Note

Determination of Pb^{II}, Cd^{II} and Bi^{III} by reversed-phase liquid chromatography of their diethyldithiocarbamate complexes with post-column ligand exchange and selective spectrophotometric detection

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Recently we reported the selective preconcentration of metal ions as their diethyldithiocarbamate[1] or diethyldithiophosphate[2] complexes, in which the selectivity was introduced via a dual pre-column system that eliminates most of the interfering compounds. As an alternative to this approach, the performance of the analytical system can be improved by using selective detection.

We have shown previously[3] that the dithiocarbamate derivative thiram (tetramethylthiuram disulphide), a notorious pesticide, can be selectively detected as its corresponding copper(II) dimethyldithiocarbamate [Cu(DmDTC)$_2$] complex, which is formed in a solid-state reactor packed with metallic copper and has a strong absorption maximum at 435 nm. During these investigations, we found that not only metallic copper and Cu^{II} ions, but also insoluble Cu^{II} salts such as copper(II) sulphide or copper(II) phosphate, react rapidly and quantitatively with thiram to form Cu(DmDTC)$_2$. This may be explained by the high complex stability of Cu(DmDTC)$_2$ (log β_2 = 21.8)[4]. The formation of copper diethyldithiocarbamate [Cu(DTC)$_2$] was also observed, *viz.*, when copper(II) phosphate was added to a solution of metal diethyldithiocarbamate (DTC) complexes such as Pb(DTC)$_2$ or Cd(DTC)^2, which possess considerably lower complex stabilities than Cu(DTC)$_2$. This ligand-exchange reaction has already been used for the determination of diethyldithiocarbamate, which was preconcentrated and chromatographed as its lead(II) complex[5]. As the ligand-exchange reaction between Pb(DTC)$_2$, Cd(DTC)$_2$ or Bi(DTC)$_2$ and Cu^{II} proceeds fairly fast, we studied the use of copper(II) phosphate and, for comparison, nickel(II) phosphate as post-column complexation reagent for the selective determination of Pb^{II}, Cd^{II} and Bi^{III}. The aim of this work was to establish a method that allows the selective single-wavelength determination of these metal ions in complex matrices.

EXPERIMENTAL

Chemicals

Sodium N,N-diethyldithiocarbamate [Na(DTC)] was supplied by EGA Chemie (Steinheim, F.R.G.). All other organic chemicals were of analytical-reagent grade

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(Baker, Deventer, The Netherlands). Copper(II) nitrate, nickel(II) nitrate, lead(II) acetate, cadmium(II), acetate, bismuth(III) nitrate, potassium hydrogenphosphate and cetyltrimethylammonium bromide (cetrimide) were Baker Analyzed Reagents.

Metal diethyldithiocarbamate complexes were prepared by adding a 1 mM solution of the corresponding metal acetate (Pb, Cd) or nitrate (Cu, Bi) to a 2 mM aqueous solution of Na(DTC) in an aqueous 10 mM phosphate buffer (pH 6.8). All experiments were carried out with fresh solutions to avoid precipitation of the metal dithiocarbamates.

Instrumentation

The high-performance liquid chromatographic (HPLC) system (Fig. 1) consisted of a Kontron (Zürich, Switzerland) LC pump and a 200 × 2.1 mm I.D. or a 100 × 4.6 mm I.D. stainless-steel column packed with 5-μm Hypersil ODS (Shandon Southern, Runcorn, U.K.). A Hewlett-Packard (Waldbronn, F.R.G.) Model 1040 diode-array detector was used at a detection wavelength of 435 nm. Trace enrichment was carried out on a laboratory-made 4.0 × 2.1 mm I.D. precolumn, which was hand-packed with a slurry of 5-μm Hypersil ODS in methanol using a syringe. The preconcentration pump was an Altex (Berkeley, CA, U.S.A.) Model 110 pump used at a flow-rate of 1.0 ml/min. For the post-column reactor the same type of precolumns of length 2.0 or 4.0 mm and 4.6 mm I.D. was used. Acetonitrile–10 mM aqueous acetate buffer (pH 6.0) containing 10 mM cetrimide was used as the mobile phase; it was degassed ultrasonically under vacuum for 20 min.
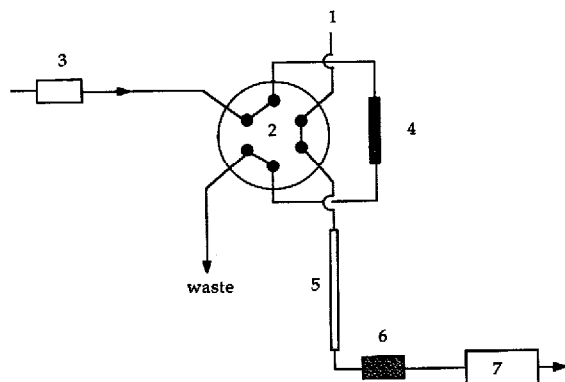

Fig. 1. Scheme of the chromatographic system. 1 = HPLC pump; 2 = six-port injection valve; 3 = preconcentration pump; 4 = C$_{18}$ precolumn; 5 = analytical column; 6 = metal phosphate post-column reactor; 7 = detector.

Preparation of the post-column reactor

The post-column reactor was a 2.0 or 4.0 × 4.6 mm I.D. column filled with copper(II) or nickel(II) phosphate, which were prepared by mixing equal volumes of a 0.1 M solution of potassium hydrogenphosphate and a 0.1 M solution of the corresponding metal nitrates. The metal phosphate precipitate was washed twice with both doubly distilled water and methanol. After treating the metal phosphate suspension in methanol ultrasonically for 20 min, it was dried on tissue paper and pressed as densely as possible into the reactor column using a micro-spatula.

Determination of Pb^{II} and Cd^{II} in urine

After filtration over a 0.2-μm membrane filter, 500 μl of aqueous 1 M acetate buffer (pH 6.0) and 500 μl of aqueous 1 mM Na(DTC) solution were added to 9 ml of a urine sample. Injections onto the C$_{18}$ precolumn (4.0 × 2.1 mm I.D.) were performed with a 1.0-ml loop connected to a Valco six-port injection valve. After preconcentration of the sample solution, the pre-column was flushed with 5 ml of 10 mM acetate buffer (pH 6.0) solution. The metal diethyldithiocarbamates were desorbed to the analytical column by means of the LC mobile phase.

RESULTS AND DISCUSSION

Chromatographic conditions

The metal diethyldithiocarbamates were chromatographed on C$_{18}$-bonded silica with acetonitrile–acetate buffer (pH 6.0) (70:30, v/v) as mobile phase. The use of methanol instead of acetonitrile led to a drastic decrease in the column efficiency. Cetrimide (10 mM) was added to the mobile phase in order to improve the peak shape of Cd(DTC)$_2$ (cf., ref. 2). Under the stated conditions, the capacity ratios of the various metal complexes were as follows Pb(DTC)$_2$ 1.9, Ni(DTC)$_2$ 2.2, Bi(DTC)$_3$ 3.2 and Cd(DTC)$_2$ 4.1.

Parameters of the reaction–detection system

Fig. 2 shows the absorption spectra of Cu(DTC)$_2$ (λ_{max} = 435 nm, ε_{max} = 13 000)[5] and Ni(DTC)$_2$ (λ_{max} = 325 nm, ε_{max} = 38 000)[5] recorded with a photodiode-array detector. Together with peak-height measurements these spectra were used to determine the degree of conversion in the metal phosphate post-column reactors. The main parameters that influence the ligand-exchange reaction between Pb-(DTC)$_2$, Cd(DTC)$_2$ or Bi(DTC)$_3$ and Cu^{II} or Ni^{II} are the reaction time and temperature, the solvent composition and the pH.

Copper(II) phosphate as post-column reagent. Fig. 3 shows the degree of conversion for Pb(DTC)$_2$, Cd(DTC)$_2$ and Bi(DTC)$_3$ at different reaction times at 20°C. Already at room temperature and at the shortest reaction time investigated (1.3 s, corresponding to a flow-rate of 1.6 ml/min), nearly complete conversion into Cu (DTC)$_2$ is obtained. This was confirmed by an evaluation of the absorption spectra for the peaks recorded after injection of Pb(DTC)$_2$ and Cd(DTC)$_2$, which were iden-
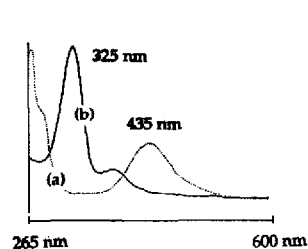

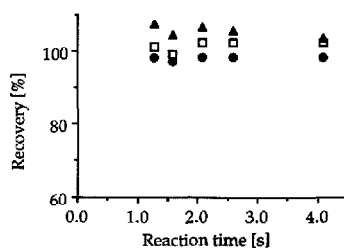Fig. 2. Absorption spectra of (a) Cu(DTC)$_2$ and (b) Ni(DTC)$_2$.

Fig. 3. Conversion of (▲) Pb(DTC)$_2$, (□) Cd(DTC)$_2$, and (●) Bi(DTC)$_3$ at 20°C as a function of the residence time using a copper(II) phosphate reactor.

tical with the spectrum of Cu(DTC)$_2$. An increase in the reaction temperature to 40°C did not result in an improved performance.

Nickel(II) phosphate as post-column reagent. Ni(DTC)$_2$ is considerably less stable than Cu(DTC)$_2$; therefore, one can expect that, with Ni^{II}, the ligand-exchange reaction with the various metal diethyldithiocarbamates will be less efficient than with Cu^{II}. Fig. 4a, b and c show the degree of conversion for Bi(DTC)$_3$ and Cd(DTC)$_2$ and Pb(DTC)$_2$, respectively, with nickel(II) phosphate at 20, 40 and 60°C. At room temperature and at the shortest reaction time investigated (about 1 s), the degree of conversion is considerably lower than in the copper(II) phosphate reactor. Cd(DTC)$_2$ and Pb(DTC)$_2$ react significantly faster than Bi(DTC)$_3$, and they are almost completely converted at room temperature if the reaction time is at least 2 s. Maximum conversion of Bi(DTC)$_3$ is only obtained at a reaction temperature of 60°C and reaction times longer than 1.2 s; this may be due to the fact that Bi(DTC)$_3$ is more stable than Cd(DTC)$_2$ and Pb(DTC)$_2$.

Effect of the mobile phase composition. The ligand-exchange reaction is not affected by the addition of common LC organic modifiers such as acetonitrile or methanol to the mobile phase. Further, no effect of these solvents on the lifetime of the metal phosphate reactor was observed. However, in order to prevent dissolution of the metal phosphates, the pH of the mobile phase has to be higher than 5.
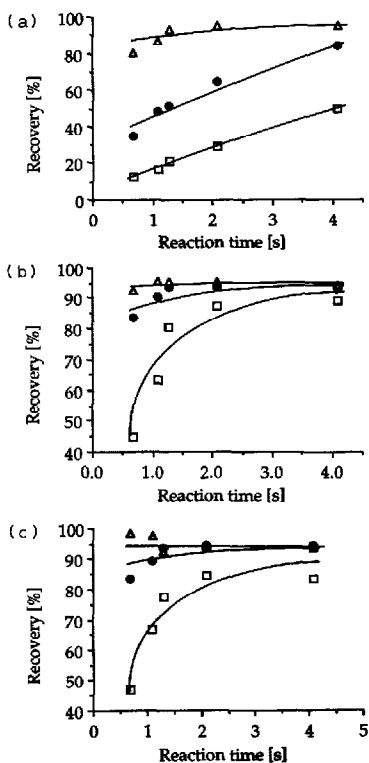

Fig. 4. Conversion of (a) Bi(DTC)$_3$, (b) Cd(DTC)$_2$ and (c) Pb(DTC)$_2$ at ($\square$) 20, ($\bullet$) 40 and ($\triangle$) 60°C as a function of the residence time using a nickel(II) phosphate reactor.

External peak broadening. The peak broadening caused by the metal phosphate reactor was tested by injecting Cu(DTC)$_2$ with and without the post-column reactor installed. It was found that the 2.0 and 4.0 $\times$ 4.6 mm I.D. reactors caused an additional peak broadening of σ = 1.0–1.5 s, which is small compared with the peak broadening of the total chromatographic system (without reactor) of σ = 5.1 s. The asymmetry factors (measured at 10% of the peak height) increased from 1.2 to 1.3 (reactor length 2.0 mm) and 1.4 (reactor length 4.0 mm). Both additional peak broadening and asymmetry factors were reproducible within 10% between different reactor packings.

Analytical data

The detection limits (signal-to-noise ratio = 3:1) for Pb^{II} and Cd^{II} were 7 ng using the copper phosphate reactor and 3 ng using the nickel phosphate reactor. The sensitivity of the system with the nickel phosphate reactor is higher because of the higher ε_{max} of Ni(DTC)$_2$. The relative standard deviation for direct-loop injections of 25 ng was less than 2.5% (n = 8). The detector response was linear over almost three orders of magnitude (r = 0.999; upper limit for the copper phosphate reactor, 2000 ng). The lifetime of the metal phosphate reactors was determined only by the injected mass of compounds which react with the corresponding metal. No effects of contaminants from biological samples on the reactor lifetime were observed.

Possible interferences from disulfiram and Ni^{II}

If metal diethyldithiocarbamates such as Pb(DTC)$_2$ are detected by UV absorbance measurement at 254 nm, quantitation is often difficult owing to interferences caused by disulfiram (tetraethylthiuram disulphide) and/or Ni(DTC)$_2$ (see Fig. 5).
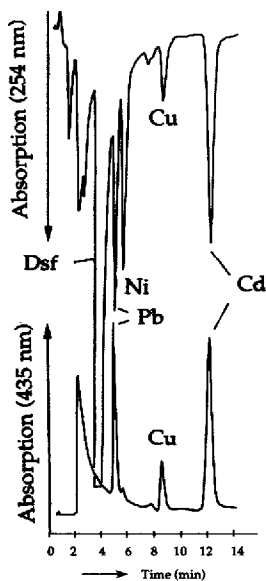

Fig. 5. Chromatogram of Ni(DTC)$_2$, Cd(DTC)$_2$, Pb(DTC)$_2$ and Cu(DTC)$_2$ with detection at (top) 254 nm and (bottom) 435 nm using the copper(II) phosphate reactor. Dsf = Disulfiram. For conditions, see Experimental.

Disulfiram is easily formed by air oxidation of diethyldithiocarbamate[7,8] and is therefore always present in samples after reaction with Na(DTC). Further, an excess of Na(DTC) can react with Ni^{II} present in the stainless-steel parts of the LC system; hence an $Ni(DTC)_2$ peak is usually found, even if no Ni^{II} was present in the sample. Both compounds, however, were found to react slowly with copper(II) phosphate. We observed that, in the presence of Cu(II), the reaction time for the quantitative conversion of disulfiram into $Cu(DTC)_2$ at 50°C is about 3 min. Under the reaction conditions chosen for the post-column ligand-exchange of $Cd(DTC)_2$ and $Pb(DTC)_2$ (room temperature, flow-rate 1.2 ml/min), disulfiram and nickel(II) therefore do not interfere with the determination of Pb^{II} (see Fig. 5).

Determination of Cd^{II} and Pb^{II} in urine

In order to demonstrate the selectivity of the detection method, urine was spiked with 200 ppb[a] of Cd^{II} and 100 ppb of Pb^{II}. After pH adjustment and the addition of Na(DTC), 1 ml of the sample was preconcentrated on a precolumn packed with C_{18}-bonded silica. Using visible detection at 435 nm after post-column ligand exchange with copper(II) phosphate, Pb^{II} and Cd^{II} can be determined with an analysis time of about 15 min, and interferences from other urine components are absent (see Fig. 6). The detection limit for the determination of these metals in urine was 10 ppb (1 ml preconcentration).

The use of the nickel(II) phosphate reactor for urine analysis was less favourable; many interferences were observed at the relatively low detection wavelength (325 nm).
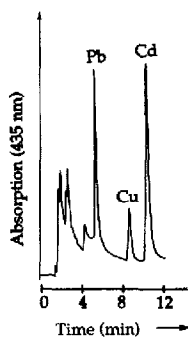

Fig. 6. Chromatogram of urine spiked with 200 ppb of Cd^{II} and 100 ppb of Pb^{II} using 1 ml preconcentration. For other conditions, see Experimental.

CONCLUSIONS

A simple detection method has been developed for the LC determination of Pb^{II}, Cd^{II} and Bd^{III}. After ligand exchange with copper(II) phosphate using a solid-state post-column reactor, both metals can be detected as $Cu(DTC)_2$ at 435 nm. Owing to the selectivity of the high detection wavelength, only a minimum of sample

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[a] Throughout this article, the American billion (10^9) is meant.

pretreatment is required if Pb^{II} and Cd^{II} have to be determined in biological and other complex samples. This result compares very favourably with that of the analysis of a urine sample by LC with UV detection at 254 nm (see Fig. 2 in ref. 4), where a broad band is observed during the first 10 min of the LC run, which makes the quantification of low amounts of metal ions impossible.

The post-column conversion of Pb(DTC)$_2$ and Cd(DTC)$_2$ into Cu(DTC)$_2$ proceeds rapidly and quantitatively at conventional LC flow-rates (*ca.* 1 ml/min) and room temperature. Because of the low solubility of copper(II) phosphate in the LC eluent, the post-column reactor can be used continuously for at least 1 week. It is therefore highly suitable for (automated) routine analysis. In addition, the elimination of interferences from the oxidation products of the ligand and undesired metal dithiocarbamates such as Ni(DTC)$_2$ or Co(DTC)$_3$ is advantageous.

Substitution at the nitrogen atom of the dithiocarbamates generally does not significantly influence the complexation properties, the wavelength of maximum absorption or the molar absorptivity of metal dithiocarbamates[9]. Therefore, the present post-column complexation technique may also be applied to other dithiocarbamate ligands frequently used in the separation of metal ions, such as bis(hydroxyethyl)[10-12] or tetramethylenedithiocarbamates[8,13,14]. The method may also be extended to the determination of other metal ions such as Zn^{II}, Mn^{II} or Se^{II}. This would require that their DTC complexes can be chromatographed by reversed-phase LC, are less stable than Cu(DTC)$_2$ and exhibit a fast ligand-exchange reaction with Cu^{II} ions.

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