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ORIGINAL PAPER

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Separation and determination of vanadium in fertiliser by capillary electrophoresis with a light-emitting diode detector

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Abstract A method has been developed for determination of vanadium, as an anionic ternary complex of vanadium(V) with 4-(2-pyridylazo) resorcinol (PAR) and hydrogen peroxide, after separation by capillary electrophoresis (CE). The optimum conditions for the formation of the ternary complex were acetate buffer (3 mmol L^{-1}) at pH 6 containing 0.15 mmol L^{-1} PAR and 7.1 mmol L^{-1} H_2O_2. The CE separation was conducted using 15 mmol L^{-1} acetate buffer at pH 6 as the background electrolyte; the separation potential was –30 kV and the injection time 100 s. The vanadium complex was detected photometrically at 568 nm, by use of a light-emitting diode (LED); the detection limit was 19 ppb. The method was applied to the analysis of vanadium in fertilisers. Clean-up of the digested fertiliser sample, with Sep-Pak C_{18} coated with tetrabutylammonium hydroxide, before analysis was used to remove matrix ions which otherwise caused electrophoretic de-stacking. Vanadium levels found in the fertiliser samples by use of the CE method were found to be comparable with results obtained by HPLC and ICP–MS.

Keywords Capillary electrophoresis · Fertiliser · Vanadium · Ternary complexes

Introduction

Of all the essential macronutrients in soil solutions, phosphorus is most often present at the lowest concentrations, typically in the range of 0.5 to 1.5 μmol L^{-1}. Phosphorus is, therefore, frequently added to the soil. The principal source of phosphorus is from phosphate rocks, which often also contain vanadium. Vanadium can have toxic effects, e.g. the death of 23 heifers out of 98 cattle within 10 days as a result of consuming hay contaminated by vanadium [1]. Vanadium accumulation in livers of cows and calves at regular slaughter has showed that levels in animals those from regions where basic slag (with 3% vanadium) has been used for many years were significantly higher than those from other regions. Vanadium also has significant effects on plant growth – uptake of trace vanadium from fertiliser causing a decrease in growth and accumulation of vanadium in all parts of the plant, especially the roots [2]. The quantities of vanadium found in several fertilisers have been reported [3].

Analytical methods currently used for the determination of vanadium are atomic absorption spectrometry (AAS) [4], inductively coupled plasma–atomic emission spectrometry (ICP–AES) [5], inductively coupled plasma–mass spectrometry (ICP–MS) [6], and ion-interaction reversed-phase high-performance liquid chromatography (ion-interaction RPHPLC) [2, 3]. The main disadvantages of AAS, ICP–MS and ICP–AES are sample matrix interferences and high cost. Ion-interaction RP HPLC for the determination of vanadium [2, 3] is a cheaper alternative to the other methods and is free from interference from sample matrix. This approach relies on the separation of the ternary V(V)-PAR-H_2O_2 complex on a C_{18} column dynamically coated with tetrabutylammonium bromide. This imposes the disadvantage of alengthy equilibration time for the C_{18} columns in order to obtain stable retention times.

The purpose of this research was to investigate the feasibility of separating and detecting the same anionic ternary complex of vanadium by capillary electrophoresis (CE). A recent description of the CE separation of standard vanadium as the 4-(2-pyridylazo)resorcinol–tartaric acid complex reported only modest sensitivity (133 ng mL^{-1}) [7]. It was expected that separation of the V(V)-PAR-H_2O_2 complex by CE would provide better results because of the high absorptivity and stability of this complex [3]; the use of a light-emitting diode as the light source was ex-

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pected to further improve sensitivity. The developed method has been applied to the determination of vanadium in fertilisers.

Experimental

Reagents and solutions

All water used was distilled and then deionised by use of a Milli-Q system (Millipore Bedford, MA, USA). Standard ammonium metavanadate (NH_4VO_3, 99.99% purity) was obtained from Aldrich (Milwaukee, WI, USA.) and a stock solution (1.000 mg mL^{-1}) was prepared in 1 mol L^{-1} HNO_3. HPLC-grade methanol was obtained from Merck (Darmstadt, Germany), 4-(2-pyridylazo) resorcinol (PAR) monosodium salt hydrate was obtained from Aldrich, and solutions of the dye were freshly prepared in water before use. Other reagents used in this study were a 20% w/v solution of poly(diallyldimethylammonium chloride) (PDDAC), molecular weight 400,000–500,000 (Aldrich), tetrabutylammonium hydroxide (TBAOH ; 0.1 mol L^{-1},; Dionex, Sunnyvale, CA, USA), AR grade aqueous ammonia (Ajax Chemicals, Sydney, Australia), AR grade acetic acid (BDH, Poole, UK), AR grade hydrochloric acid (BDH), AR grade nitric acid (Ajax), and AR grade hydrogen peroxide (30% w/v, BDH, Victoria, Australia). An N-P-K fertiliser (16-20-0) from Thailand was used in this work.

Instrumentation

Separations were performed in a 60 cm×75 μm i.d. polyimide-coated fused-silica capillary (Polymicro Technology, Phoenix, AZ, USA) coated internally with 5% PDDAC; the distance from the point of injection to the detection window was 52 cm. Analysis was performed with a Waters (Milford, MA, USA) Quanta 4000 capillary electrophoresis system, equipped with a negative power supply. Electropherograms were recorded and processed with a Millennium 2010 data acquisition system. Samples were introduced into the capillary by hydrostatic injection at 100 mm height and the injection time was 100 s. A light-emitting diode (LED) (Farnell Electronic Components, Chester Hill, NSW, Australia) provided the light source for spectrophotometric detection at 568 nm. A separation potential of –30 kV was employed. Sep-Pak C_{18} cartridges (Waters) were used for sample preparation.

Procedures

Sample digestion

N-P-K fertiliser (16-20-0) was digested by use of procedure described elsewhere [3]. In brief, sample (0.1000 g) was digested with a mixture of concentrated HNO_3 and HCl in a screw-top Teflon vial, on a hotplate, until a clear solution was obtained. The lid of the vial was then opened and the sample solution was evaporated to incipient dryness. Concentrated HNO_3 and water (a few millilitres of each) were then added to the vial, after which the lid was closed and the solution was warmed until a clear solution was obtained. The solution was diluted to 20 mL with water and filtered through a 0.45 μm filter.

Pre-column complex formation

The optimised conditions for pre-column formation of the V(V)-PAR-H_2O_2 ternary complex were modified from our previous report [3]. In brief, standard solutions of the complex were prepared by adding freshly prepared PAR solution to acetic acid and then adding a standard solution of NH_4VO_3. The pH was adjusted to 6.0 with dilute ammonia solution and H_2O_2 was added. The solution was then transferred to a 25-mL volumetric flask and diluted to volume with water. The ternary complex was then injected into the capillary. The optimised conditions for complex formation were: [PAR]=0.15 mmol L^{-1}; [H_2O_2]=7.1 mmol L^{-1}, [acetate buffer]= 3 mmol L^{-1} at pH 6.

Matrix clean-up of the V(V)-PAR-H_2O_2 complex in samples

The digested solution of N-P-K fertiliser was added to 0.75 mL acetic acid solution (0.1 mol L^{-1}) followed by 1.50 mL freshly prepared PAR solution (5×10^{-3} mol L^{-1}). The solution was adjusted to pH 6.0 with ammonia solution and 0.2 mL hydrogen peroxide (30% w/v) was then added. The solution was transferred to a 25 mL-volumetric flask and diluted to volume with water.

Methanol (3 mL) was passed through a Sep-Pak C_{18} cartridge followed by 15 mL water and then 5 mL of a solution of 3 mmol L^{-1} TBAOH in 3 mmol L^{-1} acetate at pH 6. The previously prepared vanadium complex solution (10 mL) was then passed slowly through the cartridge, followed by 15 mL water. The V(V) complex was then back flushed from the cartridge into a 5-mL volumetric flask by use of 2.25 mL 80% methanol (in 3 mmol L^{-1} acetate and 7.1 mmol L^{-1} H_2O_2 at pH 6). The solution in the volumetric flask was diluted to volume with a solution containing 3 mmol L^{-1} acetate and 7.1 mmol L^{-1} H_2O_2 at pH 6, and injected.

CE analyses

Before use new fused silica capillaries were pre-treated by flushing with 0.5% PDDAC for 5 min, water for 5 min, and electrolyte for 5 min. The V(V)-PAR-H_2O_2 complex was detected at 568 nm and peak area was measured for quantitation.

Results and discussion

Separation conditions

The ternary complex of V(V)-PAR-H_2O_2 is anionic in nature [3], so the separation was conducted with detection at the anodic end of the capillary. Use of acetate at pH 6 as background electrolyte and a bare silica capillary was found to be unsuccessful because the electrophoretic mo-

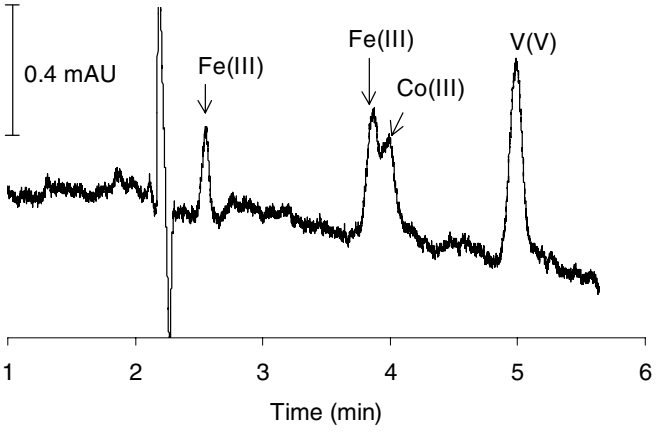

Fig. 1 Counter-EOF separation of V (0.2 mg L^{-1}), Fe (0.5 mg L^{-1}), and Co (0.5 mg L^{-1}) in a standard solution. The complexes were prepared in 10 mmol L^{-1} acetate buffer at pH 6; the separation temperature was 20 °C, the separation voltage +25 kV, the injection time 20 s, and the electrolyte 15 mmol L^{-1} acetate, pH 6. Other conditions are given in the experimental section

bility of the V(V) complex was too low compared with the electroosmotic flow (EOF) moving towards the cathode. The applied voltage was therefore changed to positive and a peak for the V(V) ternary complex was obtained at a migration time of 5.0 min, well separated from complexes formed from Fe(III) and Co(III) ions (Fig. 1). The reason for two peaks observed for Fe(III) might be that two different complexes of Fe(III) with PAR are present, but this is no cause for concern because Fe is a matrix ion and is well separated from the analyte.

This separation was then applied to the determination of vanadium in N-P-K fertiliser. The sample was cleaned up before injection to remove matrix ions present in the sample. Under these conditions, however, the migration time of the V(V) ternary complex was found to vary with each injection and the detection sensitivity was found to decrease continuously. These problems were considered to arise from adsorption of ions remaining in the sample on to the capillary wall, so modification of the capillary wall was necessary [8, 9]. A cationic polymer (poly(diallyldimethylammonium chloride), PDDAC) was therefore used to provide a semi-permanent coating on the capillary wall and to reverse the direction of the EOF.

Separation of the V(V) complex was again performed with the detection at the anodic end of the coated capillary. Preliminary experiments to determine the optimum electrolyte composition for separation of the complex were conducted within the ranges 10–20 mmol L^{-1} acetate and pH 5.5–6.5. Increasing the concentration of acetate in the electrolyte reduced the migration time, but also resulted in increased peak height of the V(V) complex. Increasing the pH of the electrolyte also reduced the migration time. After consideration of these two factors, the best conditions for obtaining both good peak shape and high detection sensitivity for the V(V) complex were use of a separation electrolyte of 15 mmol L^{-1} acetate at pH 6. The results also showed that the lower the concentration of the acetate buffer used for pre-column complex formation, the higher was the peak height obtained. As a reasonable compromise, therefore, 3 mmol L^{-1} acetate buffer was used for V(V) complex formation. The applied voltage, the injection time, and the capillary length were optimised within the ranges –10 to –30 kV, 100–150 s, and 60–80 cm, respectively. Increasing the magnitude of the negative separation voltage resulted in reduced migration times and increasing peak heights, so –30 kV was selected. Increasing the injection time had little effect on V(V) complex peak shape when a standard solution was injected but for samples broadening of the V(V) peak was observed when injection times were 150 s or more; 100 s was therefore selected. Increasing the capillary length did not significantly improve resolution of the V(V) complex from the Co(III) and Fe(III) complexes and therefore 60 cm was chosen. Fig. 2 shows an electropherogram obtained from a standard mixture of V(V), Co(III) and Fe(III) complexes under the optimised conditions.

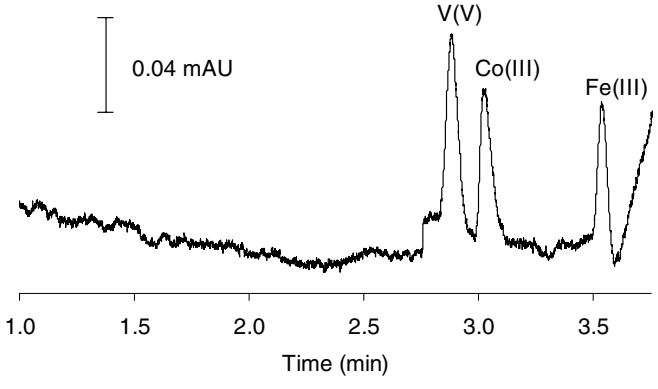

Fig. 2 Co-EOF separation of V (0.2 mg L^{-1}), Fe (0.2 mg L^{-1}), and Co (0.2 mg L^{-1}) in a standard solution. The complexes were prepared in 3 mmol L^{-1} acetate buffer at pH 6; the separation voltage was –30 kV, the injection time 150 s, and the separation temperature 20 °C; the electrolyte was as for Fig. 1

Analytical performance characteristics

Analytical performance characteristics were determined for the final method under the optimised conditions – PDDAC-coated fused silica capillary, length 60 cm (52 cm to detector), separation potential –30 kV, a background electrolyte 15 mmol L^{-1} acetic acid at pH 6, and hydrostatic injection for 100 s at 100 mm height; the sample was V(V) complex prepared in 3 mmol L^{-1} acetic acid.

The detection limit for a signal-to-noise ratio of 3 was 18.9 ppb (as V). The linearity of an external calibration plot for the V(V) complex was good up to at least 614 ppb metal ion (r^2=0.9992). Precision values (expressed as percentage relative standard deviation) for migration time and peak area for a standard solution of the V(V) complex were 0.02 and 2.24, respectively. It is important to note that the migration time of the V(V) complex could change not only as a result of the sample solution containing matrix ions but also because of progressive loss of the PDDAC layer on the capillary after numerous injections. Coating of the capillary wall was therefore repeated when any significant changes in migration time were observed.

Clean-up of the N-P-K fertiliser was required to reduce the ionic strength (described in full below). This clean-up required elution of the bound V(V) complex from a C_{18} cartridge with methanol; the final concentration of methanol in the eluted sample was 36% (v/v). Recovery of the V(V) complex from the clean-up step was determined by comparison of the peak area obtained for a standard solution prepared in 36% methanol with that obtained after elution from a C_{18} cartridge. The recovery was 100.2%.

Determination of V(V) in fertiliser samples

Digestion of the fertiliser resulted in a complex matrix of high ionic strength. Samples with conductivity higher than that of the electrolyte are generally unsuitable for CE separations because of de-stacking of the sample zone and

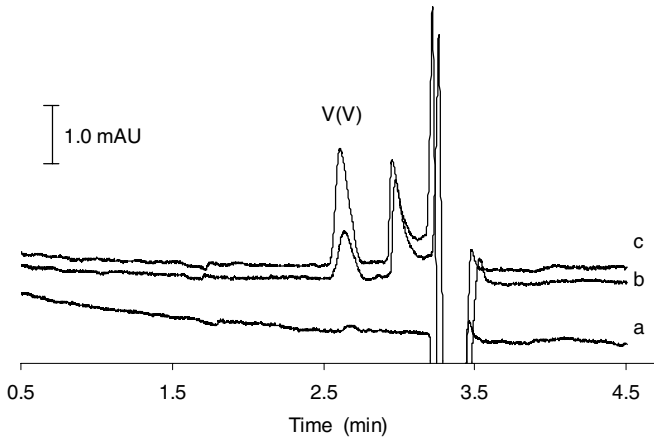

Fig. 3 Electropherogram obtained from the V(V) ternary complex from an N-P-K fertiliser sample: (*a*) blank; (*b*) unspiked N-P-K fertiliser, (*c*) N-P-K fertiliser spiked with 174 µg mL^{-1} V(V). The injection time was 100 s and other conditions as for Fig. 2

subsequent excessive peak broadening. As expected, a preliminary experiment with direct injection of the digested sample resulted in de-stacking of the V(V) zones and reduced sensitivity. The ionic strength of the sample was, therefore, reduced significantly by use of solid phase extraction with C_{18} Sep-Pak cartridges coated with TBAOH (see Experimental section). This step was used not only to clean the sample, by removing the bulk matrix ions, but also for preconcentration of the sample. This arrangement enabled the V(V)-PAR-H_2O_2 complex to bind selectively to the adsorbent, by ion-exchange and hydrophobic interactions, while much of the ionic matrix passed through the cartridge. The interstitial sample trapped in the cartridge could be removed by flushing with 15 mL water, after which the bound complex was back-flushed from the cartridge with methanol. The amount of methanol used for this purpose was minimised to reduce the impact of the methanol on detection sensitivity. A solution (2.25 mL) containing methanol (80%), 7.1 mmol L^{-1} H_2O_2, and 3 mmol L^{-1} acetate buffer at pH 6 was required to remove the V(V) complex completely. The resulting effluent was diluted to 36% methanol in the final solution with 3 mmol L^{-1} acetate buffer at pH 6 containing 7.1 mmol L^{-1} H_2O_2.

Preliminary experiments revealed that residual matrix ions in the N-P-K fertiliser sample remaining after sample clean-up slightly affected the peak area of the V(V) complex. The standard addition method of quantitation was therefore required for successful analysis of V(V). The injection time was kept to less than 100 s to avoid peak broadening. Electropherograms obtained from N-P-K fertiliser (16-20-0) before and after spiking with standard V(V) are shown in Fig. 3. The validity of the CE method was also investigated by comparing the results obtained by CE with those from HPLC and ICP–MS analysis. The result obtained for V(V) by use of CE was 171.7 mg kg^{-1}, which agreed well with those from HPLC (181.2 mg kg^{-1}) and ICP–MS (177.2 mg kg^{-1}) [3]. Advantages of the CE method are shorter analysis time, lower running costs compared to both HPLC and ICP–MS, and lower usage of organic solvents.

Conclusion

Vanadium can be determined selectively as the ternary complex V(V)-PAR-H_2O_2 after separation by capillary electrophoresis. Irreversible adsorption of matrix ions on the bare fused silica capillary can be avoided by coating the capillary wall with PDDAC. The high ionic strength resulting from sample digestion can be reduced, and preconcentration of the V(V) complex can be achieved at the same time, by use of solid phase extraction with a C_{18} Sep-Pak cartridge coated with TBAOH. This method can be applied to the analysis of V(V) in N-P-K fertiliser; results compare well with those from HPLC and ICP–MS.

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