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On-column complexation capillary electrophoretic separation of Fe^{2+} and Fe^{3+} using 2,6-pyridinedicarboxylic acid coupled with large-volume sample stacking

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Abstract

On-column complexation of Fe^{2+} and Fe^{3+} with 2,6-pyridinedicarboxylic acid (2,6-PDCA) formed anionic complexes, which were then separated by capillary zone electrophoresis with direct UV detection at 214 nm. To achieve reasonable separation selectivity and on-column complexation, the conditions such as pH, the concentration of 2,6-PCDA and the EOF modifiers in the electrolyte were examined. The electrolyte contained 5.0 mM 2,6-PDCA, 0.25 mM tetradecyltrimethlammonium bromide (TTAB) and 5% (v/v) acetonitrile at pH 4.0 was optimised for on-column complexation and the separation of $Fe[PCDA]_2^{2-}$ and $Fe[PCDA]_2^{-}$. To enhance the detection sensitivity, large-volume sample stacking (LVSS) was used for the on-line preconcentration of $Fe[PCDA]_2^{2-}$ and $Fe[PCDA]_2^{-}$. Under the optimised conditions, satisfactory working ranges (0.5–50 μM), lower detection limits (less than 0.1 μM) and good repeatability of the peak areas (R.S.D.: 5.2–7.8%, $n = 5$) was achieved using LVSS (300 s). With LVSS, the detection sensitivity was enhanced more than 50-fold compared to conventional hydrodynamic injection. The proposed method was used successfully for the determination of Fe^{2+} and Fe^{3+} in water samples.

Keywords: On-column complexation; Sample stacking; Iron; 2,6-Pyridinedicarboxylic acid

1. Introduction

The chemical form of heavy metals in the solid phase can strongly influence their behaviour such as mobility, toxicity, bioavailability and chemical interaction [1,2]. For instance, Fe plays an important role in the acidification of many soils in the temperate region. In addition, Fe can be toxic to soil organisms at higher concentration, but Fe is an essential element at lower concentration [3,4]. Separation methods for the Fe speciation mainly include ion chromatography (IC) coupled to various detection techniques [5,6], which are useful, and offer high detection sensitivity for the determination of Fe species in environmental samples. However, a simple conventional detection based on UV-Vis detection is favourable for routine analysis. In this technique, Fe species were converted into stable derivatives using suitable ligands prior to analysis, and following formation of the derivatives was separated by separation such as IC or capillary electrophoresis [7,8].

Capillary zone electrophoresis (CZE) is an alternative method to ion chromatography in the analysis of metal species [8,9]. Despite relatively poor reproducibility and lower detection sensitivity compared to IC, CZE offers the advantages such as high separation efficiency. In principle, two approaches are used in the CE separation of metals [9]. One is on-column complexation, where a soluble ligand is added to the running electrolyte and weak complexes are rapidly formed. Indirect UV detection is usually employed and carboxylic acids are usually used as the weak ligands [10,11]. Another approach is pre-column complexation, where an excess of strong ligand is added to the sample to form complexes prior to CZE analysis. This method allows for direct UV detection of the metal ions after chelating with suitable UV absorbing ligands [9,12]. In these

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approaches, significant advantages for complexation include the simultaneous determination of metal species in one run is possible because the species having a positive charge can be converted into their complexes having a negative change, and the preservation of the original oxidation states is often possible using suitable ligands [9–12].

Pre-column complexation CZE methods have recently been reported for the separation of Fe(II) and Fe(III) [13,14], which was based on UV-Vis detection. Fe(II) and Fe(III) selectively complexed with 1,10-phenanthroline and cyclohexane-1,2-diaminetetraacetic acid (CDTA) to form an anionic complex, and were separated by CZE with direct UV detection at 254 nm using a borate buffer (100 mM, pH 9.0) [13]. The detection limits of 0.06 mg/l for Fe(II) and 0.1/l for Fe(III) were obtained. Similar results for the determination of Fe species in mineral samples have been recently reported [14]. However, pre-column complexation is far from ideal and suffers from disadvantages such as incomplete derivatization and time consuming for real sample processing. In contrast, on-column complexation allows the direct injection of samples to CZE due to their complexation during electrophoresis and direct UV detection. In this approach, on-column separation of metal ions has been recently developed [15,16]. However, in both pre-column and on-column CZE separation of metal species, the detection sensitivity does not meet the requirement for the analysis of real samples containing metal species at trace level because of the small injection volume and the short optical path length associated with on-column UV detection. This problem can be addressed using on-column sample stacking techniques [17]. Large-volume sample stacking (LVSS) was proposed as a highly efficiency sample stacking in aqueous media, which included LVSS both with polarity switching and without polarity switching [18,19]. However, the polarity switching used in this technique is not possible in most commercial CE instruments. LVSS without polarity switching has been used for the separation of anions, where the addition of EOF [20] modifier to buffer or using a low pH buffer [21,22] suppressed the EOF. The detection sensitivity was enhanced in the range of 100–300-fold for small anions.

Previously, 2,6-PDCA was used as the mobile phase in ion chromatography for UV detection of inorganic anions, cations, and carboxylic acids [23–25]. Recently, 2,6-PDCA has been used as the background electrolyte (BGE) in the separation of metal ions [15,26]. These results suggested that 2,6-PDCA strongly chelates heavy metals to form anionic complexes $[M(PDCA)_2]^{2-}$, however, the detection limits for these metal ions did not meet the requirement for analysis of the real sample. In this paper, anionic Fe(II) and Fe(III) complexes on-column complexation with 2,6 PCDA were separated by co-electroosmotic capillary zone electrophoresis (co-CZE) coupled with LVSS to improve UV detection sensitivity. To achieve the aim, the following aspects included (1) the separation conditions, (2) the optimised LVSS, and (3) the demonstrated use of the proposed method for the determination of waters.

2. Experimental section

2.1. Chemicals and solutions

All reagents (analytical grade) were obtained from Sigma–Aldrich (Sydney, Australia) and dissolved in Milli-Q water without further purification. Standard solutions of Fe species were diluted daily from 10 mM stock solution using Milli-Q water and adjusted to pH 3.0 with 1 mM HNO_3. BGE required for CE were prepared by dissolution of appropriate amounts of 2,6-PDCA in Milli-Q water containing appropriate amounts of cationic surfactants. All electrolytes were filtered through a Millipore 0.45 μm membrane filter. The pH of the BGE was adjusted with 0.1 M NaOH or 0.1 M HNO_3 solution. Groundwater samples were filtered through a 0.45 μm membrane filter.

2.2. Instrumentation

All CE experiments were preformed using a Quanta 4000 instrument (Waters, Milford, USA). The system was controlled by Millennium (Waters, Milford, USA) software. Separation was carried out using a 50 cm fused-silica capillary (42.5 cm × 50 μm i.d. effective length).

2.3. Electrophoretic procedures

Prior to use, a capillary was pretreated with the following cycles: 0.1 M NaOH for 20 min, 0.01 M NaOH for 20 min, deionized water for 20 min and 10 mM the electrolyte for 30 min. The capillary was pre-conditioned with the electrolyte for 2 min before each run. Samples were injected in the hydrodynamic mode for 30 s. The capillary was held at 30 °C, and the applied constant voltage was −20 kV for co-EOF. LVSS was preformed by injection of solution of iron species using hydrodynamic mode with system raising the sample vial to a level 9.8 cm higher than that the electrolyte (pressure: 10 mbar) and with different injection time. Identification of each of the solutes was based on the migration time and was verified by spiking samples with known standards. Benzyl alcohol (0.05% (v/v)) was used as a neutral marker for the determination of the electrophoretic mobility. The electroosmotic mobility and the electrophoretic mobility of the solute and marker were calculated using the equations described previously [27]. The conversion of injection time to length of the sample zone was calculated using “expert” software ware from Beckman or was based on [27]. The relationship between the length of the sample zone and injection time is described as $y = 0.208x$ (y is the length of sample zone (mm), x is the injection time (s)) under the experimental conditions.

3. Results and discussion

3.1. On-column complexation and separation conditions

2,6-PDCA as the electrolyte in co-CZE for on-column complexation of metal ions [15] has shown that that the eluent pH and its concentration play an important role in the formation of anionic complexes and the separation selectivity. The conversion of iron species from ionic to anionic complexes can be described as [15,25,26].

$$Fe^{2+} + [2PDCA]^{2-} = [Fe(PDCA)_2]^{2-} \quad (1)$$

$$Fe^{3+} + [2PDCA]^{2-} = [Fe(PDCA)_2]^{-} \quad (2)$$

$$H_2PDCA = PDCA^{2-} + 2H^{+} \quad (3)$$

$$Fe^{2+}(Fe^{3+}) + 2OH^{-}(3OH^{-}) = Fe(OH)_2[Fe(OH)_3] \quad (4)$$

As 2,6-PDCA is an ionizable compound (pKa_1, 2.16; pKa_2, 6.92), its ligand concentration in the electrolyte depends on the pH as shown in Eq. (3). The ligand concentration is favoured with increasing pH, and consequently the degree of Fe complexation with 2,6-PDCA is increased. However, increasing the pH not only influences the metal hydrolysis as shown in Eq. (4), but also the EOF [15,16]. Consequently, pH changes affect both the separation selectivity and on-column complexation. In addition, EOF values can be manipulated by using EOF modifiers such as cationic surfactants and organic solvent [28–31]. Therefore, optimisation of the electrolyte is required to achieve the reasonable selectivity and sensitivity.

A 5 mM 2,6-PDCA electrolyte contained 0.25 mM TTAB was used as the electrolyte, and the influence of the electrolyte pH on the observed mobility was examined due to the formation of anionic Fe complexes [12,15,16]. Fig. 1 shows that the observed mobility of the Fe complexes increased as the electrolyte pH increased. The increase in the observed mobility can be attributed to the favourable formation of metal complexes as the electrolyte pH was increased, leading to an increase in negative charge on the metal complex [12,15,16]. However, the peak of $[Fe(PDCA)_2]^{-}$ reduced with increasing electrolyte pH, this is due to the formation of precipitation as described in Eq. (4). A good resolution and sharp peaks of $[Fe(PDC)_2]^{2-}$ and $[Fe(PDCA)_2]^{-}$ were obtained at a low pH. In the view of both selectivity and sensitivity, the pH of 4.0 was used in subsequent studies.

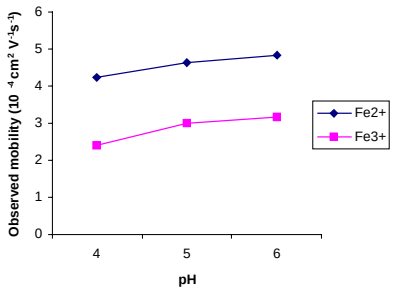

Fig. 1. Effect of pH on the mobilities of $Fe[PCDA]_2^{2-}$ and $Fe[PCDA]_2^{-}$. Conditions: capillary, fused-silica capillary 50 cm × 50 μm (effective length: 42.5 cm); electrolyte, 5 mM 2,6-PCDA and 0.25 mM TTAB. Applied potential, −20 kV; hydrostatic injection: 30 s, UV detection at 214 nm. Capillary temperature, 30 °C; 0.2 mM each solute.

From Eqs. (1) and (2), the concentration of 2.6-PDCA in the electrolyte impacts on the formation of iron complexes and the effective mobility [12,15]. With a constant concentration of 0.25 mM TTAB in the electrolyte, the concentrations of 2,6-PCDA were varied from 5 to 20 mM. The results show that the mobility of Fe complexes slightly decreased by increasing the concentration of 2,6-PCDA because of the increase in the ionic strength in the electrolyte as described previously work on the effect of electrolyte concentration on solute mobility has shown that the mobility of solutes decrease with increasing electrolyte concentration [11,28]. However, the sensitivity significantly decreased when the concentration of 2,6-PCDA increased as shown in Fig. 2. High concentration favours the formation of complexes, but at the expense of the decreased sensitivity. This can be attributed to an increase in background absorbance with an increase in the concentration of 2,6-PCDA, leading to a decrease in the sensitivity because of 2,6-PCDA with a high molar absorptivity [12,15,26]. Considering the detection sensitivity, the addition of 5 mM PDCA in the electrolyte was used.

To reduce other metal ions interference with separation of $[Fe(PDCA)_2]^{2-}$ and $[Fe(PDCA)_2]^{-}$, the separation selectivity was manipulated by controlling the EOF [28–31], which was added to TTAB and acetonitrile [27–31]. Fig. 3(a) shows the effect of TTAB concentration on the EOF. It can be seen that the EOF increased initially with increasing TTAB concentration from 0.25 to 0.75 mM, but a constant EOF over 1.0 mM TTAB was observed. This was caused by the surface of the capillary fully being coated by TTAB when its

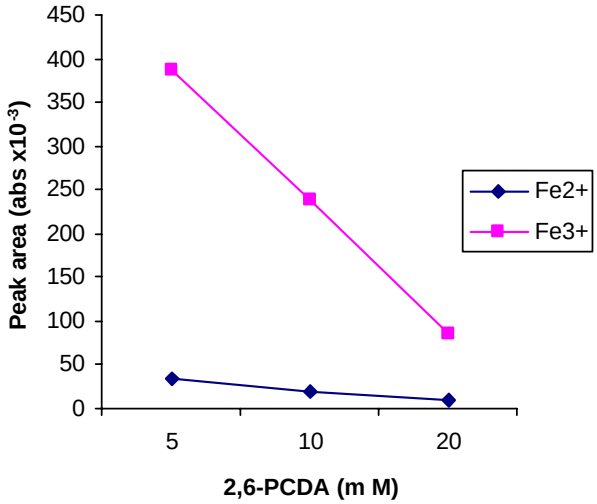

Fig. 2. Effect of 2,6-PCDA concentration on the sensitivity. The electrolyte pH at 4.0. Other conditions as in Fig. 1.

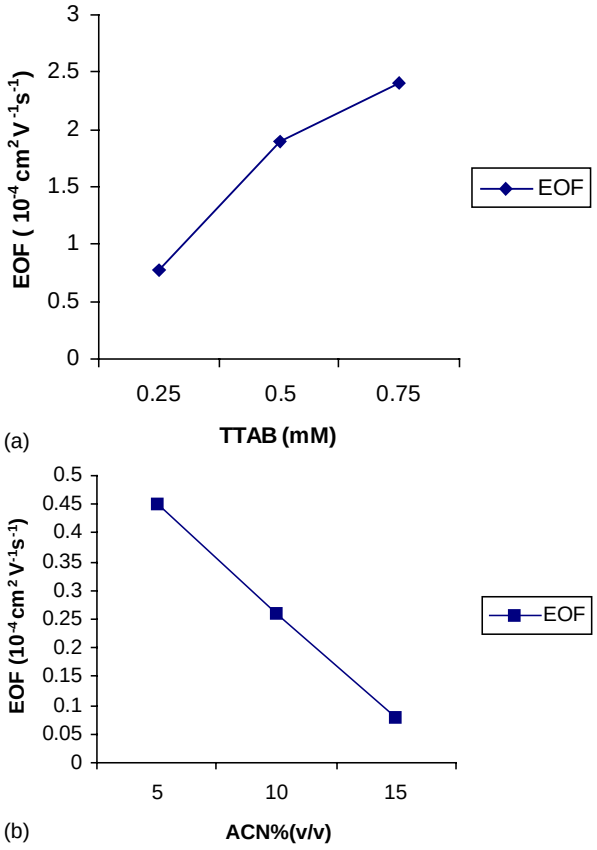

Fig. 3. (a) Effect of TTAB concentration on the EOF. The electrolyte contained 5 mM 2,6-PCDA at pH of 4.0. Other conditions as in Fig. 1. (b) Effect of acetonitrile (v/v) on the EOF. The electrolyte contained 5 mM 2,6-PCDA and 0.25 TTAB at pH of 4.0.

concentration was over 0.75 mM [32]. The influence of acetonitrile on the EOF was studied by adding acetonitrile to an electrolyte contained 5 mM PCDA and 0.25 mM TTAB at pH 4. As shown in Fig. 3(b), it can be seen that the EOF decreased with increasing acetonitrile in the electrolyte. This indicates the decrease of EOF with increasing content results mainly from a decreased dielectric constant in electrolyte, which leads to a low value for the zeta potential of the capillary wall [33]. In addition, the constant effective mobility of iron complexes was observed with increasing TTAB or acetonitrile in the electrolyte. This is due to the EOF modifiers, which do not affect the mobility as in previous reports [15,16]. On the basis of the above data, the best separation was achieved using an electrolyte containing 5 mM PDCA, 0.25 mM TTAB and 5% acetonitrile at pH 4.0, which offered the reasonable selectivity and sensitivity as shown in Fig. 4. However, the peaks of Fe^{2+} and Fe^{3+} in the electropherogram are quite broad. They could be resulted from the different complexation of two iron states. Similar results were observed on-column complexation for the separation of metal ions by CZE [15].

A number of the metal ions were tested to determine whether they interfered with separation of Fe complexes.

Table 1
The migration time, mobility and the detection sensitivity for the tested metals as their 2,6-PCDA complexes

Species (0.2 mM)	Migration time (min)	Mobility (10^{-4} cm^2 v^{-1})	Detection sensitivity (abs × 10^{-3})
Pb^{2+}	6.22	2.91	51.3
Ni^{2+}	4.07	4.47	52.9
Cu^{2+}	4.01	4.53	76.9
Co^{2+}	3.96	4.67	76.0
Cd^{2+}	4.32	4.20	24.9
Mn^{2+}	4.66	3.89	32.3
Zn^{2+}	4.13	4.39	23.5
Ca^{2+}	8.60	2.11	53.1
Al^{3+}	6.94	2.61	25.7
Fe^{2+}	4.74	4.02	33.1
Fe^{3+}	7.86	2.32	38.7

Conditions as in Fig. 4.

The mobility of the metals as their 2,6-PCDA complexes and the detection sensitivity are listed in Table 1. It can be seen that all complexes did not interfere in the separation of $[Fe(PDCA)_2]^{2-}$ and $[Fe(PDCA)_2]^{-}$ because of their various mobilities. The common metal ions in a real sample such as Al^{3+}, Mn^{2+} and Ca^{2+} complexes were 2.61, 3.89 and 2.11 (10×10^{-4} cm^2 v^{-1} s^{-1}) respectively, while there was no Mg^{2+} response for UV. The similar results were obtained in previously reports [15,26]. This indicates the proposed co-CZE conditions could be used for the separation of iron species in real samples.

3.2. Co-CZE with LVSS for the determination of Fe species

Large volume sample injection (LVSS) without polarity switching is a useful technique for on-column preconcentration of anionic solutes [34], where an EOF was added modifiers into the electrolyte to press EOF, leading to sample stacking during removal of the sample plug, and subsequent sample separation can be performed under the same negative voltage without loss of the sample. Recently, LVSS with polarity switching by the use of a low pH buffer or surfactants to suppress the EOF has been reported, where the detection sensitivity was enhanced to 100–300-fold [34,35]. On the basis of co-CZE in an acidic electrolyte containing EOF modifier such as TTAB in this case, it could be possible to use LVSS without polarity switching on-column preconcentration of $[Fe(PDCA)_2]^{2-}$ and $[Fe(PDCA)_2]^{-}$ complexes using an electrolyte containing 5 mM 2,6-PCDA, 0.25 mM TTAB and 5% acetonitrile at a pH of 4.0. Fig. 5 shows the peak areas of iron complexes increased with increasing injection time, e.g. the peak area of $[Fe(PDCA)_2]^{-}$ significantly increased from 30 (0.624 cm the length of sample zone) to 300 s (6.24 cm the length of sample zone), while the peak area of $[Fe(PDCA)_2]^{2-}$ initially increased with injection time from 30 to 250 s, but after 250 s, the peak was near constant. When the injection time was over 400 s, the peak of $[Fe(PDCA)_2]^{2-}$ reduced and peak shape

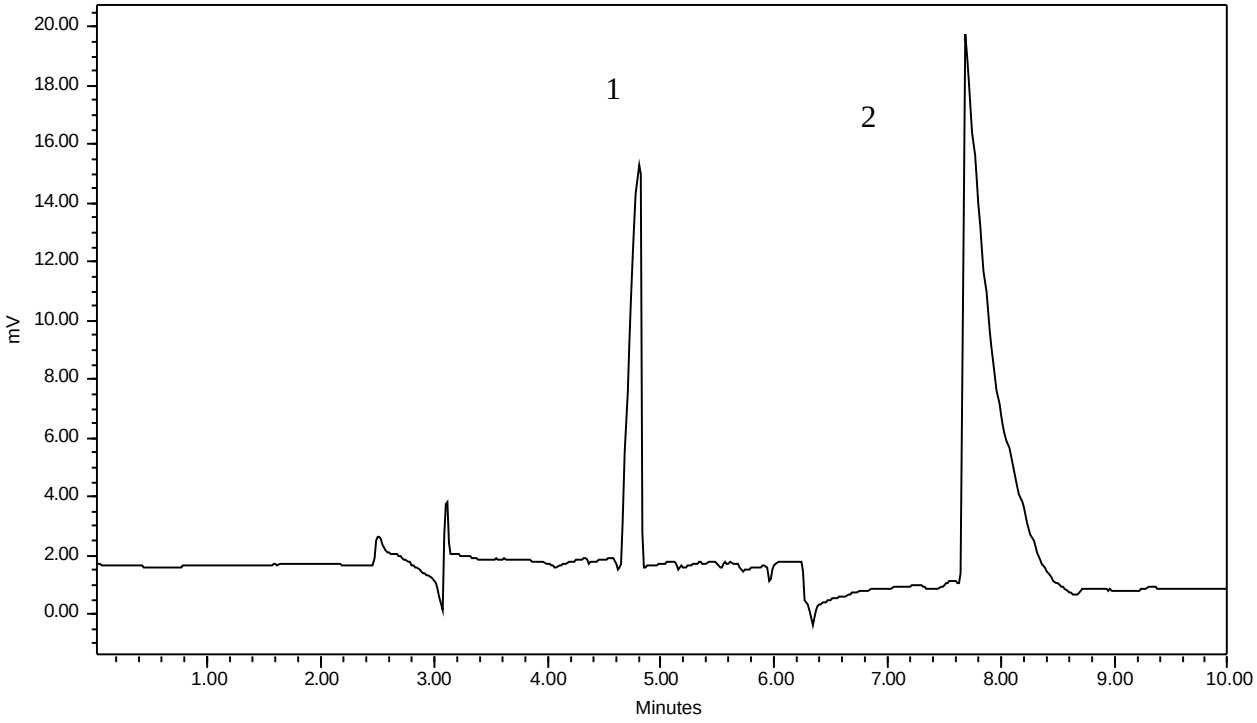

Fig. 4. Typical electropherogram obtained using an electrolyte contained 5 mM 2,6-PCDA 5% (v/v) acetonitrile and 0.25 mmTTAB, at pH of 4.0. Other conditions as in Fig. 1. (1) $Fe[PCDA]_2^{2-}$; (2) $Fe[PCDA]_2^{-}$. Other conditions as in Fig. 1.

were obtained. This is due to loss of $[Fe(PDCA)_2]^{2-}$ caused by EOF removal of sample plug and the loss of separation efficiency [24,35]. Thus, the maximum available injection time was 300 s. Fig. 6 represents the electropherograms of Fe complexes using both conventional hydrodynamic injection (10 s, Fig. 6(a)) and LVSS (300 s, Fig. 6(b)) with of 1 μM mixture of iron complexes. The detection sensitivities of $Fe(PDCA)_2]^{2-}$ and $[Fe(PDCA)_2]^{-}$ complexes were enhanced in 51- and 203-fold (peak area), respectively.

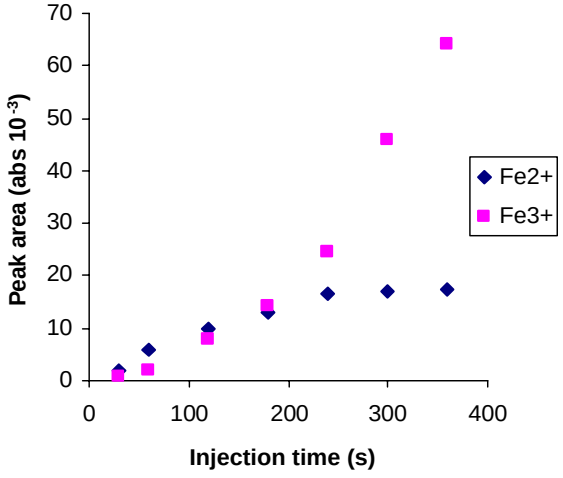

Fig. 5. The relationship between peak area and injection time. 0.01 mM for each solute, other conditions as in Fig. 1.

Detection limits of $Fe(PDCA)_2]^{2-}$ and $[Fe(PDCA)_2]^{-}$ complexes were less than 0.1 μM.

Calibration plots were obtained by plotting peak area versus concentration of $Fe(PDCA)_2]^{2-}$ and $[Fe(PDCA)_2]^{-}$ using LVSS. The linearity was in the concentration range of 0.5–50 μM. Correlation coefficients were in the range of 0.995 to 0.998. The reproducibility for the peak areas (R.S.D.%, $n = 5$) from injecting a 2 μM standard mixture ranged from 5.2–7.8% as listed in Table 2. The proposed method was used for the analysis of iron species in water. Fig. 7 shows the electropherogram of ground waters. Recoveries for the iron species were 86.5–96.3% for groundwater spiked with a 2 μM mixed standard, but depended on the samples. The results obtained in the analysis of various waters from different sites (Newcastle, NSW, Australia). Fe^{2+} and Fe^{3+} were found in the ground waters, and the concentration for Fe^{2+} and Fe^{3+} were in the range of 0.31–1.8 and 4.2–17.3 μM. In contrast, only higher concentration of Fe^{3+} was found in river water with concentration ranging from 24.2–40.5 μM. However, variable migration time for the analyte was noted when different sample matrix was injected

Table 2
The characteristics for iron species by the proposed method

Species	Regression line	Coefficient	Detection limit (μM)	Reproducibility ($n = 5$, %)
Fe^{2+}	$y = 2.79x + 7.25$	0.998	0.10	7.8
Fe^{3+}	$y = 4.67x + 10.81$	0.999	0.05	5.2

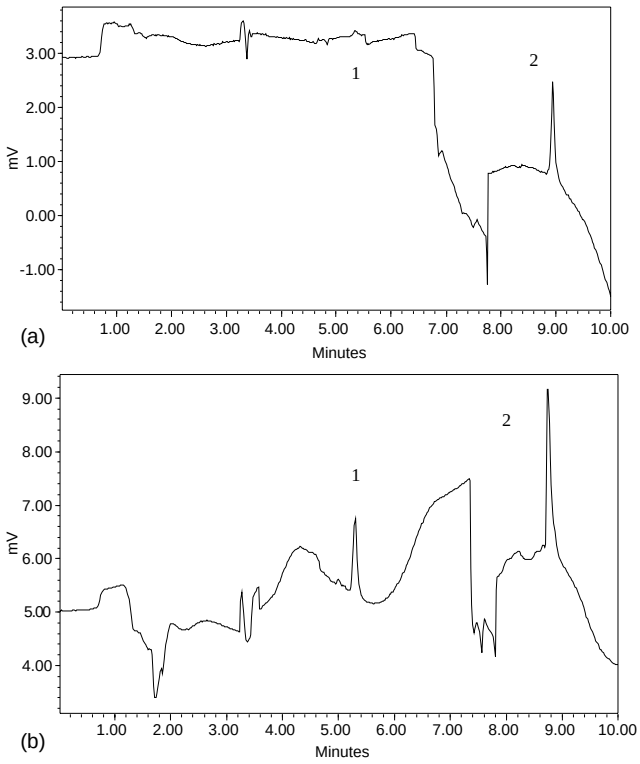

Fig. 6. Comparison of LVSS with conventional hydrodynamic injection. (a) Conventional hydrodynamic injection (10 s); (b) LVSS (300 s). (1) $Fe[PCDA]_2^{2-}$; (2) $Fe[PCDA]_2^{-}$. One μM for each solute, other conditions as Fig. 1.

using LVSS, e.g. Fig. 6(b)—standard and Fig. 7—water sample. The changes in migration time was cause by the fact that the EOF at the sample plug was significantly different that at the supporting electrolyte, for example, the pH and ionic strength of the sample plug and sample zone impacted on the EOF, and it is therefore changes in the migration time [36–38].

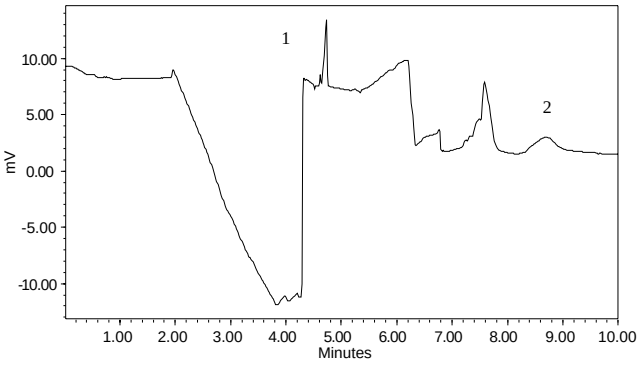

Fig. 7. The electropherogram obtained from ground water using LVSS (300 s). (1) $Fe[PCDA]_2^{2-}$; (2) $Fe[PCDA]_2^{-}$. Other conditions as in Fig. 1.

4. Conclusions

The results show that on-column complexation and separation of Fe species as their 2,6-PCDA complexes is possible, and provides a simple and rapid separation method for the determination of Fe species in real samples. The EOF modifiers such as cationic surfactants and organic solvent, as well the electrolyte pH, can be used to manipulate the separation selectivity. However, the concentration of 2,6-PCDA has significantly impacted on the detection sensitivity for on-column complexation because its high UV absorptivity. Compared with the conventional hydrodynamic injection, LVSS can be used to improve the detection sensitivity and 50–200-fold increasing was achieved in this case. The proposed method can be performed in commercial CZ instruments without polarity switching and could have potential for the determination of Fe species in real sample because of their simple procedure.

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