Short communication

Quantitative analysis of manganese, chromium and molybdenum by ion-pair reversed-phase high-performance liquid chromatography with pre-column derivatization and UV-visible detection

Huang-Ling Sun, Hui-Ming Liu, Suh-Jen Jane Tsai*

Department of Applied Chemistry, Providence University, Taichung Hsien 43301, Taiwan

Abstract

An ion-pair reversed-phase high-performance liquid chromatographic method with UV–visible spectrophotometric detection is proposed for the simultaneous determination of manganese, chromium and molybdenum. By using a C_{18}-bonded silica column, 4-(2-pyridylazo)resorcinol (PAR) chelates of Mn(II), Cr(VI) and Mo(VI) were successfully separated and accurately determined at 480 nm. Tetrabutylammonium bromide (TBAB) was used as the ion-pair reagent. Effects of pH, the buffer system, the concentration of buffer, the color developing time, the concentration of chelating reagent and the ion-pair reagent on the resolution were investigated. PAR chelates were eluted within 20 min at a flow-rate of 1.0 ml min^{-1} with a methanol aqueous mobile phase, CH_3OH–water (20:80, v/v), containing $1.0 \cdot 10^{-3}$ mol l^{-1} acetate buffer (pH 6.5), $1.8 \cdot 10^{-2}$ mol l^{-1} TBAB and $2.0 \cdot 10^{-4}$ mol l^{-1} PAR. The feasibility of the proposed method was verified with the standard reference materials of nickel-based alloys. The nickel-based alloys were analyzed chromatographically after ammonium pretreatment. Under the optimum conditions, the detection limits for the chelates of Mn(II), Cr(VI) and Mo(VI) were 0.31, 4.2 and 4.6 ng with 100 µl injection, respectively. The accuracy of the proposed chromatographic method was verified by good agreement between the values obtained by this method and certified values. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Alloys; Mobile phase composition; Manganese; Chromium; Molybdenum; Metal chelates

1. Introduction

Due to their good mechanical properties, such as temperature and stress capability, nickel-based alloys have often been used in the gas turbine engines of aircraft and the hot sections of power plants. The mechanical properties are quite susceptible to the metal content presence in the alloys. Therefore, the metal content requires precise and accurate determination. However, serious problems are often encountered when these high-temperature alloys were analyzed due to the rather complex matrices. The manganese content in alloys often ranges from 0.2 to 1.0%. The reaction between manganese and the sulfur would reduce the sulfur content. The reaction between manganese and carbon would prevent the carbon from turning into graphite. However, the content of manganese requires careful control in order to maintain the constant hardness of the alloys. Chromium (often less than 30%) could provide the high corrosion resistance for alloys. However, the anti-rupture strength would decrease when the chromium content became too high. The presence of molybdenum (often 0.2–4%) would enhance the

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*Corresponding author. Fax: +886-4-632-7554.

hardness of alloys. However, too much Mo would bring difficulty in reforming the alloys. The conventional methods are often tedious and time-consuming. Therefore, the simultaneous determination of these metal contents would fasten the quality control process.

Reversed-phase high-performance liquid chromatography (RP–HPLC) coupled with a UV–visible spectrophotometric detection has been successfully employed in the simultaneous determination of metal ions [1–5]. The sensitivity of this type of chromatographic method is susceptible to the absorptivity of the metal chelates. In addition to having a relatively strong chelating ability, the corresponding chelates should be stable during the elution to ensure a sensitive detection. Otherwise, labile chelates would dissociate during the column elution. Satisfactory results have been reported using chelating reagents such as 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (Br-PADAP) [5], 8-hydroxyquinoline (oxine) [6–9], 5-dihydroxybezene-1,3-disulfonic acid disodium salt (Tiron) [10], *N*-benzoyl-*N*-phenylhydroxylamine [11], arsenazo III [12,13] and 4-(2-pyridylazo)resorcinol (PAR) [13–21]. PAR is a heterocyclic azo dye that often forms ionic compounds with metals. With optimum chromatographic parameters, PAR chelates can be possibly resolved in spite of the their identical absorption characteristics. The relatively high solubility of PAR chelates in water is also one of the advantages. Thus, an extraction process that is often time-consuming is not necessary. Besides, less toxic organic solvents are required.

In ion-pair reversed-phase high-performance liquid chromatography (RP–HPIPC), the resolution is greatly improved by using ion-pair reagents [22–25]. In addition to ion-pair chromatography, the partitioning of metal chelates between a stationary and mobile phase accomplished with an ion interaction reagent have also been named as either ion interaction chromatography, dynamic ion-exchange chromatography or soap chromatography [26]. Alkylsulfonic acids and quaternary ammonium hydroxides have been used for neutralizing cationic and anionic species, respectively. These include heptanesulfonic acid, octanesulfonic acid, dibutylamine, tetrabutylammonium hydrogensulfate, tetramethylammonium chloride and tetrabutylammonium bromide (TBAB) [27]. The organic solvent or modifier also plays an important role in RP-HPIPC. Binary mixtures of water with an organic solvent modifier such as acetonitrile or methanol are often employed in RP-HPIPC. The resolution of analytes is also susceptible to the volume fraction of water in the mobile phase [28].

In this work, a reliable method for the determination of manganese, chromium and molybdenum has been developed. Various chelating reagents including PAR, Tiron, 1-(2-pyridylazo)-2-naphthol (PAN), Br-PADAP and oxine have been investigated. Among these, PAR gave the highest sensitivity. Both the spectrophotometric and chromatographic characteristics of PAR complexes have been investigated. After pre-column derivatization with PAR, Mn(II), Cr(VI) and Mo(VI) were determined simultaneously using reversed-phase HPLC with UV–visible detection. Silica columns with a bonded organic phase (C_{18}) were employed.

2. Experimental

2.1. Apparatus

The pH measurements were carried out with a PHM 85 precision pH meter and a combine electrode (Radiometer). The absorption spectra were obtained with a Shimadzu UV-260 UV–visible recording spectrophotometer, equipped with 1 cm quartz cells. Liquid chromatographic determinations were carried out using a Perkin-Elmer LC-235C, 250 Binary pump, 1020 integration personal computer and a Waters 486 tunable absorbance detector. A Waters μBondapak C_{18} column (No. 27324, 3003.9 mm I.D.) coupled to a Waters guard column (No. 88141) was used. A Barnstead Nanopure II system was employed for water purification.

2.2. Reagents and solutions

All of the reagents were purchased from Merck unless marked otherwise. The nickel-based alloy used, 211X11221D, was a certified reference material and was obtained from MBH Analytical (Barnet, UK). Acetonitrile, isopropanol, tetrahydrofuran and

methanol were LC grade from Mallinckrodt. The PAR solution was freshly prepared before use.

2.3. Procedure

For the pretreatment of nickel-based alloys, an amount of ca. 0.1 g of standard reference material of the nickel-based alloy, namely 211X11221D, was weighed accurately and then treated with 1.0 ml of a mixture of HCl–HNO$_3$ (4:1, v/v). For ammonium pretreatment, the sample solution prepared above was treated with 5 ml of concentrated ammonium solution. After the precipitation process was completed, the resultant aqueous mixture was centrifuged and the precipitate was decomposed with 3–5 ml diluted nitric acid (1:1, v/v). The resultant solution was heated gently to reduce it to ca. 1.0 ml, removing the excess acid, and then it was diluted to 50 ml. The corresponding blank solutions were prepared similarly. The sample and blank solutions thus prepared were filtred and were then ready for analysis by RP-HPIPC.

The solution for chromatographic detection was prepared by taking a 0.4-ml aliquot of PAR solution ($5.0 \cdot 10^{-3}$ mol l^{-1}) and gradually adding it into a calibrated 10-ml volumetric flask that contained a volume of 0.1 ml of acetic acid–sodium acetate buffer. A precise amount of a metal ion solution or an alloy sample solution was added. The pre-column chelation of the metal ions was completed after standing for 5 min. An aliquot of 100 μl was injected into the HPLC system. The metal–PAR chelates were detected at 480 nm. All of the separations were performed at a flow-rate of 1.0 ml min^{-1}.

3. Results and discussion

This work was aimed at the development of a sensitive, selective and effective method for obtaining reliable quantitative results for the determination of manganese, chromium and molybdenum simultaneously. Reversed-phase HPLC coupled with UV–visible detection was used. Various chelating reagents which included PAR, Tiron, PAN, Br-PADAP and oxine had been investigated. When Tiron was used as the chelating reagent, complicated chromatograms of

blank solutions that could not be separated from the peaks of metal chelates were obtained. PAN was slightly soluble in water and Br-PADAP was not soluble in aqueous solutions, therefore, a relatively high concentration of methanol was required in order to assure a homogeneous solution. However, a relatively broad methanol peak that was not separable from the analyte peaks was found in the chromatograms. In addition, the precipitates of metal chelates were found within 30 min with the addition of methanol. A similar problem was encountered when oxine was used as the chelating reagent. Based on previous observations, PAR was chosen as the chelating reagent.

3.1. Spectrophotometric studies

PAR was a tridentate ligand that formed colored, water-soluble chelates with various metal species [15]. Thus, the time consuming extraction process could be avoided. The equilibrium distribution of PAR (H$_2$L, p$K_{1,\text{para}}$=6.9, p$K_{2,\text{ortho}}$=12.4 [17]) species was a function of hydrogen concentration. The PAR chelates often existed as neutral species, M(HL)(L) or anions M(L)$_2$ in a slightly acidic solution [17]. Furthermore, the oxidation of Mn(II) to Mn(VI) in the basic solution, such as ammonium solution, have been reported [31].

Trace contents of Mn(II) were determined spectrophotometrically by chelating with PAR [29,30]. The chelation between metal ions and PAR was quite susceptible to the reaction conditions. In a slightly alkaline solution (pH 8.5–8.9, in triethanolamine buffer solution), Cr(III) reacted with PAR and the predominate species was anionic, [Cr(III)(L)$_2$]$^-$. Interestingly, the predominate species was susceptible to the protonation equilibria [15].

In a KH$_2$PO$_4$–Na$_2$HPO$_4$ buffer (pH 7.50), neither Cr(VI) nor Mo(VI) formed colored PAR chelates unless hydroxylamine hydrochloride was added as the reducing reagent [18]. However, Cr(III) hardly reacted with PAR as confirmed in a spectrometric determination of metal ions with PAR [31]. Mo(VI) has been studied chromatographically as derivatives of oxine [9], Tiron [10], N-benzoyl-N-phenylhydroxylamine [11] and 2,2′-dihydroxylazobenzene [22]. Interestingly, there was hardly any information on the chelation between Mo(VI) and PAR. The chro-

matographic study with the wavelenth ranging from 440 to 500 nm indicated that 480 nm was the optimum monitoring wavelength. The species of manganese, chromium and molybenum were identified as Mn(II), Cr(VI) and Mo(VI) by comparing the absorption characteristics of metal chelates obtained from the eluent to the standard metal solutions. The molar absorptivities for PAR chelates of Mn(II)–PAR, Cr(VI) and Mo(VI) chelates at 480 nm were $0.41 \cdot 10^3$ l mol^{-1} cm^{-1}, $0.15 \cdot 10^3$ l mol^{-1} cm^{-1} and $0.21 \cdot 10^3$ l mol^{-1} cm^{-1}, respectively. Chelates of Mn(II) had the highest molar absorptivity.

3.2. Chromatographic studies

Pre-column [5,7,14,18,19], on-column [4,16] or post-column derivatization [12,13] were the three most common techniques employed in the chromatographic determination of metal species. The feasibility of these derivation methods was dependent on the reactivity of the chelating ligand and the stability of metal chelates. In this work, the pre-column technique was used in order to prevent the clogging of the separation column by the precipitation of PAR chelates. Retention of PAR chelates was controlled by the properties and concentration of the organic modifiers, hydrogen ions, buffer and ion-pair reagents. The monitoring wavelength was also an important parameter. The most symmetric peaks and the best resolution for these chelates were found at 480 nm.

The pH of the mobile phase had different effects on the resolution and elution patterns of metal chelates. This was not surprising because the equilibrium distribution of neutral species, M(HL)(L) and anions, M(L)$_2$ was a function of the charge on the center metal and the chelating group and also a function of pH. Since μBondapack C$_{18}$ columns were used in all measurements, using an alkaline solution with a pH greater than 7.0 would result in the decomposition of the siloxane bond (Si–O–Si). Thus, the effect of pH on the separation, e.g. retention times, peak area and the capacity factors were studied with the pH ranging from 4.0 to 7.0. The retention times of the Mn(II) chelate and the Cr(VI) chelate were less susceptible to the variation in pH. However, the peak of the Cr(VI) chelate was split at pH 5. The peak of the Mo(VI) chelate only appeared at pH 6.0 and higher pH. At pH 6.5, PAR chelates of Mn(II) and Mo(VI) had the highest peak areas. This indicated that the highest sensitivity for these two chelates could be obtained at pH 6.5.

The resolution of metal species would be improved by including effective amounts of organic modifier in the mobile phase. This was due to the variation of solvent polarity, hydrophobicity and solvent charge. Besides, optimum solvent conditions were required in order to prevent precipitation or decomposition of metal chelates [32]. In this work, we had included different proportions of acetonitrile (ACN), methanol, tetrahydrofuran (THF) and 2-propanol in the mobile phase. These solvents have different polarities which lead to different strengths and selectivities [7]. This work showed that the metal chelates were only retained in a relatively short time. Consequently, the chromatographic peaks were seriously overlapped and could not be resolved. The poor resolution was greatly improved when methanol was included. This implied that the PAR chelates of Mn(II), Cr(VI) and Mo(VI) were relatively less polar species. When methanol instead of another organic modifier was included in the mobile phase, the PAR species reacted more strongly with the non-polar C$_{18}$ end of the column. Thus, longer retention times resulted. The methanol–water (containing TBAB) eluent proved to be effective for this separation. The PAR chelates were eluted in the following order: Mn(II), Cr(VI) and Mo(VI). This indicated that the chelates of Mo(VI) reacted most strongly with the stationary phase. The effect of the concentration of methanol on the capacity factor, k', was studied. The capacity factors of all metals are decreased by reducing the dielectric constant of the mobile phase. Based on the previous observation and the broadening and symmetry of the chromatographic peaks, methanol–acetic acid–sodium acetate buffer (20:80, v/v) eluent was employed for the following experiments.

3.3. Buffer and ion-pair reagent

The above work proved that the most effective mobile phase was a binary mixture of water with methanol as the organic modifier. The mobile phase was methanol–water (20:80, v/v), $1.0 \cdot 10^{-3}$ mol l^{-1}

acetate buffer (pH 6.5), $2.0 \cdot 10^{-4}$ mol l^{-1} PAR and $1.8 \cdot 10^{-2}$ mol l^{-1} TBAB, as the ion-pairing reagent. In this work, four different buffer systems had been studied, namely, acetic acid–sodium acetate, tartaric acid–sodium tartrate, citric acid–sodium citrate and phosphoric acid–disodium phosphate. Complicated chromatographic peaks with poor resolution for the PAR chelates were observed in the latter three buffer systems. This might be due to the competition between the dissociated anion buffer species and the PAR anion. In other words, complicated chromatograms might result from the formation of tertiary species, such as metal–buffer–PAR. Consequently, complicated chromatograms were observed for blank solutions. Therefore, acetate buffer was employed in the following experiments. Since an ion-pair separation was involved, the retention times of the PAR chelates would be a function of the acetate concentration. This was verified by monitoring the change in the capacity factor as a function of acetate concentration. The capacity factor decreased as the buffer concentration increased from 0.20 to 6.0 mmol l^{-1}. This might be due to the enhancement of the polarity of the mobile phase caused by an increasing portion of the acetate buffer. Consequently, shorter retention times resulted due to reduced retention of the PAR chelates. With regard to the resolution, all of the analysis was performed with the acetate buffer having a concentration of 1.0 mmol l^{-1}.

The role of the ion-pair reagents is to provide the exchange sites for the charged species. With the addition of the ion-pair reagents, some degree of variation in the retention time and the chromatographic selectivity was expected. The ion-pair reagents, which included quaternary ammonium hydroxides and alkylsulfonic acids, were used effectively in neutralizing anionic and cationic species, respectively [26]. Without the addition of any ion-pair reagent, PAR chelates were hardly resolved. The addition of methylsulfonic acid (CH_3SO_3H, anionic ion-pair reagent) did not bring any improvement in the resolution of the PAR chelates. The anionic characteristics of the PAR chelates was thus further verified as cationic ion-pair reagents were required in the separation. With the addition of TBAB, the analytes would react more strongly with the stationary phase through the ion-pair reaction between the PAR chelate anion and the TBAB cation. In the lower concentration range, the capacity factor increased rapidly as the TBAB concentration increased. Above a certain concentration, i.e. 15 mmol l^{-1}, it reached a plateau. In other words, the coverage of the stationary phase was more rapid and efficient in the lower concentration range. Above a certain concentration of TBAB, the slope of the curve decreased due to the near-saturation of the stationary phase with the ion-pair reagents. The concentration of 18 mmol l^{-1} was employed for all of the measurements.

3.4. Interference studies

The effects of possible interfering ions, including Co(II), Ti(IV), Al(III), Fe(III), Zr(IV), Nb(V), Ta(V), Ni(II) and Cu(II), on the recovery of chromatographic signals were studied. In order to understand the effects of interfering ions on the recovery of the chromatographic signals of the PAR chelates, the standard metal solutions were spiked with different concentrations of foreign compounds. The recoveries of the signals of the PAR chelates were more susceptible to the foreign compounds and their concentration. However, the presence of foreign ions resulted in less significant effects on the retention times of the PAR chelates. In general, these foreign ions had less effect on the Mn(II) chelates. Nb(IV) and Ta(IV) had negligible effects on all of the PAR chelates. The maximum tolerable concentration was 10 ppm for the other metal ions.

3.5. Application

In order to evaluate the proposed chromatographic method, a relatively complex substance, namely the standard reference material of a nickel-based alloy, 211X11221D, was analyzed. This certified reference material had the following composition (all values in mg g^{-1}): Si, 0.40; Mn, 2.5; Cr, 143.0; Co, 12.6; Mo, 37.2; Nb, 32.3; Ti, 15.2; Al, 63.5; Cu, 0.60; Fe, 2.90; Zr, 1.10; and Ta, 2.60. Unfortunately, Mn(II), Cr(VI) and Mo(VI) were not well resolved due to the complexity of the nickel-based alloys as shown in Fig. 1a and b. The experimental results indicated that the ammonium pretreatment worked effectively in reducing the alloy matrix effect. After treated with
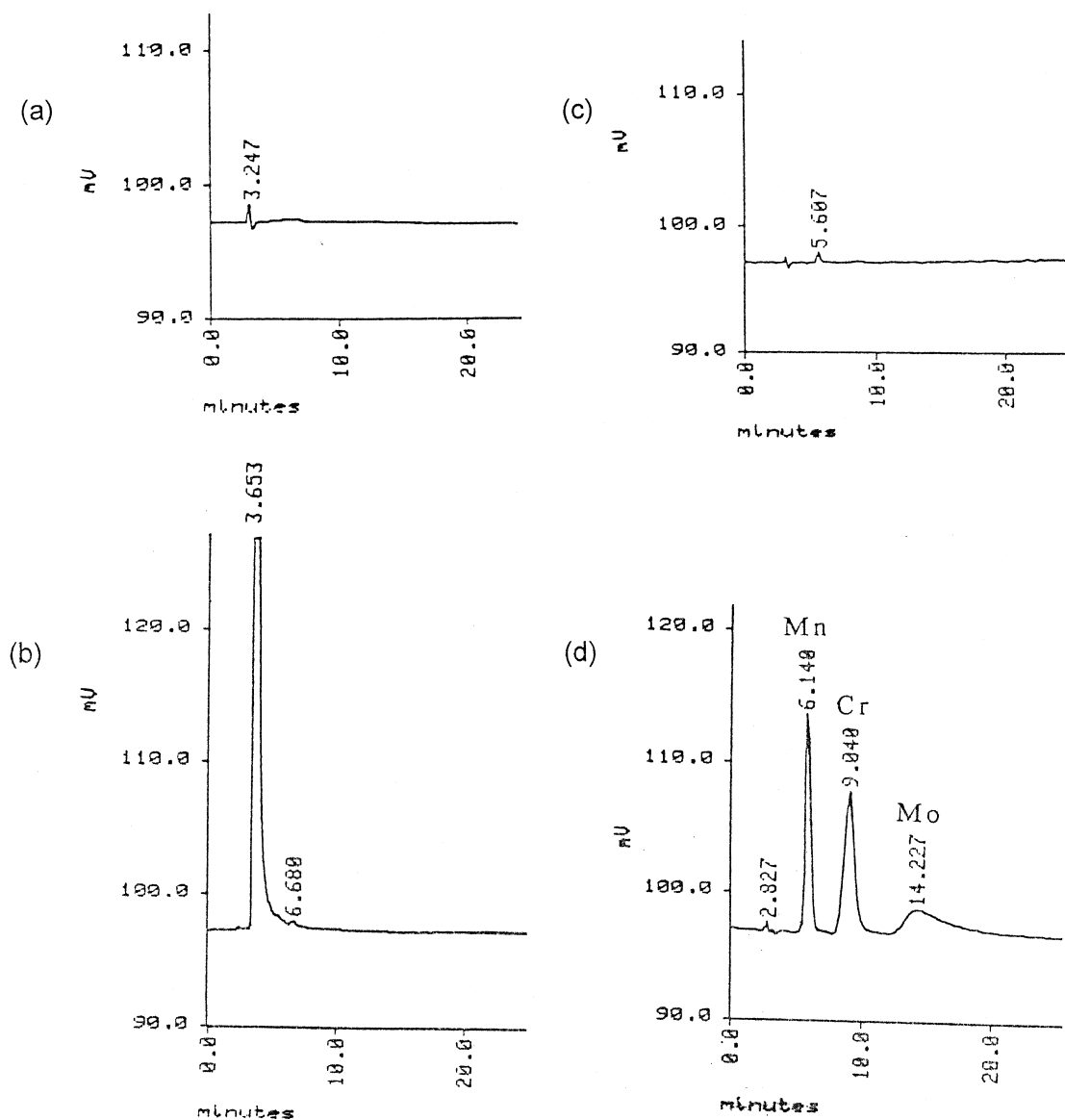
Fig. 1. Typical chromatograms of nickel-based alloys, 221x1121D, (a) blank solution obtained without sample pretreatment, (b) sample obtained without sample pretreatment, (c) blank obtained with ammonium precipitation, (d) sample obtained with ammonium precipitation. Mobile phase: methanol–water (20:80, v/v) at pH 6.5 with 1.0 mmol l^{-1} acetic acid–sodium acetate and 1.8 X 10−2 mol l-1 TBAB. Flow rate 1 ml min^{-1}; 480 nm and att 32. An amount of 17.64 mg alloy was injected. A 0 to 2.0 full scale signal corresponded to the 0 to 2 actual absorbance.

ammonium precipitation, a chromatogram with satisfactory resolution was obtained as shown in Fig. 1c and d. Each peak was identified by comparing the retention time and the absorption characteristics to the individual metal chelates. The proposed method provided a means for analyzing Mn, Cr and Mo in nickel-based alloys with detection limits of 0.31, 4.2 and 4.6 ng ($S/N=3$), respectively. With five consecutive injections of 100 μl of sample solution containing 200 ng each of Mn(II), Cr(VI) and Mo(VI), the recoveries obtained ranged from 94.3 to 105%. The experimental values obtained from the

calibration curves and by the standard additions method were compared with the certified values. The certified values for Mn, Cr and Mo in 211X11221D nickel-based alloy were 2.5, 143.0 and 37.2 mg g^{-1}, respectively. Those obtained from the calibration curves were 2.37±0.08, 106.0±3.7 and 48.1±1.4 mg g^{-1}, for Mn, Cr and Mo, respectively. Those determined by the standard additions method were 2.42±0.08, 136.0±4.6 and 40.7±1.4 mg g^{-1}, for those PAR chelates, respectively. Ion chromatography is also an important analytical method for analyzing anions and cations. However, the selection of the column and the detection methods, conductivity or photometric detection, are the most important issues. As for the fluorescence detection, the optimum conditions that give maximum signals, including the selection of the excitation and emission wavelengths, required careful investigation. With the complexity of the nickel-based alloys, it is a challenge to determine the content of one metal in the presence of numerous other metal components. Large backgrounds would be another problem when using conductimetric detection. The proposed method allowed the metal contents to be determined accurately. Although the molar absorptivities are small compared to other chromophores, the detection limits are sufficiently low to allow the determination of Mn(II), Cr(VI) and Mo(VI) in nickel-based alloys.

Acknowledgements

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