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METALLIC COPPER-CONTAINING POST-COLUMN REACTOR FOR THE DETECTION OF THIRAM AND DISULFIRAM IN LIQUID CHROMATOGRAPHY

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SUMMARY

A reaction detector has been developed for the selective detection of thiram and disulfiram. The detection is based on the post-column complexation of these analytes on a solid-state reactor packed with finely divided metallic copper to form a coloured copper complex, copper(II) N,N-dimethyldithiocarbamate, with an absorption maximum at 435 nm. The method is combined with a pre-concentration and clean-up step on a pre-column to permit the sub-ppb determination of, *e.g.*, thiram in surface water samples or disulfiram in urine. Separation is achieved by reversed-phase liquid chromatography.

INTRODUCTION

A major portion of the world fungicide use is represented by dithiocarbamates and their derivatives, the so-called thiuram disulphides[1]. Compounds such as thiram, ferbam and ziram (Fig. 1) are widely used as protective fungicides in agriculture. Other uses of dithiocarbamates are as accelerators in rubber vulcanization and, specifically, of disulfiram as a drug against alcohol abuse[2].

A review of the chemistry of this important group of compounds has been given by Thorn and Ludwig[2]. One characteristic property of the dithiocarbamates is their ability to form strong metal complexes with a wide variety of metal ions[3,4].

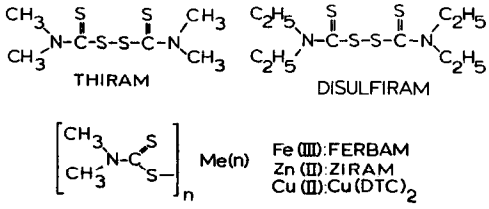

Fig. 1. Structures of the most important dithiocarbamate fungicides.

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Hence, they are widely applied in heavy metal analysis[5,6]. On the other hand, some photometric methods for the determination of dithiocarbamates and thiuram disulphides are based on their complexation reaction with metal ions such as copper(II). The intensely yellow coloured copper(II) dithiocarbamate has an analytically useful absorption maximum at 435 nm[2,7].

Gas chromatographic methods for the determination of dithiocarbamates are mostly based on the determination of carbon disulphide, which is produced by acidic digestion of the dithiocarbamates[7]. In recent years, several high-performance liquid chromatographic (HPLC) methods have been developed. Kirkbright and Mullins[8] used cetyltrimethylammonium bromide (cetrimide) in the mobile phase to achieve the separation of sodium dimethyl- and diethyldithiocarbamate and thiuram disulphides. Smith *et al.*[9] separated dithiocarbamates and thiuram disulphides as their nickel(II) and cobalt(II) complexes. Brandšteterová and co-workers[10,11] determined dithiocarbamate fungicides by normal- and reversed-phase chromatography with UV detection. Gustafsson and Thompson[12] determined thiram in food by HPLC with UV detection at 272 nm after extraction with chloroform and clean-up on a silica gel column.

Generally, the methods described are rather non-selective. UV detection, which was employed in all HPLC methods, requires several clean-up steps such as purification on silica gel, if contaminated samples are to be analysed. The aim of this work was to develop a selective analytical method for the most widely used fungicide of this group, thiram, in order to allow its determination in environmental or biological matrices without complicated sample pre-treatment. The method is based on the liquid chromatographic separation and the post-column derivatization of thiram with metallic copper. Furthermore, investigations were carried out on the pre-concentration of thiram on C_{18}-bonded silica and its stabilization in water samples to avoid losses due to complexation reactions with metal ions. In addition, some preliminary investigations were carried out to demonstrate the feasibility of this method for the determination of disulfiram in urine.

EXPERIMENTAL

Chemicals

Thiram (tetramethylthiuram disulphide) and disulfiram (tetraethylthiuram disulphide) were purchased from Fluka (Buchs, Switzerland) and were of 98% purity. Sodium N,N-dimethyldithiocarbamate [Na(DTC)] and EDTA were supplied by EGA Chemie (Steinheim, F.R.G.). All other organic chemicals were of analytical-reagent grade (Baker, Deventer, The Netherlands). Copper(II) sulphate, copper(I) chloride, calcium chloride and potassium citrate were Baker Analyzed Reagents. Sodium borohydride was of 99% purity (Baker).

Copper(II) N,N-dimethyldithiocarbamate [$Cu(DTC)_2$] was prepared by adding a 1 m*M* solution of copper(II) sulphate to a 2 m*M* aqueous solution of Na(DTC) in 10 m*M* phosphate buffer (pH 6.8). After the reaction, an aqueous 10 m*M* solution of EDTA and potassium citrate was added to avoid the formation of $Cu(DTC)^+$ due to the reaction of $Cu(DTC)_2$ with excess of Cu^{2+} ions. This positively charged 1:1 complex could not be chromatographed in a reversed-phase HPLC system. Injections of the poorly soluble $Cu(DTC)_2$ were made from fresh solutions to avoid precipi-

tation. Solutions of copper(I) chloride (10 m*M*) were made in 0.1 *M* acetate buffer (pH 4.5) containing 0.1 *M* calcium chloride [which was added to increase the solubility of copper(I) chloride] and used directly after preparation.

Instrumentation

The HPLC system (Fig. 2) consisted of a Hewlett-Packard (Waldbronn, F.R.G.) 1090 liquid chromatograph and a 200 × 2.1 mm I.D. stainless-steel column packed with 5 μm Hypersil ODS (Shandon Southern, Cheshire, U.K.). A Zeiss (Oberkochen, F.R.G.) PM2 DLC spectrophotometer or a Hewlett-Packard 1040 diode array detector was used as the detector at a detection wavelength of 435 nm. The pre-concentration pump was an Orlita (Giessen, F.R.G.) pump used at a flow-rate of 1.0 ml/min. Enrichment was carried out on laboratory-made[13] 4.0 × 2.1 mm I.D. pre-columns, which were hand-packed with a slurry of 5 μm LiChrosorb RP-18 (E. Merck, Darmstadt, F.R.G.) in methanol using a syringe. For the post-column reactor the same type of pre-columns of length 2.0 and 4.0 mm and I.D. 2.1 mm were used. In the pre-column derivatization mode, injections of the derivatization reagent were made with a 500-μl loop connected to a laboratory-made six-port injection valve. Acetonitrile–aqueous acetate buffer (10 m*M*, pH 5.0) (65–75:25–35) was used as the HPLC mobile phase.

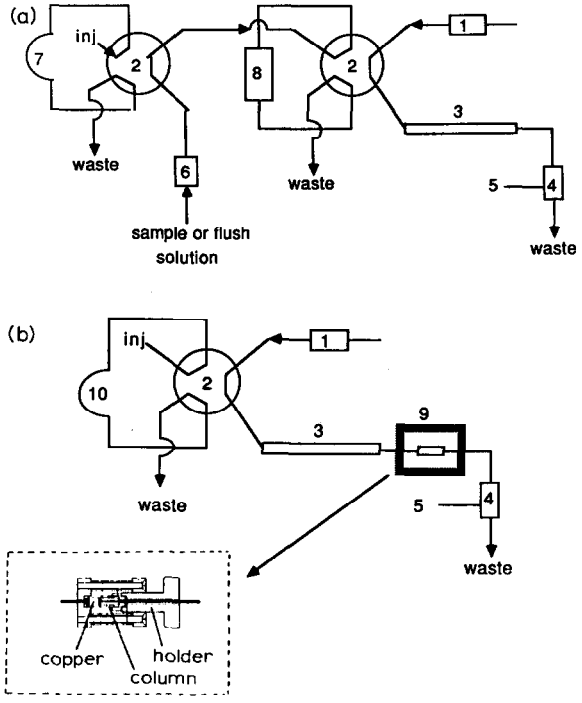

Fig. 2. Schematic diagram of the apparatus. 1 = HPLC pump; 2 = six-port injection valve; 3 = analytical column; 4 = detector; 5 = recorder/integrator. (a) Pre-column derivatization: 6 = pump; 7 = 500-μl loop; 8 = pre-column packed with C_{18}-bonded silica. (b) Post-column derivatization: 9 = copper post-column reactor; 10 = 10-μl injection loop.

Procedure for the pre-column derivatization system (Fig. 2a)

As a first step, thiram was pre-concentrated on a pre-column packed with C_{18}-bonded silica. Subsequently, 500 μl of 10 m*M* copper(I) chloride solution were injected on to the pre-column to form $Cu(DTC)_2$. After flushing the system with 10 m*M* EDTA to remove excess of copper(I) chloride, the $Cu(DTC)_2$ was eluted on-line to the chromatographic system.

Procedure for the post-column derivatization system (Fig. 2b)

Derivatization was carried out using a solid-state reactor filled with metallic copper prepared by reduction of copper(I) chloride. The reactor consisted of 2.0 or 4.0 mm long columns, which are conventionally used as pre-concentration columns. The metallic copper was prepared by carefully adding 1 g of solid copper(I) chloride to a solution of 2 g of sodium borohydride in 20 ml of doubly distilled water. The resulting (black) copper modification was washed twice with doubly distilled water and methanol to remove remaining borohydride and borate. It was suspended in methanol and treated in an ultrasonic bath to give fine particles. The slurry was then dried on tissue paper and pressed as densely as possible into the column with a micro-spatula. The post-column reactor was used immediately after preparation in order to avoid inactivation of the reactive reduced copper due to oxidation to copper(II) oxide.

RESULTS AND DISCUSSION

Derivatization principle

Fig. 3 shows the main reactions of the thiuram disulphides with copper compounds. Fredga[14] described the reaction of disulfiram with copper bronze (reaction 1), which yields $Cu(DTC)_2$. With longer reaction times and in the presence of an excess of copper bronze, Cu(DTC) was formed (reaction 2)[15,16]. This again reacts rapidly with thiuram disulphides to form $Cu(DTC)_2$ (reaction 3)[16]. The same product was obtained if copper(I) compounds such as copper(I) iodide were used instead of copper bronze (reaction 4)[16].

The copper(II) dithiocarbamate complex that is formed in this reaction has an absorption maximum at 435 nm with ε = 13000 in CCl_4 (see spectra in Fig. 4).

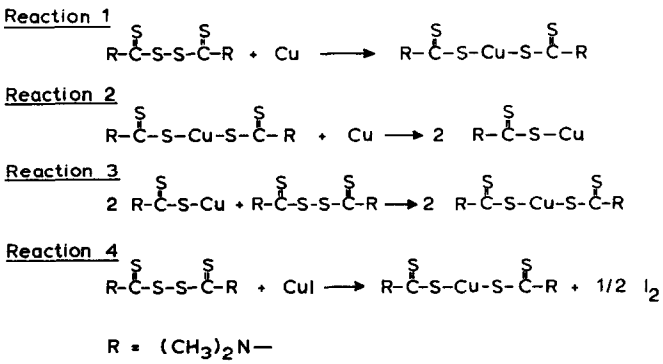

Fig. 3. Main reactions of thiuram disulphides with copper compounds.

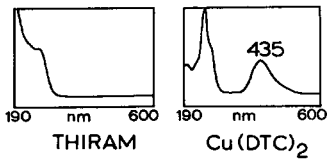

Fig. 4. UV–VIS absorption spectra of thiram and copper(II) dimethyldithiocarbamate.

Hence the transformation of the colourless thiuram disulphides into coloured derivatives offers an elegant possibility of enhancing the selectivity of the method, with almost the same sensitivity as UV detection at 254 nm.

Pre-column derivatization

Initially, copper(I) chloride was used as a derivatization reagent, as batch experiments had shown that thiram reacts rapidly and quantitatively with this compound. The formation of $Cu(DTC)_2$ was confirmed by comparison of the absorption spectrum of the reaction product with that of a standard $Cu(DTC)_2$ solution.

The above reaction was shown to be suitable for the on-pre-column derivatization of thiram (Fig. 2a). After pre-concentrating the non-polar thiram on C_{18}-bonded silica, $Cu(DTC)_2$ was easily formed by rinsing the pre-column with a small volume of a copper(I) chloride solution. Because of its low polarity, $Cu(DTC)_2$ was readily adsorbed on the pre-column; it was eluted to the chromatographic system after flushing the pre-column with 2 ml of 10 m*M* EDTA solution to remove excess of copper(I) chloride. With this method a mean recovery of 93.5% with a relative standard deviation (R.S.D.) of 9.5% ($n = 5$) was obtained. In addition to the poor reproducibility and the relatively complex instrumentation required, the pre-column derivatization has the disadvantage that no distinction can be made between thiram and other possible fungicidal metal dithiocarbamates such as ferbam or ziram, which also react with copper(I) chloride to form $Cu(DTC)_2$. In order to overcome these drawbacks, a post-column derivatization system based on a solide-state reactor was developed.

Post-column derivatization

Attempts to use copper(I) chloride mixed with C_{18}-bonded silica as a solid derivatization reagent were unsuccessful owing to the instability of copper(I) chloride towards the HPLC eluent. Further, it was found that red metallic copper reacted only slowly with thiram and therefore was not suitable in a post-column derivatization system. The reaction rate could be increased considerably, however, if the post-column reactor was packed with metallic copper prepared by the reduction of copper(I) salts with sodium borohydride. The instantaneous and quantitative formation of $Cu(DTC)_2$ using this reduced copper as a derivatization reagent was confirmed by recording the absorption spectra of the $Cu(DTC)_2$ peak using the diode array detector.

Optimization of the reactor performance

Influence of reactor bed length and eluent conditions. The reactor bed length and the pH of the eluent are the most important factors influencing the response of

the post-column reactor. Optimal results were obtained with 2.0–4.0 mm long columns (2.1 mm I.D.) and eluent flow-rates between 0.2 and 0.4 ml/min. A column longer than 4.0 mm resulted in a decreased response, possibly owing to catalytical degradation of $Cu(DTC)_2$. Flow-rates higher than 0.4 ml/min led to pressure drops greater than 300 bar for the total system and were therefore not suitable for the described chromatographic system.

The optimal pH of the eluent was between 4.5 and 6.0, under which conditions the thiram response remained almost constant. The response increased slightly if the pH was decreased; however, dithiocarbamates and thiuram disulphides are less stable at low pH[3]. pH values higher than 7.0 caused deactivation of the copper reactor, probably owing to (hydr)oxide formation. The same effect was observed when a phosphate buffer was present in the eluent [formation of copper(II) phosphate]. Acetonitrile was the only organic modifier that was used. A methanol-containing eluent led to a decreased column efficiency and to deactivation of the reduced copper. With an eluent consisting of acetonitrile and aqueous acetate buffer (10 m*M*, pH 5.0), optimal reactor performance was obtained.

Band broadening and stability of the reactor. The use of the post-column reactor did not cause substantial additional band broadening, as no retention takes place and only a small residence time is required. The increase in the peak width at half-height was 10% (with $\sigma_{reactor} = 2.1$ s). In addition, the demands on column efficiency are not too high owing to the high inherent selectivity of the detection method. If the post-column reactor is used for several days, the asymmetry factor increases from 1.3 to 2.5 or more; it can therefore be used to monitor the gradual depletion of reduced copper in the reactor. The useful lifetime of the post-column reactor is determined by the amount of thiuram disulphides injected and by the presence of oxygen, which causes passivation. Typically, with a 2.0 mm long post-column reactor that contains about 8 mg of reduced copper, more than 200 injections of nanogram amounts can be made.

Stabilization of thiram samples against complexation reactions

A problem in the determination of dithiocarbamates is their instability in aqueous solutions in the presence of metal ions[3]. In environmental samples and particularly in surface water, the reaction with metal ions such as Cu^{2+} can lead to a considerable reduction in the free dithiocarbamate concentration within a short time. Our experiments have shown that the addition of 0.1 m*M* copper(II) sulphate to a solution containing 35 ppm of thiram resulted in a 50% decrease in the thiram concentration after 20 min. As dithiocarbamates and, to a lesser extent, thiuram disulphides possess a high affinity to many metal ions[3], the presence of these ions will generally lead to a rapid decrease in the free thiram concentration. Owing to the high complex stability constants of the metal dithiocarbamates and, especially, $Cu(DTC)_2$[3,17], complexing reagents such as EDTA, tartrate or citrate cannot compete against this complexation reaction. However, it was found that a 1:1 mixture of 10 m*M* EDTA and 10 m*M* potassium citrate was able to prevent the complexation reaction of thiram with Cu^{2+}. If a thiram sample was stabilized with this mixture, no significant losses were observed, even if the sample was stored for 16 h. Water samples were thus routinely stabilized with 10 m*M* EDTA–citrate (1:1) prior to analysis.

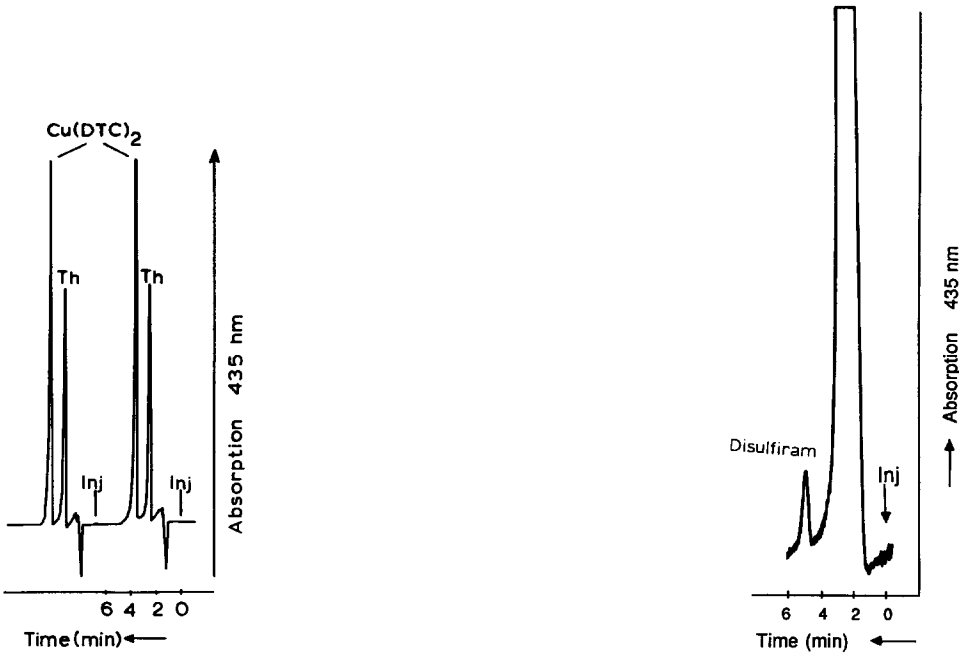

Fig. 5. Chromatogram of the duplicate injection of surface water spiked with 10 ppm of thiram and 20 ppm of $Cu(DTC)_2$. HPLC conditions: analytical column, 200 × 2.1 mm I.D. packed with 5 μm Hypersil ODS; copper reactor, 2.0 × 2.1 mm; eluent, acetonitrile–aqueous acetate buffer (10 m*M*, pH 5.0) (70:30); flow-rate, 0.3 ml/min; injection volume, 20 μl; detection wavelength, 435 nm.

Fig. 6. Determination of disulfiram in urine. HPLC conditions: analytical column, 200 × 2.1 mm I.D. packed with 5 μm Hypersil ODS; pre-column, 4.0 × 2.1 mm I.D. packed with 5 μm LiChrosorb RP-18; copper reactor, 2.0 × 2.1 mm; eluent, acetonitrile–aqueous acetate buffer (10 m*M*, pH 5.0) (65:35); flow-rate, 0.4 ml/min; detection wavelength, 435 nm (0.02 a.u.f.s.). Pre-concentration of 1.0 ml of urine spiked with 87 ppb of disulfiram (sample stabilized with 10 m*M* EDTA–citrate).

Analysis and pre-concentration of thiram samples

The calibration graph obtained using a 2.0 mm long copper-containing post-column reactor showed good linearity over almost three orders of magnitude with $r = 0.9992$. An absolute detection limit of 3.0 ng (signal-to-noise ratio = 3; 10 μl injection) was obtained. The mean R.S.D. was generally less than 2% ($n = 5$) at concentrations between 1 and 200 ppm. Fig. 5 shows the analysis of surface water spiked with 10 ppm of thiram and 20 ppm of $Cu(DTC)_2$. $Cu(DTC)_2$ is one of the main degradation products of thiram if Cu^{2+} is present. Using the copper-containing post-column reactor both compounds can be separated and detected at 435 nm.

In order to decrease the detection limit for thiram, the possibility of its pre-concentration on hydrophobic materials was investigated. As thiram is fairly non-polar, high enrichment factors can be obtained. A breakthrough volume for a 4.0 × 2.1 mm I.D. pre-column packed with 5 μm C_{18}-bonded silica of more than 50 ml was measured with a standard solution. This means that an enrichment factor of at least 5000 can be obtained compared with 10-μl injections. That is, thiram concentrations at the sub-ppb level can easily be determined. Pre-concentration of 40 ml of

a 5 ppb thiram solution resulted in a mean recovery of 93.4% with an R.S.D. of 5.0% ($n = 5$).

Determination of disulfiram in urine

The applicability of the proposed method for the determination of thiuram disulphides in urine was also studied. With the use of the copper-containing post-column reactor no complicated sample pre-treatment was required. Filtered urine was spiked with 87 ppb of disulfiram and stabilized with 10 m*M* EDTA–citrate. A 1-ml volume of the undiluted sample was pre-concentrated on a 4.0 mm long pre-column packed with C_{18}-bonded silica and then eluted to the chromatographic system with acetonitrile–aqueous 10 m*M* acetate buffer (65:35). With UV detection at 254 nm only one large band could be seen without the possibility of isolating the analyte signal. In contrast, the use of the copper-containing post-column reactor and detection at 435 nm allowed sufficient separation of the disulfiram peak for correct quantification (Fig. 6). Further work on the optimization of the method is in progress.

CONCLUSION

A post-column reactor packed with metallic copper offers the possibility of determining thiuram disulphides such as thiram and disulfiram selectively in complex samples. The conversion of thiram and disulfiram into the corresponding $Cu(DTC)_2$ is instantaneous and complete. A detection limit of 3 ng was obtained for thiram at 435 nm with a relatively old detector. Use of a modern detector will easily yield about 10-fold lower detection limits. The long lifetime of the post-column reactor allows the analysis of a large number of samples before a new reactor column is required. Owing to the selectivity of the detection wavelength, only short analysis times are required (3–5 min).

Trace enrichment of aqueous samples coupled to this detection mode can be powerful. The relatively low polarity of thiuram disulphides permits sample volumes larger than 50 ml to be pre-concentrated on 4-mm bed lengths of reversed-phase materials, yielding trace enrichment factors of over 5000. In addition, the method can be automated and, thus, permits the fast work-up of large series of samples in routine screening. The reaction principle can conceivably be used for many other chelate-forming analytes with fast reaction kinetics and favourable detection characteristics of the resulting complexes.

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