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Electrochemical detection of sulphonated azo dyes and their metal complexes in ion interaction chromatography

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Abstract

An ion interaction chromatographic method coupled to electrochemical detection was used for the separation and the determination of sulphonated azo ligands (SPADNS, Acid Alizarin Violet N and Plasmocorinth B) and their metal complexes. The aim of this work was to verify the compatibility of the separation mechanism with the electrochemical detection of the azo dyes and their metal complexes and to obtain the best experimental conditions for a highly sensitive detection of the analytes considered. The effects of the mobile phase composition (ion interaction reagent, counter-ion, organic modifier, pH) on detection efficiency was investigated and the optimum conditions were identified. The developed procedure, that enabled the determination of metal ions such as copper and iron at low μg/l levels, was applied to the analysis of natural waters. © 1998 Elsevier Science B.V.

Keywords: Water analysis; Mobile phase composition; Azo dyes; Dyes; Metal complexes; Copper; Iron

1. Introduction

The determination of traces and ultratraces of metals in aqueous samples can be performed by stripping (anodic [1,2], cathodic [3] and adsorptive [4–8]) voltammetry. The very high sensitivity, the relative low cost of the apparatus and of the analysis procedures make this method reliable and suitable for metal determination as well as other routine methods (e.g., atomic absorption and emission atomic spectrometry).

Among the other analytical techniques, chromatography represents a reliable and accurate method which can provide selective and efficient separation of metal ions even in complex matrices. A convenient and frequently used method for the determination of metal ions is through a preliminary complexation with a suitable ligand followed by the separation of the resulting coordination compounds by conventional reversed-phase [9], suppressed [10] or non-suppressed [11–13] ion chromatography. This approach is commonly used in ion interaction chromatography [14–16] (IIC), where retention is due to a proper pairing ion, added to the mobile phase, that can interact with an analyte of opposite charge in a reversed-phase stationary phase. The ligands used in IIC of metal ions should preferably be easy to detect and for this reason the ones most commonly used are UV–Vis absorbing compounds.

The electrochemical detection of metal ions coupled to liquid chromatography combines the high sensitivity of detection with the powerful selectivity capabilities of the separation system. Nevertheless, direct electrochemical detection of metal ions in liquid chromatography is not a common practise owing to their negative reduction potentials and to the electrically conductive properties of the mobile phases required [17]. Indirect amperometric detec-

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tion, instead, has been coupled to ion chromatographic separation of metal ions. In this case, the detection system involved post-column reaction of the metal species with excess of easily oxidized ligands (e.g., dithiocarbamate [18], pyrrolydinedithiocarbamate and bis(2-hydroxyethyldithiocarbamate [19]). This method enables the determination of metal ions such as Cu^{2+} and Fe^{3+} at 100–150 μg/l levels. Lower limits of detection can be obtained by an amperometric detector based on the transfer across an array of liquid–liquid micro-interfaces [20].

In this work we have evaluated the possibility to separate, by liquid chromatography, and to electrochemically detect sulphonated azo ligands [SPADNS, Plasmocorinth B (PCB), Acid Alizarin Violet N, (AVN)] whose complexation capabilities [8,21–23] and electrochemical properties [24–27] were previously studied. The chromatographic method used is based on the ion interaction mechanism. Retention and selectivity in IIC are influenced by several experimental parameters such as concentration of ion interaction reagent, ionic strength and concentration of organic modifier. A comprehensive description of the effects of these parameters on retention and of the mechanism involved has been discussed in detail elsewhere [14–16].

Owing to the complexity of this chromatographic system, one is allowed to expect that the detection conditions are strictly related to the mobile phase composition.

Considering the above remarks and the lack of available data from the current literature, the aim of this study was, therefore, to investigate the effect of the main experimental variables that control retention in IIC on the detection of the three sulphonated azo ligands and their metal complexes.

2. Experimental

2.1. Instrumentation

The chromatographic pump used was a Gilson (Middleton, WI, USA) Model 302 fitted with a Gilson Model 208 manometric module, a 7125 Rheodyne injector valve with a 100-μl sample loop.

The electrochemical detector was an ESA Coulochem II, provided with an electrolysis cell 5011 Analytical Cell Model. The cell was equipped with two porous graphite working electrodes, each coupled with a platinum counter electrode and a palladium reference electrode, enclosed in two stainless steel chambers. The detector was kept turned on overnight with the mobile phase flowing through the cell at 0.1 ml/min in order to condition the cell. The eluent was recirculated in order to reduce reagent consumption and avoid electrode fouling. Experiments were performed imposing and changing the potential at one electrode, keeping the potential at the other electrode constant at zero, unless otherwise stated.

The separation column used (10 μm, 250×4 mm I.D.) was a LiChroCART cartridge containing LiChrospher 100 RP-18 endcapped packing material. The guard column was a 5 μm (4×4 mm I.D.) LiChrospher 100 RP-18. Both guard and separation columns were from Merck (Darmstadt, Germany). An Orion (Cambridge, MA, USA) digital pH meter was used for pH measurements.

The chromatograms were recorded by AI-450 chromatographic software using an advanced computer interface ACI from Dionex (Sunnyvale, CA, USA).

All chromatograms were obtained at room temperature. The eluent flow-rate was 1.0 ml/min. Retention times were the means from triplicate injections. The dead volume was measured by injections of water.

2.2. Reagents and solutions

Eluents were prepared by dissolving analytical-reagent grade chemicals in high purity water obtained with a Milli-Q system (Millipore, Bedford, MA, USA) and filtering through a 0.22-μm filter.

2-(4-Sulphophenylazo)-1,8-dihydroxy-3,6-naphthalene-disulphonic acid, trisodium salt (SPADNS), 4-hydroxy-3-(2-hydroxynaphthylazo)-benzenesulphonic acid (AVN) and 2-(5-chloro-2-hydroxyphenylazo)1,8-dihydroxynaphthalene-3,6-disulphonic acid (PCB) were from Aldrich. Tetrabutylammonium chloride (TBACl), sodium formate were from Fluka (Buchs, Switzerland). Sodium chloride, methanol and hydrochloric acid were from Carlo Erba (Milan, Italy) while acetic acid and disodium hydrogenphos-

phate were Merck reagents. Working solutions of metal ions, namely Cu(II), Ni(II), V(IV), Co(II) and Fe(III), were prepared by dilution of concentrated standard stock solutions (Merck).

Each analyte was prepared in eluent solution.

3. Results and discussion

As a starting approach for the detection of the three ligands studied, the i/E curves were determined under the flow conditions of the chromatographic system by hydrodynamic voltammetry (Fig. 1). The mobile phase used was simply a buffered water–methanol (40:60, v/v) mixture. From the data obtained it is possible to note that AVN and PCB can be oxidized at potential values lower than SPADNS. The remarkable behaviour of AVN shows that the signal decreases for potentials higher than 800 mV, instead of reaching the plateau usually observed in the i/E curves. This behaviour observed during the experiments performed here could be due to some reactions that partially clog the electrode surface reducing its sensitivity. Some attempts have been made in order to explain the reduction of current observed. AVN has been oxidized at 1000 mV at the first electrode and then subsequently reduced at the second electrode, and the reduction current was measured at the second electrode. A symmetric negative peak was obtained instead of the broad one expected; this finding points out that adsorption of AVN, if present, is reversible. It should be noted that no decrease of current takes place if 1 μ*M* AVN is added to the mobile phase and i/E curve is determined by subtracting the signal obtained in the presence of AVN in the eluent from the one with no AVN present.

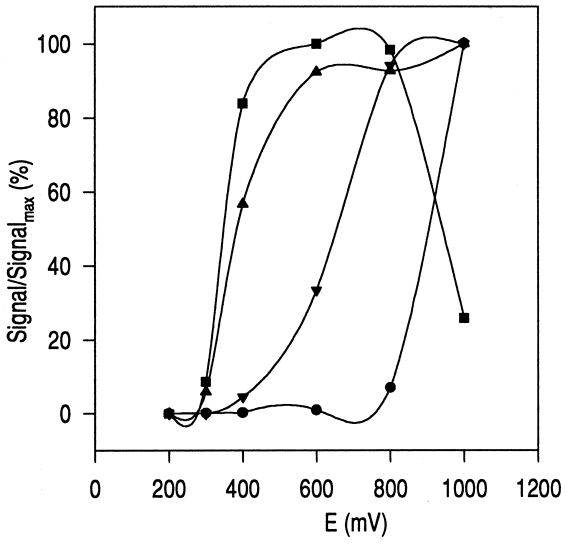

Fig. 1. i/E curves for the background and for the ligands studied. Mobile phase water–methanol (40:60, v/v), 30 m*M* acetic acid, aqueous pH 5.5. Concentrations of ligands: 6 μ*M*. – ● – Background current; – ■ – AVN; – ▲ – PCB; – ▼ – SPADNS.

At every mobile phase composition investigated, the retention order increased according to SPADNS< PCB<AVN. As the optimization of chromatographic separation for ligands was out of the aims of this study, their k' values will not be shown throughout this paper. The eluents used were chosen in order to ensure a separation of the ligands sufficient to measure the chromatographic peak areas.

The detection of the ligands has been studied taking into account the parameters of the mobile phase influencing the separation in IIC: concentration of ion interaction reagent (IIR), organic modifier (methanol), counter-ion (Cl^-) and pH. For each ligand and parameter, the detector signal has been recorded at six different potential values (150, 300, 500, 700, 800 and 900 mV), obtaining the i/E curves of the analytes under the different experimental conditions evaluated.

3.1. Effect of IIR

The detector response has been evaluated in a range included between 5 and 14.5 m*M* TBA and the results obtained are shown in Fig. 2. The general trend of the i/E curves is similar to that obtained in a simply buffered mobile phase, in fact under these conditions the decreasing signal for AVN beyond 800 mV and the shift observed for the SPADNS towards higher potentials are still present.

The presence and the increase of TBA concentrations in the mobile phase introduce a depletion of the detector response. This behaviour can be due to an increase of the viscosity of the mobile phase which reduces solutes mobility and their capability in electric charge transfer. Moreover, some interactions and partial coverage of the graphite electrode by the

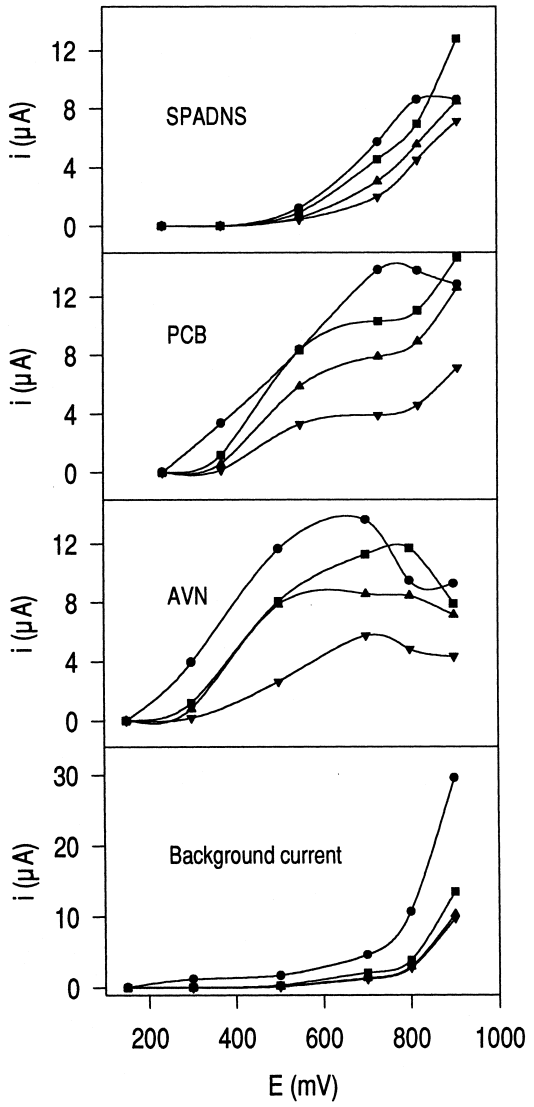

Fig. 2. Effect of TBA concentration on the i/E curves for the background and the ligands studied. Mobile phase water–methanol (40:60, v/v), 20 m*M* acetic acid, 10 m*M* NaCl, aqueous pH 5.5. Concentrations of ligands: 15 μ*M* SPADNS, 40 μ*M* PCB, 40 μ*M* AVN. – ● – [TBA]=5 m*M*; – ■ – [TBA]=7.5 m*M*; – ▲ – [TBA]=10 m*M*; – ▼ – [TBA]=14.5 m*M*.

n-butyl chains of TBA could also occur, limiting the active surface of the electrode.

From the data obtained it is possible to find the best detection conditions for the analytes studied as a function of TBA concentrations in the mobile phase. The optimal range is included between 7.5 and 10 m*M* TBA where a reduced variation in the current for different TBA concentrations is observed and a good sensitivity is still present. Standard deviations, determined for each potential imposed and for each TBA concentration, are included within a ±8% range. The following experiences were performed at 10 m*M* TBA to ensure that chromatographic peaks were resolved from the dip volume.

3.2. Effect of organic modifier

The effect of methanol on the current signal was evaluated in a range included between 45 and 60%. The study was performed only for SPADNS, because, at the low methanol concentrations, AVN and PCB would have been eluted at high retention times, making their determination hard and approximate. The range of methanol concentrations was chosen in order to have a sufficient elution strength of the mobile phase during all experiences. The results obtained at each potential value investigated for different methanol concentrations are shown in Fig. 3. Standard deviations, evaluated for each experiment, do not exceed 3%. The plots show that the

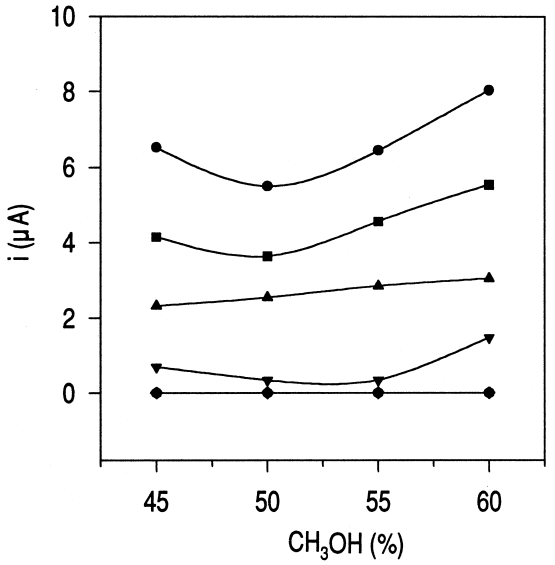

Fig. 3. Effect of methanol concentration on the i/E curves for SPADNS. Mobile phase: 20 m*M* acetic acid, 10 m*M* TBA, 10 m*M* NaCl, aqueous pH 5.5. Concentration of SPADNS: 15 μ*M*. – ● – E=900 mV; – ■ – E=800 mV; – ▲ – E=700 mV; – ▼ – E=500 mV; – ♦ – E=300 mV.

prevailing trend of the signal for SPADNS is to increase with methanol concentration. This is the result of different effects arising from the variation of methanol content in mobile phase. In fact the increase of methanol can reduce the interaction between TBA and the electrode of the detector cell, increasing the response. In addition, the response is also risen by the decreased viscosity of the mobile phase, exalting the transfer and the mobility of the analytes in the eluent. Nevertheless, the increase of methanol content changes the solvation of ionic solutes modifying their activity and their electric properties.

The experimental data showed that the highest response of the detector is obtained for the highest (60%) investigated methanol concentration. Taking into account the performance of the chromatographic separation, it is advisable to use mobile phases with a methanol content that ensures a good elution power. In fact, low percentages of methanol in the mobile phase result in too high retention times, according to the theories of reversed-phase and IIC [15,16], and in peak broadening.

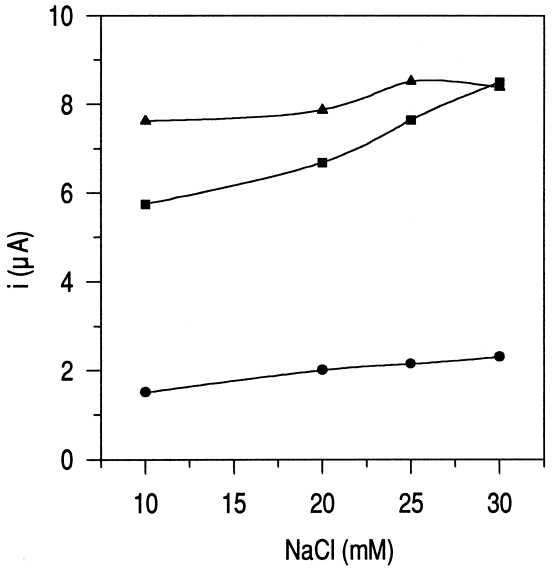

Fig. 4. Effect of NaCl concentration on the i/E curves for the background, for PCB and SPADNS. Mobile phase: water–methanol (40:60, v/v), 20 m*M* acetic acid, 10 m*M* TBA, aqueous pH 5.5. Concentrations of ligands: 15 μ*M* SPADNS, 30 μ*M* PCB. – ● – Background current; – ■ – SPADNS; – ▲ – PCB.

3.3. Effect of counter-ion

The effect of counter-ion concentration in the eluent on the detection of the ligands was performed with buffered mobile phases containing 10 m*M* TBA and 60% methanol, varying NaCl content from 10 to 30 m*M*. Experiments were carried out at six different potential values, but for simplicity, in Fig. 4, data for the background current and the ligands obtained at 800 mV are only shown. It must be remarked that during this study an instability of the signal of AVN was noted.

The experimental data show that at every potential value studied, the intensity of the signal for both the background and the ligands increases with counter-ion concentration, this trend being more emphasised for higher potentials. This behaviour should be related to the increase of the conductivity of the mobile phase that makes the charge transfer more effective. Anyway, higher salt concentration in the mobile phase is not advisable; in fact, for the ion interaction mechanism, a greater counter-ion concentration enhances the eluent strength, to the detriment of chromatographic selectivity and resolution. For the following experiences, a NaCl concentration of 30 m*M* has been chosen, owing to the good chromatographic separation and to the good detector response obtained for the analytes.

3.4. Effect of pH

The requirement to buffer the eluent in IIC comes from the need to have the analytes in a dissociated chemical form suitable for separation. In our study the ionization of ligands is complete at the pH values of the working conditions of a silica based column. Anyway, eluent pH is a crucial parameter for the complexing ability of the ligands. For this reason a detailed study of the effect of the eluent pH both on the detection of the ligands and of their metal complexes has been performed.

Experiments were done at different pH values: 3.8 by formate buffer, 4.7 and 5.5 by acetate buffer and 7.2 by hydrogenphosphate buffer, at constant ionic strength (0.03 *M*). Although we worked at constant ionic strength, it was not possible to keep the same conductivity of the mobile phase, using different buffering salts. The shape of the i/E curves obtained

under these experimental conditions is common to that previously obtained, with SPADNS oxidised at potential values higher than the other ligands. The response of SPADNS proves almost unaffected by the increase of pH up to 5.5, at every potential value investigated. In fact, beyond this value, a decrease of the signal is observed. The response of PCB is little influenced by pH changes at every potential value, but at 900 mV a maximum of signal was observed for pH 4.7. The detectability of AVN, instead, is strongly dependent on mobile phase pH; in fact, AVN signal showed a minimum at intermediate pH conditions and a maxima at extreme pH values. It should be observed that its pK_{1AVN} value is 7.0 [28] and therefore at pH 7.2 the chemical form of this ligand is changing and it results in a different detection sensitivity.

The study of the effect of pH showed that intermediate pH values (4–5) represent the best values for the detection of SPADNS and PCB; in this range the background current reaches a maximum, while formate and hydrogenphosphate provide low background.

The whole study performed allowed us to choose the best eluent conditions for the detectability of the ligands considered. The optimised values of the parameters investigated are shown in Table 1.

3.5. Separation of metal complexes

On the basis of the experiments performed, the effect of pH on metal complexes separation and detection was studied using a methanol–water (60:40, v/v) eluent containing 5 m*M* TBA, 30 m*M* NaCl and 20 m*M* of buffer solutions, at the pH values previously investigated, applying a potential of 800 mV. Attention was focused on Cu^{2+} and Fe^{3+} metal ions. According to experiments performed in our laboratory [29,30], it was not possible to detect the complexes of different metal ions (Cu^{2+}, Ni^{2+}, Fe^{3+}, Al^{3+}, Zn^{2+}, Co^{2+}) with SPADNS. In fact, at every mobile phase composition studied the injection of a precomplexed metal ion gave only one chromatographic peak at the same retention time of the ligand.

For these reasons, results obtained with the complexes of metal ions with AVN and PCB will only be shown. At each eluent pH value, 300 μg/l Fe^{3+}, and 300 μg/l Cu^{2+} in 0.2 m*M* of ligand solutions were injected (10:1 and 15:1 ligand to metal ion ratio, that assures the complex formation). Not all the mobile phases investigated gave satisfactory results, in fact at pH 3.8 the peak of Fe^{3+} is not completely resolved from the excess of both ligands, making difficult an accurate quantitative determination. Using the eluent buffered at 7.2 it was impossible to detect neither Cu^{2+}, nor Fe^{3+} as AVN complexes. The Cu^{2+} complex coeluted with the ligand, while AVN ($\log K_{FeAVN^-}$ not available from literature) competes with hydroxide ($\log K_{Fe(OH)^{2+}} = 22$ [31]) and phosphate ($\log K_{(FeHPO_4)^{2+}} = 8.3$ [31]) ions for the complexation of Fe^{3+} ion. At pH values between 4.8 and 5.5, the complexes of both metal ions with the two ligands are completely resolved and easily detectable.

3.6. Detection limits

The detection limits for Cu^{2+} and Fe^{3+} complexes with both PCB and AVN, expressed as three-times the background standard deviation were evaluated and the results obtained are shown in Table 2. The

Table 1
Optimised experimental conditions for the detection of SPADNS, PCB and AVN

Parameter	Optimal range
TBA concentration	5–10 m*M*[a]
% Methanol	50–60%
NaCl concentration	25–50 m*M*[b]
pH	4.5–5.5
E applied	800 mV

[a] As low as possible.
[b] As high as possible.

Table 2
Detection limits of Cu^{2+} and Fe^{3+}, complexed by PCB and AVN

Element	Detection limit (μg/l)	
	PCB[a]	AVN[b]
Cu	2.93	1.5
Fe	2.62	0.84

[a] $E_{appl} = 800$ mV, mobile phase: methanol–water (55:45, v/v) 5 m*M* TBA, 30 m*M* NaCl, 20 m*M* acetate, pH 4.8.
[b] $E_{appl} = 800$ mV, mobile phase: methanol–water (60:40, v/v) 5 m*M* TBA, 45 m*M* NaCl, 20 m*M* acetate, pH 4.8.

low ppb levels reached for these metal ions make the chromatographic separation coupled to electrochemical detection competitive with other techniques such as inductively coupled plasma atomic emission spectrometry.

3.7. Real samples

The method developed has been applied to the determination of Cu^{2+} in municipal drinking water. To the sample was added 20 m*M* acetic buffer (pH 4.7) and 0.1 m*M* PCB. The chromatogram obtained is shown in Fig. 5. Cu^{2+} concentration determined by the standard addition method was 386±9 μg/l (r^2=0.977). The analysis of the sample has also been performed with inductively coupled plasma atomic emission spectrometry. The result obtained, 417 μg/l, indicates the reliability and the accuracy of the quantitative analysis with the developed procedure.

The possibility to evaluate the presence of other metal ions in a water matrix has been verified with the analysis of a sample of Viverone Lake to which was added 200 μg/l Cu^{2+} and Ni^{2+}, 300 μg/l V^{4+}, Co^{2+} and Fe^{3+}. Fig. 6 shows a good separation of the metal ions and the applicability of the electrochemical detection method, without severe matrix interference.

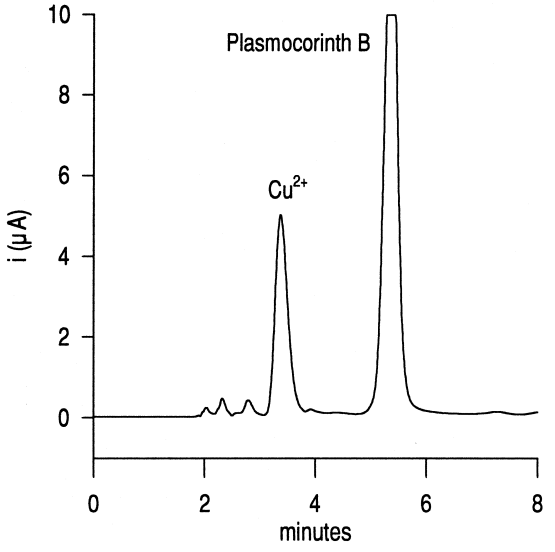

Fig. 5. Analysis of a municipal tap water sample. Mobile phase: water–methanol (45:55, v/v), 20 m*M* acetic acid, 5 m*M* TBA, 30 m*M* NaCl, aqueous pH 5.5. Oxidation potential 800 mV.

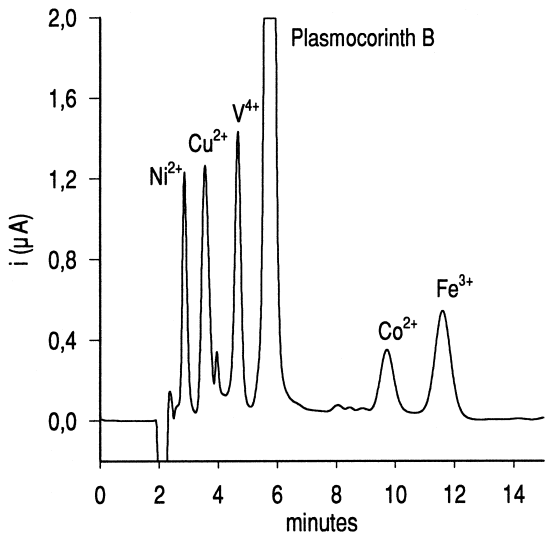

Fig. 6. Analysis of a sample from Viverone Lake water added to 0.1 m*M* PCB, 200 μg/l Cu^{2+} and Ni^{2+}, 300 μg/l V^{4+}, Co^{2+} and Fe^{3+}. Mobile phase: water–methanol (45:55, v/v), 20 m*M* acetic acid, 5 m*M* TBA, 30 m*M* NaCl, aqueous pH 4.7. Oxidation potential 800 mV.

4. Conclusions

In this work the electrochemical detection of three sulphonated azoligands (SPADNS, AVN, PCB) has been optimised as a function of the main parameters that control the retention and the efficiency of an ion interaction chromatographic separation. The study pointed out that the presence of IIR proportionally decreases the detector response for the analytes. The organic modifier (methanol) does not significantly influence the sensitivity which nevertheless increases between 50 and 60% methanol. Anyway it is important for keeping a good elution power of the mobile phase and good peak shape. The conductivity of the mobile phase must be maintained as high as possible keeping in mind the separation requirements: high counter-ion concentrations must be preferred. The best pH conditions are different for each ligand studied, but a compromising pH ranges from 4.5 to 5.5. Considering both the presence and the behaviour of the background signal, the optimal

potential value for the detection of the ligands is 800 mV.

The optimised experimental conditions have been verified for the determination of metal ions in water samples. The high separation and resolution power of the ion interaction chromatographic method, together with the good detection limits reached with the electrochemical detection (low ppb levels) make the developed method efficient and competitive towards other analytical methods such as spectroscopy techniques.

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