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Journal of Chromatography A, 939 (2001) 91–97

JOURNAL OF CHROMATOGRAPHY A

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Ultrasensitive chemiluminescence detection of sub-f*M* level Co(II) in capillary electrophoresis

Yan-Ming Liu, Er-Bao Liu, Jie-Ke Cheng*

Department of Chemistry, Wuhan University, Wuhan 430072, China

Received 3 July 2001; received in revised form 26 September 2001; accepted 26 September 2001

Abstract

A method of on-line ultrasensitive chemiluminescence detection with capillary electrophoresis for Co(II) is reported. Using our newly developed capillary electrophoresis with chemiluminescence detection system and novel mixing mode of the reagents, the effects of field-amplified injection on detection limits of metal ions were studied in detail. The sub-f*M* level ($1.3\cdot10^{-16}$ *M*, 1.6×10^{-24} mol, 1 molecule) detection of cobalt ions in ultradilute solution was performed. The catalytic behavior of the chemiluminescence reaction of luminol and hydrogen peroxide by cobalt ions and the reaction conditions, such as the concentration of luminol, H_2O_2, and pH of chemiluminescence reagent were investigated. The separation of f*M* level Co(II) and trace amounts of Ni(II) was performed successfully.

Keywords: Chemiluminescence detection; Detection, electrophoresis; Field-amplified sample stacking; Cobalt

1. Introduction

Ultrasensitive measurements have potential applications in many research areas, such as chemical analysis, DNA sequencing, molecular dynamics, nanostructured materials, and early disease diagnosis [1]. Current methods for ultrasensitive detection are primarily based on laser-induced fluorescence (LIF) because of its broad applicability and high sensitivity. However, because of the cost of LIF equipment, efforts are also made to introduce fluorophores to nonfluorescent analytes through pre- or post-column derivatization. A disadvantage of LIF is the high background due to the noise from the Rayleigh and Raman scattering and the fluorescent impurities in the solvent.

Recently, capillary electrophoresis (CE) has attracted considerable attention because of its virtues of high resolution, rapid separation, and small analyte consumption, its weakness is thought to be its detection capabilities [2,3]. Metal ion analysis is very important in a variety of research and industrial areas [4]. The analysis of metal ions with CE has recently been developing very rapidly. UV–visible absorption detection is the most commonly used method with sensitivities of 10^{-5}–10^{-6} *M*. An alternative detection method is chemiluminescence (CL), which is characterized by simple and cheap optical systems requiring no light sources, avoiding the effects of stray light and the instability of the light source, and thus providing low background with excellent sensitivity. The above-mentioned features in this approach would bring it close to an ultrasensitive

*Corresponding author. Tel.: +86-27-8768-2291; fax: +86-27-8764-7617.

E-mail address: jkcheng@whu.edu.cn (J.-K. Cheng).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)01329-2

detection system for CE. Recently, the applicability of CL detection in CE has been successfully demonstrated in analysis of amino acids [5–7], peptides [8], and proteins [9–12]. Several reviews on this technique have been published [3,13–16]. In a CE–CL instrumental configuration, the design of CE–CL interfaces, where the mixing of the analyte and the CL reagent, is a crucial factor for achieving good separation efficiencies and sensitivities [13]. Most of the interfaces of CL detection are post-column [3], which are similar to the detector originally developed by Rose and Jorgenson [17]. A new mixing mode for analyte and CL reagent for on-line CL detection of capillary ion analysis has been used by our group [18], and excellent separation efficiency and high sensitivity for analysis of metal ions have been obtained. We further improved the CE–CL apparatus [19], the grounding electrode is put in the four-way joint, instead of in the eluate reservoir. The transfer of the grounding electrode from a point downstream of the detection point to the upstream point significantly improves the performance [13]. In this paper, using the improved apparatus [19] and a novel mixing mode of the analyte and the CL reagent [18] for CE–CL, the effects of field-amplified injection on detection limits of Co(II), Cu(II), and Cr(III) were studied in detail. The catalytic behavior of Co(II) for the CL reaction of luminol with hydrogen peroxide was further studied. Sub-f*M* level (0.13 f*M*) detection of cobalt ions in ultradilute solution was obtained.

2. Experimental

2.1. Reagents and solutions

All the chemicals were of analytical-reagent grade. The 0.01 *M* stock solution of luminol was prepared by dissolving 177.16 mg luminol (analytical-reagent grade, purification by Shanxi Normal University) into 100 ml of 0.1 *M* NaOH. The 0.01 *M* Co(II) stock solution was prepared by dissolving 237.93 mg $CoCl_2 \cdot 6H_2O$ (analytical-reagent grade, Shanghai Chemical Factory) into 100 ml water (pH 3). α-Hydroxyisobutyric acid (HIBA) was obtained from Sigma (St. Louis, MO, USA). All stock solutions were stored cool and dark. All solutions were prepared daily by dilution of the stock solution to the desired concentration. In order to determine Co(II) at the f*M* level, very pure water must be used. Water (18.2 MΩ cm), triply-distilled and purified on the Water PRO PS purification system (Labconco, USA) was used for preparation of all solutions.

2.2. Apparatus

The CE–CL apparatus used consists of a conventional CE system and a CL detection system as described previously in detail [19]. A 0–30 kV power supply (Department of Chemistry, Peking University, Beijing, China) provided the separation high voltage. A capillary (52 cm×50 μm I.D.×375 μm O.D., Hebei Yongnian Optical Fiber Factory, Hebei, China) was used for separation. A 5 cm coating section of one end of the separation capillary was burned and then etched with hydrofluoric acid (HF) for 2.5 h to about 200 μm O.D. (before etch the tip of capillary is sealed by wax to avoid the inner wall is etched). The HF treated end of the separation capillary was then inserted into a reaction capillary (24 cm×530 μm I.D.). These two capillaries were held in a Plexiglass four-way joint. The required CL reagents were delivered by gravity through a reagent capillary (40 cm×320 μm I.D.). The outlet of the reagent capillary was also led to the four-way joint. Plexiglass nuts and polyimide ferrules were used to fix the above mentioned three capillaries inside the four-way joint. The grounding electrode was also put into the joint to complete the CE electrical circuit. The outlet of the reaction capillary was 2 cm lower than the other end to make the solution flow out of the reaction capillary more easily and quickly. A 1 cm detection window was formed on the reaction capillary (3 mm before the point where the inner separation capillary terminated) by burning off the polyimide coating.

The CL emission was collected with a type R928 photomultiplier tube (PMT, Hamamatsu Photonics, Japan). In order to collect the most intensive CL signal, the detection window was situated just in front of the PMT. The photocurrent was fed to a type HX-2 signal magnifier (Institute of Chemistry, Chinese Academy of Sciences, Beijing, China) and then recorded using a 3066 chart recorder (Fourth Instrumental Factory of Sichuan, China). The whole CL detection system was held in a large light-tight

box to exclude stray light. The peak height was used for the analysis.

2.3. Procedures

It is very important to obtain reproducible and uniform inner walls of fused-silica capillaries so as to generate stable electroosmotic flow (EOF) in capillary zone electrophoresis [20]. In this study, the new capillaries were rinsed sequentially with 2 *M* NaOH–CH_3OH, 1 *M* NaOH, 1 *M* HCl, and water for 30 min, and then were equilibrated with the running buffer solution for 30 min [21]. The separation capillary was filled with electrophoretic buffer while the four-way joint and reaction capillary were filled with CL reagents. The sample was injected by electroinjection for 5 s applying a voltage of 10 kV. The separation voltage was 20 kV.

The ultrasensitive detection experiments will not be successful without careful design of the experiments. One of the difficulties associated with them is contamination control [22]. There are many different sources of contamination, such as the containers, the apparatus, and the solvents. If proper precautions were taken, contamination could be controlled. All sample preparations and experimental procedures were carried out in a clean work environment with minimal air flow to disperse aerosols. A pipet was used to dispense the sample, fresh tips were used at each dilution. All solutions were shaken for 1 min after dilution. To avoid contamination, volumetric flasks and other glass containers were soaked in 3.6 *M* nitric acid for 48 h, and rinsed thoroughly with ultrapure water before use. Pipet tips and plastic vials were pretreated in $CHCl_3$, 3.6 *M* HNO_3 for 5 min by ultrasonic wave, rinsed thoroughly with ultrapure water and dried at 40°C. All solutions were carefully filtered through a 0.22 μm pore-size membrane prior to use. To avoid contamination of the running buffer, the capillary tip and platinum wire were rinsed in two different buffer-containing vials before they were placed in the final running buffer.

3. Results and discussion

3.1. Effect of field-amplified injection

On-capillary sample stacking is one of the simplest ways to improve sensitivity in CE [23–25]. Sample stacking is the process that occurs when a voltage is applied along a capillary tube containing a sample plug with a lower conductivity than that of the carrier electrolyte (field-amplified injection). The field-amplified injection has been used to enhance the detection sensitivity of various organic compounds [26–29] and metal ion complexes [30]. However, the field-amplified injection of free metal ions has not been reported. In this paper, Co(II) solutions were prepared in water and in different concentrations of HAc–NaAc solutions, the effect of concentration of HAc–NaAc on sensitivity (detection limit, DL, *M*, $S/N=3$) of Co(II) is shown in Fig. 1A. It can be seen that the sensitivities in water or low buffer concentrations are markedly higher than those obtained in high buffer concentrations. The sensitivity of Co(II) in water is six orders of magnitude higher than that in the $4\cdot10^{-2}$ *M* HAc–NaAc. The results indicate that: (i) the sensitivity of metal ions can be enhanced markedly by utilization of the field-amplified injection, (ii) a large amount of cations (Na^+) and anions (Ac^-) has a great influence on the behavior of Co(II). The lower the medium concentration (ionic strength) is, the smaller influence on Co(II), and the higher resulting sample stacking efficiency. In addition, the electrophoretic peak in water is very sharp and it will broaden in HAc–NaAc solution. Similar results as shown in Fig.

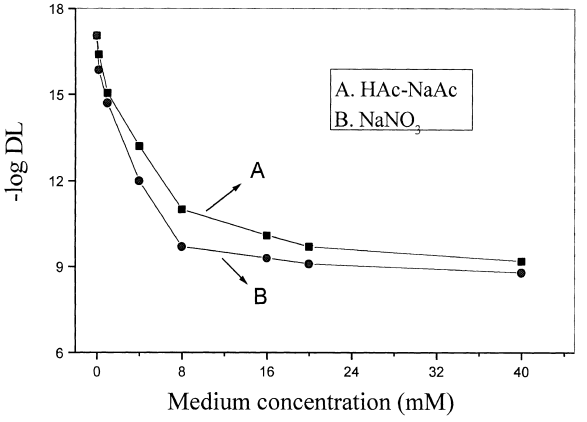

Fig. 1. Effect of concentration of HAc–NaAc and $NaNO_3$ solution on detection limit of Co(II). Conditions: electrophoretic electrolyte, $1\cdot10^{-3}$ *M* luminol+$4\cdot10^{-2}$ *M* HAc–NaAc buffer (pH 5.10); CL reagent, $2\cdot10^{-2}$ *M* H_2O_2+$2\cdot10^{-2}$ *M* Na_2CO_3 (pH 11.5); sample injection 5 s at 10 kV, separation voltage, 20 kV.

1B were also obtained for Co(II) prepared in $NaNO_3$ solution. It can be seen in Fig. 1 that the ionic strength of $NaNO_3$ solution is higher than that of the HAc–NaAc solution at the same concentration, therefore, the $NaNO_3$ solution has more influence on detection sensitivity of Co(II). To further investigate the effects of field-amplified injection on metal ions, Cu(II) was studied in different concentrations of HAc–NaAc and $NaNO_3$. Similar results were obtained, as shown in Fig. 2. The detection limit was improved five orders of magnitude and reached 10^{-11} *M*. In addition, the experimental results of Cr(III) show that the detection limit was improved approximately by six orders of magnitude and reached 10^{-14} *M*.

The effect of electrophoretic buffer HAc–NaAc concentrations on CL intensity is shown in Fig. 3. It can be seen that the rise of CL intensity is proportional to the increase of concentration of electrophoretic buffer. The increase of concentration of electrophoretic buffer results in a large difference between the conductivity of sample solution and that of carrier electrolyte, therefore, higher sample stacking efficiency and signal intensity are produced. But considerable Joule heating from the larger electrophoretic current will be also produced.

3.2. The detection of fM level Co(II)

A $1.3\cdot10^{-16}$ *M* Co(II) solution prepared with water was injected at 10 kV for 5 s, the typical electropherogram is shown in Fig. 4. No electrophoretic peak appears for both blank solution of electrophoretic buffer and ultrapure water. The relative standard deviations (RSDs) of migration time and CL intensity of nine replicate injections for $1.3\cdot10^{-16}$ *M* Co(II) are 0.9 and 26.4%, respectively. From Fig. 4 we can see that the peak is very sharp and symmetrical with a theoretical plate number of $1.9\cdot10^{5}$. These results could be attributed to: (i) sample stacking which makes the analytes stack in a

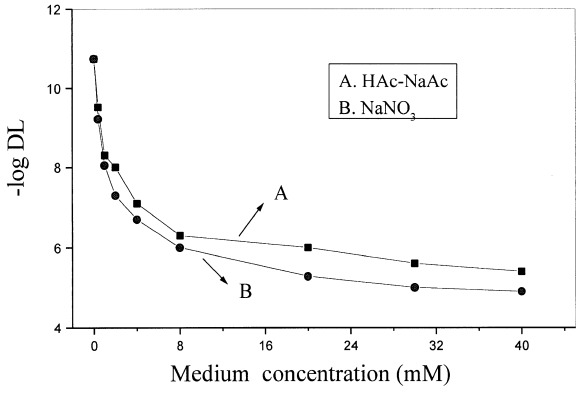

Fig. 2. Effect of concentration of HAc–NaAc and $NaNO_3$ solution on detection limits of Cu(II). Conditions as in Fig. 1.

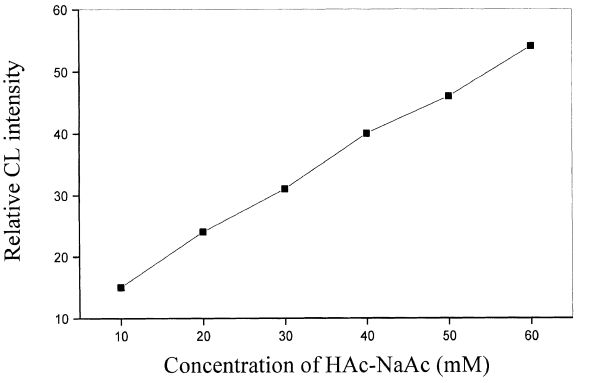

Fig. 3. Effect of concentration of electrophoretic buffer HAc–NaAc solution on CL intensity. Conditions as in Fig. 1.

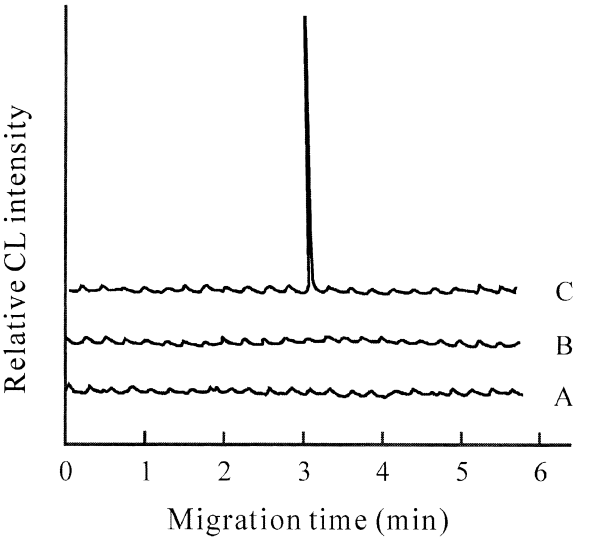

Fig. 4. Electropherogram of $1.3\cdot10^{-16}$ *M* Co(II). A (blank), acetate buffer; B (blank), water; C, $1.3\cdot10^{-16}$ *M* Co(II). Other conditions as in Fig. 1.

very thin zone, (ii) the fast CL reaction kinetics [the catalytic kinetics curve of the luminol and H_2O_2 CL reaction catalyzed by Co(II) shows that the most intensive CL signal was reached at 0.7 s, it decayed very quickly and its half-life is 1.4 s]. The light emission ceases before the analyte diffuses significantly into the reagents in bulk of the interfaces, resulting in relatively narrow peaks. According to the migration time at 10 kV, the apparent volume of injection [31] for 5 s at 10 kV is calculated to be 12.6 nl in which 1 Co(II) molecule contains. This is the highest sensitivity for metal ion analysis. In fact, the number of Co(II) molecules in injection volume is more than one because of field-amplified injection.

3.3. Relation between the concentration of Co(II) and CL intensity

We further investigated the relation between the concentration of f*M* level Co(II) and CL intensity, as shown in Fig. 5. It can be seen in Fig. 5 that the CL intensity increases with increasing the concentration of Co(II).

3.4. Effect of luminol concentration

The CL emission was found to increase with increasing luminol concentration. However, the intensity of emitted light reaches a plateau at the luminol concentration of $1\cdot10^{-3}$ *M* (Fig. 6). At concentrations higher than $4\cdot10^{-3}$ *M*, precipitates in the electrophoretic buffer sometimes appear, therefore, $1\cdot10^{-3}$ *M* luminol was used for the analysis.

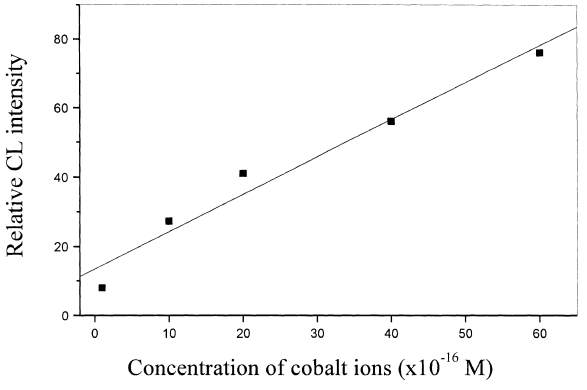

Fig. 5. Relation between the concentration of Co(II) and CL intensity. Conditions as in Fig. 1.

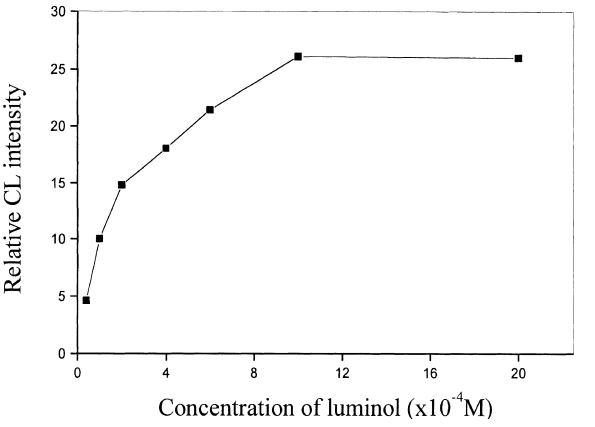

Fig. 6. Dependence of CL intensity on luminol concentration. Conditions: $1.3\cdot10^{-14}$ *M* Co(II), other conditions as in Fig. 1.

3.5. Effect of H_2O_2 concentration

Fig. 7 shows a plot of CL intensity as a function of H_2O_2 concentration. Generally, the higher the concentration of H_2O_2 is, the higher the light emission that can be obtained, but when the concentration reaches $4\cdot10^{-2}$ *M*, not only does the light emission not increase but also many bubbles are generated in the four-way joint. These bubbles lead to unstable current and large baseline noise. A

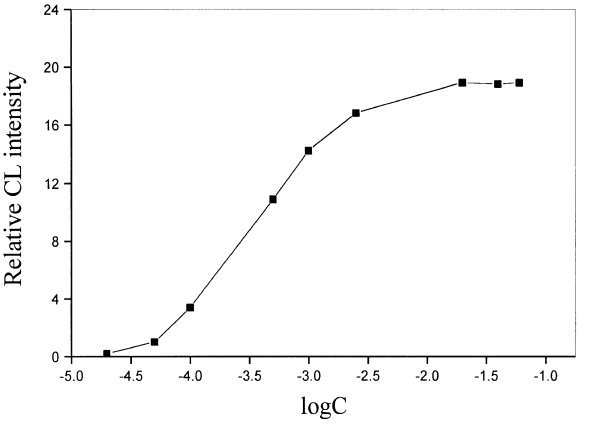

Fig. 7. A plot of CL intensity vs. H_2O_2 concentration. Conditions as in Fig. 6.

concentration of $2 \cdot 10^{-2}$ *M* H_2O_2 was chosen for further studies.

3.6. Effect of pH of CL reagent

In this study, the volume of the sample zone eluting from the 50 μm I.D. electrophoretic capillary is small enough compared to that of the reagent in the 530 μm I.D. reaction capillary, and hence the pH environment of the CL reaction is mainly dependent on the pH of reagent. Therefore the effect of pH on CL intensity was investigated. The CL intensity was found to increase with increasing pH, the maximum response was obtained at pH 11.5, as shown in Fig. 8. When the pH was further raised, however, the CL intensity decreased. It was also found that when the pH reaches 13.0, the CL signal is almost zero. Burdo and Seitz [32] and Chang and Patterson [33] studied the mechanism of cobalt ions catalysis for the reaction of luminol CL with H_2O_2; they also found that the higher pH will enhance the CL signal but that an excessively high pH will lower the CL signal or even produce no signal. Our results are similar to theirs.

3.7. The separation of fM level Co(II) and trace amounts of Ni(II)

The ultrasensitive analysis of metal ions is very important in the environmental and biological analysis. The weak complexing agent HIBA was used to enhance differences between the electrophoretic mobility of metal ions [34]. The separation of f*M* level Co(II) and trace amounts of Ni(II) was carried out. We injected a mixed solution of $5 \cdot 10^{-15}$ *M* Co(II) and $5 \cdot 10^{-7}$ *M* Ni(II) with the electrokinetic sampling; the electropherogram is shown in Fig. 9. It can be seen in Fig. 9 that Co(II) and Ni(II) are separated successfully.

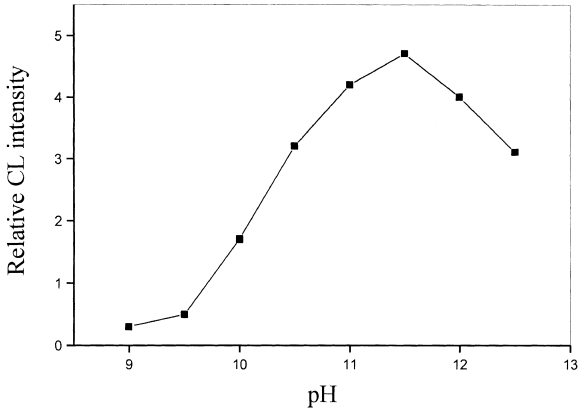

Fig. 8. Effect of pH of CL reagent. Conditions as in Fig. 6.

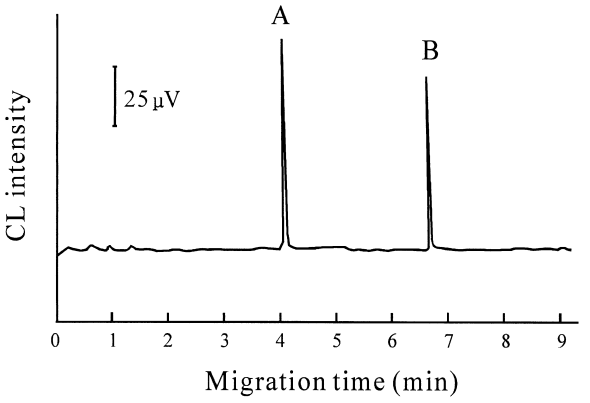

Fig. 9. Separation of $5 \cdot 10^{-15}$ *M* Co(II) and $5 \cdot 10^{-7}$ *M* Ni(II). Peaks: A, Co(II); B, Ni(II). Electrophoretic electrolyte, $1 \cdot 10^{-3}$ *M* luminol+$8 \cdot 10^{-3}$ *M* HIBA+$4 \cdot 10^{-2}$ *M* HAc–NaAc buffer (pH 4.75), other conditions as in Fig. 1.

4. Conclusion

In this paper, using on-line chemiluminescence detection with capillary electrophoresis, we have achieved the f*M* level detection of Co(II). The results obtained could be the highest sensitivity that has ever been reported up to now for metal ion analysis. The significant improvement in sensitivity is attributed to: (i) utilization of the field-amplified injection for enhancing sensitivity, (ii) Co(II) having excellent catalytic behavior for the CL reaction of luminol and H_2O_2, (iii) the CE–CL detection system including the improved apparatus, novel mixing mode of the analyte and CL reagent, and sensitive detector, etc., that has excellent performance. A more systematic study, especially for deep understanding the field-amplified sample stacking of metal ions and the mechanism of CL reaction for CE–CL in the capillary electrophoresis is in progress.

This work demonstrates that capillary electrophoresis with chemiluminescence detection could de-

velop into a simple and new method for ultrasensitive detection of metal ions.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (grant No. 20075017).

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