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THIN-LAYER POLAROGRAPHIC DETECTOR FOR THE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETECTION OF THIOUREA DERIVATIVES

ANTONÍN TROJÁNEK and MILOSLAV KOPANICA*
UNESCO Laboratory of Environmental Electrochemistry, J. Heyrovský Institute of Physical Chemistry and Electrochemistry, Czechoslovak Academy of Sciences, Jilská 16, 110 00 Prague 1 (Czechoslovakia)
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SUMMARY

A polarographic thin-layer detector for high-performance liquid chromatography, was devised. The detector has a sandwich structure with a working surface area of the mercury electrode of 0.25 mm^2. The detector was applied to the direct amperometric detection and for detection with reactivation of the electrode surface by voltage pulses. It is shown that the latter detection mode is suitable for the determination of compounds that form insoluble or complex mercury salts, *e.g.*, thiourea. Thiourea and its derivatives can be separated and determined in a wide concentration range and the detection limit for thiourea corresponds to 2 ng injected. Thiourea can be determined directly in urine.

INTRODUCTION

The use of mercury as the electrode material enables flow-through polarographic detectors to be constructed, where the problems connected with activity changes of the working electrode surface are eliminated by the renewal of the mercury surface. The periodic renewal of the mercury surface results in current oscillations, which increase the noise level and in most instance make it impossible to use voltammetric measurement modes based on accumulation of the analyte on the surface or inside the electrode. In these instances the use of a static mercury electrode is more advantageous. The static mercury electrode is renewed non-periodically, in liquid chromatography before each sample injection.

Detectors with static mercury electrode are, from the constructional point of view, similar to those with solid electrodes, among which the wall-jet[1] and thin-layer[2] types are widely applied analytically. The PAR Model 310 polarographic detector and its modifications[3,4] can be considered as the mercury counterpart of the wall-jet system. With this detector the column effluent is directed by a tube to the surface of the static mercury drop electrode, situated in a large vessel. The construction and properties of a thin-layer polarographic detector are presented in this paper.

As a practical application of the proposed detector, the separation and deter-

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mination of thiourea (TU) and some of its derivatives, such as α-naphthylthiourea (ANTU), β-naphthylthiourea (BNTU) and N,N'-diphenylthiourea (DPhTU), is described. These compounds are used as fungicides[5], herbicides[6] and rodenticides[7]. The presence of TU in urine was reported to be a non-specific indicator of cancer[8]. TU and its derivatives are toxic owing to their influence on the metabolism of carbohydrates[9]. Moreover, TU has been listed as a carcinogen since 1983[10].

EXPERIMENTAL

Chemicals

Ammonium acetate buffer solution (pH 4.6) was prepared from analytical-reagent grade chemicals. Methanol, used as the modifier of the mobile phase (60% methanol, 40% buffer), was purified by distillation with metallic magnesium. TU was of analytical-reagent grade (Lachema, Brno, Czechoslovakia); its derivatives were synthetized at the Department of Organic Chemistry, Charles University, Prague.

The mobile phase and the injected sample solutions were degassed using helium.

Apparatus

A Spectra-Physics Model SP 8770 isocratic pump, a Rheodyne 7125 sample injector and a Separon Six C 18 (5μm mean particle diameter) glass chromatographic column (150 × 3.2 mm I.D.) from Laboratorní Přístroje (Prague, Czechoslovakia) were used. In experiments where direct injection of the sample into the detector was necessary, the above injector with a 500-μl sample loop was directly connected to the detector by a stainless-steel tube (150 × 0.25 mm I.D.). To keep the pump in good operational condition in these experiments, one chromatographic column was placed before the injection valve as a pressure load. The potentiostat and circuitry for current signal processing (current follower and analogue memory with a time constant of 60 msec) were assembled using operational amplifiers.

The polarization of the working electrode by voltage pulses and current sampling was controlled by pulses derived from the mains frequency. The current signal was recorded using a TZ 4200 line recorder (Laboratorní přístroje) and a PM 3210 storage oscilloscope (Philips, Eindhoven, The Netherlands).

Detector

As shown in Fig. 1, the classic sandwich structure was preserved in the proposed detector. A PTFE spacer (1), thickness 0.2 mm, delineates a 0.25 × 3.0 mm channel (volume 0.15 μl) between two blocks made of organic glass; the sample solution flows through this channel. The liquid from the column is introduced into the detector by a stainless-steel capillary of I.D. 0.25 mm (2) and is led out by a capillary of I.D. 0.4 mm (3), which simultaneously serves as the auxiliary electrode. In the upper block is situated a chamber (7) where the aqueous saturated Ag–AgCl reference electrode is placed; this electrode is connected electrolytically with other electrodes by means of a ceramic plug (8).

Mercury is introduced into the detector from a reservoir (not shown in Fig. 1) by a PTFE tube of I.D. 0.4 mm through a simple rotary valve (4) made of organic glass and a PTFE rotor. Electrical contact to the working electrode is made by the

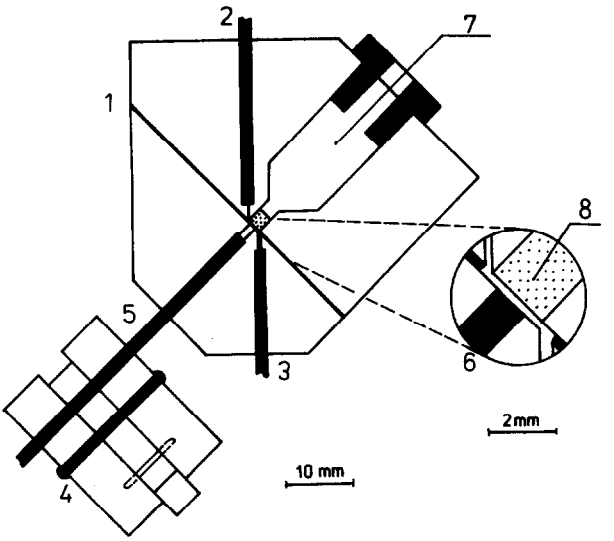

Fig. 1. Polarographic detector. 1 = PTFE spacer; 2 = inlet capillary; 3 = outlet capillary (auxiliary electrode); 4 = mercury rotary valve; 5 = mercury-supplying capillary (working electrode); 6 = hollow filled with mercury; 7 = chamber for the reference electrode; 8 = ceramic plug.

stainless-steel tube (5) between the rotary valve and the detector. To ensure contact between the end of the stainless-steel tube and the mercury column (diameter 1 mm) (6), it is advisable to cover the end of the stainless-steel tube with mercury after its previous deposition with nickel by electrolysis. The width of the channel in the PTFE spacer delineates the working surface area of the electrode exposed to the flowing solution, which is 0.25 mm^2.

RESULTS AND DISCUSSION

Detector performance

Response rate. The response rate, usually expressed as the value of the rate constant of the exponential response of the detector on a stepwise change of the input concentration, is a property of the whole detection system[11]. To characterize the detector without the contributions of other parts of the overall system, measurement was effected by direct injection of the sample into the detector by the simple arrangement described above.

Mobile phase containing dissolved oxygen from the air was chosen as the sample. The electrolytic current corresponding to oxygen reduction was recorded at constant potential of -1.00 V without electronic damping using the oscilloscope. The determined values of the time constant, τ, and the response volume, V_r (V_r is the product of τ and the volume flow-rate), are presented in Table I. It follows from Table I that the response volume is approximately 10 μl and slowly increases with increasing flow-rate.

In the pulse measurement mode the mercury electrode was polarized by rectangular voltage pulses of 100 msec duration at alternating potential values of $+0.10$

TABLE I

DEPENDENCE OF THE TIME CONSTANT AND RESPONSE VOLUME ON FLOW-RATE

Flow-rate (ml/min)	*Constant potential*		*Pulse technique*	
	τ *(sec)*	V_r *(μl)*	τ *(sec)*	V_r *(μl)*
0.2	2.7	9.0	3.3	11.0
0.4	1.4	9.3	2.1	14.0
0.6	0.95	9.5	1.6	16.0
0.8	0.72	9.6	1.5	20.0
1.0	0.6	10.0	1.45	24.2

and −1.00 V. The current was measured during an interval of 20 msec at the potential of −1.00 V at the end of the corresponding voltage pulse. The data in Table I show that the application of the pulse technique results in an appreciable decrease in the response rate. The distortion of the current signal caused by the analogue memory is highest in the region of high flow-rates, where the response rate of the detector itself is high.

Reproducibility of response. To obtain a good reproducibility of the response of the proposed detector, of greatest significance is the quality of the mercury lock. The space between the rotor of the valve and the surface of the mercury electrode should be free from any elastic element formed, *e.g.*, by an air bubble, otherwise mercury can be compressed after closing the valve, which results in a decrease in reporducibility and a decrease in the response rate. The reproducibility of the response was determined by direct injection of non-degassed mobile phase into the detector using the constant-potential (−1.00 V) measurement mode under conditions identical with those described above. Optimum results were obtained using the following procedure. The flow of the mobile phase was first stopped either using a suitable bypass or simply by turning the injection valve to the intermediate position (this is possible owing to the nature of the electronic regulation of the pump operation). During the brief opening of the mercury valve, the inner space of the detector was filled with mercury and by the subsequent introduction of the mobile phase into the detector the surplus mercury was flushed out to waste. During the period when the detector is filled with mercury, the working electrode should be disconnected to prevent short-circuiting with the auxiliary electrode. After reconnection of the working electrode and short-circuiting of the electrode (serveral tens of seconds), the detector was ready for measurement. The results of 30 measurements, each with a freshly prepared surface of the mercury electrode, gave a steady-state current response with a relative standard deviation of 0.62%.

Chromatographic detection of thiourea and its derivatives

The detection mode of TU and TU derivatives is based on the nature of the voltammetric and polarographic behaviour of these compounds[11–16]. Anodic polarographic waves of these compounds are due to the formation of mercury salts at positive potentials. In acidic or neutral media the electrode process is reversible and diffusion controlled and the product is a soluble complex of the type $Hg(TU)_2^{2+}$. In alkaline media the electrode process is irreversible and the product is insoluble HgS.

The formation of a mercury compound, which is adsorbed on the electrode surface, enables cathodic-stripping voltammetry[14] to be used for the determination, and the sensitivity of this mode can be substantially increased by adsorptive accumulation of the mercury complex in a slightly acidic medium[16]. In this determination TU yields a cathodic peak at a potential of −0.65 V (*vs.* SCE), and at this potential the mercury complex, accumulated previously on the electrode surface at a potential of +0.20 V, is stripped off.

From the above data three possible modes of voltammetric detection of TU using the mercury electrode in the flow system seem possible:

(a) direct measurement of the anodic current at a potential of approximately +0.20 V;

(b) measurement of the anodic current combined with activation of the mercury electrode surface by stripping off the adsorbed complex at a potential of −0.70 V (or more negative);

(c) accumulation of the mercury complex at a potential of approximately +0.20 V, followed by measurement of the cathodic-stripping current at a potential of −0.70 V.

The measurement mode (a) has been verified by Hanekamp *et al.*[17]. The detector used was that with a dropping mercury electrode and the detection limit for TU was 7 ng and that, for DPhTU 338 ng. In the anodic detection of TU using a static mercury electrode, the activity of the mercury surface decreases rapidly during the measurement at constant potential owing to adsorption of the mercury complex on the electrode surface. Consequently, the peak heights are mutually influenced

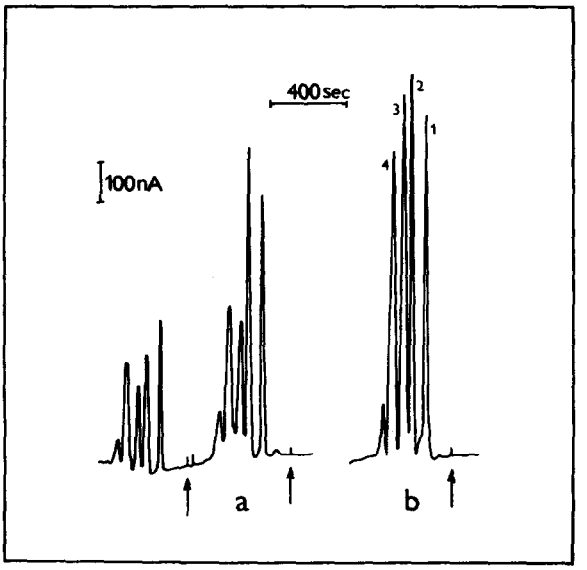

Fig. 2. Chromatograms of a mixture of thiourea derivatives. Mobile phase: 0.1 *M* acetate buffer (pH 4.6)–methanol (40:60, v/v), flow-rate 0.4 ml/min. Peaks: 1 = TU; 2 = BNTU; 3 = ANTU; 4 = DPhTU (400 ng of each). (a) Detection at a constant potential of +0.19 V, second injection performed without renewal of the electrode; (b) pulse-mode detection.

when two or more compounds of the described type are chromatographically separated (see Fig. 2).

The detection mode involving reactivation of the electrode surface [detection mode (b)] was tested using the described mobile phase, which ensure rapid separation of the derivatives mixture. Constant activity of the electrode surface was achieved by periodic changes of the potential of the mercury electrode between the working value and the value at which the adsorbed species are stripped off. In the course of the measurement the electrode was thus polarized by voltage pulses. The optimum potentials and the duration of the pulses were determined experimentally, *i.e.*, the values chosen were those at which the maximum value of the signal-to-noise ratio was found. The experiments showed optimum potential values of +0.19 V (detection) and −0.80 V (reactivation). Current measurement was carried out during the last 20 msec of the 160-msec long potential pulse at +0.19 V. Optimum results were obtained under the conditions when the durations of the two voltage pulses were identical. Prolongation of the cleaning pulses at a potential of −0.80 V was found not to improve the quality of the signal, whereas shortening of the duration of the cleaning pulses led to a decrease in the reactivation effect.

In the described detection mode the peak heights do not interfere each other and in series of repeated injections of the sample (a mixture of TU, DPhTUM ANTU and BNTU, 400 ng of each) carried out without the renewal of the mercury electrode the first three chromatograms were identical and in the fourth measurement the peak heights decreased by 15–20% of their original height. The measurement of the dependence of the concentration of the sample substances on the peak heights showed that this dependence was linear in the range corresponding to 2–1000 ng of TU injected. The slope of this dependence was 2.03 A/g, with a correlation coefficient of 0.9996. The detection limit, determined as the three times the noise amplitude measured peak to peak, was 2 ng. With DPhTU the concentration dependence was linear in the range 25–5000 ng injected, with a correlation coefficient of 0.9978, a slope of 0.16 A/g and a detection limit of 25 ng.

As far as mode (c) is concerned, it was established experimentally that detection

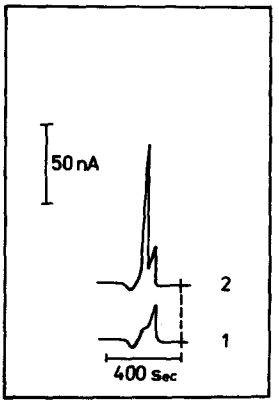

Fig. 3. Chromatograms of dilute urine. Mobile phase: 0.1 *M* acetate buffer (pH 4.6)–methanol (40:60, v/v), flow-rate 0.4 ml/min, pulse-mode detection. 1, Urine diluted with mobile phase in the ratio 1:1; 2, diluted urine spiked with TU (2 μg/ml).

based on current measurement in different intervals of a pulse during which the electrode was kept at a potential of −0.80 V (after prior accumulation of the mercury complex on the electrode surface at +0.19 V for 10–800 msec) gave no improvement compared with the above-described mode of reactivation of the electrode surface. Although the signal increased, the detection limits were not really improved owing to a higher noise level.

Determination of thiourea in urine

The high sensitivity and selectivity of the detection mode involving reactivation of the mercury electrode surface permits the direct determination of TU in urine. Urine (without any pre-treatment) was diluted with the mobile phase in the ratio 1:1 and 20 μl of the mixture was injected on to the column. A sample of non-pathological urine spiked with TU (2 μg/ml) yielded a well developed peak, which indicates the possibility of the direct determination of TU in urine at levels shown to 20 ng in 20 μl, as shown in Fig. 3.

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