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Speciation of chromium by in-capillary reaction and capillary electrophoresis with chemiluminescence detection

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Abstract

A sensitive method for the simultaneous determination of chromium(III) (Cr^{3+}) and chromium(VI) (CrO_4^{2-}) using in-capillary reaction, capillary electrophoresis (CE) separation and chemiluminescence (CL) detection was developed. The chemiluminescence reaction was based on luminol oxidation by hydrogen peroxide in basic aqueous solution catalyzed by Cr^{3+} ion followed by capillary electrophoresis separation. Based on in-capillary reduction, chromium(VI) can be reduced by acidic sodium hydrogensulfite to form chromium(III) while the sample is running through the capillary. Before the electrophoresis procedure, the sample (Cr^{3+} and CrO_4^{2-}), buffer and acidic sodium hydrogensulfite solution segments were injected in that order into the capillary, followed by application of an appropriate running voltage between both ends. As both chromium species have opposite charges, Cr^{3+} ions migrate to the cathode, while CrO_4^{2-} ions, moving in the opposite direction toward the anode, react with acidic sodium hydrogensulfite which results in the formation of Cr^{3+} ions. Because of the migration time difference of both Cr^{3+} ions, Cr(III) and Cr(VI) could be separated. The running buffer was composed of 0.02 mol l^{-1} acetate buffer (pH 4.7) with $1{\cdot}10^{-3}$ mol l^{-1} EDTA. Parameters affecting CE–CL separation and detection, such as reductant (sodium hydrogensulfite) concentration, mixing mode of the analytes with CL reagent, CL reaction reagent pH and concentration, were optimized. The limits of detection (LODs) of Cr(III) and Cr(VI) were $6{\cdot}10^{-13}$ and $8{\cdot}10^{-12}$ mol l^{-1} ($S/N=3$), respectively. The mass LODs for Cr(III) and Cr(VI) were $1.2{\cdot}10^{-20}$ mol (12 zmol) and $3.8{\cdot}10^{-19}$ mol (380 zmol), respectively.

Keywords: Chemiluminescence detection; Detection, electrophoresis; Derivatization, electrophoresis; In-capillary reaction; Chromium; Inorganic cations; Inorganic anions

1. Introduction

Chromium exists in different oxidation states in environmental water [1] and soils [2]. The determination of chromium speciation in environmental samples has become very important. Dissolved chromium is usually found in natural waters in two different oxidation states, chromium(III) and chromium(VI). Chromium(III) is an essential trace element for humans, required for the maintenance of normal glucose, cholesterol, and fatty acid metabolism. Also, chromium(III) plays a role in various enzyme reactions. On the other hand, water soluble chromium(VI), in the form CrO_4^{2-} or $Cr_2O_7^{2-}$, is highly irritating and toxic to humans and animals [3]. Its toxic effects include an immediate cardiovascular shock and later effects on kidney, liver, and blood-forming organs. Due to its toxicity and mobility, Cr(VI) has often been considered more problematic than Cr(III) as a contaminant in the environment.

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Therefore, it is necessary for risk assessment, to determine not only the total chromium in the different environmental compartments but also its different oxidation states.

UV–Vis spectrophotometry [4] was employed for Cr(VI) determination and atomic absorption spectrometry (AAS) [5] for total chromium. Other methods have been reported for the determination of Cr(III) and Cr(VI), such as bidirectional electrostacking–electrothermal atomic absorption spectrometry (ETAAS) [6], flame atomic-absorption spectrometry (FAAS) [7,8], solid-phase extraction–liquid chromatography (LC) with UV detection [9,10], and inductive coupled plasma atomic emission spectrometry (ICP-AES) [11]. However, preconcentration of analyte from the matrix prior to measurement or vaporization is necessary for these methods. Capillary electrophoresis (CE) with UV detection has been used to determine Cr(VI) and Cr(III), after chelating with organic ligands to form all anionic complexes [12]. Unfortunately, the sensitivity of this method is not sufficient.

Application of chemiluminescence (CL) for the analysis of chromium in natural water has been reported [13–16]. These methods were based on the chromium(III)-catalysed oxidation of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) by hydrogen peroxide in a basic aqueous solution. In recent years, the CE–CL detection system has received much attention. However, to our knowledge, simultaneous determination of Cr(III) and Cr(VI) with the CE–CL method has not been reported.

In-capillary reaction techniques have been applied in capillary electrophoresis [17–20]. In the in-capillary electrophoresis reaction, different electrophoretic mobilities are used to merge distinct zones of analyte and analytical reagent under the effect of an electric field. The reaction is allowed to proceed within the region of mixed reagents either in the presence or absence of an applied potential, and the product migrates to the detector under the effect of an electric field. Taga and co-workers have reported on amino acids analysis using three types of in-capillary derivatization techniques: at-inlet type derivatization [21], zone-passing derivatization [17] and throughout capillary derivatization [22]. In the zone-passing technique by the in-capillary derivatization method, for instance, a running solution zone was introduced between the sample and reagent zones and the voltage was applied immediately after the introduction of the reagent zone. The derivatization reaction occurred while the reagent zone passed the sample zone and the derivatized amino acids were separated by electrophoresis.

In CE separations performed in untreated fused-silica capillaries, the electroosmotic flow (EOF) is toward the cathode when a positive potential is applied at the injection end across the fused-silica capillary to the detection end. Consequently, cations move toward the cathode with apparent velocity (ν_{app}):

$$\nu_{app,\ cations} = \nu_{eo} + \nu_{ep} \quad (1)$$

where ν_{eo} and ν_{ep} are the electroosmotic flow velocity and the electrophoretic velocity, respectively. In the case of anions, due to the strong attraction by the positive electrode (anode), they flow toward the positive terminal against the electroosmotic flow. The apparent velocity is then expressed as:

$$\nu_{app,\ anions} = \nu_{eo} - \nu_{ep} \quad (2)$$

From Eq. (2), it can be seen that the apparent velocities of anions will be less than the electroosmotic flow velocity. However, the pH of the buffer has a significant effect on electroosmotic flow because it changes the zeta potential. As pH decreases, electroosmotic flow decreases. At pH below ~2, there is no electroosmotic flow in a fused-silica capillary because most of the silanol groups are protonated [23]. In this case, the apparent velocities of anions will be more than the electroosmotic flow velocity.

In this work, a strategy for in-capillary reduction of Cr(VI) ($Cr_2O_7^{2-}$ or CrO_4^{2-}) with acidic sodium hydrogensulfite to Cr(III) (Cr^{3+}) using the zone-passing technique was proposed. Acetate buffer was introduced into the capillary between zones of sample [Cr(III), Cr(VI)] and reductant (HSO_3^-). The voltage was applied immediately after the injection of the reductant zone. In the electric field, chromium(III) migrated to the cathode (detection window), and the chromium(VI) zone moved toward the anode and reacted quickly with the zone reductant (HSO_3^-) to form chromium(III), which then migrated toward the cathode and catalyzed luminol–hydrogen peroxide CL reaction in the detection window. Because of their migration time differences,

chromium(III) and chromium(VI) could be separated and determined.

2. Experimental

2.1. Reagents and solutions

Luminol was from Merck (Darmstadt, Germany). Hydrogen peroxide, acetic acid, sodium acetate, ethylenediaminetetracetic acid (EDTA), sodium bromide, sodium hydrogensulfite, potassium dichromate, chromium trichloride, sodium hydroxide, and hydrochloric acid were purchased from Shanghai Chemical Plant (Shanghai, China). All reagents were of analytical reagent grade purity. Ion-exchanged doubly distilled water was used for the preparation of the luminol solution, hydrogen peroxide solution, sodium acetate buffer (pH 4.7), EDTA solution, NaBr solution, $NaHSO_3$ solution and the chromium standard solutions. Standard solutions of chromium(VI) and chromium(III) were prepared by appropriate dilution from 0.01 mol l^{-1} stock solutions made from potassium dichromate and chromium trichloride, respectively.

The luminol stock solution ($1\cdot10^{-2}$ mol l^{-1}) was prepared by dissolving luminol in 1 mol l^{-1} sodium hydroxide. Hydrogen peroxide stock solution ($1\cdot10^{-2}$ mol l^{-1}) was prepared by 30% hydrogen peroxide. The chemiluminescence reagents were: (A) $1\cdot10^{-3}$ mol l^{-1} luminol, 0.1 mol l^{-1} NaBr and $1\cdot10^{-4}$ mol l^{-1} EDTA with 0.1 mol l^{-1} $NaHCO_3$–NaOH buffer to pH 11.5; and (B) $1\cdot10^{-2}$ mol l^{-1} hydrogen peroxide. Luminol solution (A) and H_2O_2 solution (B) were delivered to CL solution capillary by two microsyringe pumps. The reductant reagent was 1 mol l^{-1} $NaHSO_3$ dissolved in 0.5 mol l^{-1} HCl solution. The running buffer was composed of 0.02 mol l^{-1} acetate buffer (pH 4.7) with $1\cdot10^{-3}$ mol l^{-1} EDTA.

All solutions were filtered through a 0.45-μm membrane filter and degassed by ultrasound before use.

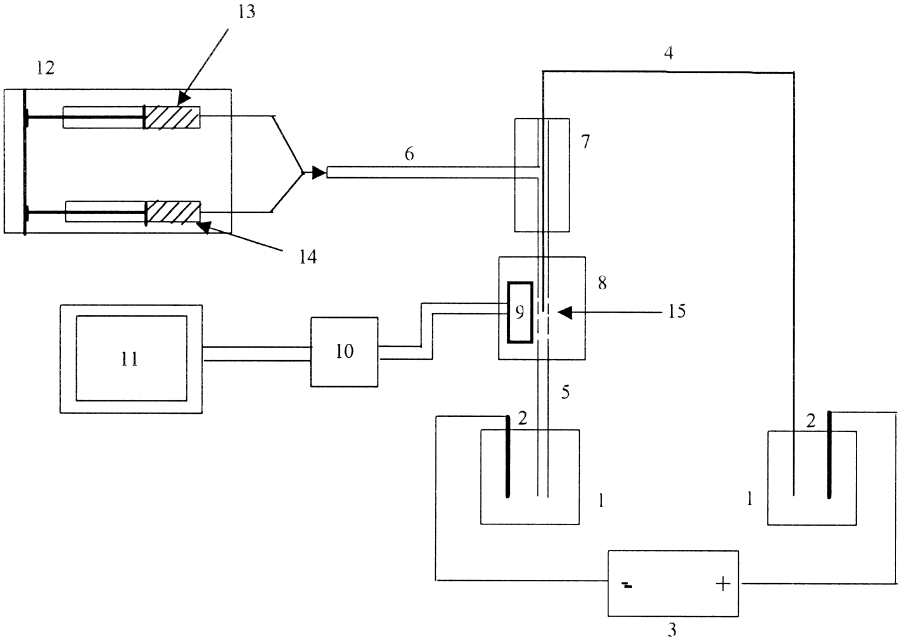
2.2. CE–CL apparatus

All the data were collected using a laboratory-built capillary electrophoresis–chemiluminescence detection system (Fig. 1A), similar to one described in the literature [24]. A 0–30-kV high voltage (HV) power supply (Tianhui Institute of Separation Science, Baoding, China) provided the separation voltage. A fused-silica capillary (60 cm×75 μm I.D.) coated with polyimide (Polymicro Technologies, Phoenix, AZ, USA) was used for separation. A 2-mm section of the end of the separation capillary was burned and then inserted into a reaction capillary (16 cm×530 μm I.D.; Hebei Optical Fiber, Hebei, China). The detection window was formed by burning 4 mm of the polyimide of the reaction capillary and setting it in front of the photomultiplier tube (PMT; Hamamatsu, Japan). The distant between the reaction capillary detection window and the PMT was 3 mm. The PMT of the detector was operated at 800–900 V. A section of the end of the separation capillary was inserted in the middle of the detection window. CL reagents were delivered by a double microsyringe pump (Shanghai Instrument Plant, Shanghai, China) and flowed through a reagent capillary (20 cm×250 μm I.D.; Lanzhou Institute of Chemical Physics, China) to the reaction capillary (Fig. 1B). CL reagent solution was fed at a rate of 10–20 μl min^{-1}. All the capillaries were fixed in place by a plexiglass or PTFE tee connector (400–600 μm I.D.). The end section of the reaction capillary exited the detector and entered a buffer reservoir to complete the circuit. Data acquisition and collection were processed using commercially available software (IFFM–D data analysis system, Xi'an, China).

The main features of the present CE–CL system are: (1) when the flowing CL reagent meets a separated sample zone in the tip of the separation capillary, the chemiluminescence reaction proceeds immediately in the detection window, and there is no dead volume in the reaction capillary; and (2) in the CE–CL system, a sample plug is driven by electroosmosis, and therefore the effects of dispersion which usually occur in flow injection analysis (FIA) are eliminated and great sensitivity can be obtained.

2.3. Preparation of capillaries

All new capillaries were initially rinsed with 0.1 mol l^{-1} NaOH for half an hour, followed by ion-exchange distilled water for 10 min, and finally with the buffer solution for 30 min. To maintain reproducible migration times, the capillary was flushed with



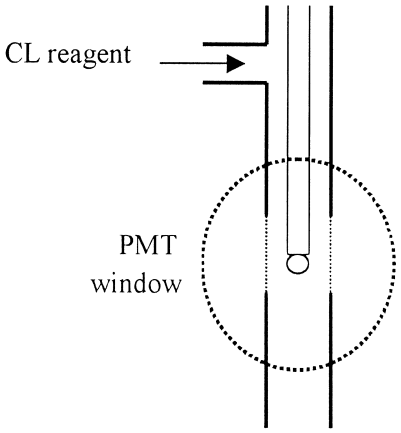


Fig. 1. Schematic diagram of the capillary electrophoresis instrument with chemiluminescence detection. (A) (1) Electrolyte reservoirs; (2) Pt electrodes; (3) high-voltage power supply; (4) electrophoretic capillary; (5) reaction capillary; (6) CL solution capillary; (7) tee connector; (8) black box; (9) PMT; (10) signal amplifier; (11) computer; (12) double syringe pumps; (13) luminol solution; (14) H_2O_2 solution; (15) detection window. (B) Schematic of CL detection interface.

0.1 mol l^{-1} NaOH for 2 min, then with the running buffer for 2 min and a voltage of 15 kV was applied to it for 120 s before each sample was injected. The capillary was filled with 0.1 mol l^{-1} NaOH overnight in order to keep the capillary wall in good condition.

2.4. CE–CL procedures

The capillary was rinsed with 0.1 mol l^{-1} sodium hydroxide, pure water and separation buffer for 2 min prior to each analysis by application of pressure (9–10 kPa). Sample, buffer and acidic sodium hydrogensulfite solutions were introduced to the capillary in that order by electrokinetic injection by applying 10-kV power for 9 s. After completion of this routine, running high voltage was applied, and the double microsyringe pumps were switched on to provide a mixed constant flow of CL reagent (luminol–H_2O_2 solution) to the reaction capillary during analysis. Electrophoretic separation was carried out at 15 kV for 8 min with current reading of ~20 μA.

Resolution (R_s) is calculated using the equation:

$$R_s = 2[(t_2 - t_1)]/(W_1 + W_2) \quad (3)$$

where t is the migration time in seconds, and W is the baseline peak width in seconds.

The theoretical plate number, N, can be obtained by:

$$N = 5.54(t/w_{1/2})^2 \quad (4)$$

where t is the migration time, and $w_{1/2}$ is half-peak width.

The sample volume injected can be calculated by [25]:

$$Volume = (\mu_{eo+}\ \mu_{ep})\pi r^2 v t/l \quad (5)$$

where μ_{eo} is the electroosmotic mobility of the sample solution, μ_{ep} is the electrophoretic mobility of the sample molecule, r is the radius of the capillary, v is the injection voltage, t is the injection time and L is the capillary total length. Sample volumes of 20 nl were injected by electrokinetic injection at 10 kV in 9 s.

3. Results and discussion

3.1. Cr(III), Cr(VI) in-capillary separation mode

In this work, Cr(III), in the form of Cr^{3+}, is a cation, but Cr(VI), in the form of $Cr_2O_7^{2-}$ or CrO_4^{2-}, has a negative charge. They move in opposite directions in the capillary when high voltage is applied. Consequently, Cr(III) and Cr(VI) can not be detected in the same detection window. On the other hand, Cr(VI) has no catalytic activity for the luminol–hydrogen peroxide CL reaction [15]. In our previous study [26], Cr(VI) was reduced to Cr(III) by hydrogen peroxide in acidic medium on solid substrate surface, and then the total chromium was detected based on solid surface chemiluminescence analysis. Using acidic sodium hydrogensulphite as reductant in this work, we proposed an in-capillary separation mode to detect Cr(III) and Cr(VI) simultaneously. The procedures are shown in Fig. 2. Sample [Cr(III) and Cr(VI)] zone, running buffer zone and reductant zone were injected into the capillary prior to applying the running voltage (Fig. 2A). After high voltage was applied, the Cr(III) moved toward the negative end and the Cr(VI) flowed toward the positive terminal against the EOF in the capillary (Fig. 2B). On the other hand, HSO_3^- flowed toward the positive terminal first, then eluted toward the cathode following the EOF. Consequently, Cr(VI) met and reacted with HSO_3^- in the buffer zone and formed Cr^{3+}, which reversed direction immediately and moved toward the cathode (Fig. 2C). Because of the migration time differences, both Cr^{3+} ions could be separated completely. Fig. 3 shows the electropherogram of Cr(III) and Cr(VI) standard solution separation. The resolution (R_s) of Cr(III) and Cr(VI) was more than 12.5. The theoretical plate numbers for Cr(III), Cr(VI) reached $1.0 \cdot 10^5$ and $4.3 \cdot 10^4$, respectively.

3.2. Optimization of CE–CL parameters

3.2.1. Effect of buffer zone on separation

The buffer zone plays a very important role in the separation of Cr(III) and Cr(VI) as well as the peak width of Cr(VI). The resolution of Cr(III) and Cr(VI) was actually decided by the length of buffer

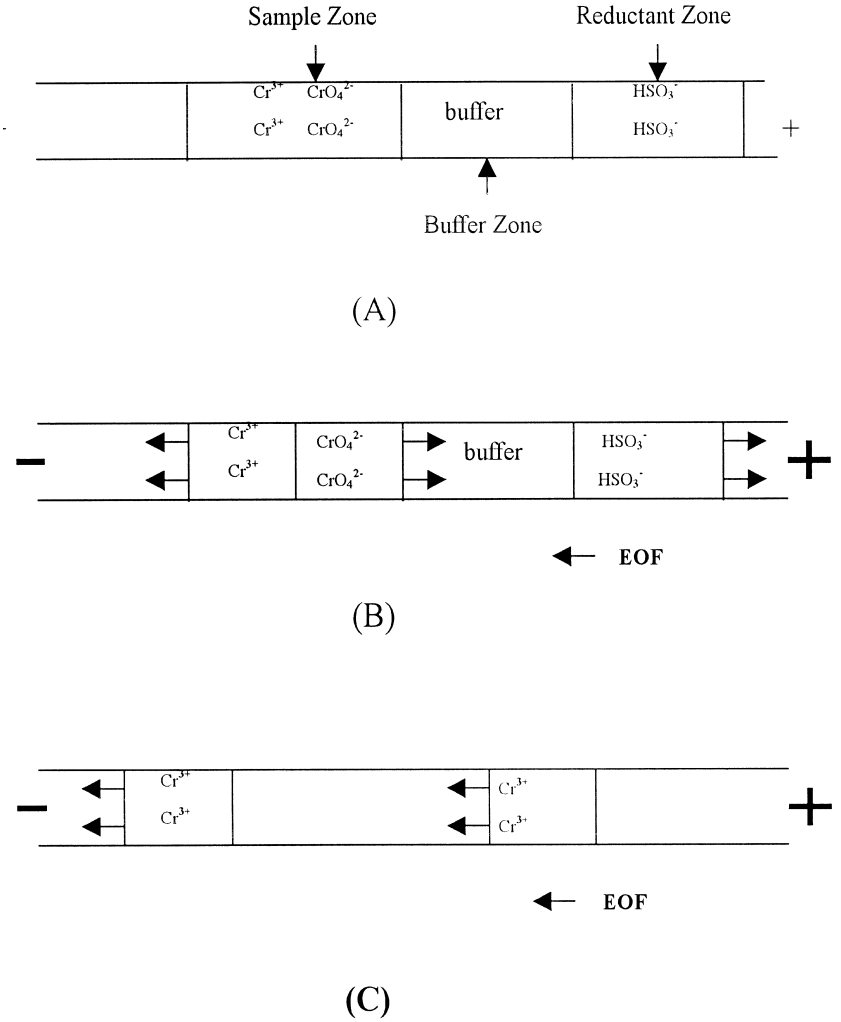


Fig. 2. Procedures of Cr(VI) reduction in-capillary and chromium speciation separation. (A) Injection order in the capillary: sample, buffer and HSO_3^-. (B) Applied high voltage, zones moving procedure and in-capillary reaction. (C) Separation of both Cr^{3+} ions. CL reaction solution: $1\cdot10^{-3}$ mol l^{-1} luminol, $1\cdot10^{-2}$ mol l^{-1} H_2O_2, 0.1 mol l^{-1} NaBr and $1\cdot10^{-4}$ mol l^{-1} EDTA (0.05 mol l^{-1} $NaHCO_3$–NaOH medium, pH 11.5–12.0). Running buffer: $2\cdot10^{-2}$ mol l^{-1} acetate buffer (pH 4.7) and $1\cdot10^{-3}$ mol l^{-1} EDTA. Injection: 9 s at 10 kV. Separation voltage: 15 kV.

zone injected. Fig. 4 shows the electropherogram of Cr(III) and Cr(VI) standard solution separation without injecting the buffer zone in the capillary. When compared with Fig. 3B,C, it can be seen that neither the migration time or the peak width of Cr(III) have changed. However, the peak of Cr(VI) is early and broad. In this case, it is possible that zones of Cr(VI) and HSO_3^- are adjacent to each other and both move to the anode at the same time, with the reaction between Cr(VI) and HSO_3^- proceeding gradually and incompletely.

3.2.2. Effect of reductant concentration on reduction of Cr(VI) to Cr(III)

The choice of reductant is very important for the Cr(VI) to be completely reduced to Cr(III) (Fig. 5). In acid medium, the reduction may be performed with S^{2-}, HSO_3^-, I^-, NO_2^- and $Fe(CN)_6^{4-}$. With the

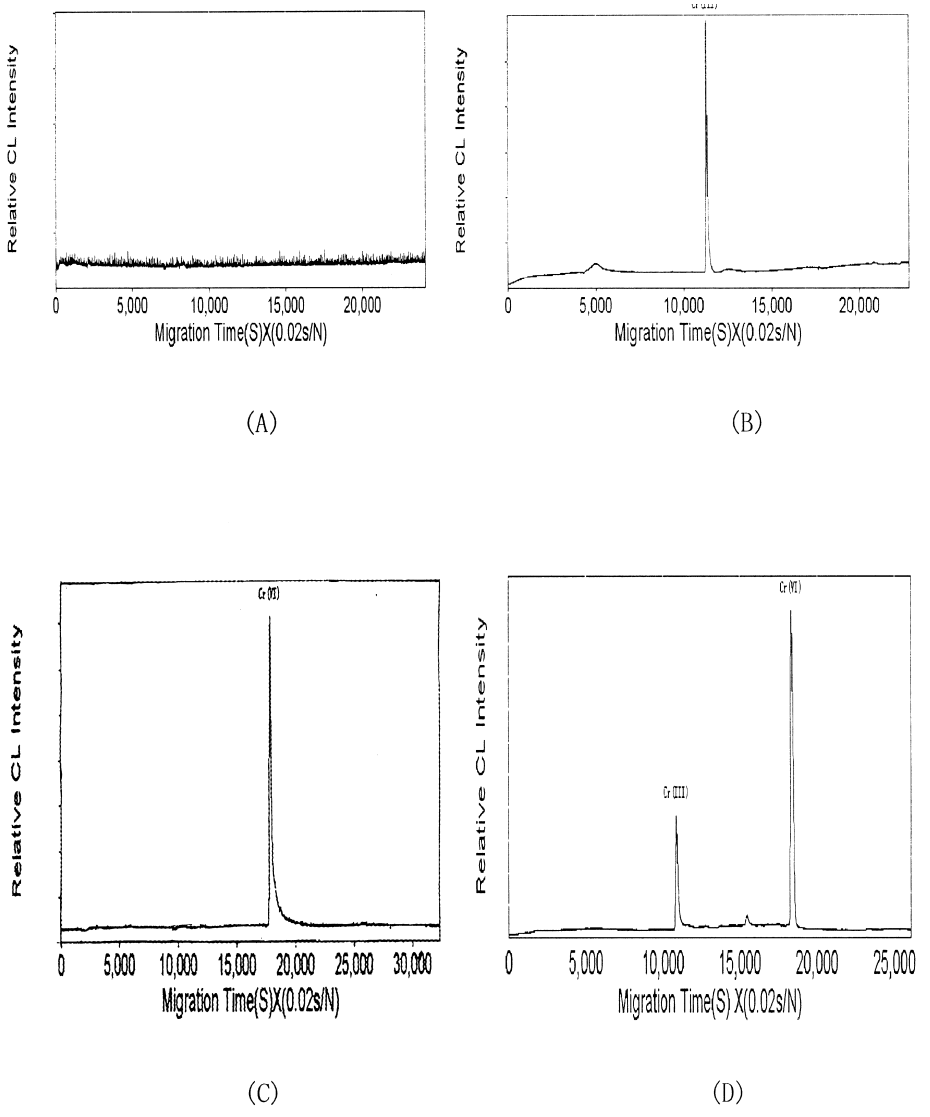


Fig. 3. Separation of Cr(III) and Cr(VI). (A) Electropherogram of blank solution. (B) Electropherogram of Cr(III) standard solution. (C) Electropherogram of Cr(VI) standard solution. (D) Electropherogram of Cr(III) and Cr(VI) mixed solution. CL reaction solution: $1\cdot10^{-3}$ mol l^{-1} luminol, $1\cdot10^{-2}$ mol l^{-1} H_2O_2, 0.1 mol l^{-1} NaBr and $1\cdot10^{-4}$ mol l^{-1} EDTA (0.05 mol l^{-1} $NaHCO_3$–NaOH medium, pH 11.5–12.0). Running buffer: $2\cdot10^{-2}$ mol l^{-1} acetate buffer (pH 4.7) and $1\cdot10^{-3}$ mol l^{-1} EDTA. Standard solution: $1\cdot10^{-9}$ mol l^{-1} Cr(III) and $1\cdot10^{-8}$ mol l^{-1} Cr(VI). Injection: 9 s at 10 kV. Separation voltage: 15 kV.

same experimental conditions, HSO_3^- achieved maximum CL intensity. The reaction may be represented by the equation:

$$2\,CrO_4^{2-} + 3\,HSO_3^- + 7\,H^+ = 2\,Cr^{3+} + 3\,SO_4^{2-} + 5\,H_2O$$

In order to reduce Cr(VI) to Cr(III) completely, the concentration of HSO_3^- was detected from 0.01 to 2 mol l^{-1}. As the experimental result shows, a measurement blank without HSO_3^- in the capillary showed no peak related to Cr(VI). A concentration of HSO_3^- greater than 0.1 mol l^{-1} achieved maxi-

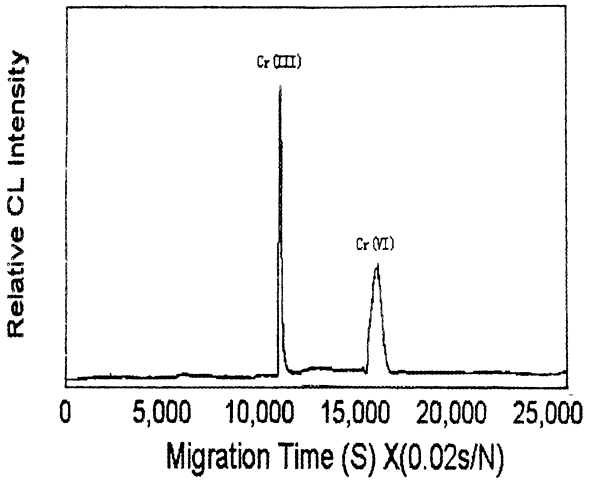


Fig. 4. Electropherogram of Cr(III) and Cr(VI) standard solution separation without injecting the buffer zone. Sample and reduction zone injection: 9 s at 10 kV. Separation voltage: 15 kV.

mum CL intensity. Considering the complete reaction and background level, 1 mol l^{-1} was chosen as the optimal concentration of HSO_3^-. A concentration of HCl solution of 0.05–1 mol l^{-1} gave the maximum response; therefore, 0.5 mol l^{-1} HCl was chosen for subsequent determinations.

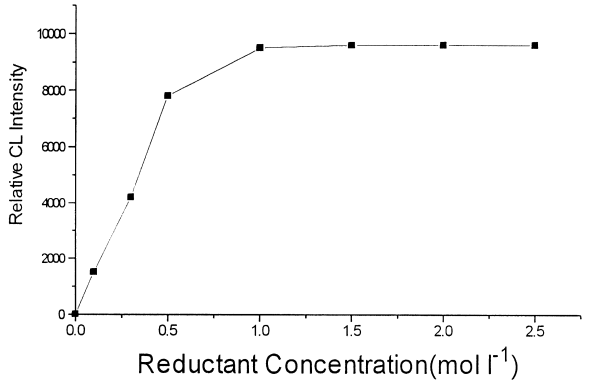


Fig. 5. Effect of reductant concentration on reduction of Cr(VI) to Cr(III). CL reaction solution: $1\cdot10^{-3}$ mol l^{-1} luminol, $1\cdot10^{-2}$ mol l^{-1} H_2O_2, 0.1 mol l^{-1} NaBr and $1\cdot10^{-4}$ mol l^{-1} EDTA (0.05 mol l^{-1} $NaHCO_3$–NaOH medium, pH 11.5–12.0). Running buffer: $2\cdot10^{-2}$ mol l^{-1} acetate buffer (pH 4.7) and $1\cdot10^{-3}$ mol l^{-1} EDTA. Standard solution: $1\cdot10^{-7}$ mol l^{-1} Cr(VI). Injection: 9 s at 10 kV. Separation voltage: 15 kV.

3.2.3. Mixing mode of the analytes with CL reagent

There were several mixing modes of the analytes with CL reagent in luminol–hydrogen peroxide CL reaction with CE: (I) both luminol and hydrogen peroxide as components of the electrophoretic carrier [27]; (II) hydrogen peroxide as a component of the electrophoretic carrier; (III) luminol as a component of the electrophoretic carrier [28,29]; and (IV) neither luminol nor hydrogen peroxide as a component of the electrophoretic carrier. That is, both luminol and hydrogen peroxide mixed together as CL reagent [13,30]. Modes I and II involved H_2O_2 in the electrolyte. However, the hydrogen peroxide can produce bubbles (oxygen) in electrolysis. The reaction may be formulated as:

$$2\,H_2O_2 = 2\,H_2O + O_2\,(g)$$

Comparing modes III and IV, we found that mode III benefited the resolution. The possible reason is that the luminol added to the running buffer changed the EOF similar to the effects of organic solvents, but the CL intensity decreased. The results are shown in Table 1 and may be due to the uneven viscosities of the luminol and analytes in the capillary. Mode IV was, therefore, finally chosen in following study.

3.2.4. Effect of luminol and hydrogen peroxide concentrations

As the chemiluminescence reagent, luminol and H_2O_2 concentrations affect the CL intensity. The

Table 1
Effects of two running buffers on CL emission[a]

Running buffer	Relative CL intensity ($n=3$)	
	Cr(VI)	Cr(III)
Acetate buffer + luminol + EDTA[b]	1260	860
Acetate buffer + EDTA[c]	3800	2530

[a] Cr(III) ($1\cdot10^{-8}$ mol l^{-1}) and Cr(VI) ($1\cdot10^{-7}$ mol l^{-1}).
[b] CL reaction solution: $1\cdot10^{-2}$ mol l^{-1} H_2O_2, 0.1 mol l^{-1} NaBr and $1\cdot10^{-4}$ mol l^{-1} EDTA (0.05 mol l^{-1} $NaHCO_3$–NaOH medium, pH 11.5–12.0).
[c] CL reaction solution: $1\cdot10^{-3}$ mol l^{-1} luminol, $1\cdot10^{-2}$ mol l^{-1} H_2O_2, 0.1 mol l^{-1} NaBr and $1\cdot10^{-4}$ mol l^{-1} EDTA (0.05 mol l^{-1} $NaHCO_3$–NaOH medium, pH 11.5–12.0).

effects of luminol and hydrogen peroxide concentration were studied. Considering the signal and signal-to-noise ratio for CL determinations, the concentration of luminol giving the best sensitivity was found to be $1 \cdot 10^{-3}$ mol l^{-1}, and the optimal concentration of hydrogen peroxide was $1 \cdot 10^{-2}$ mol l^{-1}. The results showed that the CL signal was enhanced in the presence of H_2O_2 but the signal-to-noise ratio was unfavorable because the background was also enhanced. This is because in the presence of H_2O_2 many heavy metal ions activate the luminol CL reaction. Even analytical-grade reagents and distilled water contain trace heavy metals, which may be at least a partial cause of the blank reaction when luminol and H_2O_2 are mixed. Therefore, $1 \cdot 10^{-4}$ mol l^{-1} EDTA was added to the luminol and H_2O_2 solutions to mask the metal ions. The luminol and H_2O_2 solutions were delivered to the reaction capillary at the same rate by two microsyringe pumps.

3.2.5. Effect of sodium bromide concentration

NaBr added to the luminol solution (carbonate medium) enhances the CL signal [31,32]. In this work, 0.1 mol l^{-1} NaBr was chosen. When the concentration of NaBr was higher than 0.3 mol l^{-1}, high current was generated.

3.2.6. Effect of CL reagent pH

The effect of CL reagent pH was investigated. Cr(III) catalyzes the reaction of luminol and H_2O_2 in alkaline solution. Considering CE separation, it is always better to match the pH condition of the electrophoretic medium with that of the CL reaction zone. However, the volume of the sample zone flowing from the 75 μm I.D. separation capillary is small enough compared to the volume of the reagent in the 530 μm I.D. reaction capillary, and hence the pH of the CL reaction is mainly dependent on the CL reagent pH. Several buffer solutions, such as NaOH, H_3BO_3–NaOH, and $NaHCO_3$–NaOH, were examined as a CL reaction medium. The NaOH concentration was varied in order to maximize the CL signal. The results showed that the $NaHCO_3$–NaOH solution gave larger signals than those of the other solutions, and 0.05 mol l^{-1} NaOH gave the maximum response; therefore, 0.05 mol l^{-1} $NaHCO_3$–0.05 mol l^{-1} NaOH was chosen as CL reaction reagent buffer medium (pH 11.5–12.0).

3.3. Interference studies

Chromium(III) has been determined by making use of its catalytic action on the oxidation of luminol. Other metal ions are masked with EDTA, but because the formation of the Cr(III)–EDTA complex is kinetically slow, Cr(III) can be determined selectively [14]. Using 0.02 mol l^{-1} acetate buffer (pH 4.7) with $1 \cdot 10^{-3}$ mol l^{-1} EDTA as electrophoretic carrier, the effect of foreign substances was tested by analyzing a standard solution of Cr(III) ($1 \cdot 10^{-8}$ mol l^{-1}) to which increasing amounts of interfering substances had been added. The tolerable concentration ratios for a 5% signal change are listed in Table 2. It can be seen that good selectivity can be obtained by this method.

3.4. Linearity, precision and limit of detection

We measured the linearity, reproducibility and

Table 2
Tolerable concentration ratios with respect to chromium for some interfering species

Substance	Tolerable concentration ratio
K^+, Na^+, NH_4^+, NO_3^-, F^-, Cl^-, I^-, Br^-, Ac^-	>10 000
SO_4^{2-}, HCO_3^-, CO_3^{2-}, HPO_4^{2-}, PO_4^{3-}	>10 000
MnO_4^-, $S_2O_8^{2-}$, $Fe(CN)_6^{3+}$	2000
Ca^{2+}, Mg^{2+}, Zn^{2+}, Mn^{2+}, Pb^{2+}, Hg^{2+}, As^{3+}, Sn^{2+}, Al^{3+}, Cu^{2+}	5000
Glucose, citric acid, oxalic acid, lactic acid, pyruvic acid	5000
VB_1, VB_2, VB_6, benzoic acid	5000
Co^{2+}, Fe^{2+}, Fe^{3+}, Ni^{2+}	2000

Table 3
The limit of detection (LOD), linearity and reproducibility for Cr(III) and Cr(VI) determinations

Ions	Linearity (mol l^{-1})	R	Concentration LOD (mol l^{-1})	Mass LOD (zmol)	RSD (%) ($n=3$)	
					Migration	Peak
Cr(III)	$3\cdot10^{-12}-8\cdot10^{-10}$	0.998	$6\cdot10^{-13}$	12	1.5	3.8
Cr(VI)	$8\cdot10^{-11}-5\cdot10^{-9}$	0.997	$1.9\cdot10^{-11}$	380	2.0	4.8

limit of detection for Cr(III) and Cr(VI) and the results obtained were shown in Table 3. The linear ranges were from $3\cdot10^{-12}$ to $8\cdot10^{-10}$ mol l^{-1} for Cr(III) ($R=0.9985$) and from $8\cdot10^{-11}$ to $5\cdot10^{-9}$ mol l^{-1} for Cr(VI) ($R=0.9971$). A typical calibration regression equation was $Y=141.1+7.4\cdot10^{12}X$ for Cr(III) and $Y=154.2+5.6\cdot10^{11}X$ for Cr(VI). The limits of detection (LODs) of Cr(III) and Cr(VI) were $6\cdot10^{-13}$ and $8\cdot10^{-12}$ mol l^{-1} ($S/N=3$), respectively. The mass LODs for Cr(III) and Cr(VI) were $1.2\cdot10^{-20}$ mol (12 zmol) and $3.8\cdot10^{-19}$ mol (380 zmol), respectively. The relative standard deviations (RSDs) of migration times and peak heights were less than 2.0 and 4.8%, respectively. Fig. 6 shows the electropherogram of $5\cdot10^{-12}$ mol l^{-1} Cr(III) and $1\cdot10^{-10}$ mol l^{-1} Cr(VI) standard solution separation.

3.5. Analytical application

The water samples collected from different sources (Xi'an area) were analyzed for chromium. Fig. 7 is the electropherogram of surface water. The results of using this method to analyze water samples are shown in Table 4. 1,5-Diphenylcarbazide UV–Vis spectrophotometry [4] was used as a reference method for determining Cr(VI) and total chromium. The results compared well with those obtained by the reference method. The recoveries of Cr(III) and Cr(VI) were 98 and 103%, respectively.

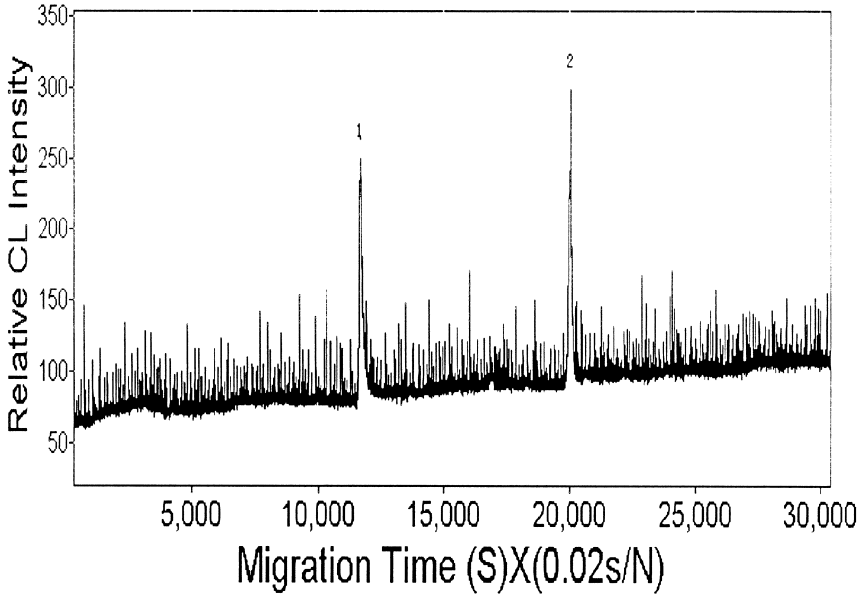


Fig. 6. Electropherogram of $5\cdot10^{-12}$ mol l^{-1} Cr(III) and $1\cdot10^{-10}$ mol l^{-1} Cr(VI) standard solution separation. Peaks: 1, Cr(III); 2, Cr(VI). CL reaction solution: $1\cdot10^{-3}$ mol l^{-1} luminol, $1\cdot10^{-2}$ mol l^{-1} H_2O_2, 0.1 mol l^{-1} NaBr and $1\cdot10^{-4}$ mol l^{-1} EDTA (0.05 mol l^{-1} $NaHCO_3$–NaOH medium, pH 11.5–12.0). Running buffer: $2\cdot10^{-2}$ mol l^{-1} acetate buffer (pH 4.7) and $1\cdot10^{-3}$ mol l^{-1} EDTA. Injection: 9 s at 10 kV. Separation voltage: 15 kV.

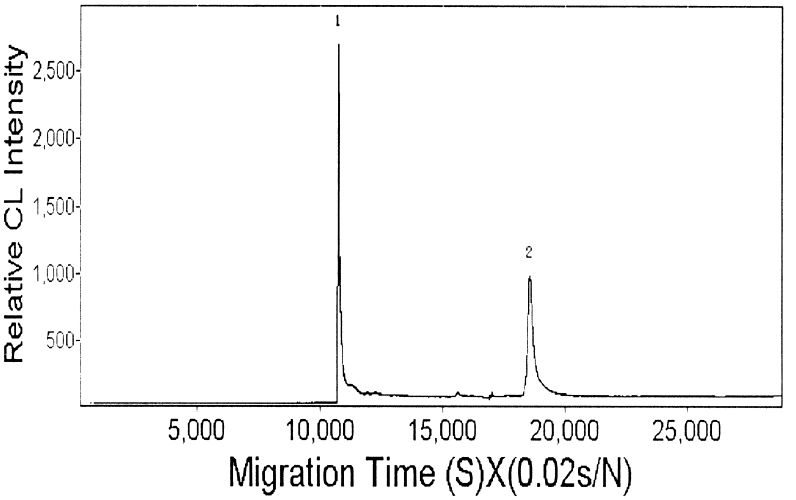


Fig. 7. Electropherogram of chromium species in water sample. Peaks: 1, Cr(III); 2, Cr(VI). CL reaction solution: $1\cdot10^{-3}$ mol l^{-1} luminol, $1\cdot10^{-2}$ mol l^{-1} H_2O_2, 0.1 mol l^{-1} NaBr and $1\cdot10^{-4}$ mol l^{-1} EDTA (0.05 mol l^{-1} $NaHCO_3$–NaOH medium, pH 11.5–12.0). Running buffer: $2\cdot10^{-2}$ mol l^{-1} acetate buffer (pH 4.7) and $1\cdot10^{-3}$ mol l^{-1} EDTA. Injection: 9 s at 10 kV. Separation *v*oltage: 15 kV.

Table 4
Determination of Cr(III) and Cr(VI) in different water samples

Sample	Results obtained by this method[a]		Reference results[b]	
	Cr(III) (mol l^{-1})	Cr(VI) (mol l^{-1})	Cr(VI) (mol l^{-1})	Total Cr (mol l^{-1})
Tap water	$6.2\cdot10^{-11}$ (±3.1%)	$5.8\cdot10^{-10}$ (±3.6%)	–	–
Surface water 1	$9.6\cdot10^{-7}$ (±2.9%)	$1.5\cdot10^{-7}$ (±3.2%)	$1.3\cdot10^{-7}$ (±2.1%)	$1.1\cdot10^{-6}$ (±2.6%)
Surface water 2	$2.1\cdot10^{-7}$ (±2.3%)	$7.3\cdot10^{-8}$ (±2.8%)	$7.9\cdot10^{-8}$ (±2.4%)	$2.9\cdot10^{-7}$ (±3.1%)
Waste water	$1.5\cdot10^{-6}$ (±3.9%)	$2.5\cdot10^{-6}$ (±3.1%)	$3.0\cdot10^{-6}$ (±1.9%)	$4.6\cdot10^{-6}$ (±2.7%)

[a] A*v*erage of three replicates (±RSD).
[b] By photometric method of diphenylcarbazide.

4. Conclusions

A strategy based on the chemiluminescent determination of Cr(III) and Cr(VI) applying in-capillary reduction with capillary electrophoresis using acidic sodium hydrogensulfite as reductant was proposed. Chromium species can be determined directly and simultaneously with high accuracy. The buffer zone introduced into the capillary between the sample zone and reductant zone is the main factor for Cr(VI) reduction to Cr(III) and chromium speciation separation. Using EDTA as a component of the electrophoretic carrier eliminated transition metal ions (such as Co^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Ni^{2+} and Mn^{2+}) interferences. The principal advantage of this method is that the inorganic cations and anions can be detected simultaneously without having to add EOF modifiers to reverse the EOF direction or reverse both electrodes. Furthermore, in-capillary reaction procedures may become much more attractive than conventional pre-capillary or post-capillary derivatization in terms of reproducibility, sensitivity and efficiency.

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