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Application of HPLC and CZE to the analysis of polyoxometalates*

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Abstract: Polyoxometalates (POM) are polymers of transition metal oxides. They are widely used as analytical reagents and reaction catalysts. Some have anti-viral properties and are being investigated as anti-HIV agents. Due to solubility and stability limitations, separation methods for POM are rare in the literatures. This paper presents a HPLC and a CZE method for the analysis of sodium tungstate, its equilibrium products and isopolyanions. The methods are simple and sensitive, and can be used to monitor the purity, stability and solution equilibria of POM.

Keywords: *HPLC; CZE; polyoxometalate; isopolyanion; tungstate; anti-viral agents.*

Introduction

Polyoxometalates (POM) are polymers of oxides of early transition metal (M) ions. The metal oxide ions are usually arranged in MO_6 octahedra, linked by shared O atoms. The POM is an isopolyanion (IPA) if M is from a single element. Otherwise, it is a heteropolyanion (HPA). The chemistry, synthesis and industrial use of POM have been extensively reviewed [1, 2]. Since Berzelius first discovered ammonium 12-molybdophosphate in 1826, hundreds of POM have been synthesized. Due to their extensive acid-base and oxidation-reduction chemistry, POM have been used extensively as analytical reagents and reaction catalysts. Recently, some POM, notably HPA-23 ($NaSb_9W_{21}O_{86}^{18-}$), have shown anti-viral properties and are being investigated as anti-HIV agents [3–10].

In aqueous solutions, POM are very unstable and undergo rapid and complex structural equilibria [1, 2]. The equilibria are both pH and concentration dependent [11]. Solution equilibria have been studied by electrochemical [12] or spectral [3, 6, 11–13] means which are time consuming, laborious, insensitive, or non-specific. The results obtained are often subject to interpretation. Due to the instability and complex solution equilibria of POM, little success has been reported in their chromatography. The TLC and HPLC methods reported by Taguchi [14] and Sakurai *et al.* [15], respectively, did not give separation of the POM due to their instability in solution. The normal phase HPLC reported by Braungart and Russel [16] is the only successful separation of HPA that has appeared in the literature. This method, however, is of limited use in pharmaceutical and biochemical applications as well as in solution equilibria studies where aqueous solutions are examined. Owing to the growing interest of POM as anti-viral agents, it is desirable to develop simple, sensitive and efficient separation techniques to analyze and to study the solution stability of this important class of pharmaceuticals.

Majority of POM are synthesized from tungsten or molybdenum. Their IPA are generated according to equation (1) [1]

$$pH^+ + q[MO_4]^{r-} \rightarrow [H_xM_qO_y]^{n-} + (4q - y)H_2O. \quad (1)$$

A simplified polymerization scheme of tungstate is presented as Fig. 1. Among the major tungsto-IPA, $W_6O_{19}^{2-}$ (II), $W_{10}O_{32}^{4-}$ (III), and $H_2W_{12}O_{40}^{6-}$ (IV) have been isolated and characterized successfully. Their solid state structures have been determined by polarography, NMR, IR, UV, and X-ray crystallography [1, 2]. However, the rapid and complex structural equilibria that often occur in solution

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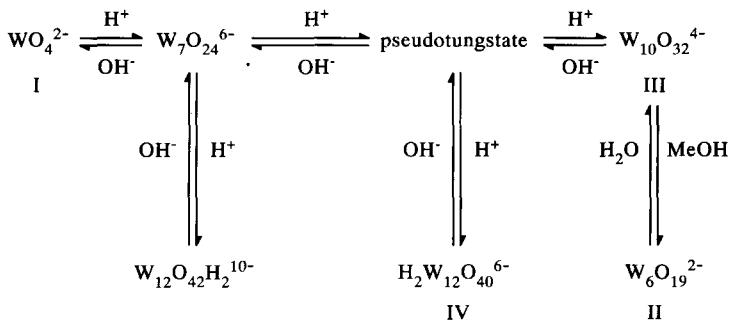

Figure 1
Simplified polymerization scheme of tungsto-isopolyanions.

cast ambiguity to their solution structures [17]. Since POM are ionic, ion-pair high-performance liquid chromatography (HPLC) and capillary zone electrophoresis (CZE) are logical methods of choice to separate them. This paper presents successful applications of HPLC and CZE to the separation of sodium tungstate, its solution equilibrium products and isopolyanions (IPA). The methods are simple and sensitive. They can be used to monitor the synthesis, purity, stability, and solution equilibria of tungsto-IPA.

Experimental

Reagents and materials

POM are stable in organic solvents but not in aqueous media. Since only organic salts of POM are soluble in organic solvents, tetra-*t*-butylammonium (tBu$_4$N) salts of the POM were used in this study. They were prepared from Na_2WO_4 and tBu$_4$NBr, and purified according to published procedures [17–19]. Na_2WO_4 and tBu$_4$NBr were purchased, respectively, from Merck & Co., Inc. (Rathway, NJ, USA) and Aldrich Chemical Co. (Milwaukee, WI, USA). Hydrochloric acid and methanol were from Mallinckrodt Inc. (Paris, KY, USA).

Tetra-*t*-butylammonium hydrogen phosphate was purchased from the Sigma Chemical Co. (St Louis, MO, USA). Sodium hydrogen phosphate was from Mallinkrodt Inc. (Paris, KY, USA). Acetonitrile (ACN) was from Baxter Healthcare Corp. (Muskegon, MG, USA). The chemicals were reagent grade. Buffer solutions were prepared with distilled water. Analytes were dissolved in H_2O, ACN or ACN-H_2O (1:1 to 3:1) to afford analyte solutions of 0.5–1.0 mg ml^{-1}.

High performance liquid chromatography (HPLC)

HPLC was performed with a Varian STAR system consisted of a model 9095 autosampler, a model 9010 pump, a model 9050 UV–Vis detector, a model 9065 photodiode array detector (PAD), and a STAR LC workstation (Palo Alto, CA, USA). The separation was achieved with an Alltech Adsorbosphere HS C18, 5 μm, 4.6 × 250 mm, stainless steel column (Alltech Assoc., Inc., Deerfield, IL, USA), using a mobile phase of 42% acetonitrile (ACN) in pH 7.0, 0.001 M tetra-*t*-butylammonium phosphate buffer at 1.0 ml min^{-1} isocratically for 25 min followed by 20 min of linear gradient to 50% ACN in buffer. Detection was by UV at 210 nm or by photodiode array detection (PAD).

Capillary zone electrophoresis (CZE)

CZE was carried out on a Biofocus 3000 Electrophoresis System using a 240 mm × 25 μm, coated fused silica capillaries (both of BIO-RAD, Hercules, CA, USA). The electrolyte was 0.1 M, pH 7.0 buffer–ACN, 70:30. The buffer was prepared with sodium hydrogen phosphate. Loading was done hydrodynamically (pressure) at 25 psi.s. The run voltage was 3.0 kV. The analyte ions migrated counter to electroosmosis, from the negative to the positive electrode. Detection, by UV at 210 nm and high speed UV scanning, was at the positive electrode. Data was collected and processed with the Biofocus 3000 Integration system (BIO-RAD, Hercules, CA, USA).

Results and Discussion

Of the various tungsto-IPA described in Fig. 1, $W_6O_{19}^{2-}$ (II), $W_{10}O_{32}^{4-}$ (III), and

$H_2W_{12}O_{40}^{6-}$ (IV) have been successfully isolated and characterized [2, 19]. Their tBu$_4$N salts are soluble and stable in organic solvents. Though unstable in H_2O, the tBu$_4$N salts are sufficiently soluble and stable in ACN-H_2O solutions for this study.

HPLC

Braungart and Russel [16] described the only reported HPLC separation of POM. Their normal phase HPLC method is not practical for pharmaceutical applications, where aqueous samples are often examined. The more common reversed-phase HPLC used in pharmaceutical analysis requires aqueous mobile phases which may cause POM decomposition during chromatography. To reduce on-column decomposition, ion-pair HPLC was chosen for method development. In ion-pair HPLC, the high concentration of organic modifier in the mobile phase would stabilize the POM during chromatography. Figure 2(a) is the chromatogram of I–IV obtained with a C18 column and an isocractic mobile phase of 42 parts ACN and 58 parts 0.001 M t-Bu$_4$NH$_2$PO$_4$ (pH adjusted to 7.0 with

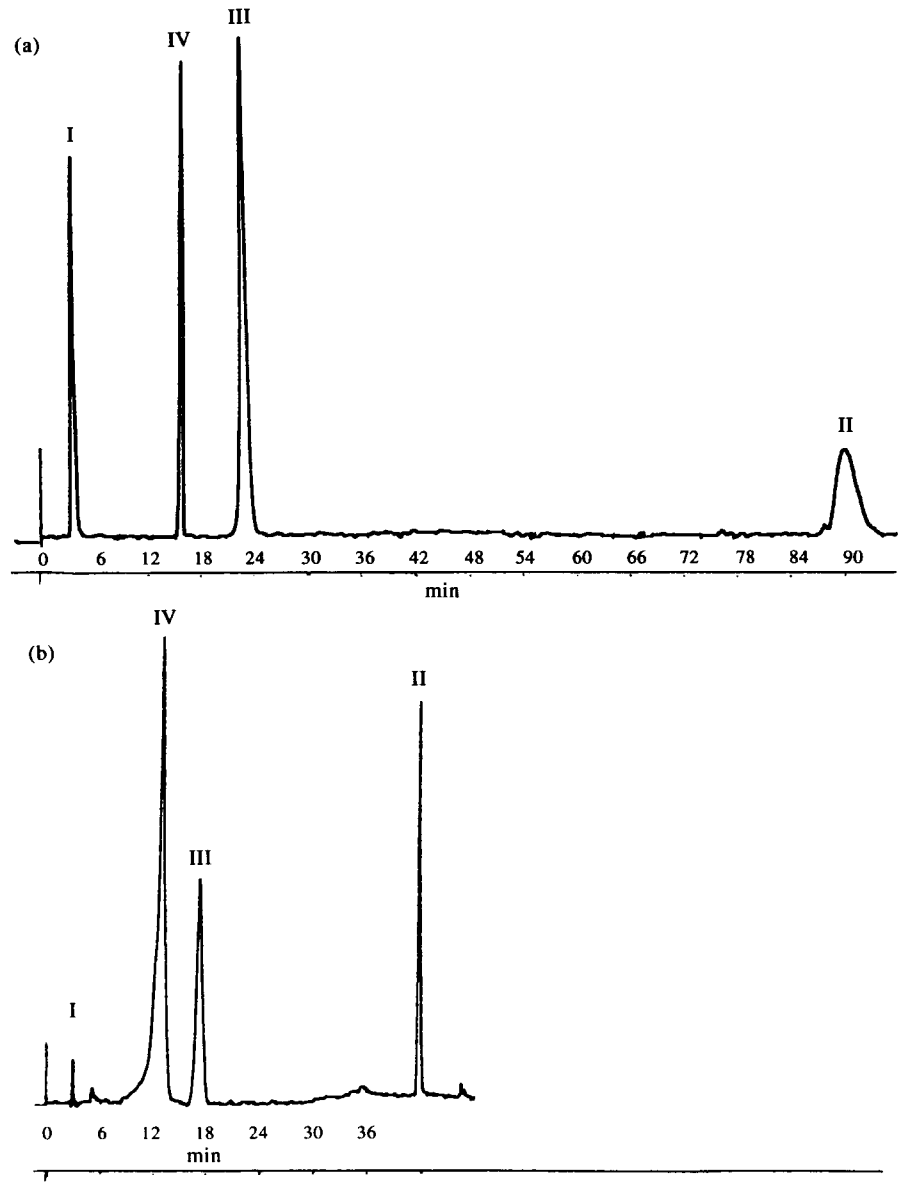

Figure 2
HPLC separation of WO_4^{2-} (I), $W_6O_{19}^{2-}$ (II), $W_{10}O_{32}^{4-}$ (III) and $H_2W_{12}O_{40}^{6-}$ (IV): (a) isocratic elution, (b) gradient elution. See text for full HPLC conditions.

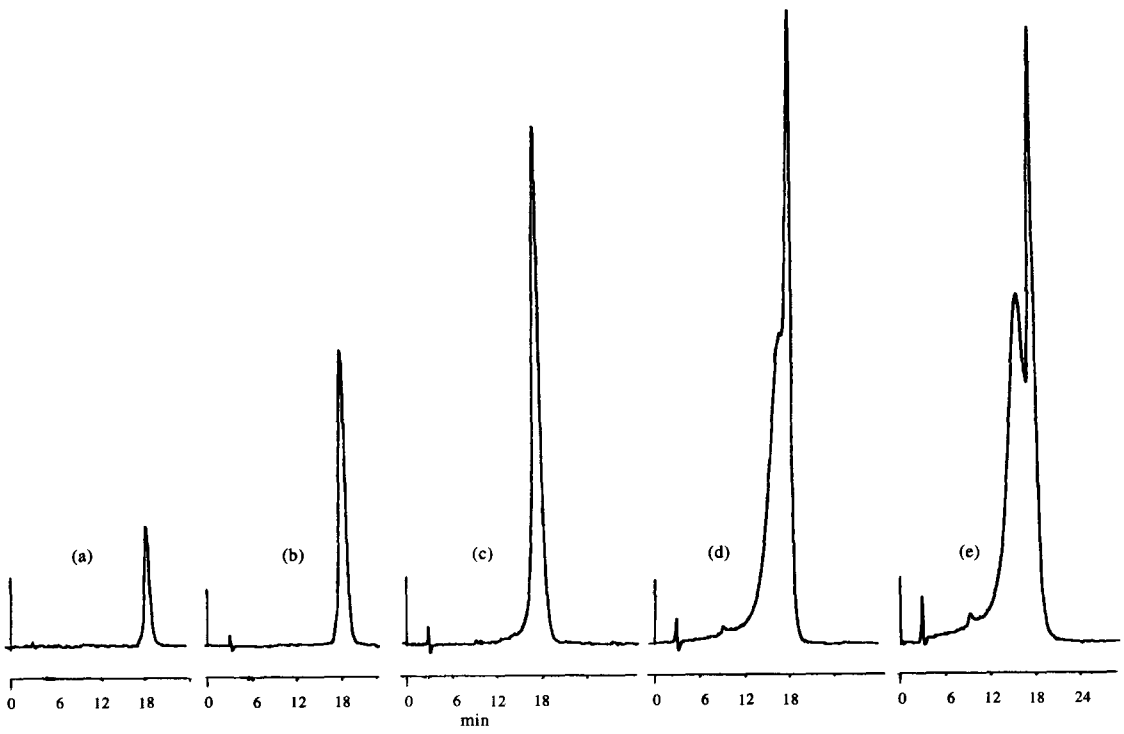

Figure 3
Effect of injection size on the HPLC peak shape of IV: (a) 2.5 μl, (b) 5 μl, (c) 10 μl, (d) 15 μl, and (e) 20 μl of a 1.0 mg ml^{-1} solution in acetonitrile.

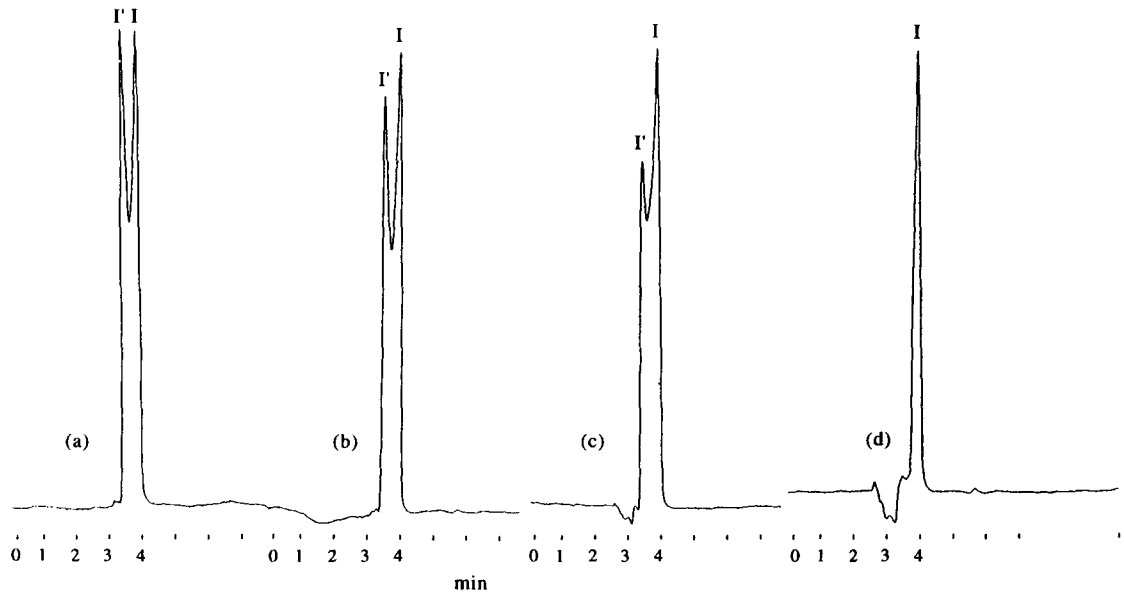

Figure 4
HPL chromatograms of sodium tungstate in H_2O: (a) 10^{-1} M, (b) 10^{-2} M, (c) 10^{-3} M, and (d) 10^{-4} M. See Experimental for HPLC conditions.

H_3PO_4). The separation was good as the peaks were baseline resolved, but took 90 min. The HPLC system has a plate number of 6000, three times that of Braungart and Russel. The retention time of hexatungstate, II, was greatly reduced from 90 to 42 min when the ACN in the mobile phase was increased from 42 to 50% linearly in 20 min after 25 min of isocratic elution (Fig. 2b). The peak shape of IV was very sensitive to injection size. Increasing the injection from 2.5 to 20 μl of a 1 mg ml^{-1} of IV in ACN caused the peak to broaden eventually to a doublet (Fig. 3). The double peak was probably caused by limited loading capacity of the HPLC system. Sodium tungstate appeared as two unresolved peaks (I and I′) near the unretained area. The relative intensities of the two peaks varied with the tungstate concentration. As shown in Fig. 4, I and I′ were of similar intensity for a H_2O solution of 10^{-1} M Na_2WO_4. I′ decreased in favour of I with reduction in tungstate concentration. At 10^{-4} M, only I was detected. This observation was consistent with the solution equilibrium model of Cruywagan and Merve for sodium tungstate in pH 7.0 buffer [11]. According to their model, the monomeric WO_4^{2-} is in equilibrium with the polymeric $W_6O_{20}(OH)_2^{6-}$, $W_7O_{24}^{6-}$ and $H_2W_{12}O_{42}^{10-}$ in concentrated (10^{-1} M) tungstate solutions. In more diluted solutions (10^{-3} M), WO_4^{2-} was the predominant species. However, their model was based on computation of potentiometric titration data and not on direct measurement of individual species. The ability of the HPLC method to detect directly the equilibrium products of sodium tungstate in solution demonstrates that the method can be a valuable tool to study solution equilibria of POM.

Figure 5 presents the UV spectra of I, I′, II, III, and IV obtained with PAD. The spectrum of I, with end absorption only, is transparent above 210 nm and is identical to that of a dilute H_2O solution of Na_2WO_4 (4×10^{-3} M). Spectra of I′, II and IV show absorption maxima at 220, 275 and 260 nm, respectively. As reported [20], III has a distinctive absorption maximum at 325 nm, in addition to the one at 265 nm.

ACN solutions of II–IV are stable at ambient temperature for at least 10 days, as evidenced by invariant HPLC profiles obtained during a 10-day period. When the ACN solutions were diluted with H_2O, decomposition started to occur after several hours (Fig. 6). One decomposition product, V, was common to aged ACN-H_2O solutions of II, III and IV. Its UV profile was identical to that of IV. While IV generated only V; II and III generated IV as their decomposition or transformation products as well. Absence of decomposition products in chromatograms obtained from freshly prepared ACN–H_2O solutions of II–IV confirmed that the IPA were stable during chromatography.

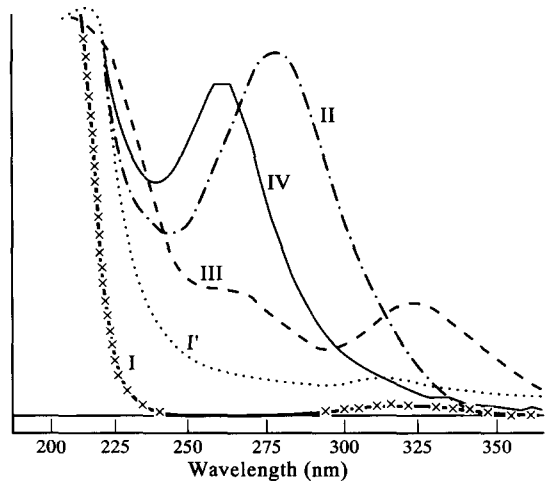

Figure 5
UV profiles of WO_4^{2-} (I & I′), $W_6O_{19}^{2-}$ (II), $W_{10}O_{32}^{4-}$ (III), and $H_2W_{12}O_{40}^{6-}$ (IV); obtained by HPLC-PAD.

Concentrations of as low as 30 μg ml^{-1} of the IPA can be easily detected by this HPLC method. This sensitivity is much better than the currently used electrochemical and spectral methods. Thus, the HPLC system presented offers a simple and sensitive technique to analyse tungsto-IPA and to monitor their synthesis and stability.

CZE

Though CZE is a powerful separation technique for ionic compounds, its application to polyanionic POM has not been reported. The lack of application of CZE or electrophoresis to the POM analysis is due to the insolubility or instability of the POM in aqueous buffers required by the techniques. Since tBu$_4$N salts of tungsto-IPA II–IV are soluble and stable in organic solvents, the feasible use of CZE in their analyses was explored. To stabilize the IPA, 30% ACN was added to the electrolyte buffer (pH 7.0, 0.1 M sodium phosphate).

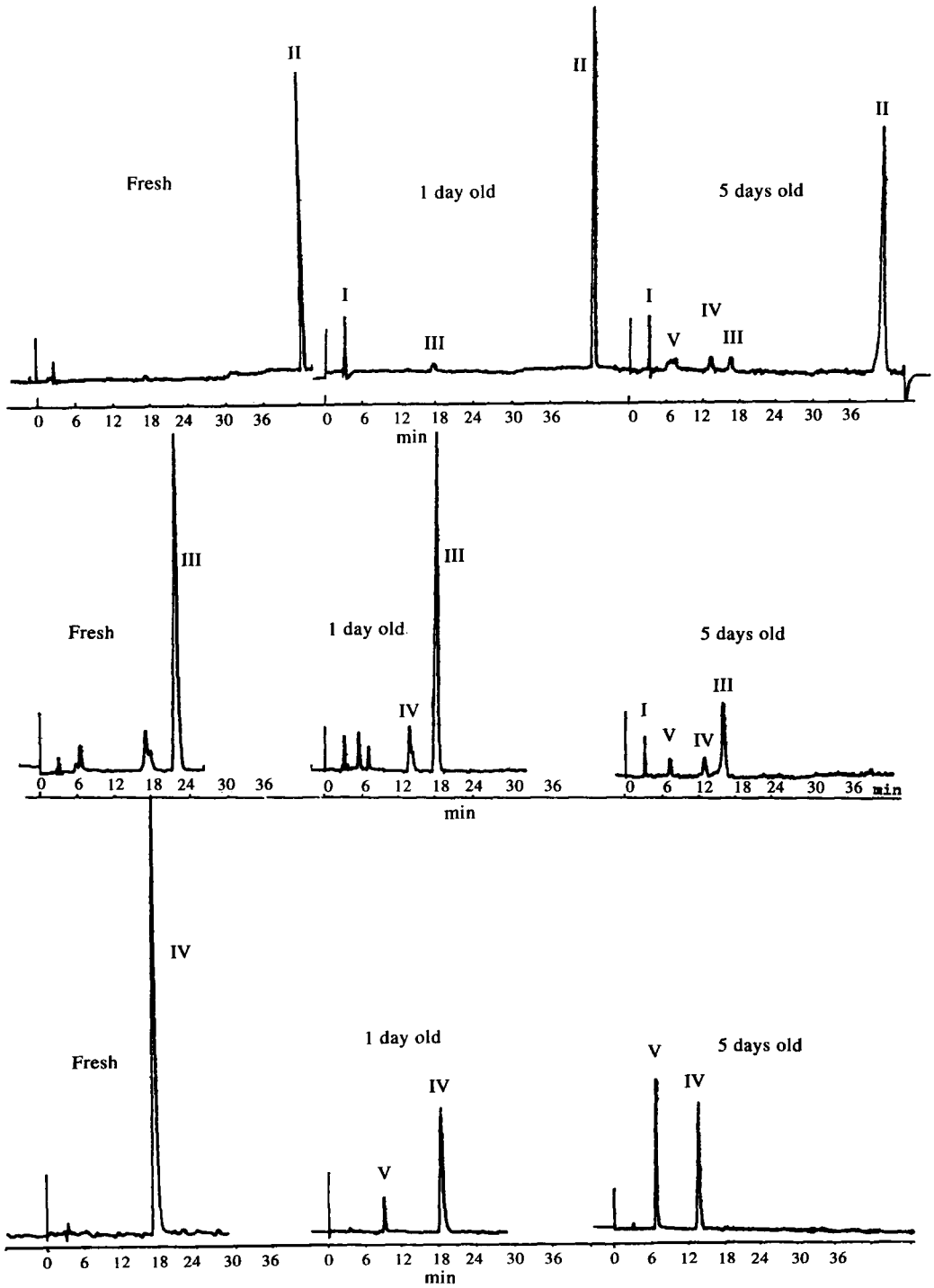

Figure 6
HPL chromatograms of fresh and aged acetonitrile–water (1:1) solutions of tungsto-IPA: (top) II, (middle) III, and (bottom) IV. See Experimental for complete experimental conditions.

Figure 7 shows the electropherograms of fresh ACN-H_2O solutions of I–IV. Based on the tallest peak of each electropherogram, the number of theoretical plates was in excess of 60,000 for the system, 10 times that of the HPLC.

The electropherogram of a 3×10^{-3} M (1 mg ml^{-1}) aqueous solution of I, sodium

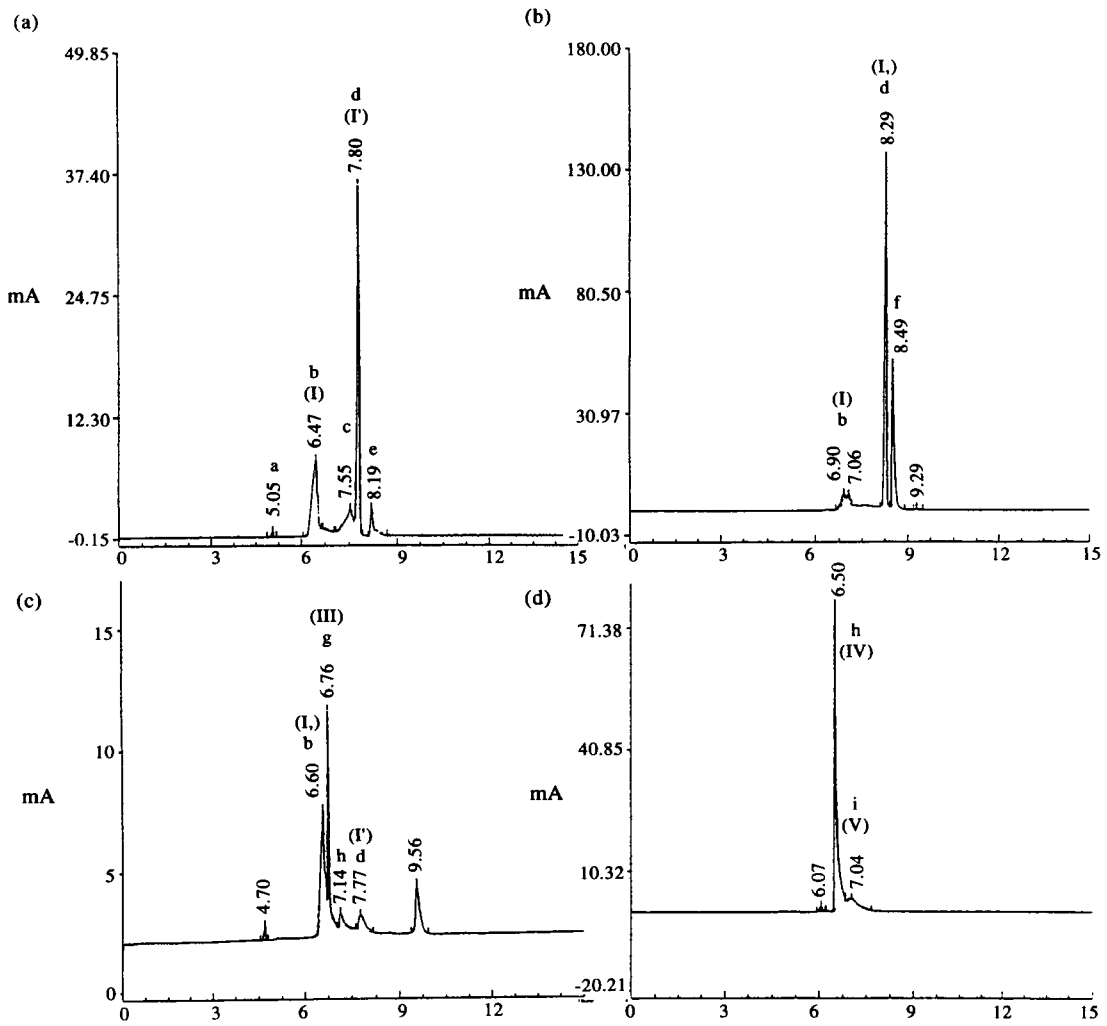

Figure 7
CZ electropherograms of (a) 1 mg of I in 1 ml H_2O, (b) 3 mg of II in 1 ml ACN–H_2O, 3:1, (c) 2 mg of III in 1 ml ACN–H_2O, 1:1, and (d) 2 mg of IV in 1 ml ACN–H_2O, 1:1. See Experimental for details.

tungstate shows two major and three minor peaks (Figure 7a). Similar to HPLC observations, the intensities of the major peaks, b and d, were concentration dependent. At high tungstate concentration (10^{-1} M), the intensity of b was reduced in favour of d (Fig. 8). As the tungstate concentration decreased, so were peaks c, d and e, with comcomitant increase of a and b. At 5×10^{-5} M tungstate, a and b were the only significant peaks left in the electropherogram (Fig. 9). Their UV spectra and their interconversion with tungstate concentration indicate that b and d are, respectively, I and I′. Peak a, therefore, is another solution equilibrium product of tungstate that is favoured in extreme dilution solutions but not detected by HPLC. Compared to HPLC, CZE appears to be a superior technique for solution equilibrium studies of tungstate.

Electropherograms of II and III are more complex than their corresponding chromatograms. It appears that both II and III have been decomposed during electrophoresis. None of the three peaks in the electropherogram of II, Fig. 7(b), corresponded to the hexatungstate II. Peaks b and d were identified, respectively, as I and I′ by their migration times and UV spectra. Of the six peaks detected in the electropherogram of III, Fig. 7(c), only peak g has the UV profile identifiable with III. The UV profiles of peaks b, d and h corresponded to those of I, I′ and

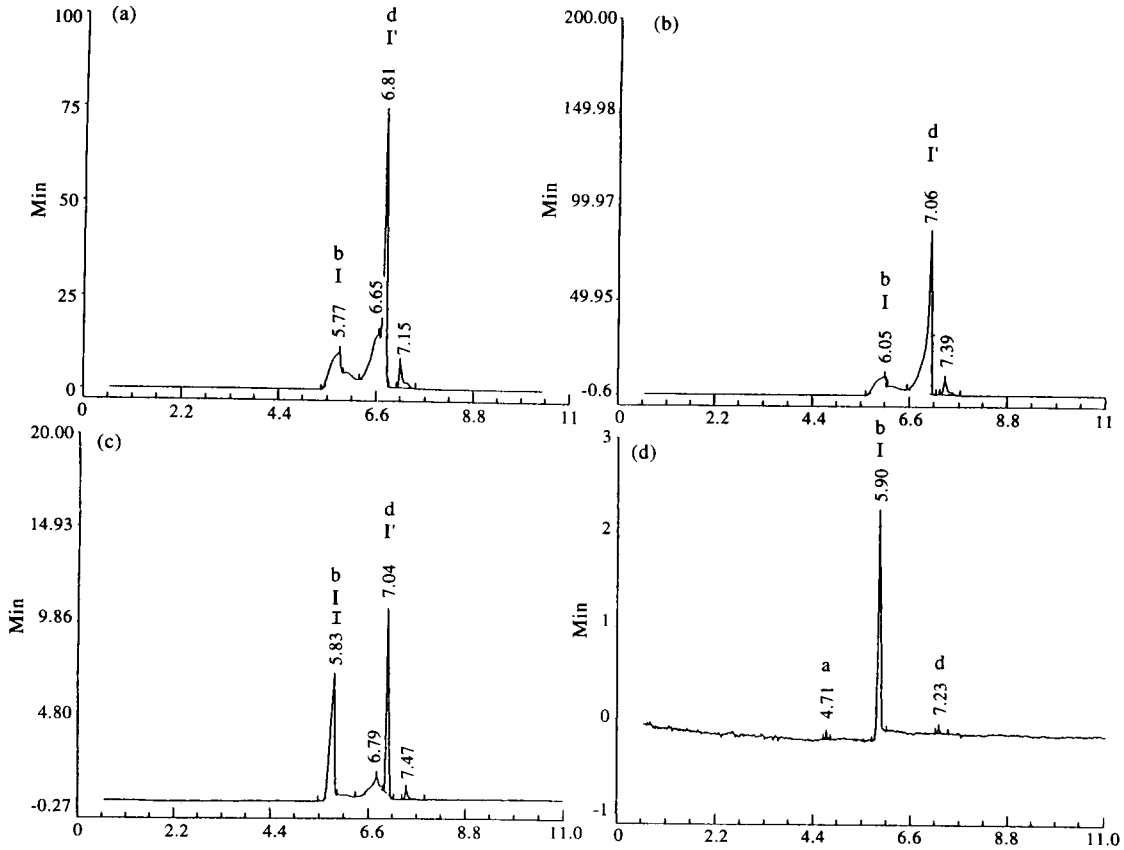

Figure 8
CZ electropherograms of sodium tungstate in H_2O: (a) 10^{-1} M, (b) 10^{-2} M, (c) 10^{-3} M, and (d) 10^{-4} M. See Experimental for CZE conditions.

IV, respectively. The cause for the decomposition of II and III during CZE is not yet understood.

The electropherogram of IV (Fig. 7d) shows a major peak h and a minor peak i, both having UV spectra identical to that of IV. Peak h was converted exclusively to i upon ageing of the solution of IV. Therefore, i was identified as V. When an ACN solution of IV was electrophoretically loaded, only the major peak h was detected suggesting that IV was stable during CZE.

Though CZE offers excellent separation efficiency, its application to tungsto-IPA analysis may be limited by the instability of some of the IPA during electrophoresis. However, its ability to resolve the solution equilibrium products suggests that CZE can be a valuable tool in the solution equilibria studies of sodium tungstate.

Conclusion

In this paper, an ion pair reversed-phase HPLC and a CZE method are presented. The HPLC system gave excellent separation of sodium tungstate and its IPA. The method is simple and sensitive. It can be used to monitor the synthesis, purity and stability of tungsto-IPA. It also has the ability to monitor the solution equilibria of sodium tungstate. The CZE method has a separation efficiency 10 times that of the HPLC method. Due to chemical instability, not all tungsto-IPA can be analysed by the CZE method. Owing to better separation for the solution equilibrium

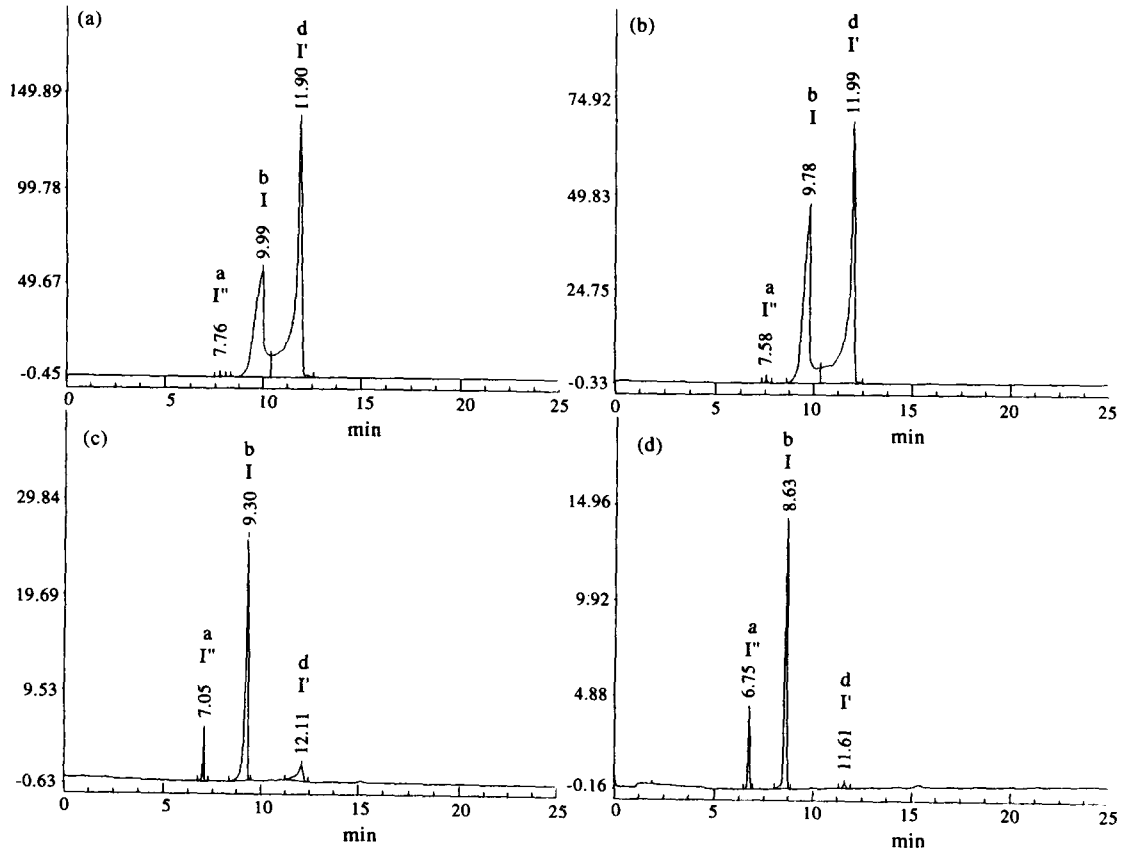

Figure 9
CZ electropherograms of sodium tungstate in H_2O: (a) 10^{-3} M, (b) 5×10^{-4} M, (c) 10^{-4} M, and (d) 5×10^{-5} M. See Experimental for CZE conditions.

products compared to HPLC, CZE is a better technique for solution equilibria studies of sodium tungstate.

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