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On-column chemiluminescence detection of rare earth ions with capillary electrophoresis

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Abstract

Dual effects of rare earth ions on a chemiluminescence (CL) reaction of luminol with H_2O_2 enhanced by Co^{2+} are first reported. Under static CL conditions, rare earth ions could complex with luminol and inhibit CL emission; on the other hand, they could catalyze the CL reactions second transformation from luminol free radical to aminophthalate and enhance CL emission in capillary electrophoresis (CE) with on-line chemiluminescence detection (CE–CL). The cause for the difference between static CL and on-column CE–CL is that luminol free radical are preceded by rare earth ions migrating into reaction capillary under CE–CL conditions. The enhancement behavior in CE–CL was used to separate and directly detect rare earth ions. La^{3+} , Ce^{4+} , Pr^{3+} and Nd^{3+} were successfully separated and detected under the optimum CE–CL conditions. The detection limits were 33, 27, 42 and 50 fmol for La^{3+} , Ce^{4+} , Pr^{3+} and Nd^{3+} respectively. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Chemiluminescence detection; Detection, electrophoresis; Rare earth ions; Luminol; Hydrogen peroxide; Metal ions

1. Introduction

Metal ion analyses are very important in a variety of research and industrial areas [1]. Recently, capillary electrophoresis (CE) has attracted considerable attention as an alternative to traditional ion chromatography techniques [2,3]. Capillary zone electrophoresis (CZE) has the capability to separate very small quantities of sample when small inner diameter capillary columns are used, but detection of samples remains a challenge. Conductivity detection [4], UV absorbance detection [1,2,5–7], fluorescence detection [8–10], X-ray [11] and inductively coupled

plasma mass spectrometry (ICP-MS) [12,13] are used when metal ions are separated by CZE. UV absorbance and fluorescence detection are the most widely used methods for CE.

Depending on the spectroscopic properties of the ligand, direct or indirect detection is used. Indirect detection is used when neither the complex nor the ligand are UV or fluorescence active. Generally, indirect detection is more widely used. The indirect UV absorbance detection with CE technique, where the analyte ions replace a UV-absorbing ion of the same charge (electrolyte co-ion) [14], requires the molar absorbance of the co-ion to be high and the background absorption should be adjusted to be close to maximum value for the linear range of the

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detector in order to give the maximum linear calibration range. Therefore the detection sensitivity of the indirect UV-absorbing technique is always subject to the background co-ion. When CZE with indirect fluorescence detection is used, a fluorescing running buffer is used. When the solute ions of the sample elute past the detector, they displace the fluorescent buffer ions. So a reduction in the background fluorescence intensity occurs and the metal ions are detected as negative peaks. The indirect detection with CE technique requires the concentration ratio of electrolyte ion to background co-ion be suitable to optimize the separation and detection conditions. The chemical nature and the concentration of the background co-ion are also factors that influence the separation and detection limit in indirect photometric detection [15].

The detection volume of CE is small, therefore the detection method used with CE is of importance in order to obtain the highest possible sensitivity. Displaying high detection sensitivity and bearing small detection volume, the chemiluminescence (CL) technique is reasonably eligible for this demand. Recently, the feasibility of CL reaction as a detection scheme in CE has been demonstrated. Transition metal ions [16], amino acids [17–19], peptides [20] and even proteins [21–26] have been analyzed using this mode. Several reviews on this technique were recently published [27,28].

The separation of rare earth metal ions by CE with indirect UV absorbance detection was successfully investigated [5,29,30]. Although CL detection with flow injection analysis (FIA) [31] and extraction chromatography [32] were used for rare earth metal ion analysis, CL detection with CE for rare earth metal ion analysis has not yet been reported. Employing CL detection with CE as an on-column

analysis method, we find that rare earth metal ions can actually enhance a background CL produced by a Co^{2+} -catalyzing reaction of luminol and H_2O_2 . The second enhancement behavior of rare earth metal ions is first reported in this paper and applied to the direct detection of rare earth metal ions as well.

2. Experimental

2.1. Apparatus

The CE–CL apparatus used here consisted of a conventional CE system and a CL detection system as described previously in detail [16]. The separation capillary of 56 cm×50 μm I.D. was inserted into a reaction capillary of 25 cm×320 μm I.D. Via a reagent capillary of 40 cm×120 μm I.D. the CL reagents, hydrogen peroxide and luminol, were siphoned into a tee. From the tee the CL reagents flowed down to the detection window. The detection window was a length of burned reaction capillary where the CL reagents and eluents from the separation capillary mixed and reacted. The schematic of the CE–CL system is shown in Fig. 1. The CL emissions from the detection window were collected by a photomultiplier tube (PMT, Mamatsu, Iwata-Gun, Japan) from which signals were directed to a HX-2 chemiluminometer (Institute of Chemistry, Chinese Academy of Science, Beijing, China). The signals were at last recorded by the 3066 chart recorder (The Fourth Instrumental Factory of Sichuan, China). All of the capillaries were purchased from Hebei Optical Fiber Factory (Hebei, China).

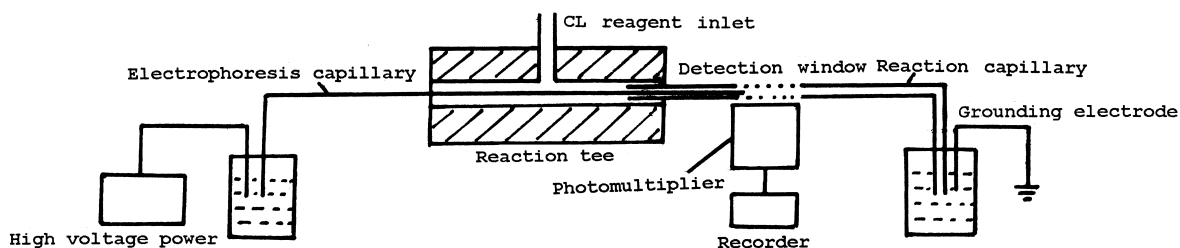


Fig. 1. Schematic drawing of the CL detector using in CE.

2.2. Reagents

The stock solution of luminol was prepared by dissolving 177.16 mg luminol into 100 ml of 0.1 mol/l NaOH. As hydrogen peroxide may breakdown and form bubbles in basic medium, the 30% (w/w) hydrogen peroxide was diluted with 3 mmol/l of nitric acid to prepare H₂O₂ solution in different concentrations, the acid conditions helped stabilize the hydrogen peroxide solution. The hydrogen peroxide was added to 3 mmol/l nitric acid carefully and slowly. Oxides of La, Ce, Pr and Nd were weighed accurately and dissolved in 1:1 (v/v) hydrochloric acid to prepare standard solution of rare earth ions. Hydroxyisobutyric acid (HIBA) was purchased from Sigma (St. Louis, MO, USA). The reagents were analytical grade and commercially available.

The electrophoretic buffer was a 10 mM phosphate buffer, pH 4.5. The CL reagent buffer was a 10 mM phosphate buffer (pH 11.0). All solutions were prepared by double distilled water and filtered through 0.22- μ m pore-size membrane filter before being used.

2.3. Procedures

The capillaries were cleaned with 0.1 mol/l NaOH, distilled water and electrophoretic buffer in sequence. The separation capillary was filled with electrophoretic buffer while the tee and reaction capillary were filled with CL reagents. The sample was injected by electroinjection for 10 s at a voltage of 10 kV. High power with 18 kV voltage was applied to the CE–CL system for separation.

3. Results and discussion

3.1. CE–CL results

Several buffer solutions were tested in the process of exploring the CL conditions for detection of rare earth metal ions. The results are presented in Table 1. Both Co²⁺ and Cr³⁺ could catalyze the CL reaction of luminol with H₂O₂ and the catalysis efficiency of Co²⁺ was better than that of Cr³⁺, as shown in tests 1 and 2. Tests 3 and 5 (and test 6) were designed to make on-column investigations on the influence of La³⁺ on the CL reaction. La³⁺ in test 3 was added into the CL reagent before the CL reaction was initiated by Co²⁺. La³⁺ in this mode could suppress the CL reaction in comparison with that in test 1 (and test 2). In tests 5 and 6, Cr³⁺ and Co²⁺ were added into the electrophoretic buffer to produce a stable background CL and La³⁺ was injected from the separation capillary. Bringing the diversity to the result of test 3, the injected La³⁺ in test 5 (and test 6) was found to promote the background CL reaction. The results mean that La³⁺ could suppress or promote the CL reaction on different conditions. The most apparent difference between tests 3 and 5 (and test 6) was that the CL reaction of luminol with H₂O₂ in test 5 (and test 6) had been catalyzed by Co²⁺ (and Cr³⁺) before La³⁺ migrated into the reaction capillary. The difference of results in tests 4 and 5 clearly shows that the catalysis of Co²⁺ beforehand was the prerequisite for the promotive behavior of La³⁺ in the CL reaction. On the basis of the results of Table 1, the preconditions of La³⁺ promoting CL reaction of luminol with

Table 1
Results of CE–CL detection

Test No.	Sample	Electrophoretic buffer (pH 4.0)	CL reagent buffer (pH 11.0)	Δ CL intensity	Result
1	Co ²⁺	PBS + HIBA	PBS + luminol + H ₂ O ₂	1654	Sharp-pointed peak in symmetry
2	Cr ³⁺	PBS + HIBA	PBS + luminol + H ₂ O ₂	1586	Fairly sharp-pointed and symmetric peak
3	Co ²⁺ /Cr ³⁺	PBS + HIBA	PBS + luminol + H ₂ O ₂ + La ³⁺	530	Weakened peak
4	La ³⁺	PBS + HIBA	PBS + luminol + H ₂ O ₂	0	No peak
5	La ³⁺	PBS + HIBA + Cr ³⁺	PBS + luminol + H ₂ O ₂	1174	Broadened peak in symmetry
6	La ³⁺	PBS + HIBA + Co ²⁺	PBS + luminol + H ₂ O ₂	985	Fairly sharp-pointed peak in symmetry

PBS = Phosphate buffer.

H₂O₂ were (1) La³⁺ does not interact with the CL reagent in advance; (2) the CL reaction was catalyzed by Co²⁺ beforehand.

Besides La³⁺, Ce⁴⁺, Pr³⁺, and Nd³⁺ could also promote the CL reaction of luminol with H₂O₂ under the above listed preconditions appropriate for La³⁺. The enhancement effects of CL are summarized in Table 2. Similar to test 4 in Table 1, test 1 in Table 2 also clearly shows that catalyzing of Co²⁺ the CL beforehand is of significance for rare earth metal ions' promoting the CL reaction.

Furthermore, with the increase of background CL, the signal-to-noise ratio for the peaks of rare earth metal ions was amplified. This tendency is demonstrated in Table 3.

3.2. Mechanism

Burdo and Seitz [33] put forward the mechanism of a CL reaction, luminol with H₂O₂ enhanced by Co²⁺. In their opinion, luminol was reduced to luminol free radical by Co²⁺ and further to aminophthalate ion by H₂O₂. CL emission took place during the second transformation.

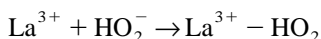
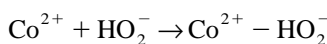
In our CE–CL experiments, the above-mentioned CL reaction was used to produce a background CL. Co²⁺ and luminol/H₂O₂ were introduced to the reaction capillary, respectively, from the separation capillary and reagent capillary. As rare earth ions sample migrated to the reaction capillary, an enhanced CL signal came out. However, this phenomenon occurred only if the background CL was present. This result also justified a conclusion that it was the second transformation of the background CL reaction that rare earth ions promoted. Associated with the mechanism of the background CL emission, a possible mechanism is shown below:

Table 3

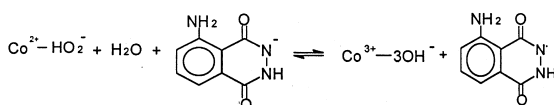
Relationship between background CL and response signal exemplified in La³⁺

Background CL	500	700	900
Peak height	2034	2394	3063

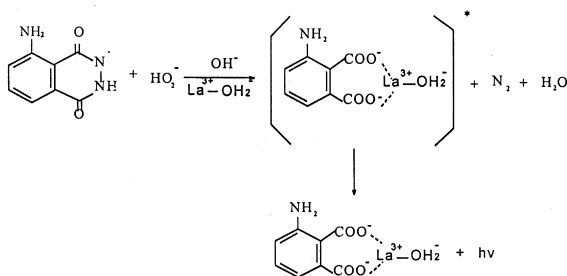
Initial step:



The first transformation:



The second transformation:



Under CE–CL conditions, CL reagents (H₂O₂ and luminol) and Co²⁺ flowed into the detection window in incessant stream, and hence there frequently existed fresh reagents to react. The background CL emissions were constantly produced there. As the background CL tended to be stable, a constant concentration of luminol free radical was presented. When a rare earth ion migrated into the detection window from the separation capillary, it was greeted

Table 2

Summary of enhancement behavior of rare earth ions in CE–CL

Test No.	Sample	Electrophoresis buffer (pH 4.0)	CL reagent buffer (pH 11.0)	Peak height (cm)
1	La ³⁺ , Ce ⁴⁺ , Pr ³⁺ , Nd ³⁺	PBS	PBS + H ₂ O ₂ + luminol	No peak
2	La ³⁺	PBS + Co ²⁺	PBS + H ₂ O ₂ + luminol	15.0
3	Ce ⁴⁺	PBS + Co ²⁺	PBS + H ₂ O ₂ + luminol	14.5
4	Pr ³⁺	PBS + Co ²⁺	PBS + H ₂ O ₂ + luminol	13.2
5	Nd ³⁺	PBS + Co ²⁺	PBS + H ₂ O ₂ + luminol	12.2

PBS: 0.01 mol/l; H₂O₂: 0.05 mol/l; Co²⁺: 1.0 · 10⁻⁶ mol/l; luminol: 2.5 · 10⁻⁵ mol/l; rare earth ions: 5.0 · 10⁻⁵ mol/l.

with a luminol free radical and generated a second-enhanced CL signal. The stronger the background CL was, the more the luminol free radical existed and hence the induced response signed of the rare earth metal ions' was more intense. The results of La^{3+} under on-column CE–CL conditions and the CL experiments of La^{3+} under static conditions were compared. The CL reaction in static conditions was performed in LKB1251 Chemiluminometer by injecting 0.2 ml solution into 0.2 ml solution B in Table 4. Table 4 makes it apparent that La^{3+} suppressed the CL reactions of H_2O_2 –luminol under static CL circumstances, whether the CL reactions were enhanced by $\text{Co}^{2+}/\text{Cr}^{3+}$ in advance or not. The decrease in CL emission was presumably caused by a reduction of the free luminol due to the rare earth ion complexing with luminol.

Under static CL circumstances, the unstable luminol free radical in the CL reaction vanished quickly due to its fast kinetic features, thereby the luminol free radical could not exist in an effective concentration and could not be captured by the added rare earth ions. In contrast, these rare earth ions could complex with luminol. This complexing interaction would lead to a decrease of luminol free radical and would result in the weakened CL signals.

FIA–CL and extraction chromatography–CL employed by Zhang et al. [31,32] were similar to the above mentioned static mode. In this mode, the CL reagents, rare earth ion and the metal ion that enhanced CL reaction were mixed and reacted a few seconds before the CL emission was measured. Obviously, the unstable luminol free radical in the CL reaction could not be maintained for long enough. This detection delay glossed over the instant state of the CL reaction. Different from the followed static detection modus, the CL detection window in

CE–CL is just suited in the joint of CL reagent and elutes from the separation capillary. This design ensured that the CL emission from the fast kinetic reaction could be collected in full. CL detection in CE can also utterly trace the whole dynamic course of the CL reaction.

3.3. Optimizing CE–CL conditions

The effects of the electrolyte pH on the separation of rare earth ions firstly display in the interaction between metal ion and HIBA. The interaction between the rare earth metal ion and HIBA can be converged by the overall conditional formation constant K [2]:

$$K = \alpha^n K_f \quad (1)$$

where K_f is the conditional formation constant at infinite dilution and α is the degree of protonation of HIBA. Decreasing pH, the value of α is decreased and hence the concentration of the complex decreases. In contrast, with the increase of pH the concentration of the dissociated HIBA^- increases. Consequently the average complexation degree of metal ion increased, then both gross charge and gross electrophoretic mobility of metal ion decreased. This should cause longer migration time. Moreover, with the increase in HIBA^- , the complexation degree of metal ion–HIBA should be diversified. Therefore, the peak of metal ion in CE became tailing for complexes of metal ion–HIBA with different complexation degrees had different electrophoretic mobilities. The second point of interest about the effect of the electrolyte pH, is that minor change in metal ion mobility displayed due to the EOF change. The

Table 4
Results of static CL

Solution A (pH 11.0)	Solution B (pH 11.0)	Strength of CL emission
H_2O_2	Luminol + Co^{2+}	5076
	Luminol + Cr^{3+}	4168
	Luminol	1990
	Luminol + La^{3+}	370
	Luminol + Co^{2+} + La^{3+}	366
	Luminol + Cr^{3+} + La^{3+}	374

Luminol: $1.0 \cdot 10^{-5}$ mol/l; H_2O_2 : $1.0 \cdot 10^{-3}$ mol/l; Cr^{3+} : $5.0 \cdot 10^{-8}$ mol/l; Co^{2+} : $5.0 \cdot 10^{-8}$ mol/l; La^{3+} : $1.0 \cdot 10^{-6}$ mol/l.

velocity of ion migration in CE, ν_{obs} , is given by the combination of μ_{app} and μ_{EOF} :

$$\nu_{\text{obs}} = (\mu_{\text{app}} + \mu_{\text{EOF}})E \quad (2)$$

where μ_{app} is the apparent mobility of metal ion in electrolyte, μ_{EOF} is the electroosmotic flow mobility, and E is the electric field. μ_{EOF} is dependent of the electric on double layer adjacent to the capillary wall. The range of the electrolyte pH from 2.0 to 5.5 was studied to optimize the separation pH. With the increase in electrolyte pH, the baseline noise increased, which may have been caused by excessive Joule heat due to an increase in the current as a result of the greater mobility of the hydronium ions [1]. At pH greater than 5.0, the peaks of lanthanide ions began to broaden, possibly due to hydrolysis of metal ions. A pH of 4.5 was chosen to optimize both baseline stability and resolution.

Although the apparent mobilities of metal ions may be altered by modifying the electrolyte pH, the difference of the apparent mobilities between metal ions is achieved to great extent by the addition of a complexing agent. The electrophoretic mobilities of cations, μ_i , directly affects the selective separation of cations [34]. The Lanthanide group have similar μ_i compared to other metal groups, and hence metal ions belonging to this group are not readily separated. One frequently used strategy to separate lanthanide group ions is based on complexation. Since each ion has a unique affinity for the complexing agent, their mobilities are altered to different extents, providing the difference in mobilities are altered to different extents. The difference in mobility is needed for separation [2].

The choice of complexing agent is dependent on application. However, if too strong a complexing agent is used, ion mobility is decreased to a great degree and the advantage of speed provided by CE is impaired. Weak complexation can take the advantage of efficient separation over strong complexation for the mobility of each cation can be modulated by diversifying its complexation degree with a weak ligand.

HIBA is most commonly used in separation of lanthanides [35]. Generally, several different lanthanide–HIBA complexes can exist simultaneously. The apparent electrophoretic mobility, μ_{app} , can therefore be assumed to be a combination of the electro-

phoretic mobilities of the various forms of the ion present [2,6]:

$$\mu_{\text{app}} = x_M \mu_M + x_{ML} \mu_{ML} + x_{ML_2} \mu_{ML_2} + \dots \quad (3)$$

where μ_M , μ_{ML} and μ_{ML_2} are mobilities of the free metal ion, 1:1 HIBA complex, and 1:2 complex; and x_M , x_{ML} and x_{ML_2} are the mole fractions of each species in the capillary. These mole fractions depend on the electrophoretic buffer parameter. A significant effect on the separation of lanthanides can be seen by altering the concentration of HIBA when pH is maintained at 4.5. Fig. 2 shows the relationship between the concentration change of HIBA in electrophoretic solution and the separation effect. As the concentration of HIBA is increased, the equilibrium of metal ion–HIBA is preferable to form a complex with high complexation degree. The increase in HIBA concentration could lead to the decrease in EOF due the decrease in ion strength [2]. The formation of complexion with high complexation degree reduced the gross charge of metal ion. Both the decrease in gross charge and in EOF could result in slower electrophoretic mobilities and a longer analysis time. The formation of metal ion–HIBA

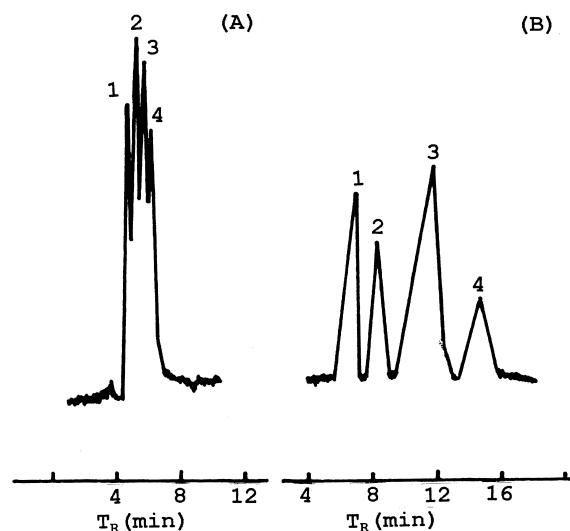


Fig. 2. Separation of the rare earth ions with different concentrations of HIBA in electrophoretic buffer. Electrophoretic buffer: 0.01 mol/l PBS + $6.4 \cdot 10^{-5}$ mol/l Co^{2+} ; CL reagent buffer: 0.01 mol/l PBS + 0.05 mol/l H_2O_2 + $2.5 \cdot 10^{-5}$ luminol. 1 = La^{3+} ; 2 = Ce^{4+} ; 3 = Pr^{3+} ; 4 = Nd^{3+} . (A) 2 mM HIBA, (B) 10 mM HIBA.

complexes with different complexation degrees could also lead to broadened peaks. The concentration of HIBA in electrophoretic solution not only affected the separation of rare earth ions, but also had an influence on the background CL. HIBA could suppress the background CL for its complexation interaction with Co^{2+} . The optimum concentration of HIBA for separating La^{3+} , Ce^{3+} , Pr^{3+} and Nd^{3+} is about 8 mmol/l, which is demonstrated in Fig. 3.

Free Co^{2+} in electrophoretic solution is essential to obtain a stable background CL. However, its concentration should not be too high, or else the baseline noise produced by the background CL becomes unstable. A stable baseline could be obtained when the concentration ratio of Co^{2+} to HIBA was $8 \cdot 10^{-3}$. Since the optimum concentration of HIBA was 8 mM, the optimum concentration of Co^{2+} was $6.4 \cdot 10^{-5}$ mol/l. Both Co^{2+} and Cr^{3+} could catalyze the CL reaction of H_2O_2 with luminol to produce background CL. However, the catalysis efficiency of Co^{2+} was better than that of Cr^{3+} as shown in Table 1. The detectability and the peak symmetry deteriorated when Cr^{3+} was substituted for Co^{2+} to produce the background CL as shown in Fig. 4.

Four rare earth ions La^{3+} , Ce^{4+} , Pr^{3+} and Nd^{3+} ,

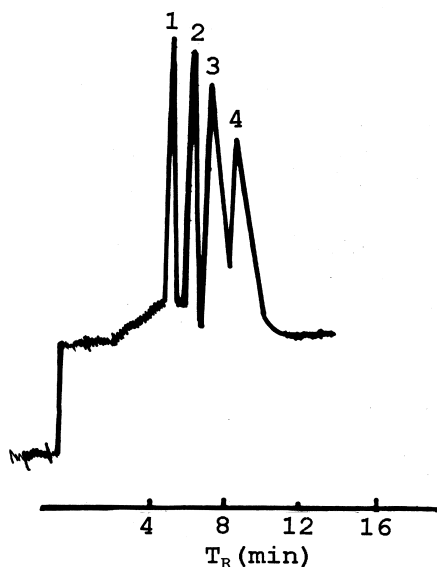


Fig. 3. Electropherogram of rare earth ions. Conditions were as in Fig. 2 except the concentration of HIBA was 8 mM. 1= La^{3+} ; 2= Ce^{4+} ; 3= Pr^{3+} ; 4= Nd^{3+} .

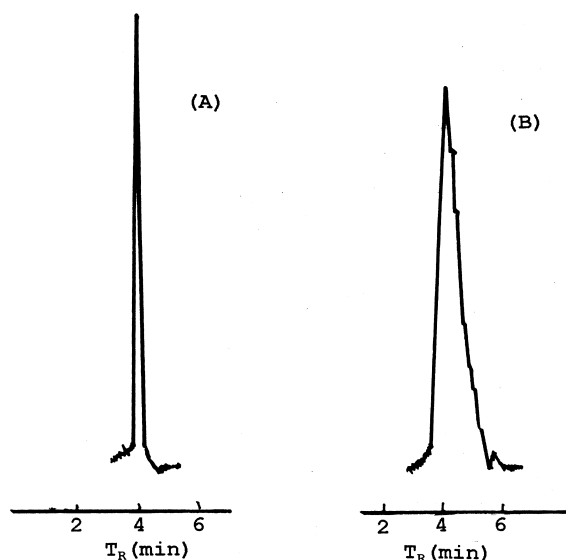


Fig. 4. The different effects of Co^{2+} (A) and Cr^{3+} (B) used as the enhancing ion. Conditions as in Fig. 1.

were successfully separated under the foregoing optimizing CE–CL conditions. The following detection limits were obtained: La^{3+} 33 fmol ($2.0 \cdot 10^{-6}$ mol/l), Ce^{4+} 27 fmol ($1.8 \cdot 10^{-6}$ mol/l), Pr^{3+} 42 fmol ($2.0 \cdot 10^{-6}$ mol/l) and Nd^{3+} 50 fmol ($2.2 \cdot 10^{-6}$ mol/l).

Acknowledgements

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