Short communication

Separation of chromate and tungstate by reversed-phase high-performance chromatography using rutin as chelating reagent

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Abstract

Rutin was used as a chelating reagent to form ternary complexes with metal ions and hexadecyltrimethylammonium bromide (cetyltrimethylammonium bromide, CTAB). The retention behaviour of ternary complexes of Cr(VI) and W(VI) on a Nucleosil C_{18} column were examined. For chromatographic analysis the sample solution had to be changed because these ternary complexes did not give any peaks in aqueous medium. A solid-phase extraction step with polyethylene as sorbent and methanol as eluent was introduced. Cr(VI) and W(VI) complexes in methanolic medium gave sharp peaks in the chromatogram and were separated from each other and other metal ions by using a mobile phase of 42.5% (v/v) tetrahydrofuran containing 0.003 M CTAB. The method was applied to the analysis of chromate in geological samples.

1. Introduction

The application of surfactants in the photometric measurement of metal ions has been studied intensively for many years. The introduction of surfactants greatly increases the solubility of the metal complexes and the sensitivity of the photometric method; however, the selectivity of the method is not considerably improved.

High-performance liquid chromatography (HPLC) has the advantages of high sensitivity, selectivity and the possibility to simultaneously detect several analytes and has been widely used for the separation and determination of binary (metal ion and ligand) metal-ion complexes. Many metal ions that suffer from interference by

other ions in spectrophotometry, can be separated and determined by HPLC. However, very little information is available in the literature on the direct HPLC analysis of ternary complexes with surfactants [1], although in some studies surfactants have been introduced into the mobile phase to increase the solubility of the metal complexes [2–5].

Rutin can form ternary complexes with Cr(VI) and W(VI) in the presence of cetyltrimethylammonium bromide (CTAB) [6,7]. Because their maximum absorption coefficients are very similar, Cr(VI) and W(VI) complexes can not be determined by spectrophotometry. The aim of this work was to examine the application of ion-pair reversed-phase chromatography to the analysis of these two ternary complexes. After solid-phase extraction the Cr(VI)- and W(VI)–rutin–CTAB ternary complexes in methanolic medium were separated with a mobile phase of

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42.5% (v/v) tetrahydrofuran containing 0.003 M CTAB. CTAB, a surfactant with positive charge, was added to the mobile phase to improve the selectivity of the separation, whereas solid-phase extraction was used to change the sample medium in order to reduce the retardation of the ternary complexes.

2. Experimental

2.1. Apparatus

The liquid chromatograph consisted of a Bischoff 2200 pump, an injection valve with an injection volume of 20 μl, a Bischoff Lambda 1000 UV–Vis detector and a Shimadzu C-R6A integrator. A Nucleosil C_{18} column (250 × 4 mm I.D.) (Macherey-Nagel) was employed.

The spectral measurements were carried out in a Spectronic 20 D Model spectrophotometer (Milton Roy Company). The pH was adjusted with an E516 pH meter (Metrohm, Herisau).

A cartridge (plastic container, 60 × 9 mm I.D.) pre-packed with 250 mg of polyethylene was used for solid-phase extraction. The cartridge was connected to a SPE-21 vacuum pump (J.T. Baker), and the solutions were aspirated through. The cartridge was pre-conditioned by passing 2 ml methanol, then 2 ml acetic acid–sodium acetate buffer. At this stage the polyethylene in the cartridge had to be maintained moist.

2.2. Reagents

Rutin (Sigma) was dissolved in methanol to give 0.001 M solutions. Hexadecyltrimethylammonium bromide (CTAB) was obtained from Merck. A 0.01 M solution of CTAB was prepared by dissolving the solid in water. Acetic acid–sodium acetate (0.2 M, pH 5) buffer was used to control the pH of the pre-column derivatization. All chemicals used were of analytical-reagent grade.

2.3. Method

To a solution containing 5–100 μg CrO_4^{2-} and WO_4^{2-} were added 2 ml of 0.001 M rutin, 2.5 ml of 0.01 M CTAB and 2.5 ml of 0.2 M acetic acid–sodium acetate buffer. The mixed solution was diluted to 25 ml with deionized water and kept for 15 min in an ultrasonic bath for colour development.

A 5-ml volume of the above solution containing metal-ion complexes was aspirated through the pre-conditioned cartridge. The cartridge was then flushed with 2 ml of buffer. After drying of the column, the rutin–metal–CTAB ternary complexes were slowly eluted from the cartridge with 2 ml of methanol.

2.4. High-performance chromatography

A 20-μl methanolic sample solution was injected onto the liquid chromatographic system. The complexes were eluted with a mobile phase of 42.5% (v/v) [for the separation of Cr(VI) and W(VI)] or 40% (v/v) [for the determination of Cr(VI)] tetrahydrofuran containing 0.003 M CTAB at a flow-rate of 1 ml min^{-1}. The complexes were detected by a UV–Vis detector at 408 nm.

2.5. Determination of chromate in geological samples

A 0.1–0.5 g amount of sample was fused in an aluminium oxide crucible with 4 g of sodium peroxide at 700°C for 7 min. The melt was cooled and dissolved in 50 ml of water. The hydrogen peroxide was removed by boiling the solution for 10 min. After cooling, the solution was transferred to a volumetric flask and diluted to 100 ml with deionized water. A 5-ml volume of sample was transferred to a 25-ml flask and neutralised with diluted acetic acid. By using the above mentioned method chromate complexes were formed and then transferred to the methanolic medium by solid-phase extraction. Finally a 20-μl aliquot of the methanolic sample solution was injected onto the HPLC system.
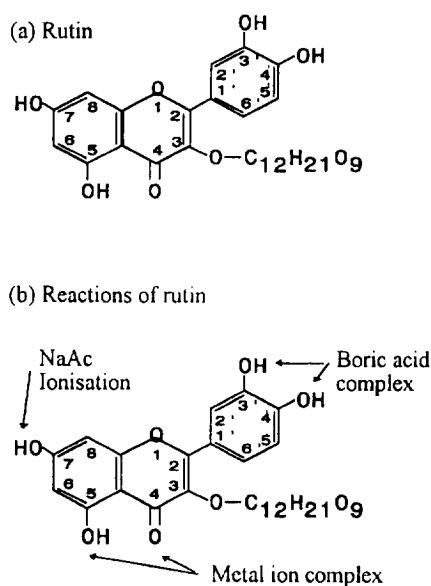
(a) Rutin

(b) Reactions of rutin

Fig. 1. Structure and reactions of rutin.

The amount of chromate in the sample solution was determined by measuring the peak height and comparison with calibration curves.

3. Results and discussion

3.1. Pre-column derivatization and solid-phase extraction of rutin complexes

The structure and reaction characteristics of rutin are shown in Fig. 1 [8]. With the hydroxyl group on C5 and the keto group on C4 rutin can form stable complexes with metal ions [9]. If the medium is not very acidic, the sugar moiety is stable [6] and the hydroxyl groups at the 3', 4' and 7 positions of rutin can be deprotonated

differently. Therefore, rutin complexes are negatively charged and can form micellar ion-association compounds with CTAB. Table 1 shows some properties of the Cr(VI) and W(VI) ternary complexes. These two complexes are relatively stable and their extinction is stable for a period of at least two hours. The retention properties of the Cr(VI), W(VI) and other metal-ion complexes are discussed in Ref. [6].

Direct injection of non-pretreated aqueous solutions of Cr(VI) and W(VI) complexes did not yield any chromatographic peaks. This could be due to the strong hydrophobic character of the complexes. To reduce their adsorption on the stationary phase, the complexes were transferred to a methanolic medium by means of solid-phase extraction with polyethylene as the sorbent. The methanolic sample solution was injected onto the chromatographic system and separated peaks of the Cr(VI) and W(VI) complexes were obtained.

3.2. Chromatographic separation of ternary complexes of Cr(VI) and W(VI)

Table 1 shows that the ternary complexes of Cr(VI) and W(VI) have not only a similar λ_{max} but also a similar optimal pH. This non-selectivity of the complexation reactions leads to difficulties in the spectrophotometric detection but is a prerequisite for the simultaneous determination of metal-ion complexes by means of HPLC.

Separation of the Cr(VI) and W(VI) ternary complexes could be achieved by reversed-phase chromatography when the concentrations of tetrahydrofuran and CTAB were suitably selected. The rutin peak eluted early in the chromatogram and did not interfere with the solute peaks

Table 1
Properties of ternary complexes of rutin with metal and CTAB

Metal	λ_{max} (nm) (water)	λ_{max} (nm) (methanol)	pH$_{opt}$	Metal/ligand (molar-ratio)
Cr(VI)	429	416	5.0	1:2
W(VI)	417	408	4.4–5.1	1:2

because the rutin ternary complexes were more strongly retained by the column than non-complexed rutin.

The concentration of tetrahydrofuran in the mobile phase had a significant effect on the separation of the Cr(VI) and W(VI) complexes. The Cr(VI) and W(VI) complexes could be separated with a tetrahydrofuran content of 40% (v/v), but the retention time of the W(VI) complex was very long (15 min) and the peak was very wide. When the tetrahydrofuran content was higher than 45% (v/v), the Cr(VI) complex peak overlapped with the system peak and the Cr(VI) and W(VI) complexes could not be separated. A concentration of 42.5% (v/v) tetrahydrofuran was chosen for the separation of the Cr(VI) and W(VI) complexes. The mobile phase was carefully handled in a ultrasonic bath to prevent volatization of tetrahydrofuran.

The effect of CTAB in the mobile phase on the retention of Cr(VI) and W(VI) complexes was examined. With 42.5% tetrahydrofuran but no CTAB in the mobile phase the chromatographic peaks of the Cr(VI) and W(VI) complexes could not be resolved from each other and from the solvent peak. In contrast to the usual behaviour described in the literature [5], the addition of CTAB did not result in a decrease, but in an increase of the retention of the metal-ion complexes on the stationary phase. CTAB can act as counter ions in this system forming ion-pairs with complexes containing opposite charge. CTAB has a large hydrophobic group and can be adsorbed on the surface of the stationary phase. In this way a positively charged layer is formed on the surface of stationary phase. When the sample solution together with the mobile phase flows through the column, this positively charged layer competes with the CTAB in the mobile phase for the negatively charged binary rutin–metal-ion complexes. The higher the concentration of CTAB in the mobile phase, the more CTAB will adsorb on the stationary phase and the stronger the retention characteristics of the stationary phase. Because the W(VI) complexes were more sensitive to changes in the concentration of CTAB than the Cr(VI) complexes, the two ternary complexes could be separated. However, a too high concentration of CTAB gave an inferior baseline and resulted in a turbid mobile phase due to the limited solubility of CTAB. A concentration of 0.003 M CTAB was appropriate for the separation of the Cr(VI) and W(VI) complexes.

The pH of the eluent was 5.85. An increase or decrease of the pH neither improved the peak shape nor reduced the retention. Thus the eluent was used without pre-adjustment of the pH. In contrast to the results found with other ion-pair reversed-phase chromatographic separations of metal-ion complexes the addition of ligand and buffer to the mobile phase did not lead to any noticeable improvement in the separation of the complexes; it did however result in deformed peak shapes. Therefore, only 42.5% (v/v) tetrahydrofuran containing 0.003 M CTAB was chosen as the mobile phase.

It must be emphasised that the complexes of Cr(VI) and W(VI) could be not separated on a new column. Even when the tetrahydrofuran content in the mobile phase was increased to 60% (v/v), no W(VI)-complex peak did appear, whereas the Cr(VI) complex eluted together with other components with less retention. With the ageing of the column the retention of the Cr(VI) complex did not decrease substantially, whereas the retention of the W(VI) complex decreased dramatically. Thus, the Cr(VI) and W(VI) complexes could be separated on a used column with 42.5% (v/v) tetrahydrofuran containing 0.003 M CTAB.

An interesting phenomenon was observed in this experiment. When Cr(VI) and W(VI) were first mixed, coloured and then handled by solid-phase extraction, the peak height of tungstate was smaller than that of chromate, although the concentration of tungstate was 4-fold higher than that of chromate (Fig. 2a). When Cr(VI) and W(VI) were coloured separately, extracted and then mixed, a completely different chromatogram was obtained (Fig. 2b). The peak heights of the W(VI) and Cr(VI) complexes were almost identical, although the concentration of tungstate was only twice that of chromate. The reason could be that the chromate complex is more stable than the tungstate complex. Thus, the
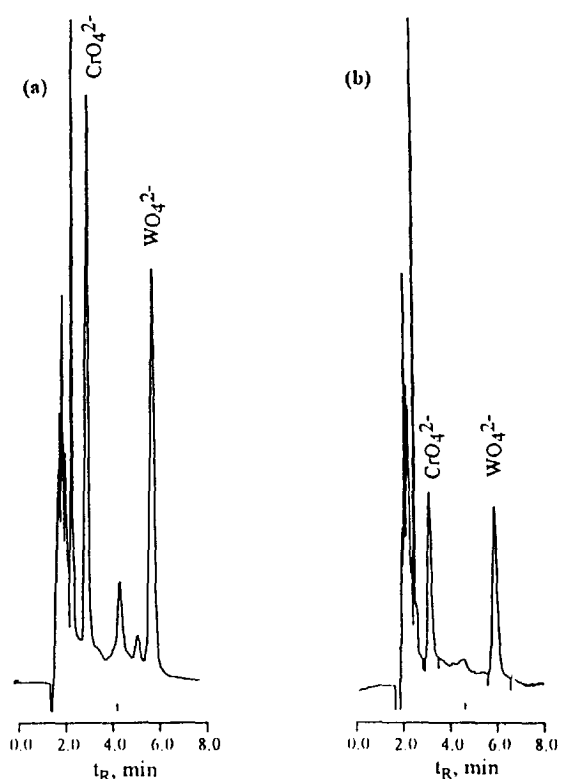
Fig. 2. Separation of the ternary complexes of Cr(VI) and W(VI). (a) Cr(VI), W(V) were first mixed, coloured and then handled by solid-phase extraction; concentration: 2 mg l⁻¹ (chromate), 8 mg l⁻¹ (tungstate); (b) Cr(VI), W(VI) were coloured first respectively, extracted and then mixed; concentration: 1 mg l⁻¹ (chromate); 2 mg l⁻¹ (tungstate). Mobile phase: 42.5% (v/v) tetrahydrofuran containing 0.003 M CTAB; column: Nucleosil C_{18}, 250 × 4 mm I.D.; flow-rate: 1 ml min⁻¹; detection: Vis (408 nm).

relatively stable complex between rutin and chromate is eluted first. Only excess of rutin is able to complex the tungstate completely; this will lead to a low absorbance of tungstate.

3.3. Determination of chromate in geological samples

Because no samples containing detectable W(VI) could be found, only the ion-pair reversed-phase chromatographic determination of the chromate–rutin–CTAB complex was performed as an example of the practical application of the method developed above (section 3.2). In order to obtain a more accurate measurement of the peak height, the concentration of tetrahydrofuran in the mobile phase was reduced to 40%.

This method was very sensitive. Even at a chromate concentration of 5 μg l⁻¹, the chromate peak could be still detected. The calibration curve is linear at least over the range 0.02–0.2 mg l⁻¹ of chromate in the initial aqueous solutions.

Four geological samples were analysed and the results are summarised in Table 2. In order to evaluate the quantitative performance of the HPLC method, a photometric method [10] was used to compare the results. The results from the two methods show good agreement for the determination of chromate in geological samples.

The proposed HPLC method should be applicable to the determination of tungstate in the appropriate samples.

Table 2
Comparison of results for the determination of chromate in geological samples by HPLC and spectrophotometry [10]

Sample	Chromate content[a] (mg kg⁻¹)	
	Proposed HPLC method	Spectrophotometry
Ore	174 ± 1.50	172 ± 0.52
Sediment	214 ± 2.07	230 ± 0.61
Grey soil	282 ± 3.49	260 ± 0.54
Red soil	910 ± 0.57	990 ± 0.09

[a] Mean ± R.S.D.% ($n = 5$).

4. Conclusions

A new ternary complex system has been developed for the separation of Cr(VI) and W(VI) by reversed-phase chromatography. Solid-phase extraction was used to transfer the ternary rutin–metal–CTAB complexes from an aqueous to a methanolic phase. Thus the interaction between the complexes and the stationary phase was reduced and separation of the complexes could be achieved. Due to the limited availability of samples the method was only applied for the determination of chromate in geological samples. When suitable samples are available the method should be applicable to the determination of tungstate or the simultaneous determination of chromate and tungstate.

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