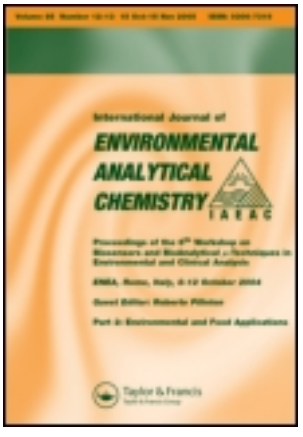

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Simultaneous determination of phosphate and calcium in river water samples by capillary zone electrophoresis with UV detection

Qing-Ping Wang [a b], Zu-Liang Chen [b], Guo-Nan Chen [a] & Jin-Ming Lin [a c]

[a] Ministry of Education Key Laboratory of Analysis and Detection for Food Safety, Fuzhou University, Fuzhou, Fujian, China

[b] School of Environmental Science and Engineering, Fujian Normal University, Fuzhou, Fujian, China

[c] Department of Chemistry, Tsinghua University, Beijing, China

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Simultaneous determination of phosphate and calcium in river water samples by capillary zone electrophoresis with UV detection

Qing-Ping Wang[ab], Zu-Liang Chen[b]*, Guo-Nan Chen[a] and Jin-Ming Lin[ac]

[a]*Ministry of Education Key Laboratory of Analysis and Detection for Food Safety, Fuzhou University, Fuzhou, Fujian, China;* [b]*School of Environmental Science and Engineering, Fujian Normal University, Fuzhou, Fujian, China;* [c]*Department of Chemistry, Tsinghua University, Beijing, China*

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Capillary zone electrophoresis (CZE) with UV detection at 214 nm was developed for the simultaneous determination of phosphate and calcium in waters, where Ca^{2+} reacted with 2,6-pyridinedicarboxylic acid (2,6-PDCA) in the electrolyte to form anionic $Ca[PDCA]_2^{2-}$. Consequently, HPO_4^{2-} and $Ca[PDCA]_2^{2-}$ were simultaneously separated by CZE using an electrolyte containing 10 mM 2,6-PDCA and 0.75 mM tetradecyltrimethylammonium bromide (TTAB) at pH 7.0. The results showed that reasonable resolution with low interference from other ions in the water was achieved. Linear calibration curves were obtained in the concentration range of 0.01–0.5 mM with hydrodynamic injection. The detection limits ($S/N = 3$) were 5 μM for $[Ca(PDCA)_2]^{2-}$ and 2 μM for $[HPO_4]^{2-}$. The separation of $[HPO_4]^{2-}$ and $Ca[PDCA]_2^{2-}$ occurred within 6 min with small relative standard deviation of peak areas (<5%). The method was successfully applied to the determination of HPO_4^{2-} and Ca^{2+} in river water samples, and provided a fast and simple procedure that required no modification of the CE instrument.

Keywords: on-column complexation; phosphate and calcium; co-EOF CZE; water

1. Introduction

Since phosphorus is added directly to the land as a fertiliser as a part of many agricultural practices, it discharges directly to rivers via surface run off and impacts directly on water quality [1]. Phosphorus enrichment in rivers can result in excessive growth of aquatic plants, which leads to a decline in water quality due to an increase in outbreaks of toxic algal blooms and consequential reductions in dissolved oxygen levels. High phosphorus concentrations can also inhibit calcium carbonate precipitation [2]. The determination of phosphate concentrations in natural waters therefore provides essential information for assessing the health of aquatic ecosystems and investigating biogeochemical cycling.

The simultaneous separation and detection of anions and cations in real samples by ion chromatography (IC) has received much attention in recent years because it provides additional information with cost-effectiveness and shorter analysis times when compared to conventional IC approaches which require two different sets of conditions, and

*Corresponding author. Email: zlchen@fjnu.edu.cn

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two different runs, for the separation and detection of both cationic and anionic species. Thus, simultaneous determination of anions and cations in a single run is highly advantageous and both multi-column [3] and single column [4–6] IC systems have been developed for the simultaneous determination of anions and cations in real samples. Many of these systems have recently been extensively reviewed [7], but all suffer from relatively low separation efficiency.

Due to its high separation efficiency and short analysis time, capillary zone electrophoresis (CZE) is becoming a popular alternative to IC. Many CZE methods have been developed for the separation and detection of anions or cations using a range of background electrolytes [8–10]. However, there are some reports on the simultaneous separation and detection of both anions and cations by CZE with direct and indirect UV detection since these ions tend to migrate in opposite directions under a given electrical field. Several different approaches have been developed to examine the possibility of simultaneous separation and detection of both anions and cations using CZE. First, a high electro-osmotic flow (EOF) can be used to force the cations and anions to co-migrate towards the detector [11–15,16]. Secondly, Xiong *et al*. [14,15] developed new background electrolytes (BGEs) containing two UV-absorbing probes that were used to separate and detect alkali metals and organic acids simultaneously in real samples. Thirdly, Kuban *et al*. [17,18] and Padarauskas *et al*. [19–21] developed special CE systems, where the sample was injected from both ends of the capillary and both cations and anions migrated in opposite directions from each end, and the ions were detected at the detector window after applying voltage. While most of the common anions and cations can be separated using this approach [17–21], it does require the modification of a commercially available CE instrument, and therefore limits some applications [22]. Finally, metal cations were converted to their anionic chelates and were separated simultaneously with some inorganic anions by CZE [23]. This latter approach seems to be more applicable to commercially available CE instruments, since it does not require the modification of CE instrumentation and has consequently been adopted by many users for the simultaneous separation and detection of cation and anions [23–26].

Previously, 2,6-PDCA has been added to the mobile phase in ion chromatography [6,27] or as one of the electrolyte component in CZE [28,29] for UV detection of inorganic anions, organic acids and cations. These studies suggest that 2,6-PDCA chelates heavy metals to form anionic complexes $[M(PDCA)_2]^{2-}$. Since 2,6-PDCA can also be used as a background electrolyte (BGE) for both indirect UV detection of anions and direct UV detection of metal ions [24,27–29], it could potentially be used for the simultaneous determination of anions and cations. In this paper, a method for the simultaneous determination of HPO_4^{2-} and Ca^{2+} was developed using 2,6-PCDA as the BGE and natural water samples were used to validate the utility of the developed method.

2. Experimental

2.1 *Chemical and sample preparation*

All reagents were of analytical grade, obtained from Sigma and Aldrich (Sydney, Australia), and were used without further purification. Standards for the anions and calcium were prepared daily from 10 mM stock solutions using deionised water. The background electrolyte was prepared by dissolving the appropriate amount of 2,6-PDCA in deionised water, which also contained an appropriate amount (0.75 mM) of

tetradecyltrimethylammonium bromide (TTAB). All electrolytes were filtered through disposable Millipore 0.45 μm membrane filters and degassed in an ultrasonic bath prior to use. Background electrolyte pH was adjusted using 0.1 M NaOH or 0.1 M HCl. River water samples were collected from different sampling sites in the Minjian River, Fuzhou City, China, where the industrial population has rapidly increased in the last 20 years, and wastewater and sewage contribute to the river. The water samples were stored in the dark room at 4°C until the analysis. Water samples were filtered through a Millipore 0.45 μm membrane filter before injection into the CZE system. Ion chromatography was also used for the analysis of phosphate and calcium in river water samples [30,31].

2.2 *Instrumentation*

All CE experiments were performed using a Quanta 4000 instrument (Waters, Milford, USA). The system was controlled by Millennium (Waters, Milford, USA) software. Separation was carried out on 55 cm (48.5 cm × 50 μm I.D) fused-silica capillaries with UV detector at 214 nm.

2.3 *Electrophoretic procedures*

Prior to use, the capillary was conditioned with the following cycles: 1 M NaOH for 10 min, 0.1 M NaOH for 10 min, deionised water for 20 min and the electrolyte for 20 min. The capillary was also rinsed with the BGE for 2 min between each run. Samples were injected hydrodynamically at a pressure of 40 mbar for 30 s. The capillary was held at 30°C, and the applied voltage was constant at −20 kV. Identification of each of the solutes was initially 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 [32].

3. Results and discussion

3.1 *On-column complexation and separation conditions*

Previous work using 2,6-PDCA as the electrolyte for CZE has shown that the electrolyte pH plays an important role in determining separation selectivity and the formation of anionic metal complexes [27,28]. The conversion of cationic Ca^{2+} to an anionic complex on the column is influenced by the electrolyte pH as described below by Equations (1)–(3) [28,29,33].

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

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

$$Ca^{2+} + 2OH^- = Ca(OH)_2 \quad (3)$$

Since 2,6-PDCA is ionisable (pka_1 2.16; pka_2 6.92), the ligand concentration in the BGE depends on the pH as shown by Equation (2). An increase in the electrolyte pH favours

increasing concentrations of anionic ligand concentrations, and therefore increases the degree of calcium chelating with 2,6-PDCA. In addition, the formation of $Ca(OH)_2$ decreases with an increase in pH because Ca^{2+} chelating with 2,6-PDCA is the dominant reaction, which was observed in our previous reports [28,29,34]. Consequently, optimisation of the electrolyte pH is required to balance these competing effects to ensure high separation efficiency. The influence of electrolyte pH on the effective mobility was examined under co-EOF mode using a BGE containing 10 mM 2,6-PDCA solution and 0.75 mM TTAB. While the effective mobility of both $[HPO_4]^{2-}$ and $[Ca(PDCA)_2]^{2-}$ increased with increasing electrolyte pH, the most significant increase in mobility of $[HPO_4]^{2-}$ occurred between pH 6–8 (Figure 1). This is because phosphate speciation changed in this range and $[H_2PO_4]^-$ was ionised to $[HPO_4]^{2-}$ (pka_2: 7.20). In contrast, the effective mobility of $[Ca(PDCA)_2]^{2-}$ slightly increased with electrolyte pH since the charge of $[Ca(PDCA)_2]^{2-}$ was not significantly affected by a change in the electrolyte pH and the EOF was invariant with pH because under the separation conditions the capillary surface was completely saturated with adsorbed TTAB. A pH effect on EOF was observed under the co-EOF mode [35].

2,6-PDCA can also potentially complex other metal ions present in samples depending on the relative association complexes of the ligand-metal pair [28,29,33]. Since these anionic solutes also migrate in the capillary column and potentially absorb significantly at 214 nm, they also interfere with the separation of $[Ca(PDCA)_2]^{2-}$. Thus, a number of the metal ions, including Mg^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+} and Fe^{3+} were tested using an electrolyte containing 10 mM 2,6-PDCA at pH 7.0, while most of the

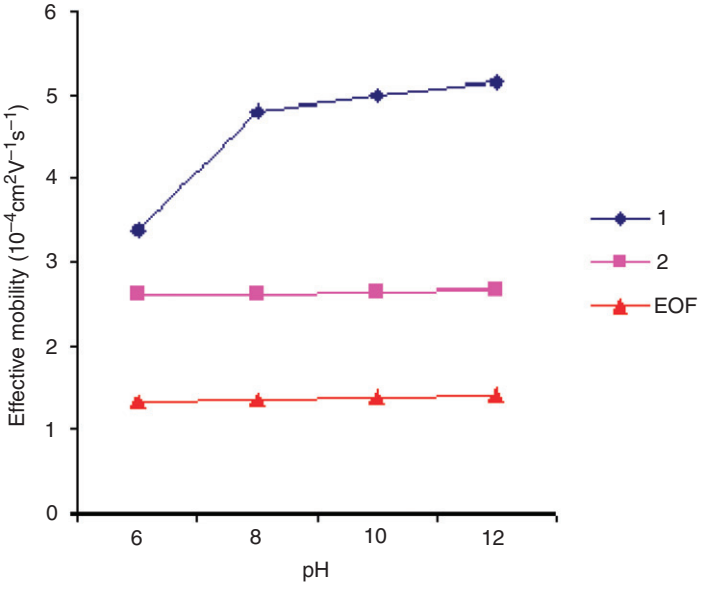

Figure 1. Effect of electrolyte pH on the mobility and the EOF. (1) HPO_4^{2-}; (2) $[Ca(PDCA)_2]^{2-}$ Conditions: fused-silica capillary 55 cm × 50 μm (effective length: 48.5 cm); electrolyte, 10 mM 2,6 PDCA + 0.75 mM TTAB. Applied potential, –20 kV; hydrodynamic injection: 30 s, UV detection at 214 nm. Capillary temperature, 30°C, the concentration of each solute: 0.1 mM.

metals gave some UV response due to the formation of metal anionic complexes, they did not interfere with the separation of $[Ca(PDCA)_2]^{2-}$ because they had different mobilities [25,28,29]. Likewise anions did not interfere with the separation of $[HPO_4]^{2-}$ (Figure 2). The results show that simultaneous separation of phosphate and calcium was possible without interference from other metals under the conditions used.

3.2 *Analysis of phosphate and calcium in waters*

Electropherograms showed that the target compounds (Cl^-, SO_4^{2-}, $[HPO_4]^{2-}$ and $[Ca(PDCA)_2]^{2-}$) could be separated with good separation of anions using 10 mM 2,6-PDCA at pH 7.0 (Figure 2), anions (Cl^-, SO_4^{2-}, HPO_4^{2-}) were indirectly detected at 214 nm since these anions have relatively weak absorption in the UV region [8], while $[Ca(PDCA)_2]^{2-}$ was detected directly at 214 nm since $[Ca(PDCA)_2]^{2-}$ has strong UV absorption [6]. Calibration plots obtained by plotting peak area *versus* concentration were linear in the concentration range of 0.01–0.5 mM having correlation coefficients (r^2) > 0.998. The detection limits (S/N = 3) were 5 μM for $[Ca(PDCA)_2]^{2-}$ and 2 μM for $[HPO_4]^{2-}$. The precision of the peak area (relative standard derivation, $n = 5$) from injecting a 0.1 mM standard mixture were 3.1 and 4.4% for $[Ca(PDCA)_2]^{2-}$ and $[HPO_4]^{2-}$, respectively. Analytical characteristics of the test solutes using the proposed method, including the calibration range, precision and detection limit, are listed in Table 1.

The developed method was used for the determination of the target compounds in river water which were collected from sites with agriculture activity and where some phosphate had been applied. Water samples were diluted ten-fold with Mili-Q water prior to injection into the CZE system. The results indicate that Cl^-, SO_4^{2-}, HPO_4^{2-} and $[Ca(PDCA)_2]^{2-}$ were all detected in Figure 3. Satisfactory reproducibility for the peak

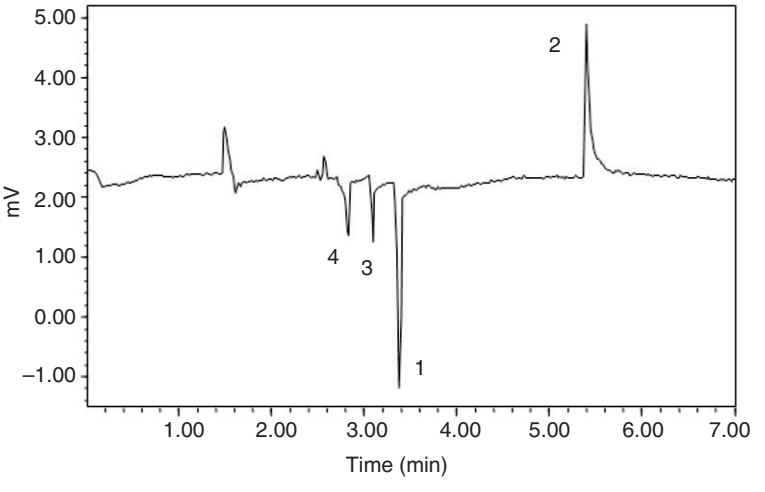

Figure 2. Typical electropherogram. (1) HPO_4^{2-}; (2) $[Ca(PDCA)_2]^{2-}$, (3) SO_4^{2-}; (4) Cl^- Conditions: fused-silica capillary 55 cm × 50 μm (effective length: 48.5 cm); electrolyte, 10 mM 2,6 PDCA + 0.75 mM TTAB at pH 7.0. Applied potential, –20 kV; hydrodynamic injection: 30 s, UV detection at 214 nm. Capillary temperature, 30°C, the concentration of each solute was 0.1 mM.

Table 1. The characteristics for the proposed method.

Species	Calibration range (mM)	LOD(S/N = 3) (μM)	Peak area	RSD (%, $n = 5$) migration time
$[Ca(PDCA)_2]^{2-}$	0.01–0.5	5	4.4	0.51
$[HPO_4]^{2-}$	0.01–0.5	2	3.1	1.02

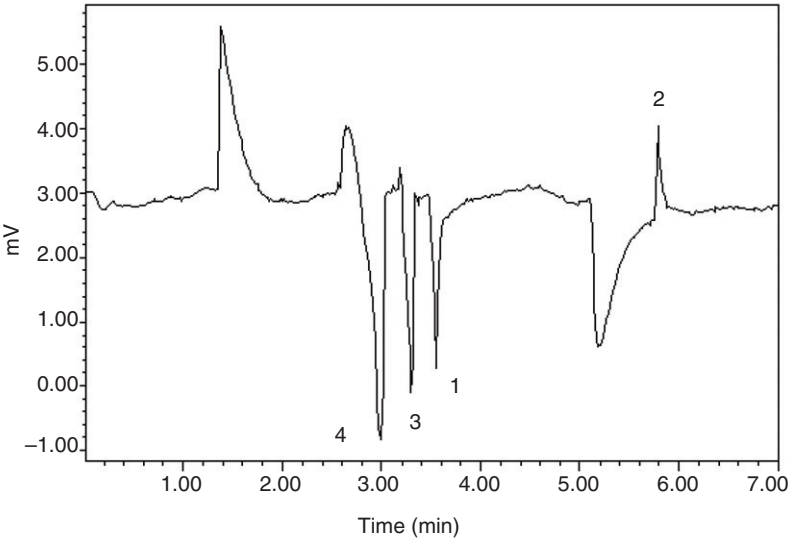

Figure 3. A river water sample. Conditions as in Figure 2. (1) HPO_4^{2-}; (2) $[Ca(PDCA)_2]^{2-}$, (3) SO_4^{2-}; (4) Cl^-.

Table 2. The real samples data obtained from proposed method and IC method (mM).

	The proposed method		Ion chromatography	
	$[Ca(PDCA)_2]^{2-}$	$[HPO_4]^{2-}$	Ca^{2-}	$[HPO_4]^{2-}$
Sample 1	0.03 ± 0.01	0.07 ± 0.01	0.035 ± 0.01	0.06 ± 0.01
Sample 5	0.15 ± 0.02	–	0.16 ± 0.02	–

Note: – not detectable.

areas of the solutes were obtained with 3.3–4.4% R.S.D. ($n = 5$). Recoveries of 95.3 to 101.5% in samples spiked at 0.1 mM were also obtained. $[HPO_4]^{2-}$ was found in the waters in the concentration range of 0.02–0.14 mM, while Ca^{2+} ranged from 0.24–0.45 mM. To validate the proposed method, two water samples were analysed using an ion chromatography as listed in Table 2 [30,31] and found the concentration of $[HPO_4]^{2-}$ and Ca^{2+} in agreement between the two methods.

4. Conclusions

Co-EOF capillary electrophoresis with UV detection can be used for the simultaneous determination of $[HPO_4]^{2-}$ and Ca^{2+} in water samples without modification of the commercial CZE instrument. 2,6-PDCA can be used for both on-column complexation of Ca^{2+} and as the background electrolyte. The selectivity for the tested solutes depends mainly on the electrolyte pH. The proposed method was used for the analysis of both $[HPO_4]^{2-}$ and Ca^{2+} in water samples and offers the advantages of simplicity of sample preparation and fast analysis.

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