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Journal of Chromatography A, 696 (1995) 227–234

JOURNAL OF CHROMATOGRAPHY A

Limitations of ion chromatography with post-column reaction for determination of heavy metals in waters containing strong chelating agents

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First received 20 July 1944; revised manuscript received 15 December 1994; accepted 23 December 1994

Abstract

A published method for the determination of heavy metals was applied to the determination of Cu(II), Ni(II), Zn(II) and Mn(II) in synthetic solutions that contained one of the following ligands (L): citrate, nitrilotriacetate (NTA), ethylenediaminetetraacetate acid (EDTA) and cyclohexylenediaminetetraacetate (CDTA). The method involves on-column derivatization with 2,6-pyridinedicarboxylic acid (PDCA) and ion-exchange separation, followed by post-column reaction with 4-(2-pyridylazo)resorcinol (PAR) to form metal–PAR chelates, which can be sensitively monitored by spectrophotometric detection at 520 nm. Solutions with 12.6 μmol/l of Cu(II), Ni(II), Zn(II) and Mn(II) and 12.6 or 25.2 μmol/l of L were analysed. A 100% recovery was obtained for all metals with citrate or NTA, for Cu(II), Zn(II) and Mn(II) with EDTA and only for Mn(II) with CDTA. The recoveries in further cases were Ni(II)–EDTA $\leq$68%, Ni–CDTA $\leq$80%, Cu(II)–CDTA $\leq$20% and Zn(II)–CDTA $\leq$87%. To interpret these results, simpler solutions with 12.6 or 6.3 μmol/l Cu(II) and Cu:L ratios (R) in the range 4.2–0.26 were analysed. For both citrate and NTA an almost 100% recovery of Cu(II) was found for all values of R. For EDTA, 100% recovery was only observed for $R \geq 1.0$. For CDTA the recoveries were between 76% ($R = 4.2$) and 0% ($R \leq 0.52$). Speciation calculations showed that only kinetic factors were responsible for the inefficiency of both the on-column and the post-column derivatizations. Analytical implications of the results are discussed.

1. Introduction

One of the most satisfactory methods for the determination of transition metals by high-performance liquid chromatography (HPLC) involves a cation-exchange (or a mixed cation- and anion-exchange) separation followed by post-column complexation with 4-(2-pyridylazo)resorcinol (PAR), to form chelates which can be sensitively monitored by spectrophotometric detection [1]. To facilitate transition metal separation without the need for concentrated eluent solutions, complexing agents are also included in the mobile phase to provide on-column (or in situ) derivatization. 2,6-Pyridinedicarboxylic acid (PDCA) is one of the most commonly used reagents for this purpose.

When a sample solution which contains metal ions in free (hydrated) form is injected into a flow of eluent which contains PDCA, metal

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0021-9673/95/$09.50
SSDI 0021-9673(94)01295-4

complexes are formed (electric charges were omitted for the sake of simplicity):

$$M + PDCA \rightleftharpoons M\text{–}PDCA \quad (1)$$

Anion exchange in the column, conditioned by the relative affinity of the complexes to the resin, is responsible by the separation of the different complexes:

$$M\text{–}PDCA + resin\text{–}PDCA \rightleftharpoons resin\text{–}(M\text{–}PDCA) + PDCA \quad (2)$$

After elution, the complexes with PDCA are converted into coloured complexes in the post-column reaction system, by reaction with PAR, and detected by spectrophotometry at 520 nm:

$$M\text{–}PDCA + PAR \rightleftharpoons M\text{–}PAR + PDCA \quad (3)$$

The suitability of this method depends on the following chemical factors: (i) high thermodynamic stability of the complexes formed in reactions 1 and 3, and (ii) rapid kinetics of ligand substitution in both reactions. PDCA and PAR, which are used in very large excess relative to the metal, satisfy these requirements.

One of the main fields of application of this ion chromatographic (IC) method is the determination of heavy metals in natural or residual waters. In the case of industrial effluents, the waters may contain aminopolycarboxylic acids, e.g., nitrilotriacetic acid (NTA) and ethylenediaminetetraacetic acid (EDTA), which are widely used in industrial processes and as additives in detergents [2–4]. These chelating agents produce complexes of high thermodynamic stability and low rate of dissociation. However, little attention has been paid to establishing whether strong chelating agents such as these in the sample interfere in the IC method.

The purpose of this work was to evaluate the interference of citrate (Cit), nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA) and cyclohexylenediaminetetraacetic acid (CDTA) in a procedure recommended by Dionex to be performed with their equipment [5], which involves on-column (with PDCA) and post-column (with PAR) derivatizations. These ligands (L) were selected because they form complexes with very diverse stability constants. First, the method was applied to a mixture of Cu(II), Ni(II), Zn(II) and Mn(II) and severe interferences of EDTA and CDTA were found. As the interpretation of the results was very difficult, owing to the complexity of the chemical system, a more detailed study was performed only for copper(II), selected because in the Irving–Williams series it is the metal that forms the most stable complexes.

The efficiencies of (i) the overall procedure, e.g., on-column plus post-column reactions, and (ii) of only the post-column derivatization were studied. For the latter purpose, the set of separation columns were replaced by a simple tube which transports quickly and without separation the sample from the injection loop to the post-column reaction system. The interpretation of the experimental results is based on both thermodynamic and kinetic considerations. For this purpose, speciation calculations based on stoichiometric stability constants from the literature were performed for the systems Cu–PDCA–L or Cu–PAR–L in the ranges of concentrations used in the study. Further, kinetic measurements were carried out to estimate the rate of dissociation of the Cu–L complexes in the chromatographic system. Some important consequences of the results are discussed.

2. Experimental

2.1. Reagents, solutions and apparatus

All reagents were of analytical-reagent grade. All solutions were prepared with deionized water with conductivity <0.1 μS/cm. Standard solutions of metallic cations were in the nitrate form. The compositions of the buffer solutions used for the several types of experiments are given in Table 1.

A DX 300 IC system from Dionex was used. The system included a 50-μl loop, an IonPac CG5 guard column (50×4 mm I.D.), an IonPac CS5 analytical column (250×4 mm I.D.) and a post-column reaction (PCR) system composed of a DQP-1 isocratic peristaltic pump and a mem-

Table 1
Buffer solutions

Buffer	Composition	pH
T1	5 mmol/l NaOAc–5 mmol/l HOAc	4.5
T2	6 mmol/l PDCA–50 mmol/l NaOAc–50 mmol/l HOAc	4.4
R1	0.2 mmol/l PAR–3 mol/l NH_3–1 mol/l HOAc	10.4
R2	0.2 mmol/l PAR–50 mmol/l NaOAc–50 mmol/l HOAc	4.6

brane reactor (MR). After the membrane reactor, a reaction coil of length 234 mm was used. A Model 204 UV–Vis detector from Konik with a 9-μl cell was used.

2.2. Separation and detection

The following two IC configurations were used: IC(A), Dionex columns for separation and T2 buffer solution (Table 1) as mobile phase; IC(B), similar to IC(A) but the columns were replaced with a 450 mm × 0.25 mm I.D. polyether ether ketone tube. Four standard solutions with metal concentrations between 3 and 16 μmol/l prepared in buffer T1 were used for calibration.

2.3. Kinetics

To determine the rate constant of the Cu–L dissociation (see discussion below), 500 μl of a pre-equilibrated 1:1 Cu–L solution which was 400 μmol/l in copper(II) were mixed with stirring with 20 ml of buffer R1 (Table 1) to attain a final solution with [Cu–L]/[PAR] ratios $\geq$1:20. This solution was immediately ($t = 0$ s) injected into the spectrophotometer cell with stopped flow, and the rate of appearance of Cu–PAR at 508 nm (absorbance maximum of that complex) was measured with time (t) at constant ln t intervals. The data were collected in a PC and stored to be processed later. For the calculation of the rate constant of the reaction under study, the absorbance of the $Cu(PAR)_2$ after each period of time (A_t) was subtracted from the final absorbance (A_∞). The experiments were repeated for solutions in which buffer R1 had been replaced with buffer R2 (Table 1) to study the influence of both the pH and solution composition on the kinetic behaviour.

3. Results and discussion

3.1. Heavy metal determination

A widely used procedure [5–7] [IC(A) configuration] was applied to determine total heavy metal concentrations in synthetic solutions with and without L at pH 4.5. Results were obtained for solution containing a pre-equilibrated mixture of 12.6 μmol/l each of Cu(II), Ni(II), Zn(II) and Mn(II) and 12.6 or 25.2 μmol/l of L (L = CDTA, EDTA, NTA, Cit). For Cit and NTA, 100% recoveries were found for all the metals at both L concentrations. However, as Fig. 1 shows, for EDTA low recoveries were observed for Ni(II) viz., 68% and 36% for 12.6 and 25.2 μmol/l, respectively. For CDTA, low recoveries occurred for Cu(II) (19% and 0%, respectively), Ni(II) (83% and 68%) and Zn(II) (87% and 51%), but not for Mn(II). The complexity of the sample, where competition of several chemical equilibria takes place, rendered the interpretation of the results very difficult.

Therefore, simpler solutions containing copper(II) only and Cu:L ratios (R) between 4.2 and 0.26 were used as synthetic samples for a more detailed study. A single chromatographic peak for a retention time (t_r) of 7.86 ± 0.02 min ($n = 58$, $P = 0.05$) was observed for both "samples" and standards, which indicates that the peak corresponds to the metal that was retained in the separation column (hydrated form plus labile complex on the time-scale of the chromatographic separation). The recoveries of cop-

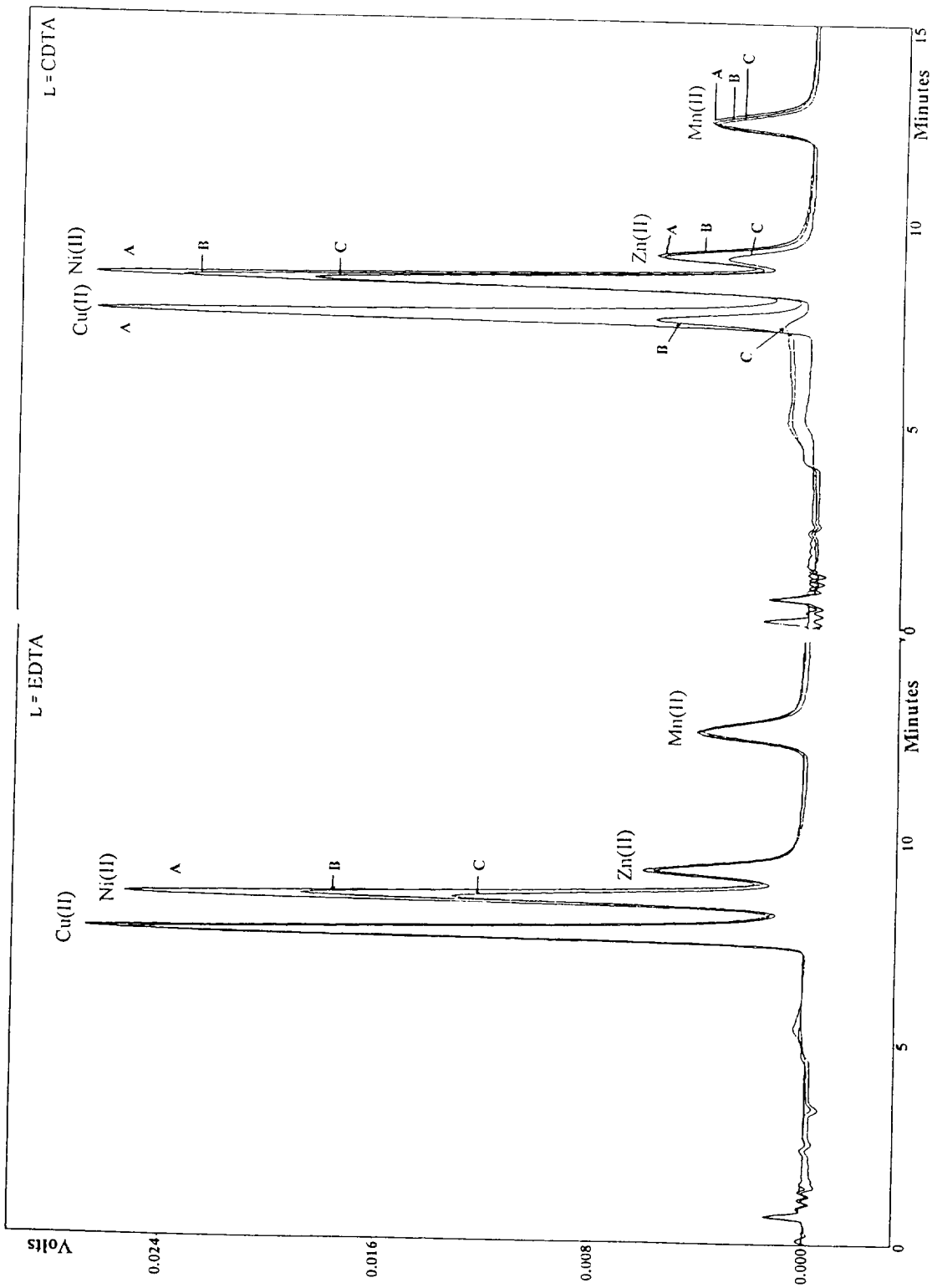

Fig. 1. Overlapping of typical chromatograms obtained with the IC(A) configuration for a mixture of 12.6 μmol/l of heavy metals (A) without and with (B) 12.6 μmol/l and (C) 25.2 μmol/l EDTA (left) or CDTA (right), prepared in buffer T1 (pH 4.5). Eluent, 6 mmol/l PDCA–50 mmol/l sodium acetate–50 mmol/l acetic acid; flow-rate of eluent, 1 ml/min; separation column, IonPac CG5 + IonPac CS5; post-column reagent, 0.2 mmol/l PAR–3 mol/l NH_3–1 mol/l acetic acid; flow-rate of post-column reagent, 0.71 ml/min; loop volume, 50 μl.

Table 2
Copper recovery (%) obtained with procedure IC(A)

Cu(II)[a]:L	CDTA		EDTA		NTA		Cit
	T1	T2	T1	T2	T1	T2	T1
4.20	74.6 ± 5.2		100.3 ± 1.1		104.5 ± 1.1		99.7 ± 5.7
2.10	71.4 ± 2.0		100.5 ± 1.1		103.7 ± 1.1		101.9 ± 10.1
1.05	12.7 ± 2.0		100.3 ± 5.0		99.7 ± 8.0		102.9 ± 9.9
0.53	ND[b]		92.1 ± 5.2		99.7 ± 8.2		102.4 ± 9.9
0.26	ND	99.9 ± 0.75	89.7 ± 5.2	101.4 ± 3.8	102.4 ± 12.0	101.1 ± 1.2	100.0 ± 10.4

Mean values and confidence limits ($P = 0.05$, $n = 3$) are given.
[a] [Cu(II)] = 12.6 μmol/l.
[b] ND = Not detected.

per(II) are presented in Table 2, which shows that no inferences of Cit or NTA were found even when the concentration of any of these was about four times higher than that of the metal. Interference of EDTA was observed when $R \leq 0.5$, and marked interference of CDTA was found even with a ligand deficiency: for instance, for $R = 4.2$, the copper(II) recovery was 76% and total suppression of the chromatographic peak occurred for $R < 0.5$. Similar results were obtained for 6.3 μmol/l Cu(II) and an identical R range.

These results are a consequence of the fact that when the analyte is in the form of a very stable complex in the "sample", the reaction which takes place on-column is not that in Eq. (1) but

$$M\text{–}L + PDCA \rightleftharpoons M\text{–}PDCA + L \quad (4)$$

The extension of the ligand substitution on the time-scale of the chromatographic procedure depends on the thermodynamic and kinetic factors.

Speciation calculations (performed by the program SPECIATE, Microsoft Basic V7 version, developed according the COMPLEX [8] algorithm program) based on stoichiometric stability constants from the literature [9–11] were performed for the different chemical Cu–PDCA–L systems (and also for the other metals used in this study) as pH 4.5 for the ranges of concentrations used. For all types of L and all values of R, the Cu–PDCA complexes predominate under equilibrium conditions. These results show that the low copper(II) recoveries observed were mainly due to kinetic factors. Indeed, when PDCA was added to the sample before the analysis, e.g., when buffer T1 was replaced with buffer T2 (see Table 1), the copper(II) recovery became virtually 100% in all cases (Table 2). Instead of the batch inclusion of PDCA in the sample, on-line precolumn derivatization with PDCA (in a coil of length 234 mm) was also carried out, but although the efficiency increased, low Cu(II) recoveries were still found: for instance, for $R = 0.26$, the recovery was only 91.01 ± 0.91% (confidence limits, $P = 0.05$, $n = 3$) for EDTA and 1.63 ± 0.25% for CDTA. Longer precolumn reaction coils were not used, because they induce dispersion of the analyte in the eluent and broadening of the peaks, which reduces the efficiency of the process.

3.2. Performance of the detection system

The occurrence of a single chromatographic peak, which appeared for a t_r identical with that for the standard solutions [all the copper(II) in the hydrated form], indicate that the copper(II) fraction in the Cu–L (L = EDTA or CDTA) form not substituted on-column was not detected. Therefore, the replacement of L by PAR in the post-column reactor:

$$Cu\text{–}L + PAR \rightleftharpoons Cu\text{–}PAR + L \quad (5)$$

also did not occur, again for kinetic reasons. Indeed, speciation calculations [8] similar to those mentioned above for Cu–PAR–L systems showed that virtually 100% of the copper was in the Cu–PAR form when equilibrium conditions were attained.

The efficiency of the post-column reaction system to provide complete conversion of Cu–L to Cu–PAR (Eq. 5) was also evaluated separately. For this purpose, the separation column was replaced with a simple tube [IC(B) configuration] to ensure that all the copper(II) from the sample (independently of its chemical form) reached the detection system at virtually the same time. Experiments were performed for Cu–EDTA solutions with R between 4.12 and 0.26. It was found that when EDTA was in excess relative to the metal, the recovery of copper(II) was much lower than 100% (78.5% for $R = 0.52$ and 65.4% for $R = 0.26$), corresponding to incomplete replacement of EDTA by PAR.

3.3. Kinetic aspects

Another set of experiments was carried out to obtain information about the rate of dissociation of the different Cu–L complexes. Cu–L solutions (1:1) (e.g., with $R = 1$) were mixed with a solution of PAR, with a large excess relative to L ([PAR] = 20[L]), and the absorbance of Cu–PAR in the mixture was measured with time (experimental details as above). If pseudo-first-order kinetics relative to Cu–L are assumed, the rate of formation of the Cu–PAR complex, d[Cu–PAR]/dt, is given by

$$\frac{\mathrm{d[Cu\text{–}PAR]}}{\mathrm{d}t} = {}^{*}k_{\mathrm{d}}[\mathrm{Cu\text{–}L}] \qquad (6)$$

where ${}^{*}k_{\mathrm{d}}$ is the pseudo-first-order rate constant of reaction 5.

The formation of the Cu–PAR complex is almost instantaneous [12], hence it can be assumed that the rate-determining step of reaction 5 is the dissociation of the Cu–L complex [12,13]:

$$\mathrm{Cu - L} \xrightarrow{k_{-1}} \mathrm{Cu + L} \qquad (7)$$

Therefore, if PAR is in large excess, the determination of the rate constant of reaction 5, ${}^{*}k_{\mathrm{d}}$, will provide an estimate of the rate constant of reaction 7, k_{-1}.

Kinetic experiments were performed at two pH values: 10.4 (buffer solution R1), and 4.6 (buffer solution R2). The pH of 10.4 was that used in the post-column reagent in the chromatographic determinations. However, as the mobile phase in procedure IC(A) had a pH of 4.4 (buffer T2), measurements at a similar pH (buffer R_2, pH 4.6) could provide an estimate of the on-column (Eq. 4) dissociation rate of Cu–L.

The rate constant of reaction 5 could not be determined for Cu–NTA and Cu–Cit because the reactions were too fast to allow the measurement of A_t with the rudimentary device used in this work. In fact, A_∞ (e.g., for stabilized signal) had already been attained when the solution arrived at the detector cell. Therefore, it was concluded that ${}^{*}k_{-1} > 0.2\ \mathrm{s}^{-1}$ (calculated assuming that the equilibrium position was attained for $t = 30$ s). For Cu–EDTA at pH 10.4, although slower than for the Cu–NTA and Cu–Cit complexes, reaction 5 was still too fast to allow accurate results. However, for Cu–EDTA at pH 4.6 and especially for Cu–CDTA at both pH values, the reaction was sufficiently slow for measurement.

Fig. 2 illustrates the results obtained for Cu–CDTA (for Cu–EDTA similar behaviour was observed). The function $\ln(A_\infty - A_t) = f(t)$ was almost linear (correlation coefficient of the adjustment by least squares >0.99), which suggests that pseudo-first-order kinetics describe reaction 5 and, therefore, reaction 7. From the slopes, k_{-1} values were calculated (Table 3). The k_{-1} values are lower for Cu–CDTA than for Cu–EDTA at both pH values, and are compatible with the results obtained by the IC(A) procedure. The rigid and voluminous cage structure of CDTA, which causes steric hindrance, prevents direct attack of the metallic centre of Cu–CDTA by PAR.

Table 3 also shows that ${}^{*}k_{\mathrm{d}}$ is about one order of magnitude higher at pH 10.4 than at pH 4.6 for Cu–EDTA, whereas for Cu–CDTA ${}^{*}k_{\mathrm{d}}$ is similar at both pH values. This is probably due to the presence of 3 mol/l of ammonia in the R2 buffer solution (pH 10.4). Reactions with am-

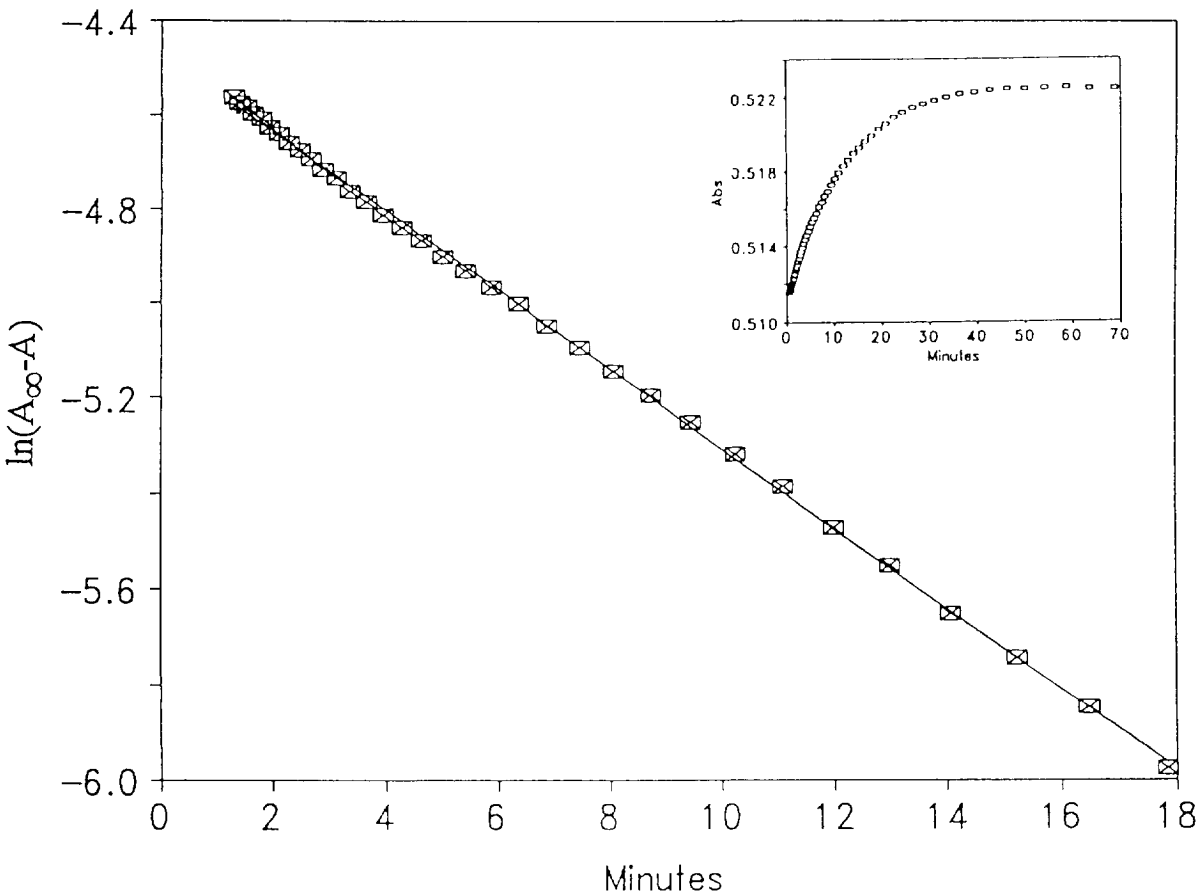

Fig. 2. Kinetic curves for the reaction of replacement of CDTA by PAR (Eq. 5) obtained for [Cu] = 9.8 μmol/l, [Cu]:[CDTA]:[PAR] = 1:1:20 and pH 4.6 (buffer R2). Regression parameters: $\ln(A_\infty - A_t) = -1.40 \cdot 10^{-3}\ (\pm 1 \cdot 10^{-5})t - 4.47\ (\pm 5 \cdot 10^{-3})$, $R = 0.9998$, $n = 34$ (confidence limits, $P = 0.05$ are given).

monia (and polyamines) are much faster for Cu–EDTA than for Cu–CDTA [14]. Ammonia can complex with copper without displacing the EDTA or the CDTA, and the mixed complexes formed [14] are probably intermediate species in reaction 5. The more basic nitrogen atoms and cage-like structure of CDTA contribute to a smaller rate constant, because both stabilize the Cu–CDTA complex but not the transition state [14].

Table 3
Pseudo-first-order rate constants [$^*k_1$ (s^{-1})] calculated for ligand substitution reaction (Eq. 5)

Ligand	pH	
	4.6	10.4
EDTA	$(6.1 \pm 5.1) \cdot 10^{-1}$ ($n = 3$)	>0.2
CDTA	$(1.40 \pm 0.13) \cdot 10^{-3}$ ($n = 4$)	$(1.29 \pm 0.49) \cdot 10^{-3}$ ($n = 4$)

Mean values and confidence limits ($P = 0.05$) are given.

4. Conclusions

IC with on-column derivatization with PDCA followed by post-column reaction with PAR and spectrophotometric detection [5–7] was applied to heavy metal determination. It was found that in samples which contain very stable (both

thermodynamically and kinetically) complexes, interferences in the determination of the metals may occur. For instance, for Cu(II), an EDTA interference was observed when this was in excess relative to the metal, and for CDTA even when it was deficient. For Ni(II), severe EDTA interference was found even with a ligand deficiency. The low metal recoveries are mainly due to kinetic factors, which prevent complete replacement of L by PDCA in the mobile phase or by PAR at the post-column reactor. Low metal recoveries are also expected for other metals not included in this work, e.g., Co(III) (d^3) and Cr(III) (diamagnetic d^6), due to the large crystal field activation energies for the substitution reactions [15]. Therefore, when strong chelating agents are suspected to be present in the sample, a batch derivatization with PDCA should be performed before the analysis. Alternatively, digestion of the sample (e.g., in acid or using UV irradiation) to destroy the ligand can be used.

However, these procedures are time consuming and not easily compatible with, for instance, automated on-line analysis, which are frequently used in industrial plant operation and control [16]. Even with more acuity, these procedures preclude the use of a concentration column for collecting a ppb-level sample in the field and returning it to the laboratory for analysis [17]. In such cases the development of instrumentation and procedures for on-line metal collection and/or the determination of heavy metals free of interferences is required. The results in this paper may be helpful for this purpose.

Acknowledgements

The authors thank JNICT(Lisbon)–PROGRAMA CIÊNCIA, project M-27/9/20, for financial support for acquisition of the Dionex ion chromatograph and the Konik detector. Professor A.A.S.C. Machado (Chemistry Department, Faculty of Science, University of Oporto, Oporto, Portugal) is thanked for helpful discussions.

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