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Simultaneous capillary electrophoretic separation and detection of P(V) and As(V) as heteropoly-blue complexes

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Abstract

A capillary electrophoretic (CE) method was developed for the simultaneous determination of P(V) and As(V). A Mo(VI)–ascorbic acid reagent reacted with a mixture of trace amounts of P(V) and As(V) to form the corresponding heteropoly-blue complexes in 0.05 *M* acetate buffer (pH 3.5). When 0.05 *M* malonate buffer was used as a migration buffer, the peaks due to their migrations were well separated in the electropherogram, and the pre-column complex-formation reaction was applied to the simultaneous CE determination of P(V) and As(V) with direct UV detection at 220 nm. With the proposed method, the calibration curves were linear in the concentration range of $5\cdot10^{-7}$–$1\cdot10^{-4}$ *M*, with a detection limit of $1\cdot10^{-7}$ *M* (a signal-to-noise ratio of 3). Interference from foreign ions was also discussed.

Keywords: Complexation; Phosphorus; Arsenic; Inorganic anions

1. Introduction

The so-called molybdenum-blue method has been widely used for the colorimetric determination of inorganic oxoanions such as P(V) and As(V) [1–3]. Since the molybdenum-blue method is not specific for each oxoanion, separation techniques such as extraction and ion chromatography are necessary when they exist together in a sample solution [4–10]. Flow injection methods are also applied to the determination of P(V) and As(V) in their mixtures [11,12]. For the determination of P(V), As(V) is selectively reduced to As(III) with proper reducing reagents [13–15], because As(III) does not produce heteropoly-blue species. However, most of these methods require time-consuming and tedious procedures.

Capillary electrophoresis (CE) has proved to be a powerful technique for the determination of ionic species. For the separation of inorganic cationic species, many research works have utilized pre-column complex-formation with various complexing reagents [16–19]. Based on the pre-column complex-formation of inorganic species with a Mo(VI)–P(V) reagent, we have recently developed sensitive CE methods with direct UV detection. The Mo(VI)–P(V) reagent reacts with V(V) and V(IV) to form the corresponding V-substituted complexes: $[P(V^{V}Mo_{11})O_{40}]^{4-}$ and $[P(V^{IV}Mo_{11})O_{40}]^{5-}$, which is the basis for the simultaneous CE determination of V(V) and V(IV) [20]. In the Mo(VI)–P(V) reagent

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method, V(V) and V(IV) are incorporated in the peripheral structure of a lacunary molybdophosphate complex. The formation of the so-called Anderson-type complexes with a Mo(VI) reagent is also the basis for the simultaneous CE determination of Cr(III) and Cr(VI), and of IO_4^- and IO_3^- [21,22].

Recently, the presence of CH_3CN as an auxiliary solvent made it possible to develop a sensitive CE method for the determination of P(V) or As(V) [23,24]. However, the Mo(VI) reagent method, based on the formation of the Keggin-type $[PMo_{12}O_{40}]^{3-}$ and $[AsMo_{12}O_{40}]^{3-}$ complexes, was unsuccessful for the simultaneous determination of P(V) and As(V) in their mixtures.

In the present study, we have found the formation of heteropoly-blue complexes of P(V) and As(V) with a Mo(VI)–ascorbic acid reagent in aqueous media of pH 2–5. The present study was undertaken to develop the Mo(VI)–ascorbic acid reagent method for the simultaneous CE determination of P(V) and As(V), because both blue species possess different mobilities and high molar absorptivities in the UV region. The Mo(VI)–ascorbic acid reagent method brings about an increase in the sensitivity by an order of magnitude, as compared with the so-called indirect method [25–27].

2. Experimental

2.1. Instrumentation

The CE experiments were carried out with a P/ACE MDQ system (Beckman Instruments, Fullerton, CA, USA) equipped with a built-in UV diode-array detector. All separations were performed on a fused-silica capillary (GL Sciences, Tokyo, Japan) of 60 cm (50 cm effective length)×75 μm I.D. Samples were injected at the cathodic end of capillary by applying a pressure of $3.4 \cdot 10^3$ Pa for 20 s. The temperature of the capillary was kept at 25 °C using a liquid-cooling system. The separation voltage was set at −20 kV, and the migrated heteropoly-blue anions were detected at 220 nm. For the indirect method, UV detection was made at 254 nm, due to chromate ions added to the running buffer. UV–visible spectra were recorded on a Hitachi Model U-3000 spectrophotometer.

2.2. Chemicals

A Mo(VI) stock solution was prepared by dissolving $Na_2MoO_4 \cdot 2H_2O$ in water. Standard solutions of P(V) and As(V) were prepared by dissolving $NaH_2PO_4 \cdot 2H_2O$ and $Na_2HAsO_4 \cdot 7H_2O$ in distilled water, respectively. $[(n\text{-}C_4H_9)_4N]_4[H_3PMo_{11}O_{39}]$ and $[(n\text{-}C_4H_9)_4N]_4[H_5AsMo_{10}O_{37}]$ were prepared according to our previous procedures [28,29]. All other reagents were of analytical grade and were used as received.

3. Results and discussion

3.1. UV–visible spectra for blue molybdophosphate and -arsenate complexes

Fig. 1a shows a UV–visible spectrum for a $2 \cdot 10^{-3}$ *M* Mo(VI)–$1 \cdot 10^{-3}$ *M* ascorbic acid–0.05 *M* acetate buffer system (pH 3.5). With the addition of $2 \cdot 10^{-4}$ *M* P(V), as shown in Fig. 1b, the colorless solution turned blue with the appearance of a new absorption maximum around 320 nm. Similarly, a blue molybdoarsenate complex was also formed with the

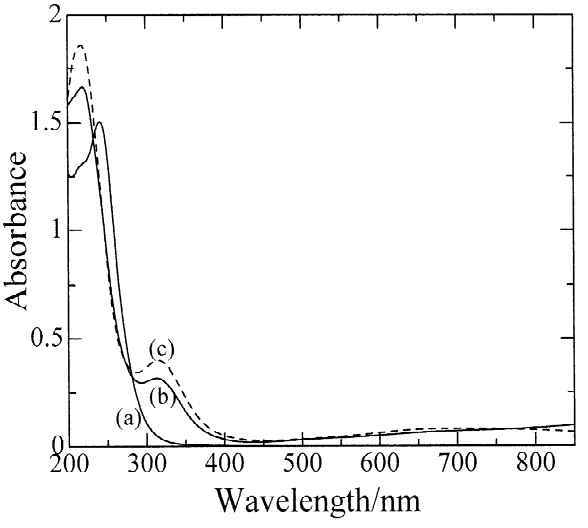

Fig. 1. UV–visible spectra for (a) $2 \cdot 10^{-3}$ *M* Mo(VI)–$1 \cdot 10^{-3}$ *M* ascorbic acid–0.05 *M* acetate buffer (pH 3.5); (b) (a)+$2 \cdot 10^{-4}$ *M* P(V); (c) $2 \cdot 10^{-4}$ *M* $[(n\text{-}C_4H_9)_4N]_4[H_3PMo_{11}O_{39}]$+$1 \cdot 10^{-3}$ *M* ascorbic acid in CH_3CN. Path length: 1.0 mm.

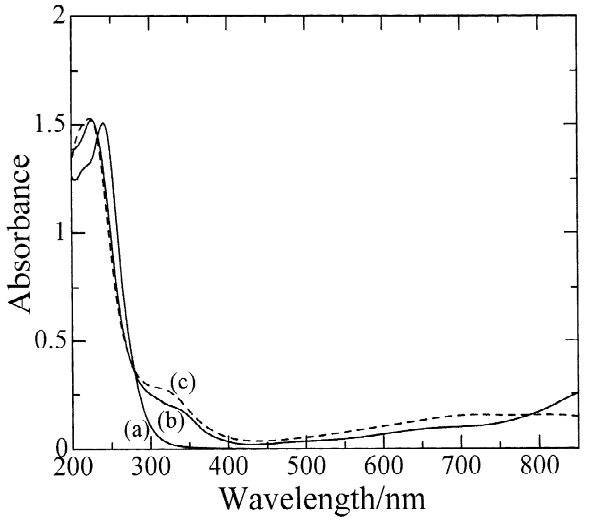

Fig. 2. UV–visible spectra for (a) $2\cdot10^{-3}$ *M* Mo(VI)–$1\cdot10^{-3}$ *M* ascorbic acid–0.05 *M* acetate buffer (pH 3.5); (b) (a)+$2\cdot10^{-4}$ *M* As(V); (c) $2\cdot10^{-4}$ *M* $[(n\text{-}C_4H_9)_4N]_4[H_5AsMo_{10}O_{37}]$+$1\cdot10^{-3}$ *M* ascorbic acid in CH_3CN. Path length: 1.0 mm.

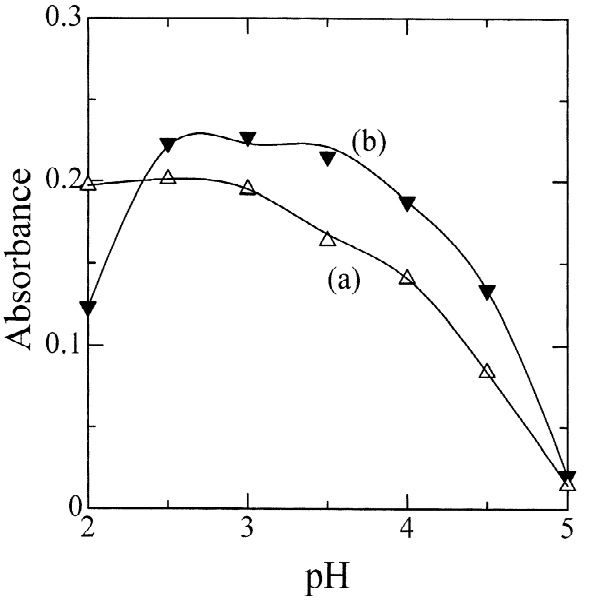

Fig. 3. Variation of the absorbance value at 450 nm as a function of pH for a $2\cdot10^{-3}$ *M* Mo(VI)–$1\cdot10^{-3}$ *M* ascorbic acid–0.05 *M* acetate buffer containing $2\cdot10^{-4}$ *M* P(V) (a) or As(V) (b). Path length: 1.0 cm.

addition of $2\cdot10^{-4}$ *M* As(V) to the $2\cdot10^{-3}$ *M* Mo(VI)–$1\cdot10^{-3}$ *M* ascorbic acid–0.05 *M* acetate buffer system (pH 3.5) (Fig. 2b). These behaviors can be ascribed to the formation of the reduced forms of $[H_3PMo_{11}O_{39}]^{4-}$ and $[H_5AsMo_{10}O_{37}]^{4-}$ which occur predominantly under these conditions [28–30]. It should be noted that the Keggin-type $[PMo_{12}O_{40}]^{3-}$ and $[AsMo_{12}O_{40}]^{3-}$ complexes are not formed at any appreciable amount in aqueous media of these weak acidities and of these low concentrations of Mo(VI) and P(V) or As(V). For comparison, Figs. 1c and 2c show UV–visible spectra for the $(n\text{-}C_4H_9)_4N^+$ salts of $[H_3PMo_{11}O_{39}]^{4-}$ and $[H_5AsMo_{10}O_{37}]^{4-}$ in CH_3CN containing ascorbic acid, respectively.

In order to obtain the formation condition of the reduced form of $[H_3PMo_{11}O_{39}]^{4-}$ or $[H_5AsMo_{10}O_{37}]^{4-}$, the absorbance values at 450 nm were plotted against pH for the $2\cdot10^{-3}$ *M* Mo(VI)–$1\cdot10^{-3}$ *M* ascorbic acid–0.05 *M* acetate buffer system (pH 3.5) containing $2\cdot10^{-4}$ *M* P(V) or As(V); no absorption due to excess of the corresponding oxidized forms occurs at this wavelength. As shown in Fig. 3, the reduced polyoxomolybdate complexes were formed in the pH range of 2–5.

A wavelength of 220 nm, where the maximum absorbance was obtained, was chosen as best for the CE determination of P(V) and As(V).

3.2. Optimization of the CE conditions

3.2.1. Effect of ascorbic acid

Fig. 4 shows electropherograms for $2\cdot10^{-3}$ *M* Mo(VI)–$1\cdot10^{-5}$ *M* P(V)–$1\cdot10^{-5}$ *M* As(V)–0.05 *M* acetate buffer (pH 3.5). In the absence of ascorbic acid, no peaks were assigned to $[H_3PMo_{11}O_{39}]^{4-}$ or $[H_5AsMo_{10}O_{37}]^{4-}$; a broad peak around 7 min is due to isopolymolybdate species (Fig. 4a). With the addition of $1\cdot10^{-3}$ *M* ascorbic acid to the solution, two sharp peaks were observed: peaks (i) and (ii) are due to the migration of the blue molybdo-phosphate and -arsenate complexes, respectively (Fig. 4b).

At Mo(VI) concentrations $>3\cdot10^{-3}$ *M*, the isopolymolybdate peak overlapped with peak (ii), and peak (i) split into two or more components at ascorbic acid concentrations $>5\cdot10^{-3}$ *M*.

3.2.2. Choice of running buffer

The selection of an appropriate buffer was ex-

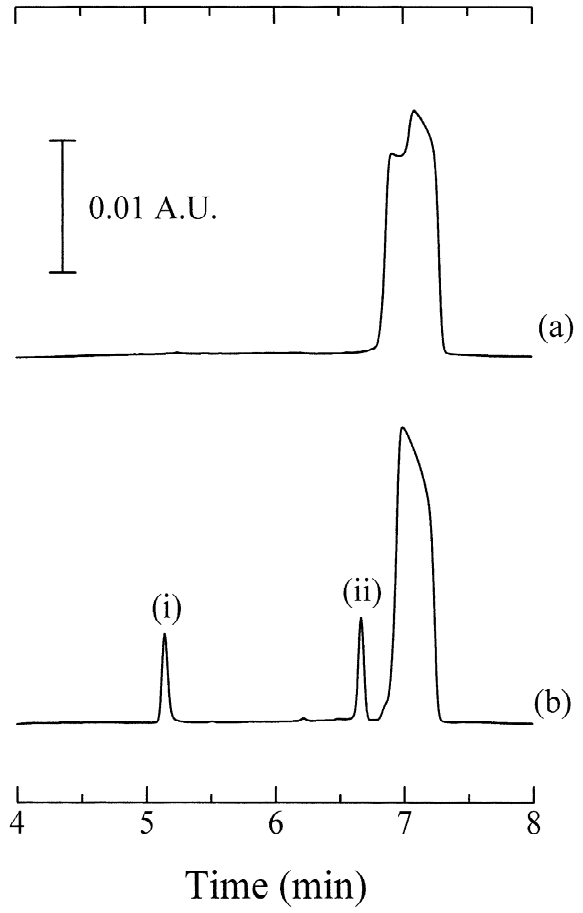

Fig. 4. Electropherograms for (a) $2 \cdot 10^{-3}$ *M* Mo(VI)–0.05 *M* acetate buffer (pH 3.5) containing $1 \cdot 10^{-5}$ *M* P(V) and $1 \cdot 10^{-5}$ *M* As(V); (b) (a)+$1 \cdot 10^{-3}$ *M* ascorbic acid. Running buffer: 0.05 *M* malonate buffer (pH 3.5). Recorded after standing at 25 °C for 80 min. Applied voltage: −20 kV. (i) The blue molybdophosphate complex; (ii) the blue molybdoarsenate complex.

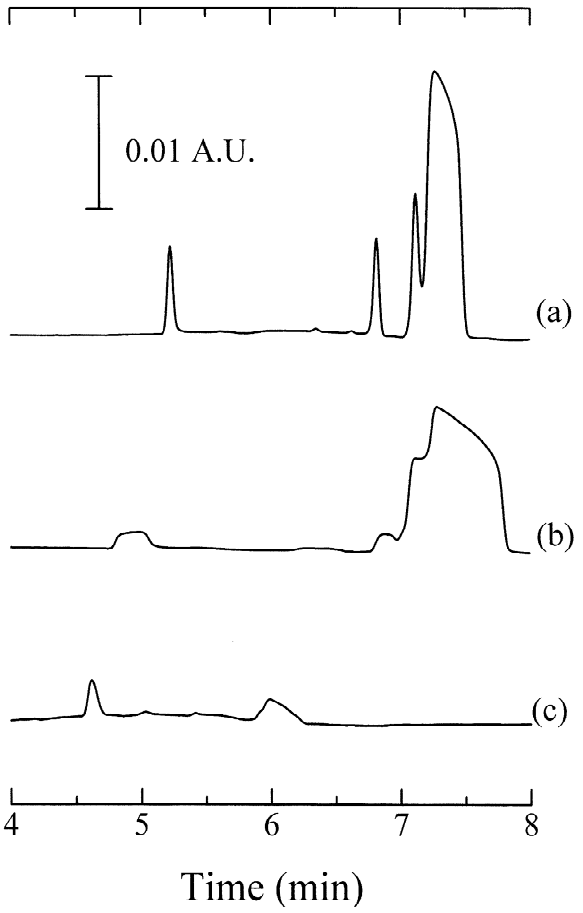

Fig. 5. Electropherograms for $2 \cdot 10^{-3}$ *M* Mo(VI)–$1 \cdot 10^{-3}$ *M* ascorbic acid–$1 \cdot 10^{-5}$ *M* P(V)–$1 \cdot 10^{-5}$ *M* As(V) (pH 3.5). (a) 0.05 *M* acetate buffer as a sample buffer and 0.05 *M* malonate buffer as a running buffer; (b) 0.05 *M* malonate buffer as both sample and running buffers; (c) 0.05 *M* acetate buffer as both sample and running buffers.

tremely important to the CE separation of P(V) and As(V), and several buffer systems were studied to find out a proper buffer system. As shown in Fig. 5a, the best results regarding the sensitivity and peak-shapes were obtained with a 0.05 *M* acetate buffer as a sample buffer and a 0.05 *M* malonate buffer as a running buffer. Both peak-shapes were unsatisfactory using malonate buffer or acetate buffer as both sample and running buffers (Fig. 5b,c). This behavior can be ascribed to transitional isotachophoresis [31]. Since the heteropoly-blue complexes are kinetically stable and no degradation occurs during migration in the capillary, addition of the Mo(VI)–ascorbic acid reagent to the running buffer is not required.

3.2.3. Effect of buffer pH

In order to find out the optimum buffer pH, the electropherogram was recorded for a $2 \cdot 10^{-3}$ *M* Mo(VI)–$1 \cdot 10^{-3}$ *M* ascorbic acid–$1 \cdot 10^{-5}$ *M* P(V)–

$1 \cdot 10^{-5}$ *M* As(V)–0.05 *M* acetate buffer system, and the results are shown in Fig. 6. At buffer pH values <3.0, the peaks corresponding to the migration of the blue species split into several components (Fig. 6a). Both blue species showed well-defined migration peaks in the pH range of 3.3–3.8 (Fig. 6b). At buffer pH values >3.8, the isopolymolybdate species migrated faster than the reduced molybdoarsenate species (Fig. 6c). Therefore a buffer pH of 3.5 was chosen as optimum for the simultaneous CE determination of P(V) and As(V).

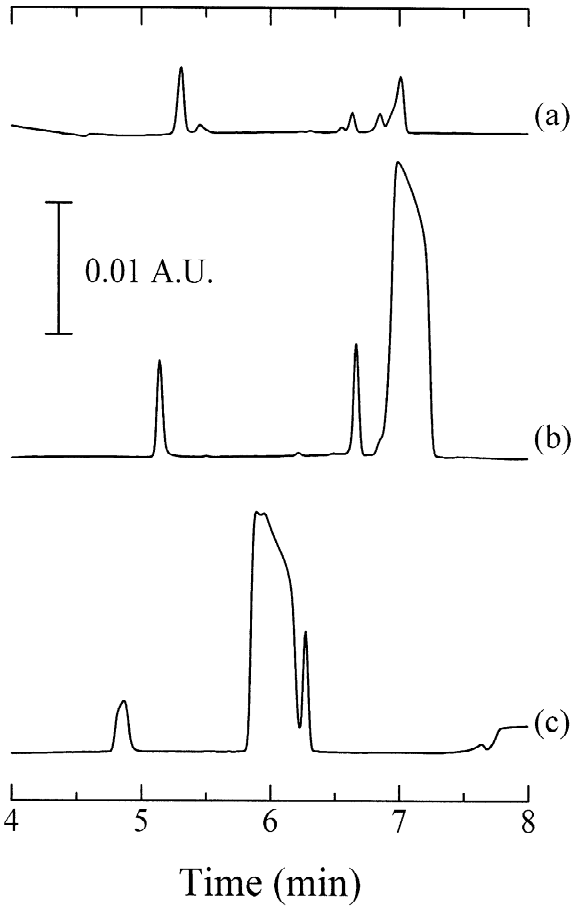

Fig. 6. Electropherograms for $2 \cdot 10^{-3}$ *M* Mo(VI)–$1 \cdot 10^{-3}$ *M* ascorbic acid–0.05 *M* acetate buffer containing $1 \cdot 10^{-5}$ *M* P(V) and $1 \cdot 10^{-5}$ *M* As(V). pH values: (a) 2.5; (b) 3.5; (c) 4.0. Running buffer: 0.05 *M* malonate buffer (the buffer pH was adjusted to be the same as the sample buffer).

On the basis of these findings, the pre-column complexation of P(V) and As(V) was made in the $2 \cdot 10^{-3}$ *M* Mo(VI)–$1 \cdot 10^{-3}$ *M* ascorbic acid–0.05 *M* acetate buffer (pH 3.5) system.

3.3. Recommended procedure

First, a complexing reagent consisting of 0.02 *M* Mo(VI)–0.01 *M* ascorbic acid–0.50 *M* acetate buffer (pH 3.5) was prepared; the Mo(VI)–ascorbic acid reagent gave reproducible results during 12 h when stored in a refrigerator. Prior to CE measurement, the capillary was filled with 0.05 *M* malonate buffer (pH 3.5). An appropriate amount of a sample to be analyzed was placed in a 20-ml volumetric flask. After the addition of 2 ml of the complexing reagent, the solution was diluted to the mark with distilled water. The peak area for peak (i) was reproducible even if the sample solution was introduced into the capillary immediately after preparation. On the other hand, peak (ii) grew with time and the peak area attained a constant value in ~80 min.

After the sample solution was left standing for 80 min at room temperature, the electropherogram was recorded. Calibration curves were thus constructed by varying the concentrations of P(V) and As(V). The peak areas showed a linear dependence on the concentration of P(V) or As(V) in the range of $5 \cdot 10^{-7}$–$1 \cdot 10^{-4}$ *M*; the RSD ($n=10$) values were 2.7 and 2.5%, respectively. The detection limit of $1 \cdot 10^{-7}$ *M* was achieved for each oxoanion (a signal-to-noise ratio of 3).

3.4. Comparison of the developed and indirect methods

In order to demonstrate the utility of the developed method, the present results were compared with those obtained by the indirect method with the use of chromate as the UV absorbing probe. Under the conditions of Fig. 7a, the indirect method permits the simultaneous detection of P(V) and As(V). Despite the advantage with respect to simplicity, the indirect method was not sufficiently sensitive owing to high noise; detection limits: $2 \cdot 10^{-6}$ *M*. Thus, the sensitivity is improved at least ten-fold compared to the usual indirect method.

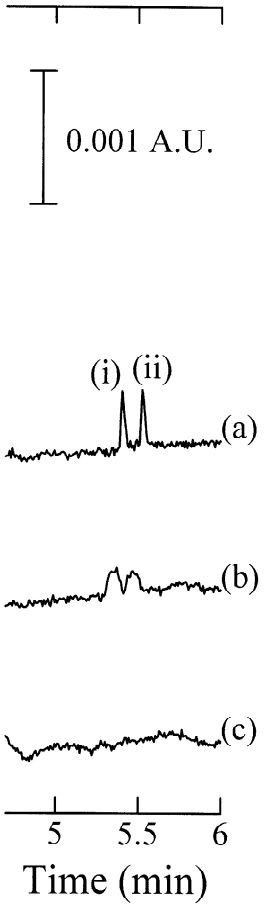

Fig. 7. Electropherograms for (a) $1\cdot10^{-5}$ *M* P(V)+$1\cdot10^{-5}$ *M* As(V); (b) (a)+$1\cdot10^{-2}$ *M* NaCl; (c) (a)+$5\cdot10^{-2}$ *M* NaCl. Running electrolyte: $3\cdot10^{-3}$ *M* K_2CrO_4–$2\cdot10^{-4}$ *M* tetradecyltrimethylammonium bromide (TTAB) (pH 10.0). Applied voltage: −15 kV. Detection wavelength: 254 nm. (i) P(V); (ii) As(V).

3.5. Interference from foreign ions

Interference from foreign ions was investigated with the proposed method, and the results are given in Table 1. Under the recommended conditions, the Mo(VI)–ascorbic acid reagent can react with Si(IV) and Ge(IV) to form the corresponding heteropoly-blue anions. However, their presence causes no serious errors, because their mobilities are different from those of the blue molybdo-phosphate and -arsenate complexes. The remaining Fe(III), Ni(II), Cu(II), Co(II) and Mn(II) ions do not interfere because no complex-formation occurs under the proposed CE conditions.

In contrast to the indirect method (Fig. 7b,c), the proposed method permits the presence of NaCl in concentrations up to $5\cdot10^{-2}$ *M*.

Table 1
Effect of foreign ions on the determination of P(V) and As(V)

Ions added as	Concentration (*M*)	Relative error (%)	
		P(V)	As(V)
NaCl	$5\cdot10^{-2}$	3.3	−4.6
	$1\cdot10^{-2}$	2.3	−3.2
$MgSO_4$	$1\cdot10^{-2}$	−4.0	4.0
KNO_3	$1\cdot10^{-2}$	−4.5	0.8
Na_2SiO_3	$1\cdot10^{-5}$	2.0	0.8
GeO_2	$1\cdot10^{-5}$	3.2	−2.8
$FeCl_3$	$1\cdot10^{-4}$	3.9	0.5
$NiCl_2$	$1\cdot10^{-4}$	−2.9	−2.9
$CuSO_4$	$1\cdot10^{-4}$	−1.5	1.7
$CoCl_2$	$1\cdot10^{-4}$	2.3	3.3
$MnCl_2$	$1\cdot10^{-4}$	−0.9	3.2

Sample solution: $2\cdot10^{-3}$ *M* Mo(VI)–0.05 *M* acetate buffer (pH 3.5)–$1\cdot10^{-3}$ *M* ascorbic acid–$1\cdot10^{-5}$ *M* P(V)–$1\cdot10^{-5}$ *M* As(V). Running buffer: 0.05 *M* malonate buffer (pH 3.5). Measured after standing at 25 °C for 80 min. Wavelength: 220 nm. Injection: 20 s at $3.4\cdot10^{3}$ Pa. Applied voltage: −20 kV.

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