Improvement in the Determination of Mancozeb Residues by the Carbon Disulfide Evolution Method Using Flow Injection Analysis

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The sample decomposition of the carbon disulfide evolution method for the determination of dithiocarbamate residues was carried out in a closed vial in the presence of hexane. The evolved carbon disulfide was extracted by the organic solvent and injected in a flow system for its quantification as copper complex. The conditions for batch decomposition, flow injection determination, and association of both were investigated with sodium diethyldithiocarbamate as model substance. An one-channel flow system was employed where the carrier stream was the ethanolic ethylenediamine/copper solution. The determination range was of 0.01−1.26 μg of CS_2, with a relative standard deviation of 0.06% ($n = 10$), with a sample throughput of 45 samples/h. The association of the batch decomposition with the flow system was carried out with the fungicide mancozeb and was applied to the analysis of its residue in potato, lettuce, cucumber, and green bean crops. The approach allowed the analysis of 11 samples in triplicate in 2 h, with recoveries between 85% and 92% and relative standard deviation about 2%.

Keywords: *Residue analysis; dithiocarbamates; carbon disulfide; FIA*

INTRODUCTION

Dithiocarbamates (DTC) form an important class of pesticides for broad-spectrum control of a variety of fungal diseases on growing crops. Various methods have been developed for the analysis of the DTC residues (Scaroni, 1996), which include principally gas chromatography (Committee for Analytical Methods for Residues of Pesticides and Veterinary Products in Foodstuffs of the Ministry of Agriculture, Fisheries and Food, 1981; Maini and Boni, 1986) and liquid chromatography (Miles and Zhou, 1991; Lehotay el al., 1992). However, the most accepted method for this analysis is the one proposed by Keppel (Keppel, 1971; Cunniff, 1995), based on the degradation of the DTC in acid medium in the presence of $SnCl_2$ to form CS_2, which is subsequently determined spectrophotometrically as cupric complexes of *N*,*N*-bis(2-hydroxyethyl)dithiocarbamic acid. Nevertheless it is a time-consuming procedure, since each sample must be heated in a distillation apparatus for at least 1 h for the evolution of the CS_2. Since time and temperature for the decomposition are not absolutely identical for all samples, the reproducibility and precision of the method can be affected.

The purpose of this work was to adapt the Keppel method to a flow injection (FI) system, associating a batch decomposition/extraction procedure for the CS_2 with a flow system for its colorimetric quantification as copper complex, to process several samples simultaneously.

The sample decomposition was carried out in the presence of a nonpolar solvent (hexane), so that the liberated CS_2 was extracted by this solvent. The decomposition took place in a gastight tube filled with liquids, and the CS_2 was directly sampled (hexane layer) with a syringe and injected in the channel of the flow system, which had the color solution of the Keppel method as carrier stream. The formed copper complex was spectrophotometrically detected.

The method was first developed with the standard sodium diethyldithiocarbamate (NaDEC) (Cunniff, 1995) and then applied to mancozeb, which has a polymeric structure ($(C_4H_6N_2S_4Mn)_xZn_y$) and an unknown molecular mass. The elemental analysis of mancozeb gave its sulfur content and provided a mancozeb−CS_2 conversion factor for recovery calculations. The developed method was applied to the determination of residues of mancozeb in potato, lettuce, cucumber, and green bean crops.

EXPERIMENTAL PROCEDURES

Apparatus. *General.* A Gilson Minipuls-3 peristaltic pump (France) with 1-mm i.d. Tygon tubes, a Rheodyne (USA) injection valve with a sample loop of 100 μL, a Schimadzu SPD 10 AV UV/VIS detector (Japan), an ECB 201 recorder (Brazil), a Perkin-Elmer Lambda 16 spectrophotometer (Germany), a Colora MB 33-644 water bath (Germany), a Sonorex Super RK510 H ultrasound bath (Germany), an Elementar Analysensysteme GmbH elemental analyzer (Germany), and a Digital DM 20 (Brazil) pH meter were used.

CS_2 Evolution Apparatus. As described by Keppel (Keppel, 1971), the apparatus consisted of a 500-mL round-bottom, three-neck boiling flask, heated by mantle controlled with variable transformer, with the reflux condenser in the center neck. Two traps were connected in series with the upper part of the reflux condenser: the first trap, containing 50 mL of 5% (m/v) lead acetate solution, to remove H_2S and other volatile interferences from gas stream, and the second trap containing the color reagent (15 mL of color reagent solution described under Reagents) to react with the evolved CS_2. A vacuum line was attached to the exit of the CS_2 trap to draw air through the system.

Reagents. All chemicals were of analytical reagent grade. All aqueous solutions were prepared with distilled and deionized water that was further purified by a Milli-Q high-purity

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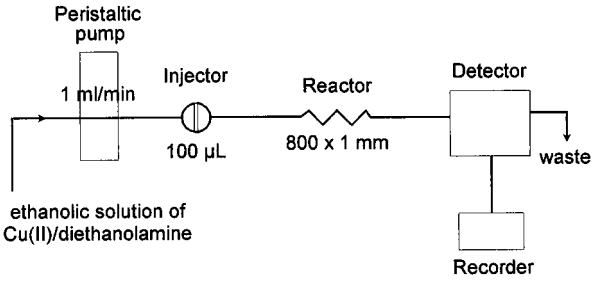

Figure 1. Flow diagram of the system for the CS_2 quantification as copper complex.

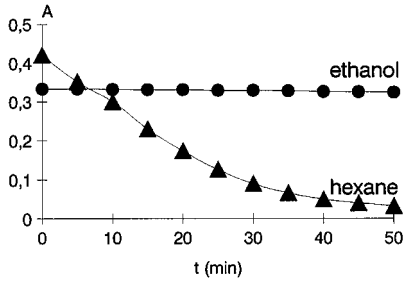

Figure 2. Stability of the copper complex in ethanol and in hexane/ethanol (1/1.5) mixture; absorbance measured at 435.0 nm.

water device (Millipore, USA). A stock standard CS_2 solution containing 5.04 mg/mL was prepared with 0.1 mL of CS_2 (99.9%, $d = 1.26$ g/mL; Merck) in 25.0 mL of absolute ethanol (Merck), and working standard solutions were prepared by suitable dilutions of the stock solution in absolute ethanol or in hexane. The HCl/$SnCl_2$ mixture was prepared dissolving 5 mL of a 40% (m/v) $SnCl_2 \cdot 2H_2O$ (Merck) solution in 20 mL of HCl (36%, $d = 1.14$ g/mL) (Merck) and diluting with water to 200 mL. Solution for solubilization and extraction of mancozeb: 45 mL of 0.2 mol/L ethylenediamine tetraacetic acid disodium salt (EDTA) mixed with 5 mL of 10% (m/v) NaOH, pH 9.5. Color reagent and carrier of the flow system: 12 mg of copper(II) acetate monohydrate (Merck) was dissolved in 25.0 mL of diethanolamine (Merck) and diluted to 250 mL with absolute ethanol. Test substance: sodium diethyldithiocarbamate trihydrate (Merck, 99%). Mancozeb: Dithane M-45, 800 g/kg (Rohm and Haas).

Flow System for the Determination of CS_2. The FI setup is schematically shown in Figure 1. It consisted of a liquid stream with the color reagent, in which the CS_2 from standards and samples, dissolved in hexane, was directly injected. The copper complex was detected spectrophotometrically at 435.0 nm and the signal recorded as peak height. The concentrations of the standard solutions were from 0.1 to 12.6 mg/L. Flow rates between 0.38 and 2.0 mL/min and reactors with 50−200 cm length were tested for a standard solution containing 5.0 mg/L CS_2.

Sample Decomposition and CS_2 Extraction. As mancozeb is insoluble in water (Hartley and Kidd, 1987) it was converted into nabam, sodium salt of this DTC, by action of an alkaline EDTA solution. For this purpose 30 mg of mancozeb was mixed with 50.0 mL of the EDTA/NaOH solution and shaken for 10 min. The best pH for this reaction was investigated by addition of NaOH to the EDTA solution in order to give solutions with pH from 5 to 13. The presence of nabam was investigated by measuring the absorbance of the solution at 252.0 nm, wavelength of the maximal absorbance. The stability of nabam at pH 9.5 was also determined measuring the absorbance of the solution at 252.0 nm, at intervals of 5 min for 90 min.

The decomposition of the samples (NaDEC and mancozeb) was carried out in glass tubes of 16.5 mL with plastic screwcaps (Brand, Germany), in which 4.0 mL of the solution or suspension of the DTC was mixed with 4.5 mL of the acid solution and 8.0 mL of hexane. The tubes were placed in the water bath and heated for 55 min at 80−85 °C. Every 10 min the tubes were hand-shaken. At the end of this time the tubes were cooled and opened, and an aliquot of hexane (upper layer) was immediately injected in the FI system. As many tubes can be placed together in the water bath, the simultaneous decomposition of samples was possible.

Stability of the Copper Complex in Hexane. Two solutions of 10 mg/L CS_2 were prepared in ethanol and in hexane, respectively. For each 10 mL of the solutions, 10 mL of color reagent solution was added and the volume was completed up to 25 mL with the respective solvent. The absorbance of the copper complex formed in these solutions was measured at 435.0 nm at intervals of 60 s for 50 min.

Keppel Method. Samples containing from 60 to 360 μg of mancozeb, in suspension and after dissolution in the EDTA/NaOH, pH 9.5, solution, were mixed with 25.0 mL of the HCl/$SnCl_2$ solution in the round-bottom boiling flask and heated under reflux. In the first trap 50 mL of the lead acetate solution was added and in the second 15 mL of the color reagent solution. After 1 h the heating mantle current was shut off, the vacuum line disconnected, and the content of the CS_2 trap drained into a 25.0-mL volumetric flask. The volume was completed until the mark with ethanol, and the absorbance was measured at 435.0 nm.

To check the efficiency of the system, the decomposition was also carried out with 360 μg of NaDEC (Cunniff, 1995).

Application of the FI Method to the Analysis of Vegetables. The samples were potato (peel), lettuce, cucumber (peel), and green bean, which were cultivated free of dithiocarbamates or other pesticides. They were stored at −18 °C until analyzed. For the analysis, about 150 g of each vegetable was well-chopped in a cutting machine (Walita, Brazil) and four portions of 25 g were weighed. In three portions 1.0 mL of a suspension with 30 mg/L mancozeb (30 μg) was added and mixed with the chopped vegetable. In all four portions 49.0 mL of the EDTA/NaOH solution was added and, by means of an ultrasound bath, mixed for 10 min. The extracts were filtered (qualitative filter paper, Whatman No. 3; England), and 4.0 mL of each was placed in the glass tubes with 4.5 mL of the HCl/$SnCl_2$ solution and 8.0 mL of hexane. After heating for 55 min at 80−85 °C and cooling, aliquots of the hexane layers were injected in the flow system. For the determination by the Keppel method, 60 μg of mancozeb (2.0 mL of the suspension with 30 mg/L) was added to 25 g of the chopped vegetables, well-mixed, placed in the round-bottom boiling flask, and heated under reflux as described above.

Interferences. Interferences from ethylenethiourea (ETU), ethylenediamine (EDA), and sulfide ion were investigated in the decomposition/extraction procedure and in the flow system. For this purpose 4.0-mL aqueous solutions containing 250 mg/L of each substance were placed in the closed vials with the HCl/$SnCl_2$ solution and hexane. They were placed in the water bath and heated for 55 min at 80−85 °C. After cooling, absorption spectra of the hexane phase were recorded from 190 to 500 nm, and an aliquot was injected in the flow system.

RESULTS AND DISCUSSION

FI System for the CS_2 Quantification. The formation and stability of the copper complex in a hexane/ethanol mixture was investigated since in the FI system, the CS_2 dissolved in hexane was injected in the ethanolic solution of copper and diethanolamine. After 10 min the absorbance of the complex decreased about 23% in the solution containing hexane, and it remained constant when only ethanol was present (Figure 2). However the instability of the complex in hexane does not affect its measurement, since the residence time of the sample in the flow system is about 1.3 min and good repeatability can be obtained in FI measurements even at nonequilibrium conditions.

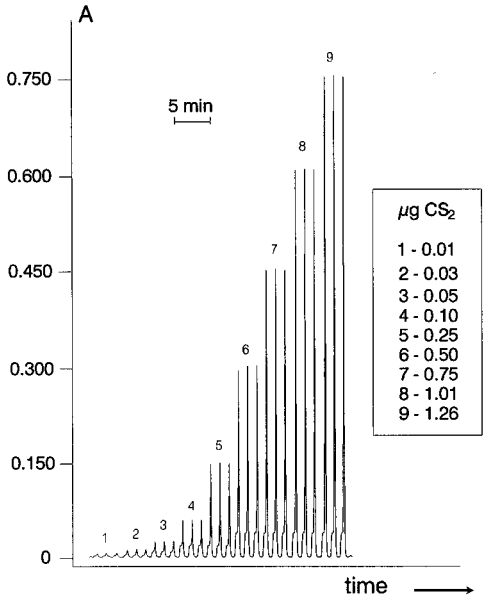

Figure 3. FI signals obtained for different CS_2 concentrations, injected in triplicate.

To find a compromise between sensitivity and sampling frequency, the effects of flow rate, mixing coil length, and injection volume were investigated. Good results were obtained with a flow rate of 1.0 mL/min, a coil length of 80 cm, and an injection volume of 100 μL. Flow rates <1 mL/min increase the dispersion of the sample, and higher rates lead to a reduction of the analytical signal. A small peak, observed immediately prior to sample signals, is caused by the ethanol/hexane interface due to Schlieren effect (Fang, 1993), and it is present even when there is no CS_2 in the sample. However, it does not cause interference since the baseline is quickly restored when coils longer than 80 cm are used. Sample volume of 100 μL was chosen to minimize the Schlieren effect too. Under the optimized conditions the injection frequency was about 45 samples/h. The relative standard deviation for 10 determinations of a 12.6 mg/L CS_2 solution was 0.06%, and the analytical calibration function, for a concentration range of 0.1−12.6 mg/L CS_2, was $A = 0.614m - 2.92 \times 10^{-3}$, where A is absorbance and m is the mass of CS_2 injected in μg. The correlation coefficient was 0.9999. The FI signals for this calibration are shown in Figure 3.

Conversion of Mancozeb into Nabam. The mixture of EDTA/NaOH solubilizes the mancozeb due to the conversion of the insoluble manganese/zinc salt into the sodium salt. The measure of the absorbance at 252.0 nm of this solution in a pH range between 5 and 13 showed that the best pH for this reaction is 9.5, as the absorbance reaches its maximal value between pH 9 and 10.5. The nabam formed at this pH is stable at least for 90 min, time interval during which the absorbance was measured. It allows one to solubilize mancozeb simultaneously in several samples prior to putting them in the water batch for the decomposition.

Decomposition of Mancozeb and Extraction of CS_2 in Hexane. In a collaborative study organized by the Committee for Analytical Methods for Residues of Pesticides and Veterinary Products in Foodstuffs (Ministry of Agriculture, Fisheries and Food, U.K., 1981) the use of organic solvents, to absorb the CS_2 liberated by the decomposition of the DTC, was suggested. Some laboratories that participated in a study used hexane, heptane, and 2,2,4-trimethylpentane as solvent for the extraction, and they found recoveries of about 75% at the 1.0 mg/kg level from spiked lettuce samples. In this proposed procedure, hexane was chosen as solvent because it did not show interference when injected in the FI system and did not extract degradation products of the DTC (such as ETU, EDA, and H_2S), which could be present in the sample or be formed during the decomposition process (Newsome, 1980).

Table 1. CS_2 Produced by the Decomposition of Different Quantities of NaEDC and Mancozeb with the FI Method from Suspensions and Solutions in EDTA ($n = 3$)

	mass of CS_2 (μg) (100 μL)		
mass (μg) (4 mL)	theor	exptl	recovery rate (%)
	NaEDC		
0	0	0	
8.0	0.034	0.030	88.7 ± 0.4
8.0	0.034	0.027	79.8 ± 0.6
16	0.068	0.056	83.3 ± 0.6
16	0.068	0.054	79.8 ± 0.7
20	0.081	0.073	90.1 ± 0.6
40	0.169	0.137	81.5 ± 0.5
40	0.169	0.132	78.3 ± 0.6
	Mancozeb/Suspension		
0	0	0	
2.4	0.012	0.010	85.8 ± 1.7
6.0	0.029	0.024	82.5 ± 0.8
12	0.058	0.045	77.3 ± 0.8
24	0.115	0.106	91.7 ± 0.7
48	0.231	0.206	89.3 ± 0.5
	Mancozeb/Solution		
0	0	0	
4.0	0.019	0.016	85.3 ± 0.6
12	0.058	0.045	78.7 ± 0.5
20	0.096	0.073	75.8 ± 0.5
40	0.192	0.169	88.1 ± 0.6
48	0.231	0.190	82.2 ± 0.5

The results of the elemental analysis showed that the analyzed mancozeb contains 32.34% sulfur, 14.68% carbon, 8.79% nitrogen, and 2.04% hydrogen. Considering that all sulfur in the sample will form CS_2 and that the product Dithane M-45 contains 80% mancozeb, the conversion factor $\mu g\ of\ product \times 0.384 = \mu g\ of\ CS_2$ was established. This conversion factor was used through this work to calculate the efficiency of the procedure and recoveries. The best conditions for the mancozeb decomposition were a water bath temperature of 80−85 °C for a time interval of 55 min; longer time and higher temperature did not increase the amount of CS_2 formed.

Table 1 shows the recovery rates of the decomposition/extraction procedure for the test substance NaDEC and for mancozeb. After the decomposition, the quantification of CS_2 was done by FI. Table 1 also shows the results obtained from mancozeb suspensions and after its solubilization with EDTA. It can be seen that there is no difference between the results from both procedures. The solubilization of mancozeb can be advantageous because, after chopping, some vegetables can cause degradation of the fungicide (Vuik et al., 1992); according to the authors 50% of thiram is decomposed after 0.5 h in chopped lettuce; additionally the solubilization allows smaller and more representative aliquots of samples to be taken for analysis.

Determination by the Keppel Method. Table 2 shows the results of the quantification of NaDEC and mancozeb samples by means of the Keppel method. The same conversion factor (μg of product × 0.384 = μg of CS_2) was used to calculate the recoveries of mancozeb,

Table 2. CS_2 Produced by the Decomposition of Different Quantities of Mancozeb with the Keppel Method from Suspensions and Solutions in EDTA

mass (μg)	mass of CS_2 (μg) theor	exptl	recovery (%)
	NaDEC		
360	121.5	98.78	81.3
	Mancozeb/Suspension		
60	23.04	25.08	108.8
150	57.60	60.03	104.2
210	80.64	78.32	97.1
300	115.20	124.73	108.3
360	138.24	125.18	90.6
	Mancozeb/Solution		
100	38.40	29.65	77.2
150	57.60	45.61	79.2
250	96.00	74.79	81.0

Table 3. Results of the Analysis of Fortified Vegetable Samples with the FI Method and with the Keppel Method ($n = 3$)

vegetable	mancozeb added (to 25-g sample) (μg)	mancozeb found (μg)	recovery (%) FI method	Keppel method
lettuce	0			
	30	26.43	88.1 ± 2.2	
cucumber	0			
	30	25.62	85.4 ± 1.8	
	60	47.42		79.0 ± 1.6
potato	0			
	30	26.25	87.5.5 ± 1.6	
	60	42.92		71.5 ± 5.1
green bean	0			
	30	25.50	91.6 ± 1.8	
	60	59.14		98.6 ± 12.6

either from suspensions or from solutions. The reproducibility of the Keppel method, for a sample containing 100 μg of the product, carried out with a suspension and in triplicate, was 7.6%.

Results from Interference Tests. The interference of ETU and EDA, subproducts of the decomposition of mancozeb (Newsome, 1979; Newsome et al., 1975; Brandsteterová et al., 1986), and the formation of sulfide ions during the decomposition procedure were investigated. ETU and EDA were not extracted by hexane from aqueous solutions. Absorption spectra of the hexane extracts, from 190 to 500 nm, did not show characteristic signals of these species. The transformation of ETU and sulfide ions in CS_2 and their extraction by hexane during the decomposition did not occur. The injection of a hexane aliquot in the FI system, after the batch decomposition/extraction procedure with these substances, showed no peaks.

Application of the FI Method in the Analysis of Vegetables. Table 3 shows the results of the quantification of mancozeb from fortified samples of vegetables determined by the FI method as well as by the Keppel method. The samples were fortified with 1.2 mg/kg for the FI method and with 2.4 mg/kg for the Keppel method, since the residue limit for mancozeb in these crops is about 1 ppm (ILSI Brazil, 1996). The recoveries of the FI method were calculated with a calibration curve of CS_2 standards injected alternately with the samples in the FI system. For both methods, FI and Keppel, the conversion factor of 0.384 (product → CS_2) given by the elemental analysis was used.

The results of both methods are in agreement, and recoveries of the FI method, between 85.4% and 91.6%, show that it can be useful in residue analysis. It can also be advantageous for routine analysis: by the classical Keppel method, with one distillation apparatus, all vegetable samples required 33 h for the complete analysis (1 h for each one), whereas by the FI method the analysis of the same number of samples was carried out in 2 h.

Conclusion. The CS_2 evolved by the decomposition of DTC in acid medium can be extracted by hexane and determined spectrophotometrically in a flow system by means of its reaction with copper and diethanolamine to form a complex that absorbs at 435.0 nm. The FI and classical methods showed equivalent results, but the FI method has a higher sample throughput and better reproducibility. The method was tested for mancozeb, but given the similarity in the chemical properties between the compounds, it is conceivable that the FI method can also be applicable to other dithiocarbamates.

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