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Pre-evaluation of metal ions as a catalyst on chemiluminometric sequential injection analysis with luminol–H_2O_2 system

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ABSTRACT

Catalytic effect of metal ions on luminol chemiluminescence (CL) was investigated by sequential injection analysis (SIA). The SIA system was set up with two solenoid micropumps, an eight-port selection valve, and a photosensor module with a fountain-type chemiluminescence cell. The SIA system was controlled and the CL signals were collected by a LabVIEW program. Aqueous solutions of luminol, H_2O_2, and a sample solution containing metal ion were sequentially aspirated to the holding coil, and the zones were immediately propelled to the detection cell. After optimizing the parameters using 1×10^{-5} M Fe^{3+} solution, catalytic effect of some metal species was compared. Among 16 metal species examined, relatively strong CL responses were obtained with Fe^{3+}, Fe^{2+}, VO^{2+}, VO_3^-, MnO_4^-, Co^{2+}, and Cu^{2+}. The limits of detection by the present SIA system were comparable to FIA systems. Permanganate ion showed the highest CL sensitivity among the metal species examined; the calibration graph for MnO_4^- was linear at the concentration level of 10^{-8} M and the limit of detection for MnO_4^- was 4.0×10^{-10} M (S/N = 3).

1. Introduction

Sequential injection analysis (SIA) has been proposed by Ruzicka and Marshall [1], and it is known to be the second generation of flow injection analysis (FIA). The principle of SIA is that the liquid zones sequentially aspirated by a multi-position valve are propelled to any kind of detector and detected. Chemical reactions would accompany during the operations of the valve and the solution sending. Many research have been reported on the basis of the SIA concept [2,3]. According to the zone handling technique in SIA, instability of the baseline necessarily accompanied when the detection uses external light source. The detection sensitivity, therefore, is restricted by the baseline fluctuation, and limit of detections are usually inferior to FIA.

Chemiluminescence (CL) is one of the powerful detection techniques in analytical chemistry, and various types of reagents and reactions have been proposed [4]. Tucker et al. demonstrated the availability of chemiluminescence in SIA at early stage using luminol as a CL reagent and with a fountain-type detection cell [5]. As for the CL detection in SIA, Min et al. combined enzyme reaction immobilized in a certain port of the multi-position valve to detect glucose and lactic acid [6], and Liu and Hansen also reported the detection of D-glucose with an immobilized enzyme [7]. Immobilized enzyme was also used for the detection of diamines [8]. Economou et al. used soluble enzyme to generate H_2O_2 for the luminol CL reaction, where Co^{2+} was used as a metal catalyst [9,10]. Semi-SIA, where a liquid flow of luminol solution was merged in the SIA flow, was also proposed for the assay of antioxidative activity [11,12]. Magnetic microbeads were also utilized in a sense of bead injection for the assay of vitellogenin [13] and nonionic surfactant [14]. In the SIA using luminol CL as a detection reaction, hydrogen peroxide was mainly aimed to detect, and Co^{2+} was usually used as a metal catalyst.

In this study, we aimed at evaluating the catalytic effect of several metal ions on luminol CL in sequential injection analysis. The SIA parameters were investigated using 1×10^{-5} M Fe^{3+} as a model metal ion catalyst. Among 16 metal species examined, relatively strong CL responses were obtained with Fe^{3+}, Fe^{2+}, VO^{2+}, VO_3^-, MnO_4^-, Co^{2+}, and Cu^{2+}. The limit of detection for MnO_4^- reached down to 4.0×10^{-10} M. Availability of chemiluminescence detection in SIA using luminol as a CL reagent has been demonstrated. The detection sensitivity by the present SIA was compared to the FIA detection systems.

2. Experimental

2.1. Apparatus

An SIA system was set up with two solenoid micropumps (Bio-Chem Valve 120SP1225, Bio-Chem Fluidics, Boonton, NJ, USA), an eight-port selection valve (Hamilton MPV, Reno, NV, USA), and a photosensor module (Hamamatsu Photonics H5784, Hamamatsu, Japan). A fountain-type flow cell with its size of 1.0 cm $\phi \times 2$ mm depth made of transparent acryl block was attached on top of the

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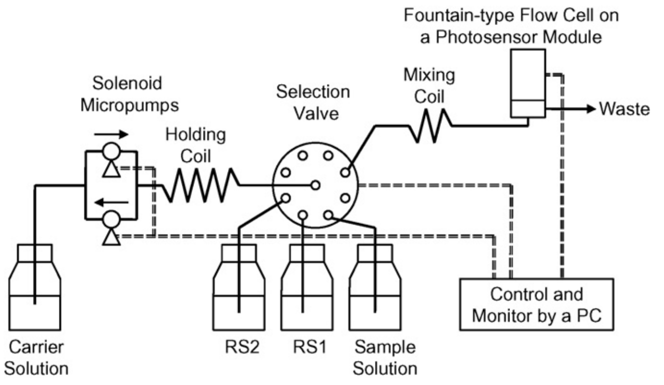

Fig. 1. Sequential injection manifold for the detection of metals by luminol chemiluminescence. RS1: H_2O_2 solution, 5×10^{-5} M. RS2: luminol solution, 5×10^{-3} M in 0.1 M borax-NaOH (pH 9.5). Holding coil: 0.75 mm i.d. × 5.6 m (2.5 mL); mixing coil: 0.75 mm i.d. × 0.35 m (0.15 mL). Aspiration volumes and the sequence: 150 μL RS2, 150 μL RS1, and 200 μL sample solution. Flow rate: 2.5 mL min^{-1}. Other conditions are written in the text.

window of the photosensor module. Teflon tubing (0.75 mm i.d.) was used to connect the components and to flow the solutions in it. The components were connected to a personal computer with a USB-6008 interface (National Instruments, Austin, TX, USA). A laboratory-made program written by LabVIEW software Version 7.1 (National Instruments) controlled the components and collected the measurement data. Chemiluminescence signal from the photosensor module was collected every 0.5 s at 0.7 V control voltage of the photosensor module, and was recorded by the LabVIEW program. The SIA system is schematically illustrated in Fig. 1.

2.2. *Reagent*

Luminol and hydrogen peroxide solution (30%) were obtained from Kanto Chemical (Tokyo, Japan). Sodium tetraborate was from Wako Pure Chemical Industries (Osaka, Japan). Metal standard solutions (100 mg L^{-1} or 1000 mg L^{-1}) were obtained from Wako. Metal salts of $Al(NO_3)_3 9H_2O$, $Cr(NO_3)_3 9H_2O$, $MnSO_4 5H_2O$, $Fe(NH_4)_2(SO_4)_2 6H_2O$ and $VOCl_2$ were also from Wako, and used after dissolving in 0.1 M nitric acid solution or water. The potassium permanganate obtained from Wako was used after recrystallization in water. The metal solutions were used after dilution with the purified water. Other reagents were of analytical grade. Water used was ion-exchanged, distilled, and treated with Milli-Q Labo system (Nihon Millipore, Tokyo, Japan).

2.3. *Procedure for the sequential injection analysis*

A luminol solution with its pH adjusted with 0.1 M borate buffer solution, a hydrogen peroxide solution, and a sample solution containing a certain amount of metal ion were sequentially introduced into the holding coil using the particular ports of the selection valve; one of the solenoid micropumps was used to aspirate the solutions. The solutions held in the holding coil were immediately propelled toward the detection cell by using another solenoid micropump; purified water was used as the carrier stream. When the propelling was started, the chemiluminescence was detected in a flow cell and the CL response was recorded by the LabVIEW program.

3. Results and discussion

3.1. *Set up of the SIA system*

Two solenoid micropumps were used in this SIA system. The pumps were used individually; one is for the aspiration of the solutions through the eight-port selection valve, and the other is for propelling the solution toward the CL detection cell. The stepwise volume of the propelling solution by the solenoid micropump was set at 25 μL. The volume of the holding coil was 2.5 mL, which was enough for the sequential aspiration of the solutions, and relatively large volume of the holding coil can reduce the pulse of the flow. Three ports among the eight on the MPV selection valve was used for the sample solution, reagent solution (luminol + borate buffer), and H_2O_2 solutions, respectively. One port of the MPV selection valve was connected to the flow cell as is illustrated in Fig. 1.

3.2. *Optimization of the SIA parameters*

Luminol itself shows the chemiluminescence at alkaline solution in the presence of hydrogen peroxide. Iron(III) ion was used as a model metal ion to examine the catalytic effect of metal ions on luminol chemiluminescence. Effect of pH on chemiluminescence was firstly examined in the absence and presence of Fe^{3+} in the sample solution. The results are shown in Fig. 2. The chemiluminescence signal was weak at neutral pH region even in the presence of Fe^{3+}, and the blank signal was observed at alkaline pH conditions. When the solutions were aspirated to the holding coil in the order of luminol, H_2O_2, and Fe^{3+} solutions at pH 9.5, Fe^{3+}-catalyzed luminescence signal were obtained in the presence of Fe^{3+}, as is shown in Fig. 3b, while a negligible signal was observed in the absence of Fe^{3+} (Fig. 3a). The reproducibility was much worse at higher pH conditions because of the hydrolysis of the metal ion. The pH condition at 9.5 was chosen; a 0.1 M borax–NaOH buffer was used to control the pH afterward.

The effect of H_2O_2 concentration on the CL intensity was examined in the concentration range between 5×10^{-6} M and 1×10^{-4} M; the results are shown in Fig. 4. The highest CL intensity was observed at 5×10^{-5} M H_2O_2 with a negligible blank signal, the CL intensity further decreased at H_2O_2 concentration over 1×10^{-4} M which is the highest concentration plotted in Fig. 4. The H_2O_2 concentration was set at 5×10^{-5} M. The effect of luminol concentration was also examined in the concentration range between 1×10^{-4} M and 1×10^{-2} M. Stable chemiluminescence was observed at luminol concentration over 1×10^{-3} M with a negligi-

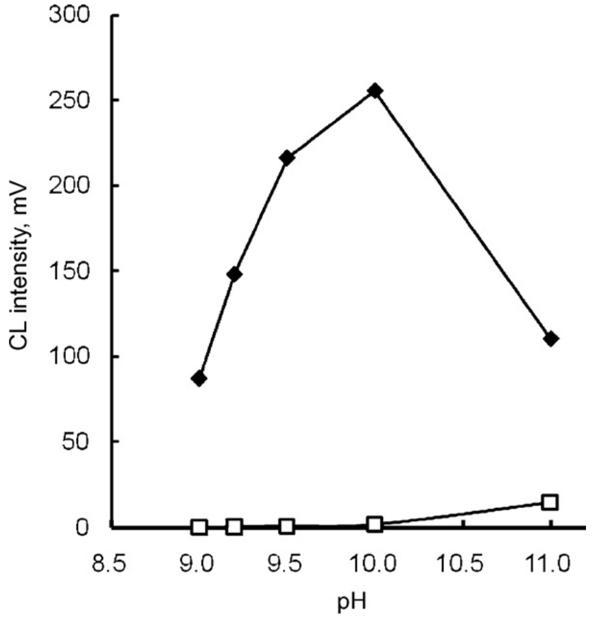

Fig. 2. Effect of pH on chemiluminescence intensity. [Fe^{3+}]: (□) none; (◆) 1×10^{-5} M. The SIA conditions except pH of the luminol solution are the same in Fig. 1.

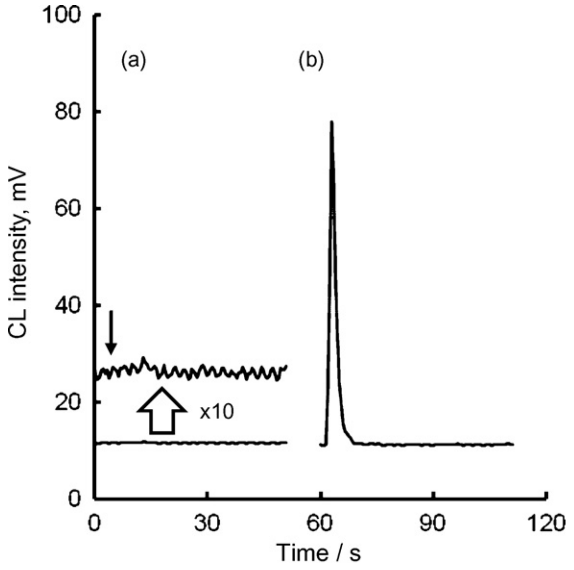

Fig. 3. SIA signals obtained with a luminol solution with its pH at 9.5. [Fe^{3+}]: (a) none—the propelling started at 0 s. (b) 1×10^{-5} M—the propelling started at 60 s. Signal around 4 s for the blank solution, at which time a metal ion-catalyzed signal would be expected, is indicated with an arrow.

ble blank signal. Therefore the concentration of luminol was set at 5×10^{-3} M.

In the SIA system applying three reagent zones, the order of the aspiration of the reagent solutions is also one of the important parameters as is pointed out [5]. The results are summarized in Table 1. Metal ions work as reaction catalysts on the luminol CL reaction, and overlapping of the solution zones by mixing/diffusion is predominant on the CL intensity. When the solutions were introduced into the holding coil in the order of Fe^{3+}, H_2O_2, and luminol solutions, highest CL intensity was observed, as can be read in Table 1; sufficient mixing around Fe^{3+} zone would have given the most intense CL signal. However, the blank signal was observed and the Fe^{3+}-catalyzed signal was a broad and tailing one; the CL signal started rising about 10 s from the start of the solution propelling and returned to the baseline around 35 s. It would be attributed to the zone broadening of Fe^{3+} zone, which is more deeply aspirated into the holding coil. On the other hand, relatively high CL signal was also observed when the solutions were aspirated in the order of luminol, H_2O_2, and Fe^{3+} solutions. In this aspiration order, the zone of Fe^{3+} reached to the detection cell in the earliest, and then the other solutions followed. Thus the CL response was fast and the signal was narrow. The results also indicated that catalyzed chemiluminescence emitted around Fe^{3+} zone. The difference in the CL intensity between the two sequences would have reflected the sufficient mixing of the three zones. On the compromise between the intense CL signal and the CL signal broadening, the aspiration sequence of luminol, H_2O_2, and Fe^{3+} solutions was chosen.

Table 1
Sequence of the aspiration of solutions on CL intensity.

Sequence of aspiration	CL intensity/ mV	Blank CL/ mV	Note
H_2O_2–luminol–sample	36	0	
Luminol–H_2O_2–sample	217	0	Good reproducibility
H_2O_2–sample–luminol	94	0	
Luminol–sample–H_2O_2	25	7	
Sample–luminol–H_2O_2	15	13	High blank signal
Sample–H_2O_2–luminol	357	2	Blank signal with tailing

Concentration of Fe^{3+} in the sample solution: 1×10^{-5} M. Volume: 150 μL each.

The volumes of the zones were also examined in the range between 50 μL and 300 μL, at which the order of the aspiration was luminol, H_2O_2, and Fe^{3+} solutions by changing the aspiration time by the LabVIEW-based SIA program. The CL signal increased with increasing volumes of the luminol and the sample solutions; the CL signals got almost plateau over 150 μL and the aspirating volume of 150 μL and 200 μL were chosen for the luminol and the sample solutions, respectively. Since the H_2O_2 solution is sandwiched between the luminol and the sample solutions, the volume of H_2O_2 solution would be most effective on the CL intensity; the volume affects the mixing of the zones/diffusion of the reagents. The results are shown in Fig. 5. When the sample volume was small, the amount of H_2O_2 would be insufficient and CL intensity was

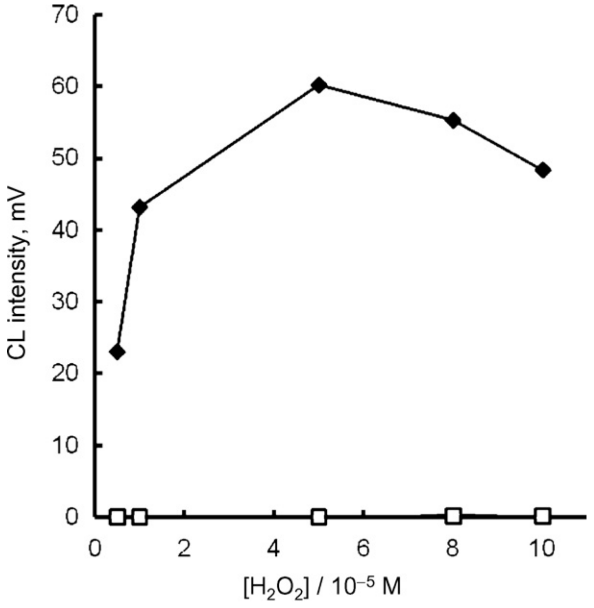

Fig. 4. Effect of the concentration of H_2O_2 in RS1 on CL intensity. [Fe^{3+}]: (□) none; (♦) 1×10^{-5} M. Other conditions are the same in Fig. 1.

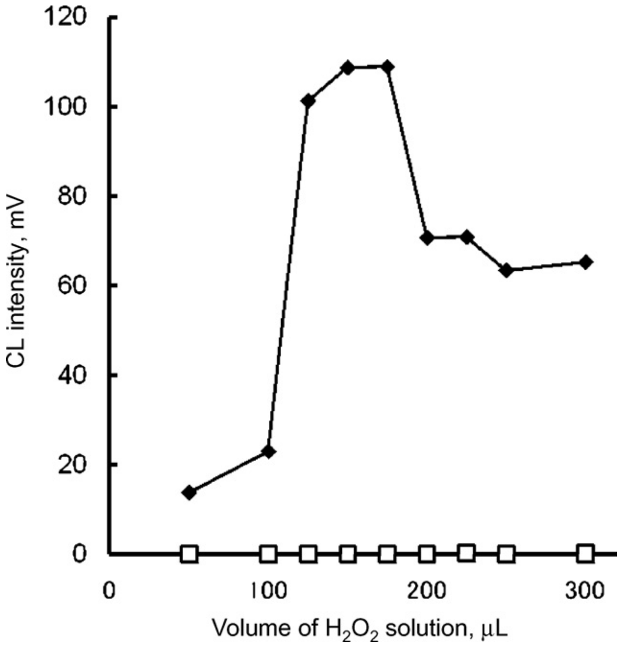

Fig. 5. Effect of the volume of H_2O_2 solution (RS1) on CL intensity. [Fe^{3+}]: (□) none; (♦) 1×10^{-5} M. Other conditions are the same in Fig. 1.

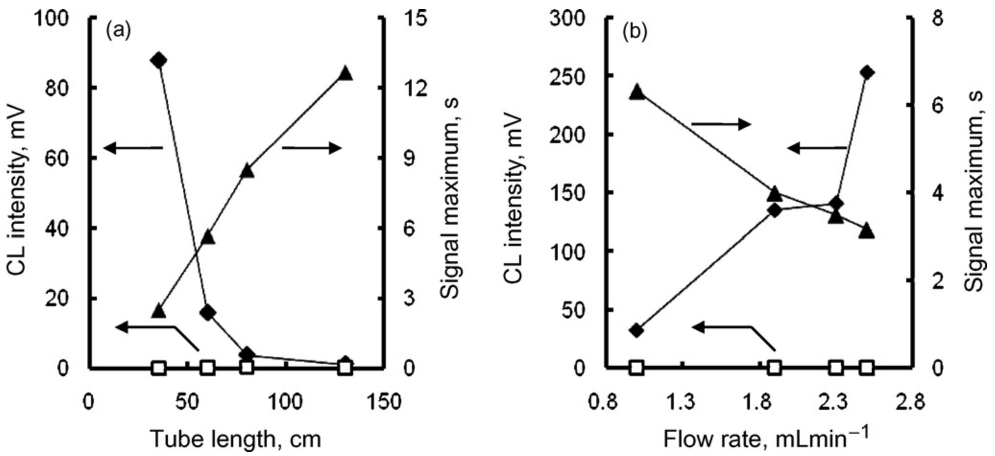

Fig. 6. Effect of the tube length from the selection valve to the detection cell (a) and its flow rate (b). [Fe^{3+}]: (□) none; (♦) and (▲) 1 × 10^{-5} M. Other conditions are the same in Fig. 1.

weak. The intensity got stronger with increasing volume of the H_2O_2 solution around 150 μL, but got weaker beyond the volume. The result would be attributed to the effective mixing/diffusion of the three reagent zones. When the zones of Fe^{3+} and luminol solutions were apart more than needs by the volume of the H_2O_2 solution, the mixing/reaction would be insufficient. The volume of the fountain cell used in this study was 150 μL, and the cell volume would also affect the mixing of the zones and/or the diffusion of the reagents. As a result, the volume of H_2O_2 solution was set at 150 μL.

It is known that luminol chemiluminescence is a fast reaction, and the transfer of the aspirated zones to the fountain detection cell is a key factor to obtain the intense chemiluminescence. Tucker et al. used different diameters of the transferring tube to control the carrying time to the detector [5], and a couple of seconds are shown to be effective for the detection of luminol luminescence. We examined the carrying time by changing the tube length and the flow rate. The results are shown in Fig. 6. A short length of the transfer tube, 35 cm from the selection valve to the fountain cell, and fast flow rate, 2.5 mL min^{-1}, showed the intense chemiluminescence. The carrying time was within a couple of seconds, which agreed with the fast reaction of the luminol chemiluminescence.

The optimized conditions are summarized in Table 2.

3.3. Calibration graph for iron(III)

Calibration graph for Fe^{3+} was drawn at the concentration range of 10^{-5} M level; a linear one was obtained with a correlation coefficient of $r^2 = 0.9884$. Iron(III) can be detected every 50 s with a fair reproducibility. The limit of detection corresponding to the signal-to-noise ratio of three was estimated to be 1.1 × 10^{-7} M. The detection sensitivity in SIA by photometric detections is generally inferior to flow injection analysis; because the zones reach to the detection cell without sufficient mixing and schlieren phenomenon necessarily accompanies. The chemiluminescence detection, however, does not need any external light source, and stable baseline can also be obtained by SIA. Therefore, chemiluminescence reaction is advantageous as a detection tool in SIA.

Table 2
Optimized parameters for Fe^{3+} by the CL-SIA.

Parameter	Optimized value	Examined range
pH of the luminol solution	9.5	9.0–11.0
Sequence of aspiration	Luminol–H_2O_2–sample	
H_2O_2 concentration	5 × 10^{-5} M	5 × 10^{-6}–1 × 10^{-4} M
Luminol concentration	5 × 10^{-3} M	1 × 10^{-4}–1 × 10^{-2} M
Volume of sample solution	200 μL	50–300 μL
Volume of H_2O_2 solution (RS1)	150 μL	50–300 μL
Volume of luminol solution (RS2)	150 μL	50–300 μL
Mixing coil length	35 cm	35–130 cm
Flow rate	2.5 mL min^{-1}	1.0–2.5 mL min^{-1}

3.4. Comparison of metal ions on CL intensity and the limit of detection

Various metal species are known to catalyze the luminol chemiluminescence. Catalytic effect of several metal ions on SIA-luminol chemiluminescence was investigated in this study at the metal concentration of 1 × 10^{-5} M. Iron(III) was used as a comparison standard: the results are summarized in Table 3. Among the 16 metal species examined in this study, relatively strong CL responses were obtained with Fe^{3+}, Fe^{2+}, VO^{2+}, $VO_3{}^-$, $MnO_4{}^-$, Co^{2+}, and Cu^{2+}. The CL intensity with iron is different between Fe^{3+} and Fe^{2+}; Fe^{2+} showed much intense signal than Fe^{3+}. Ussher et al. utilized the CL response for the speciation of iron by FIA [15]. Similar phenomenon was also observed in this SIA study.

Table 3
Comparison of the CL intensity at metal concentration of 1 × 10^{-5} M and the limit of detection.

Metal ion	Relative CL intensity	Limit of detection[a]/M	LOD by FIA/M (Ref.)
Fe^{3+}	1.0 (std)	1.1 × 10^{-7} (6.0)	
Fe^{2+}	17.5	5.0 × 10^{-9} (0.28)	5.0 × 10^{-10} M (Ref. [17])
Al^{3+}	0.01	–	
VO^{2+}	3.1	4.1 × 10^{-8} (2.7)	
$VO_3{}^-$	2.5	4.2 × 10^{-8} (4.2)	1.1 × 10^{-7} M (Ref. [16])
Cr^{3+}	0.29	–	
$CrO_4{}^{2-}$	0.01	–	
Mn^{2+}	0	–	
$MnO_4{}^-$	280[b]	4.0 × 10^{-10} (0.05)	
Co^{2+}	38.6	3.0 × 10^{-9} (0.18)	2.4 × 10^{-10} M (Ref. [17])
Ni^{2+}	0	–	
Cu^{2+}	20.9	2.0 × 10^{-9} (0.13)	
Zn^{2+}	0	–	
Ag^{+}	0	–	
Cd^{2+}	0.01	–	
Pb^{2+}	0.01	–	

[a] S/N = 3; values in parentheses are the ones at μg L^{-1}.

[b] Comparison of the slope of the calibration graphs.

Calibration graphs for the sensitive metal ions were drawn and the limit of detection (LOD) was estimated on the basis of signal-to-noise ratio of three. Satisfactorily results including the linearity and the reproducibility were obtained. As an example, calibration graph for MnO_4^- was linear at the concentration range at 10^{-8} M level with a correlation coefficient of $r^2 = 0.9858$. The LODs for some metal species are also summarized in Table 3; some of them were compared to the ones reported in flow injection analysis without any enhancing reagent. The limits of detection in the present SIA study are almost comparable to the ones by FIA. The chemiluminescence detection is proved to be advantageous as a detection system in sequential injection analysis rather than absorptiometry.

4. Conclusions

In this study, advantages of luminol chemiluminescence are indicated as a detection tool to realize high sensitivity in sequential injection analysis; no necessity of external light source realized the stable baseline even in the presence of the sequential zones. Some variables contributing to the intense chemiluminescence have been investigated, and catalytic effect of several metal ions on luminol chemiluminescence has also been demonstrated in the present SIA system. Metal species of Fe^{3+}, Fe^{2+}, VO^{2+}, VO_3^-, MnO_4^-, Co^{2+}, and Cu^{2+} showed strong CL response among 16 species examined, and a calibration graph at 10^{-8} M level was drawn for MnO_4^- with its limit of detection of 4.0×10^{-10} M.

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