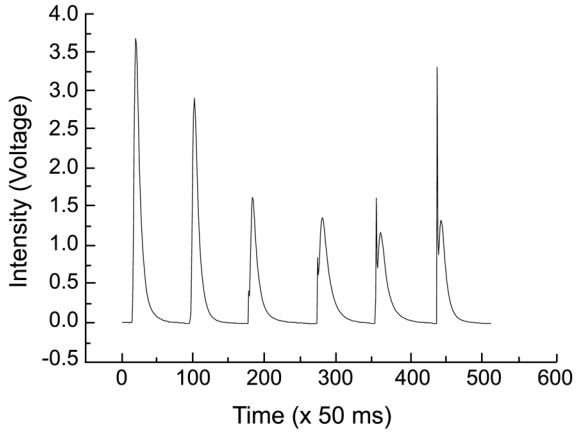

Figure 6. Peaks of epinephrine (1×10^{-5} g/mL) when Fe^{3+} ions of 0, 1, 2.5, 5, 10, and 20 μg/mL were added in turn.

change of emission peaks of epinephrine in the presence of Fe^{3+}. The signal intensity of epinephrine was decreased when the concentration of Fe^{2+} or Fe^{3+} ion was increased. Noticeably, peaks of Fe^{3+} were appeared faster and sharper compared to epinephrine. Conclusively the reaction rates of them were different in luminol-iodate solution and the peaks

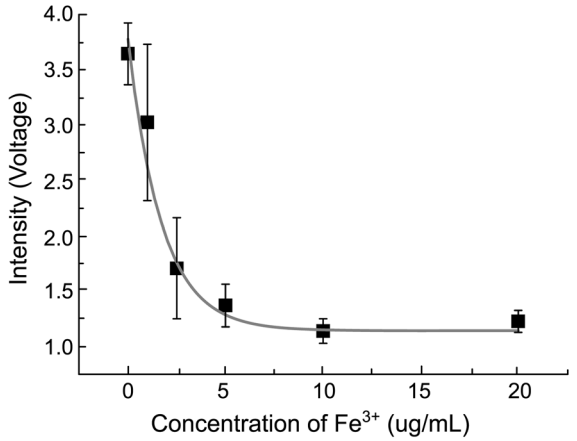

Figure 7. Interference effect of Fe^{3+} ion on the integrated peak area of epinephrine; PMT 600 V, op amp 10^5, 0.01 M luminol (in phosphate buffer), 0.01 M $NaIO_4$, epinephrine (1×10^{-6} g/mL).

were partially separated for quantification.

Figure 7 is showing the interference effect of Fe^{3+} ion on the integrated peak area of epinephrine. The error bars are representing the reproducibility of the integrated peak area when 4 measurements were performed. As shown in the figure, the signal intensity of epinephrine was significantly decreased up to 5 μg/mL of Fe^{3+}, and then stabilized at higher concentration probably due to matrix buffer effect.

In conclusion, luminol-iodate system was successfully applied to determine epinephrine in chemiluminescence. The limit of detection was obtained below 10^{-9} g/mL at the condition of 0.01 M sodium iodate, and phosphate buffer. Since the background was decreased with time, background subtraction should be employed for quantification. Interference effect of Fe ions was studied. The peak of epinephrine can be partially separated from the Fe^{3+} peak.

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