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DESIGN AND SELECTIVE APPLICATION OF A DROPPING MERCURY ELECTRODE AMPEROMETRIC DETECTOR IN COLUMN LIQUID CHROMATOGRAPHY

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SUMMARY

The design of a polarographic detector is described and its performance is compared with that of a commercial solid-state amperometric detector using a glassy carbon electrode and with a UV detector. For the dropping mercury electrode (DME) detector, conically ground capillaries and reference electrodes are used in order to improve the dead volume and the response. The application of this detector to thiourea herbicides, making use of the specific complexation of these organosulphur compounds with the mercury surface, is discussed. In artificial solutions of the thioureas the performance of the DME detector is about the same as that of a UV detector but inferior to that of a solid-state detector. For urine analysis the DME detector profits from its high inherent selectivity and the absence of surface poisoning, which makes it the detector of choice for this type of application.

INTRODUCTION

Electrochemical detection in high-performance liquid chromatography (HPLC) has been recognized as a sensitive means of determining a wide range of compounds[1]. Although most attention has been paid to solid-state detectors, the dropping mercury electrode (DME) detector possesses some advantages, especially the continuous renewal of the electrode surface and the wide negative potential range.

Fleet and Little[2] discussed the requirements of a good electrochemical detector for HPLC. They recommended a three-electrode system and for DME detectors the use of conical capillaries to obtain a better geometry and a lower dead volume.

The problem of constructing a good DME detector has been partially solved by using a horizontal mercury capillary[3], resulting in fast drop rates. Another improvement was achieved by controlling the drop rate with a moveable pin[4]. Based on the experience gained with this latter detector[4,5], we constructed a new DME

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detector, which has been compared with some other detectors and has proved to be a useful addition to the range of available detectors[6].

In this paper it will be shown that not only the sensitivity, but also the selectivity, of this detection mode can be of importance. The detection of thiourea and its derivatives was compared at a high potential on a glassy carbon electrode and with a low and hence more selective potential on a DME. The selectivity was further increased by using specific complexation of the compounds on the mercury-drop surface. In addition, thiourea and its derivatives and particularly ethylenethiourea (ETU) were chosen for this study, because they form an important group of pesticides and breakdown products.

The need for more selective and sensitive techniques for this group of compounds has been pointed out by Cochrane[7], who surveyed currently available gas chromatographic technology in this area. ETU is of particular importance in this group[8,9] as it is suspected to have mutagenic and carcinogenic properties. The aim of this work was therefore also to develop an alternative method to gas chromatography in order to permit more selective and simple handling of large-scale sample series containing these pesticides. The feasibility of HPLC and electrochemical detection for the determination of ETU in urine samples was also studied.

EXPERIMENTAL

The DME detector

In the design of the DME detector (see Fig. 1) special attention was paid to decreasing the cell volume and optimizing the flow pattern. Another important aspect was the correct positioning of the three electrodes.

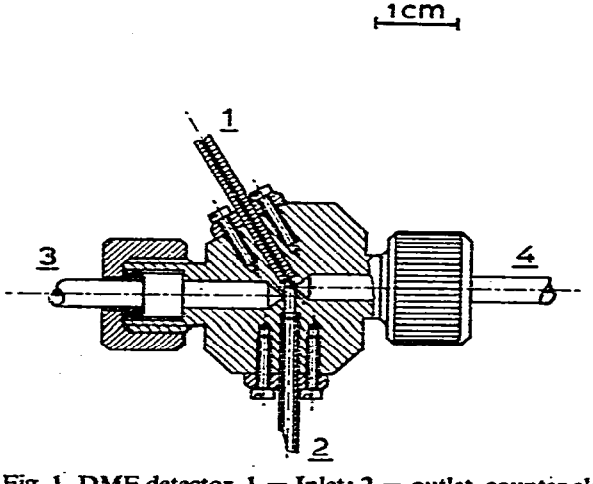


Fig. 1. DME detector. 1 = Inlet; 2 = outlet, counter electrode; 3 = reference electrode; 4 = DME.

In order to reduce the cell volume, the end of the horizontally placed mercury capillary and also the glass connecting tube of the reference electrode were ground conically. As a result the cell volume could be minimized, while the conical ends of these capillaries permitted excellent sealing of the cell.

The small cell volume also made the correct positioning of the three electrodes possible. They are positioned in such a way that a large *IR* drop is avoided. This aspect permits the use of eluents that contain low concentrations of supporting electrolyte; hence the level and noise of the background current can be reduced.

The horizontal position and the conical end of the mercury capillary gives very short drop times, of the order of 10 msec. Such a drop time is obtained by using a short (2.5 cm) capillary with a conical end and a mercury height of *ca.* 35 cm. In an earlier paper[5] we showed that it is advantageous to use short drop times in order to improve the signal-to-noise ratio. The drop time in this design is barely influenced by the flow-rate. The efficiency of the detector is also enhanced by directing the eluent stream on to the mercury drop, which stimulates the mass transport to the electrode due to greater convection.

Apparatus

The chromatographic system consisted of an Orlita TW 1515 pump (Orlita, Stephan-Werke, Hameln, G.F.R.) with a damping device, an injection valve (Rheodyne, Berkeley, Calif., U.S.A.) and a pressure gauge (Chrompack, Middelburg, The Netherlands).

The column (28 cm × 4.6 mmI.D.) was packed with LiChrosorb RP-8, 10μm (Merck, Darmstadt, G.F.R.). The electrical currents were measured with a home-made potentiostat/amplifier, controlled on a Tektronix 5103N oscilloscope (Tektronix, Beverton, Ore., U.S.A.) and recorded with an HP 7046 dual-pen XY recorder (Hewlett-Packard, San Diego, Calif., U.S.A.). We further used a wall-jet electrode detector (EA 1096/2, Metrohm, Herisau, Switzerland) and a UV detector (LC 3 U.V., Pye Unicam, Cambridge, Great Britain). The mercury capillary was supplied by Metrohm (1091/1). The applied voltage was always measured *versus* a home-made Ag | AgCl | 1 *M* LiCl, methanol | water (50:50) reference electrode. The counter electrode was made of platinum and the DME detector compartment of Plexiglass.

Chemicals and procedure

In every measurement water–methanol mixtures were used as the eluent, which contained 0.1 *M* potassium nitrate and 0.02 *M* nitric acid for the DME detector and a concentration of 0.01 *M* potassium nitrate and $5 \cdot 10^{-3}$ *M* nitric acid for the wall-jet electrode detector and the UV detector.

The structures of the thiourea compounds studied are shown in Table I. Methanol, silver chloride, lithium chloride, nitric acid, potassium nitrates, N-phenylthiourea and N,N'-diphenylthiourea were Baker "Analyzed" chemicals (J. T. Baker, Phillipsburg, N.J., U.S.A.). Thiourea and allylthiourea were supplied by Merck and 4-methylthiosemicarbazide by Janszen Pharmaceutics (Beerse, Belgium). ETU was received from the Food and Drug Administration, Ottawa, Canada. Urine samples were directly injected without further preparation.

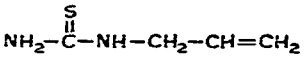
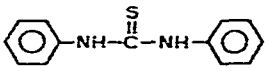
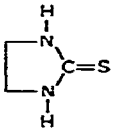
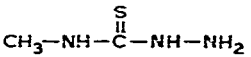
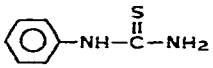
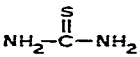
RESULTS AND DISCUSSION

The DME detector

The characterization of the detector (Fig. 1) was based on several testing

TABLE I

STRUCTURES OF THE THIOUREA COMPOUNDS STUDIED

Compound	*Structure*	*Molecular weight*
Allylthiourea		116.20
N,N′-Diphenylthiourea		228.32
Ethylenethiourea		102.16
N-Methylthiosemicarbazide		105.17
N-Phenylthiourea		152.23
Thiourea		76.12

criteria such as the time constant, the response volume, the dependence of the drop time on the flow-rate, the linearity of response as a function of concentration, the linearity of response as a function of concentration, the sensitivity and detection limit. Nitrobenzene was used as a test compound and the results were compared with those obtained with other detectors[6].

The most important results can be summarized as follows. The detector has a favourable time constant for all flow-rates higher than 0.5 ml/min; at these flow-rates the cell seems to behave as a mixing chamber. The calibration graph computed via linear regression revealed an excellent linear dynamic range of 3–4 orders of magnitude. The detection limit was computed for a signal-to-noise ratio of 2 and was 3 ng with no damping applied and 1 ng with a time constant of 0.3 sec.

The reaction

For the special application of the detector to thiourea (TU) compounds, advantage was taken of the specific complexation of the thiocarbonyl functional group with the mercury drop surface. The overall anode reaction is:

$$Hg^0 + 2\,TU \rightarrow Hg(TU)_2^{2+} + 2e$$

As a result of this reaction a polarographic wave is obtained, the limiting current of which is proportional to the concentration of the complexing species. A relatively low pH is required. The half-wave potential of the resulting polarographic wave is shifted towards more negative potentials compared with the free formation of mercury(II) ions.

The extent of this shift is dependent on the stability of the complex formed. All of the compounds of the thiourea group which were tested gave a good wave and hence lend themselve swell to this mode of detection.

As a favourable working potential +190 mV was chosen. This low applied positive potential has the advantages of not being critical with regard to oxygen, of giving a low noise and of providing a good selectivity.

Comparison of detectors

The performance of the DME detector for TU compounds was compared with those of a commercially available solid-state glassy carbon detector and UV detector.

Fresh stock solutions were prepared for these measurements and diluted for immediate determination. The stock solutions of thiourea, 4-methylthiosemicarbazide and ETU were prepared with water and the others with methanol. By varying the methanol content in water, the chromatographic conditions were chosen such that the k' value remained at approximately unity for all compounds tested.

The glassy carbon wall-jet electrode detector and the UV detector were used in series and their signals were recorded simultaneously with a dual-pen recorder. The measurements with the DME detector were performed separately. The indication potential for the wall-jet electrode detector was set at +1050 mV and for the DME detector at +190 mV. The UV detector was operated at 254 nm.

Calibration graphs of peak height *versus* concentration were computed for the given range by linear regression (least-squares method), with sensitivity A and regression coefficient R. From these calibration graphs the detection limits were calculated for a signal-to-noise ratio of 3. The noise was measured as the peak-to-peak variation in the baseline. The results for these detectors are given in Table II.

With the UV detector some problems were encountered in detecting 4-methylthiosemicarbazide, N-phenylthiourea and thiourea in this system. The solvent peaks disturbed the detection of these compounds, so only rough estimate of the detection limit was obtained.

From Table II it is obvious that the detection limits for the wall-jet electrode are superior to those for the DME detector. The results obtained with the glassy carbon electrode detector are comparable to the findings of Cox and Przyjazny[10].

The high detection limits for the arylthioureas with the DME detector can be explained only by the lower complexation constant, resulting from the use of a high percentage of methanol in the eluent (70% for N,N-diphenylthiourea and 25% for N-phenylthiourea) and possibly also by steric effects (see Table I). However, the detection limits in moles are not substantially higher than for other compounds. For the most important compounds (thiourea and ETU) the detection limits differ by about 20–50-fold between the two electrochemical detectors and are about the same for the DME and the UV detector.

Determination of ETU in urine

Although the sensitivity of the DME detector does not seem very attractive, it is the selectivity of this detection mode which seemed particularly worth pursuing. Monitoring of ETU in urine samples, which is an important problem and which so far has not been solved satisfactorily, was hence chosen as a practical example. ETU

TABLE II.

COMPARISON OF DETECTOR PERFORMANCE FOR DIFFERENT THIOUREA COMPOUNDS

Compound	*DME detector*				*Solid-state detector*				*UV detector*		
	R	*A (nA/ng)*	*Detection limit*		*R*	*A (nA/ng)*	*Detection limit*		*R*	*Detection limit*	
			ng	*mole*			*ng*	*mole*		*ng*	*mole*
N-Allylthiourea	0.999	0.016	74	$0.64 \cdot 10^{-9}$	0.999	1.4	1.1	$9.2 \cdot 10^{-12}$	0.998	2	$10 \cdot 10^{-12}$
N,N-Diphenylthiourea	0.994	0.0013	338	$1.48 \cdot 10^{-9}$	0.997	2.5	0.5	$2.2 \cdot 10^{-12}$	0.997	5	$10 \cdot 10^{-12}$
Ethylenethiourea	0.998	0.043	16	$0.16 \cdot 10^{-9}$	0.989	1.3	0.9	$8.8 \cdot 10^{-12}$	0.998	20	$0.2 \cdot 10^{-9}$
4-Methylthiosemi-carbazide	0.995	0.018	34	$0.32 \cdot 10^{-9}$	0.98	2.8	1.1	$10.4 \cdot 10^{-12}$	—	11	$50 \cdot 10^{-12}$
N-Phenylthiourea	0.997	0.0019	155	$1.02 \cdot 10^{-9}$	0.997	1.2	1.0	$6.8 \cdot 10^{-12}$	—	6	$40 \cdot 10^{-12}$
Thiourea	0.998	0.086	7	$0.09 \cdot 10^{-9}$	0.998	19.5	0.15	$2.0 \cdot 10^{-12}$	—	5	$60 \cdot 10^{-12}$

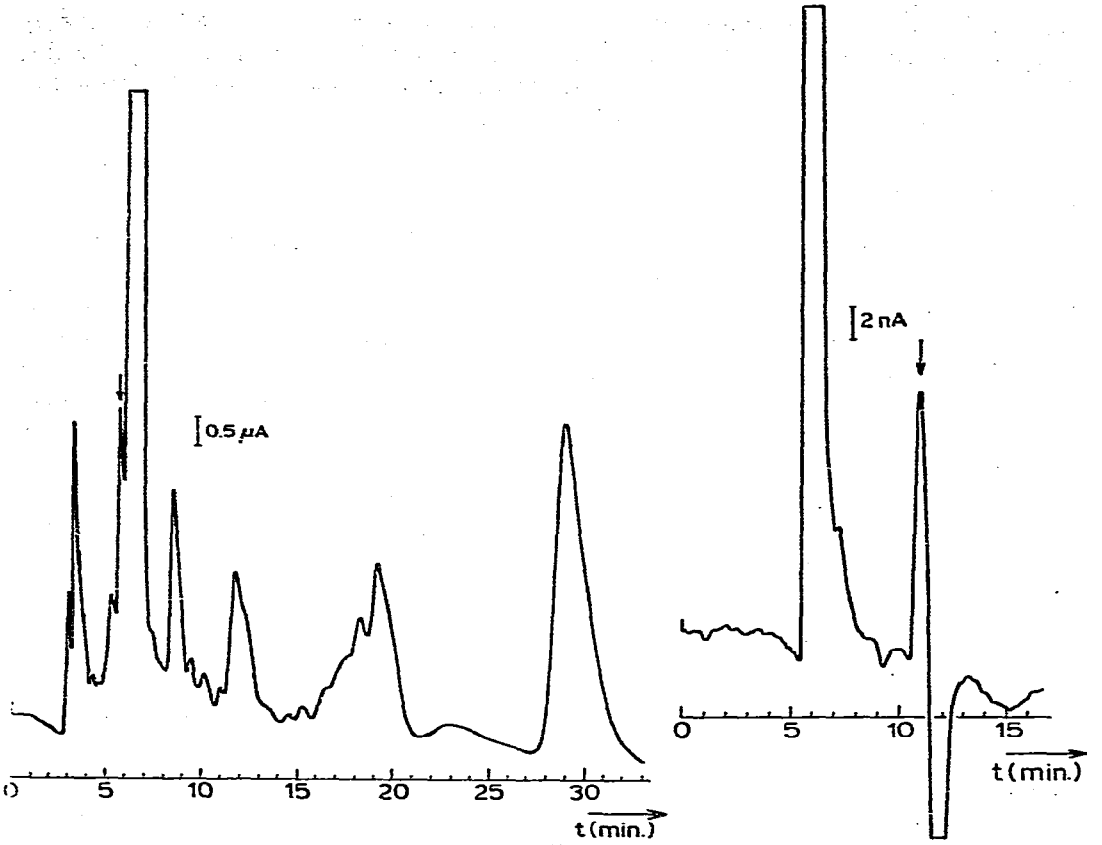


Fig. 2. Wall-jet electrode detector: chromatogram of urine (20 μl injected) spiked with 1.0 μg of ETU (indicated peak). $E_{ind} = +1050$ mV. Chromatographic conditions: flow-rate, 1.2 ml/min; 1% methanol in water, 10^{-2} *M* KNO_3, $5 \cdot 10^{-3}$ *M* HNO_3; column, 28 cm × 4.6 mm I.D., 10-μm LiChrosorb RP-8.

Fig. 3. DME detector: chromatogram of urine (20 μl injected) spiked with 1.1 μg of ETU (indicated peak). $E_{ind} = +190$ mV. Chromatographic conditions: flow-rate, 0.9 ml/min; 1% methanol in water, 0.1 *M* KNO_3, 0.02 *M* HNO_3; column as in Fig. 2.

was determined directly in urine under the same chromatographic conditions as in the measurements above (see Figs. 2 and 3).

Owing to its high selectivity, the DME detector enables the amount of ETU in urine to be measured without sample preparation, whereas with the wall-jet electrode detector under otherwise identical conditions it is hardly possible to determine ETU in spite of its high sensitivity. Another problem with the solid-state detector is caused by the decrease in its sensitivity as a result of poisoning of the electrode surface after multiple injections of urine. Even after one injection the response was observed to decrease significantly. The linearity, sensitivity and detection limit of the DME detector from a calibration graph for urine samples spiked with ETU are comparable to those in artificial solutions.

The detection limit was 10 ng for a signal-to-noise ratio of 3 with a noise of

0.1 nA. A regression coefficient of 0.999 for a linear range from 0.092 to 1.108 μg and with a sensitivity of 0.032 (nA/ng) was observed. The reproducibility for this detection mode was 2.1% (relative standard deviation) ($N = 10$) and linearity was usually observed over 2 or 3 orders of magnitude.

CONCLUSIONS

With regard to the overall performance in artificial solutions or samples with a simple matrix, the DME detector described here can at best be comparable to a UV detector. For the thiourea compounds studied it is significantly inferior to the glassy carbon detector, particularly with regard to sensitivity. However, in a comparison of selectivity and surface problems, particularly in a complex matrix such as urine, then the DME principle will be advantageous. The selectivity of the complexation reaction of the thioureas with mercury can therefore make it an interesting detection principle for many other organosulphur compounds where the sulphur exhibits good complexation properties, *i.e.*, a suitable oxidation state and steric configuration. A further advantage is the lack of interference from oxygen as one is working in the positive voltage range.

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