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Determination of metal ions by on-line complexation and ion-pair chromatography

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ABSTRACT

An ion-pair chromatographic method utilizing on-line complexation and ion-pair formation in a post-column reactor was developed for the determination of copper, palladium, cobalt and iron in mixtures. The system features a reversed-phase column and a second eluent line feeding the ligand reagent, connected after the column via a T-piece, to a mixer and through that to a knitted tubing reactor. The ion-pair former was added to the eluent before the column and the ligand after it. The separation was studied using a binary eluent system containing cetyltrimethylammonium bromide (CTMABr) or tetradecyltrimethylammonium bromide (TDTMABr) in water–methanol (99:1, v/v) as ion-pair former and methanol. In addition, water–methanol (99:1, v/v) containing 1-nitroso-2-naphthol-6-sulphonate (126NNS) as ligand was added to the eluent, through the T-piece, after the column. Mixing of the two eluents took place in the mixer. Methanol was used both isocratically and in gradient addition. Selective UV–VIS detection of the metal–126NNS ion pairs was at wavelengths 230, 260, 310 and 400 nm and their identification was effected in wavelength range 190–600 nm. The metal complex formation in the aqueous methanol eluent evidently governed the retention of the ion pairs, while the selectivity of the method was provided by the different rates of reaction of the metal, the ligand and the ion-pair former in the mixer–reactor system. The detector response for copper, palladium, cobalt and iron was linear up to concentrations of 10 μM. In spiked water–methanol samples the detection limits for these metals ranged from $1 \cdot 10^{-3}$ to 1 mg/l. When the on-line complexation and ion-pair formation method was tested with nickel, mercury and zinc, the results proved that these ion pairs were unstable. Because of the insufficient reproducibility of the absorption intensities of these metal ion pairs, their qualitative study could be performed only in the pH range 7–8. The method was successfully applied to real samples after removal of the organic material.

INTRODUCTION

A number of papers have been published on the use of ion-pair chromatography (IPC) for the separation and determination of metals [1–8]. Because metals in a complex matrix are difficult to detect directly in low concentrations, on the basis of their UV–VIS absorption, pre- and post-column derivatization with an absorbing ligand is often applied as an effective approach to the problem [4].

The mechanism by which metal complex ion pairs are separated in ion-pair chromatography is complicated owing to both metal complex and ion-pair formation. Separation depends on the extraction constants of the ion pairs (K_{ex}), on the ratio of the solid phase and the eluent in the column (V_s/V_m) and, via pH, on the dissociation constant of the ligands (pK_a), the ionic strength of the eluent and temperature.

Column application of strong ligands, such as those containing nitrogen and sulphur atoms in the molecule, offers new possibilities for metal analysis. If the ligand is added after the column, metal complex stability is enhanced and ion-exchange reactions and insolubility effects are minimized. By optimizing separation and detection independently, greater freedom and effectiveness can be obtained in analytical methods [4,6].

Derivatization on-line within the system has a number of advantages over off-line derivatization, but steps need to be taken to avoid broadening in the on-line reactor. There have been several discussions in the literature on the theoretical basis for minimizing band broadening, such as when open-tubular

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reactors are geometrically deformed by helical coiling of the tube to give minimum retention of the components [9,10]. A knitted reactor has the added advantage that peak tailing is minimized.

In this study, we introduced an on-line derivatization method for metal ions, in which the metals are injected into an eluent containing a quaternary ammonium bromide in methanol–water solution and after the column are further combined with a ligand solution. The metals are separated as their ion pairs, consisting of metal–1-nitroso-2-naphthol-6-sulphonate complexes combined under carefully controlled elution conditions with an organic ammonium compound.

The method was developed using mixtures of Cu(II), Pd(II), Co(II) and Fe(II) ions and tested using mixtures of Cu(II), Co(II), Fe(II), Ni(II), Hg(II) and Zn(II) ions. The tests were performed with two real samples, namely nutrient sticks and health drinks containing several of the metal ions. The ligand used for the complex formation, 1-nitroso-2-naphthol-6-sulphonate (126NNS) (as the sodium salt), is UV–VIS absorbing and the ion-pair former, either cetyltrimethylammonium bromide (CTMABr) or tetradecyltrimethylammonium bromide (TDTMABr), is UV absorbing.

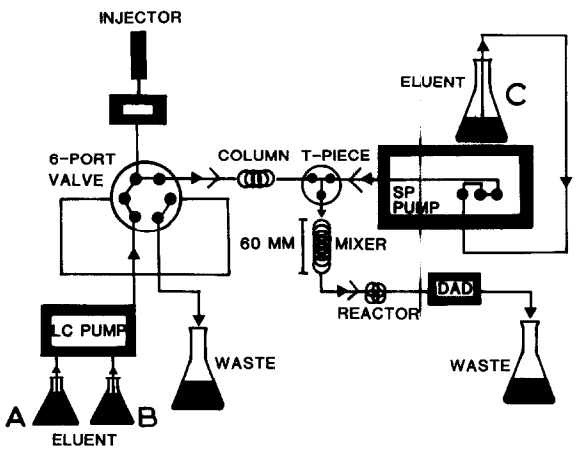

Fig. 1. Schematic diagram of the on-line derivatization technique. Eluent system: (A) ion-pair former solution, (B) methanol and (C) ligand solution. Components: LC pump (HP1090), SP pump (Spectra-Physics), six-port switching valve, column, mixer tube filled with acid-washed glass-wool, reactor (PTFE tubing, 3 or 5 m) and a diode-array detector (DAD).

EXPERIMENTAL

Apparatus

A Hewlett-Packard Model 1090 liquid chroma tograph fitted with an HP 1040A diode-array de tector was used together with an HP 85B microcom puter. An HP 3392A integrator was employed fo data storage and reporting. A PTFE (Habia PTF tubing, transparent, 300 or 500 mm × 0.3 mm I.D. × 0.86 mm O.D.) laboratory designed reacto was maintained at 25°C in the column oven. Th mixer, a steel tube (30 mm × 4.6 mm I.D. was laboratory made and filled with glass-woo (Supelco, acid washed). An SP8810 precision iso cratic pump (Spectra-Physics) was used on-lin for the ligand flow. The columns were an H LiChrosorb RP-18 (30 mm × 3 mm I.D., film thickness 5 μm, N = 38 100 m^{-1}) and an Asahipa ODP-50 (150 mm × 4.6 mm I.D., film thickness μm, N = 16 800 m^{-1}) (Fig. 1).

Standard metal solutions

Stock solutions were prepared by dissolving cop per(II) perchlorate (G. F. Smith, Quality 1) an palladium (II), iron(II), cobalt(II), zinc(II), chrom um(II), nickel(II), mercury(II), magnesium(II) an (II) chloride (Merck, analytical-reagent grade) i distilled, demineralized water. The samples wer filtered before use.

Ligand solution

The sodium salt of 1,2-naphthaquinone-6-sulpho nate was synthesized as described in the literatur [11]. A stock solution was prepared in water methanol (99:1, v/v) and diluted before use.

Mobile phases in HPLC

The eluents were (A) 0.008 mM CTMABr in wa ter–methanol (99:1, v/v) or 0.008 mM TDTMABr i water–methanol (99:1, v/v) [4], (B) methanol and (C 0.165 mM sodium 126NNS in water–methano (99:1, v/v) containing 0.008 mM ion-pair form (CTMABr or TDTMABr) (see Fig. 1). Eluents wer bubbled with helium for 15 min and filtered throug 0.45-μm fluoromembranes (ACRO LC 13) befo use.

Chromatographic procedures

The UV–VIS diode-array detector was operate

at 230, 260, 310 and 400 nm, with a reference wavelength of 550 nm. Identifications of the metal ion pairs were made using the wavelength range 190–600 nm. The oven temperature was 25°C. The samples were manually injected using a 5-μl loop capillary. The flow-rate of the mobile phase from the first pump (LC pump) was 0.5 ml/min, and that of the ligand solution from a second pump (SP pump) was 0.3 ml/min. When the flow-rate of the mobile phase was changed to 0.7 ml/min, that of the ligand solution was increased to 0.5 ml/min.

Calculations. Chromatographic retention times were calculated as the averages of triplicate determinations. The retention time, t_0, of water–methanol (50:50, v/v) was taken as the unretained peak. The capacity factor, k', was calculated from the retention time of the solute, t_R, according to the equation $k' = (t_R - t_0)/t_0$.

Pretreatment of real samples. Health drinks (Pfrimmer, Germany), containing (per 100 g) 0.47 mg of Cu, 4.24 mg of Fe, 85 mg of Mg, 0.59 mg of Mn and 0.70 mg of Cr, and nutrient sticks (Kemira, Espoo, Finland), containing (per kg) 1 mg of Cu, 1 mg of Zn and 1 mg of Fe, were wet digested with 2 ml of concentrated perchloric acid plus 5 ml of concentrated nitric acid on a sand-bath at 195°C for 5 h. The reagent blank solutions were prepared in the same way as the samples. The samples were eluted through prewashed (with methanol and water) Sep-Pak C_{18} columns (Millipore–Waters).

RESULTS AND DISCUSSION

Retention behaviour

Preliminary studies with liquid–liquid extraction and spectrophotometric detection [12] showed that copper, palladium, cobalt and iron easily form complexes with 126NNS (pK_a 7.29) in different molar ratios depending on the pH of the aqueous solution. The metal complexes were easily extracted as ion pairs into organic solvents, but quantitative extraction was obtained only in a controlled pH range [12]. In addition, all the metal complexes were simultaneously extracted into organic solvents in the pH range 7–8.

As the ligand solution was introduced only after the column (see Fig. 1), the metals could not have been retained on the column in the form of metal complexes. The column material was coated with the ammonium compound which, like the metal ions, was in cation form. It therefore seems improbable that the metals were separated from each other during their passage through the column. They nevertheless appeared to be retained longer when polymer material was used, which suggests that the material had some anionic ability to retain them, independent of their molecular weight or ionic charge. These considerations led us to conclude that the retention and separation of the metal complex ion pairs were primarily dependent on the metal complex and ion-pair formation constants. Possibly, also, there was a thin layer of ammonium compound covering the material of the mixer and the PTFE tubing and having some retention effect.

Formation of the metal complexes was registered on the monitor: the metal complex ion pairs were detected one by one as they eluted to the diode-array detector, and their UV–VIS spectra were compared with library spectra. The purities of the absorption spectra were checked from the slopes and the apex of the spectra to confirm that the chromatographic conditions were good.

The chromatographic behaviour of the ion pairs depended essentially on the properties of the mobile phases and the formation and stability of the metal–126NNS complexes [4]. In this and our earlier studies [4,12–14], the reaction conditions for on-line formation of metal–126NNS complex ion pairs were optimized by manipulations of solvent effects, pH and ionic strength of the mobile phase mixture, and the concentrations of metal, ligand ions and the ammonium salt. Flow-rates of the eluent containing methanol and the ion-pair former (A and B in Fig. 1) and of the ligand solution containing the ion-pair former (C in Fig. 1) influenced the conditions to be optimized for the quantitative complexation and the metal complex ion-pair formation. The pH of the HPLC eluent was maintained between 7 and 8 to ensure optimum metal complex formation with 126NNS. This pH range also provided the best conditions for the formation of metal complexes in the eluent in desired molar ratios [12].

The eluent composition has a clear effect on the retention of the ion pairs (Figs. 2–5). Fig. 3 shows the capacity factors of the copper–, palladium–, cobalt– and iron–126NNS ion pairs to decrease linearly with increasing methanol concentration of the eluent. As the hydrophobicity of the ion pairs

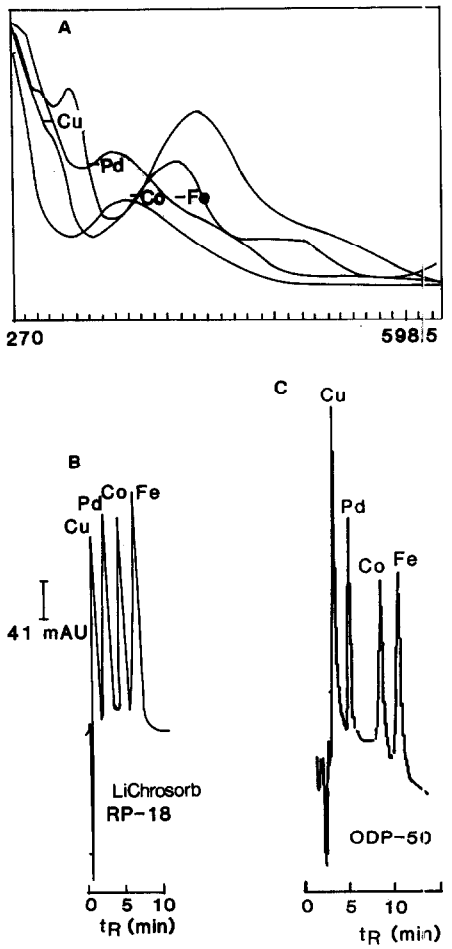

Fig. 2. Liquid chromatogram of metal ion pairs separated on ODP-50 column. (A) UV–VIS spectra of the metal ion pairs; corresponding chromatograms on (B) LiChrosorb RP-18 and (C) ODP-50. Eluent system as described under Experimental; methanol gradient, 95% (5 min) to 100% (3 min); flow-rates, (B) from LC pump 0.7 ml/min and from SP pump 0.5 ml/min; (C) from LC pump 0.5 ml/min and from SP pump 0.3 ml/min; ion-pair former, TDTMABr; length of the reactor, 5 m; detection wavelengths, 310 and 400 nm.

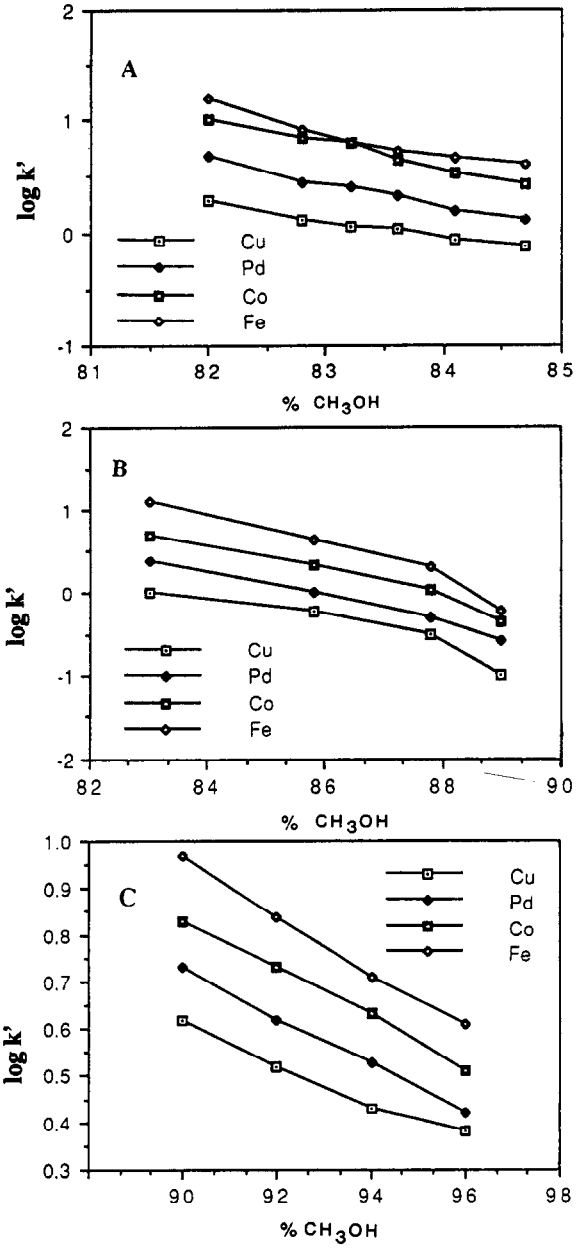

Fig. 3. Dependence of capacity factors (log k') on percentage of methanol, (A) with LiChrosorb RP-18 column; (B) without column; (C) with ODP-50 column. On-line derivatization of metal cations. Flow-rates of the eluents as in Fig. 2B. Ion-pair former, CTMABr; length of the reactor, 3 m; detection wavelength, 260 nm.

decreases from iron to copper, the solute retention increases in this order. The longer retention of the compounds with increased concentration of ion-pair former and with decrease in the methanol concentration was independent of the column material, *i.e.*, of octadecyl-substituted silica (LiChrosorb RP-18) and the polymer-based material end-capped with octadecyl groups (ODP-50). The ion pairs eluted more slowly, however, when the polymer material was used (Fig. 3), and a higher methanol concentration was needed to produce the same effect. If the ion-pair former concentration was higher than 0.200

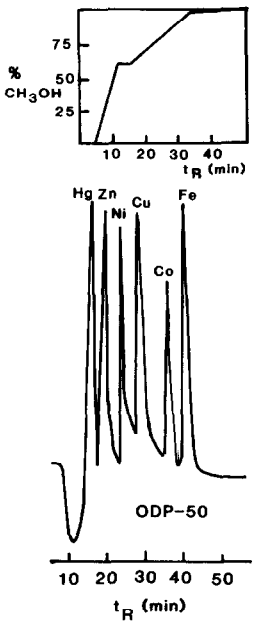

Fig. 4. Liquid chromatogram of separation of metal cations complexed with 126NNS anions and ion-paired with CTMABr. Column material, ODP-50; flow-rates of the eluents as in Fig. 2B; length of the reactor, 5 m; detection wavelength, 310 nm.

m*M*, the compounds did not elute in a reasonable time. The ligand concentration was kept as low as possible to minimize the background absorbance.

In reversed-phase ion-pair chromatography, addition of an organic ion to the system easily disturbs the chemical equilibrium in the mobile phase. This results in migrating zones of the different eluent compounds involved in the disturbance. If, for example, only one eluent component is visible to the detector, these zones will appear as negative system peaks in the visible absorption region (wavelengths below 450 nm); usually these peaks are seen in the first part of the chromatograms as in Figs. 2 and 4–6. The UV–VIS absorption of a system peak is negatively orientated on the monitor.

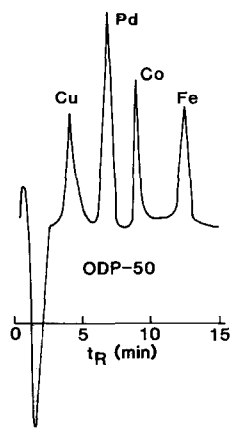

Fig. 5. Liquid chromatographic separation of metal ions as ion pairs on ODP-50 column. Gradient elution with methanol from 85% (3 min) to 95% (5 min); flow-rates, from LC pump 0.5 ml/min, from SP pump 0.3 ml/min; length of reactor, 5 m; detection wavelength, 310 nm.

Samples and technique

Conditions for the study were optimized by injecting spiked water samples into the line. The eluent flowing through the column contained only water, the ion-pair former and methanol; the ligand solution with the organic ammonium salt was continuously added to the system, through a T-piece, after the column (see Fig. 1). We assume, then, that the metal complexes, and also the ion pairs, were formed in the mixer–reactor system. The metal complexes formed after the column where the metal ions were combined with the ligand solution containing the ion-pair former. The different metal complexes, completed as ion pairs, were then separated according to their extractability [12], which was confirmed with liquid–liquid extraction studies by calculating their log K_{ex} values: Cu (13.79) > Pd (12.22) ≈ Co (12.39) > Fe (11.81).

On-line methods

As seen in Fig. 2, cobalt, copper, iron and palladium ions introduced as their salts were separated as their ion pairs by the IPC reactor system on LiChrosorb RP-18 and ODP-50 columns. It can be assumed, however, on the basis of our systematic studies and the literature [15], that the metal cations were not noticeably separated from each other in the column, although slight separation of copper and palladium was evident on the ODP column. The column worked more as a pressure regulator than a separator, providing the low flow-rates necessary for the formation of metal–126NNS complexes.

The reactor knitted from PTFE tubing provides good separation of the metal complex ion pairs, evidently because the reaction time in the mixer and the knitted reactor is long enough for metal complex formation [12] and subsequent ion-pair formation.

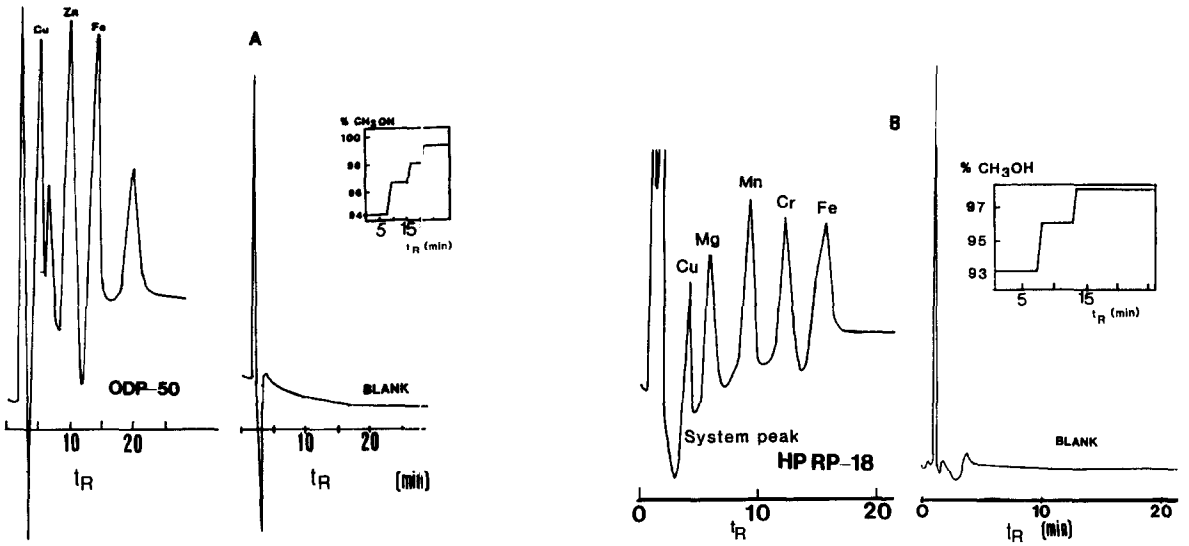

Fig. 6. Liquid chromatographic separation of metal ions by on-line derivatization technique. (A) Nutrient sticks and the reagent blank (see Experimental); column, ODP-50; flow-rates, from LC pump 0.7 ml/min and from SP pump 0.5 ml/min. (B) Health drinks and the reagent blank (see Experimental). Column, LiChrosorb RP-18; flow-rates, from LC pump 0.5 ml/min and from SP pump 0.3 ml/min; length of the reactor, 5 m; detection wavelength, 260 nm. Concentrations of metals: (A) 1 mg/l and (B) 4.7 mg/l Cu, 42 mg/l Fe, 850 mg/l Mg, 5.9 mg/l Mn and 7.0 mg/l Cr.

As can be seen in Fig. 3, the LiChrosorb RP-18 and ODP-50 columns improve the separation of the metal compounds, without effecting significant separation of the metal ions as shown by tests without a column. However, the selectivity is better with than without the column. Moreover, methanol affects the capacity factors less with the column than without it, as can be seen from the slopes of the straight lines. Although the LiChrosorb RP-18 column was good and short enough for our work, it was replaced with a five times longer ODP-50, column, which decreased the flow-rate of the mobile phase. The mixer contributes by homogenizing the eluents containing the ligand solution and the ion-pair former in water and methanol. Without the mixer the metal complex formation would be inefficient and detection of the compounds poor.

Elution

The elution order was copper, palladium, cobalt and iron. In isocratic elution the copper ion pair eluted within a few minutes and the iron ion pair in about 20 min. With an ammonium compound and methanol both present in the eluent, it was also possible to carry out gradient experiments in which the percentage of methanol was gradiently increased.

Equilibrium requirements

No attempt was made to mask the excess of ligand in the eluent by the addition of extra metal ions because the complex formation stability would have been totally confused [4,12,14]. An increased metal concentration would have changed the ionic strength, and the metal–126NNS complex ion pairs would not have been formed quantitatively because of the competition between the metals. Moreover, if a masking agent had been used, its absorbance might have been so strong as to cover the absorption of interest. The background absorption due to excess of the ligand was instead eliminated through careful stabilization of the system and zero setting of the detector.

The ion-pair former was not used in excess in the eluent because it disturbed the ion-pair equilibrium in the system and prevented the ion-pair elution, as observed with the unstable baseline on the monitor of the chromatograph and the split peaks in the chromatograms.

Gradient elution was the main method used.

However, under the conditions optimized for the formation and separation of the metal ion pairs the proportion of methanol had to be greater than 50%, otherwise, the peaks were broad and retention times long. The more water in the eluent, the less likely was the metal ion pair to elute as a single peak, and conversely, the more methanol the eluent contained, the shorter was the analysis time. A short analysis time is essential for routine methods.

Detection limits

For the purposes of this work, the detection limit was considered to be the minimum concentration at which the minimum abundance for the detector (1.0 mAU) was exceeded. Using the detector at 260 nm, the detection limit (signal-to-noise ratio 2) for the cobalt–126NNS ion-pair was 69 n*M*, for palladium 50 n*M*, for copper 43 n*M* and for iron 76 n*M*. The response was linear from the detection limit up to the maximum concentrations tested, *i.e.*, for copper, palladium, cobalt and iron ion pairs 43–870, 50–950, 69–980 and 76–1120 n*M*, respectively. The relative standard deviation at the detection limit was 4.3, 5.0, 6.9 and 7.6 n*M* for copper, palladium, cobalt and iron, respectively. The reproducibility was within 1–3%. The results of the method applied to simulated samples are summarized in Table I and show that the on-line derivatization is useful even when small concentrations are to be detected.

In addition to copper, cobalt, palladium and iron we studied nickel, mercury and zinc. As seen in Fig. 4, all three metals were eluted within 25 min under gradient elution conditions. All seven metals formed low-UV absorbing complexes with 126NNS, but with nickel coloured metal complexes with different metal to ligand molar ratios were formed, depending on the eluent content and its pH.

Applications

Our on-line complexation and ion-pairing system with optimized gradient elution was tested on health drinks and nutrient sticks. Before injection into the chromatograph, the organic materials were carefully acid hydrolysed to eliminate background absorbance. The chromatograms obtained are shown in Fig. 6A and B and standard addition plots are presented in Fig. 7. To study the usefulness of the method, four samples were prepared of each matrix and ten injections were made for each sample. Compared with the precolumn method described previously [14], the conditions for this study proved more difficult to optimize. The present method is nevertheless suitable for routine use and, once the chromatographic conditions have been optimized to produce stable metal ion pairs and to separate them, provides sufficient sensitivity for detection. The selectivity of the method can be exploited by modifying the detection wavelength according to the compound of interest. As the column pressure is low even in sequential runs, and elution occurs at pressures below 160 bar, the ion-pair compounds are eluted more slowly than in the precolumn system [14]. Accordingly, the peaks in the chromatograms tend to be broad.

TABLE I

THE RESULTS OF THE METHOD APPLIED TO SIMULATED SAMPLES

Metal	Metal concentration (μg/ml)	Metal-complex ion concentration (μg/ml)	Recovery (μg/ml) (mean ± S.D.)	Recovery (%)
Copper	2.73	42.3	36.3 ± 1.9	85.8
	553	857	852 + 7.7	99.5
Palladium	5.32	54.1	48.8 ± 3.4	90.2
	101	1028	1022 ± 30	99.4
Cobalt	4.06	105	95.9 ± 5.8	91.3
	57.7	1492	1489 ± 29	99.8
Iron	4.24	116	97.7 ± 5.2	84.2
	62.6	1702	1695 ± 20	99.6

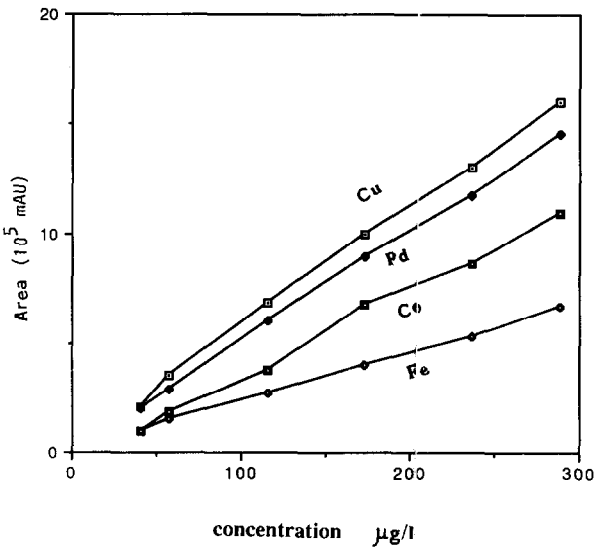

Fig. 7. Standard addition plots for copper, cobalt, iron and palladium.

CONCLUSIONS

The on-line method described here is useful for the determination of copper, cobalt, iron and palladium in mixtures. The equilibria of metal complex and ion-pair formations in the mobile phase governed the retention of the ion pairs. Therefore, the ion pairs were formed simultaneously and quantitatively only in the pH range 7–8. The selectivity of the method was enhanced by the reaction of the metal, the ligand and the ion-pair former in the mixer–reactor system. The detector response for copper, palladium, cobalt and iron was linear up to concentrations of 10 μM. In spiked water–methanol samples the detection limits for these metals ranged from $1 \cdot 10^{-3}$ mg/l to 1 mg/l. The method can also be applied to samples containing zinc, nickel and mercury. Our present and earlier [4,14] findings show the on-line complexation and ion-pairing techniques to be more useful than time-consuming liquid–liquid extraction of ion pairs and metal complex formation before injection. By optimizing the eluent flow-rate and the methanol content, band broadening in the on-line mixer–reactor system was minimized and the peak tailing of the ion pairs was avoided.

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