POLAROGRAPHIC AND VOLTAMMETRIC DETERMINATION OF 6-MERCAPTOPURINE AND 6-THIOGUANINE*

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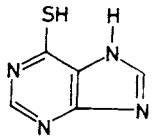
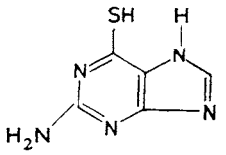
Conditions were found for the determination of 6-mercaptopurine (*I*) and 6-thioguanine (*II*) by TAST polarography, differential pulse polarography and fast-scan differential pulse voltammetry at a hanging mercury drop electrode. The detection limits were 10^{-6}, $8 . 10^{-8}$, and $6 . 10^{-8}$ mol l^{-1}, respectively. A further lowering of the detection limit to $2 . 10^{-8}$ mol l^{-1} was attained by preliminary accumulation of the determined substances at the surface of a hanging mercury drop.

Substances 6-mercaptopurine (*I*) and 6-thioguanine (*II*), which have been successfully used in the therapy for different forms of leukemia[1], are considered as potential chemical carcinogens[2] because of their ability to inhibit DNA and RNA synthesis[3].



I



II

A DC polarographic determination of 6-mercaptopurine has been described, based on the anodic wave corresponding to the formation of a compound with mercury[4,5], or cathodic waves corresponding to a 2-step reduction, first to 1,6-dihydropurine involving 4-electron exchange and then to 1,2,3,6-tetrahydropurine with 2-electron exchange[6,7]. Cathodic stripping voltammetry was employed for the determination of sub-micromolar concentrations of 6-mercaptopurine[8]. Attention has also been paid to the AC polarographic[9] and oscillopolarographic[10] determinations of this substance as well as to the mechanism[11] and analytical use[12] of the electrochemical oxidation and reduction of some purine derivatives.

As the sensitivity of DC polarography is relatively low, this study deals with the more sensitive determination of 6-mercaptopurine and 6-thioguanine by TAST polarography, differential pulse polarography (DPP) and fast scan differential pulse voltammetry (FSDPV) at a hanging mercury drop electrode (HMDE).

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EXPERIMENTAL

Reagents

The 1 mmol l^{-1} stock solutions of 6-mercaptopurine and 6-thioguanine in 0·1 mol l^{-1} H_2SO_4 were prepared by dissolving an exactly weighed amount of the pure substance (supplied by International Agency for Research on Cancer, Lyon). The low concentration solutions were prepared by exact dilution of the stock solutions with 0·1 mol l^{-1} H_2SO_4. The Britton–Robinson buffer solutions were prepared conventionally[13]. All the other chemicals were of reagent grade purity (Lachema Brno). Water was doubly distilled in a quartz apparatus.

Apparatus

A PA 3 polarographic analyzer interfaced to an XY 4105 recorder and a static mercury dropping electrode with a capillary diameter of 0·153 mm (all from Laboratorní přístroje, Prague) were used. TAST and DC polarography were carried out at a classical dropping mercury electrode. The electrode parameters at $h = 36$ cm were $m = 5{\cdot}77$ mg s^{-1} and $t = 2{\cdot}05$ s at a potential of 0 V *vs* SCE in 0·1 mol l^{-1} KCl. A three-electrode arrangement with saturated calomel reference and Pt auxiliary electrodes was employed. Unless otherwise stated, the mercury column height was 36 cm, controlled drop time 1 s, rate of polarization 5 mV s^{-1} and pulse amplitude −50 mV (DPP). The FSDPV measurements at the HMDE were carried out at the largest possible drop size obtained by opening the valve for 160 ms, with a polarization rate of 20 mV s^{-1} and pulse amplitude of −50 mV. Oxygen was removed from the solutions by passage of nitrogen, purified by passing through an alkaline solution of sodium anthraquinone-2-sulfonate and a solution of chromium (*II*) ions in dilute hydrochloric acid, both in contact with zinc amalgam. The pH was measured with a PHM 62 digital pH-meter (Radiometer, Copenhagen) with a combined glass and saturated calomel electrode.

RESULTS AND DISCUSSION

TAST Polarography

The formation of 2 waves for 6-mercaptopurine was observed at pH $\leqq$ 2 (Britton–Robinson buffer). At higher pH's the waves coalesce and shift towards negative potentials. Simultaneously, the TAST curves are distorted by the formation of sharp maxima, resulting in a typical peak shape. These waves cannot be recorded at pH > 5 as they are obscured by the supporting electrolyte background current. Even at pH $\leqq$ 2, substance 6-thioguanine exhibited only one wave with a height corresponding to the sum of both waves of 6-mercaptopurine. The pH dependence of $E_{1/2}$ and I_{lim} (or the values corresponding to the above mentioned peaks) is given in Table I.

As the best developed waves were obtained at pH 2, the use of 0·1 mol l^{-1} sulfuric acid as a supporting electrolyte was investigated. In this medium, 6-mercaptopurine yields two waves ($E^1_{1/2} = -970$ mV and $E^2_{1/2} = -1\,120$ mV). The height of the first wave is a linear function of the concentration, while the second wave has no analytical importance. Substance 6-thioguanine is reduced in a single wave ($E_{1/2} =$

TABLE I

The effect of pH on TAST and DP polarograms for 10^{-5} mol l^{-1} solution of 6-mercaptopurine and 6-thioguanine

pH	6-mercaptopurine				6-thioguanine			
	$E_{1/2}$, mV	I_{lim}, nA	E_p, mV	I_p, nA	$E_{1/2}$, mV	I_{lim}, nA	E_p, mV	I_p, nA
1·99	−1 020[a]	390[a]	−1 030[a]	270[a]	−1 040[a]	570[a]	−1 050[a]	840[a]
	−1 145[b]	180[b]	−1 135[b]	200[b]				
2·50	−1 220[c]	170[c]	−1 165	655	−1 165[c]	450[c]	−1 085	950
3·00	−1 260[c]	455[c]	−1 225	1 290	−1 240[c]	960[c]	−1 145	1 340
3·50	−1 310[c]	350[c]	−1 275	1 140	−1 270[c]	1 800[c]	−1 235	2 090
4·07	−1 320	510	−1 340	380	−1 335[c]	2 275[c]	−1 335	3 275
4·50	−1 360	410	−1 400	200	−1 420[c]	850[c]	−1 360	1 820
5·00	—[d]	—[d]	−1 445	225	−1 480	190	−1 400	340

[a] Values corresponding to the first wave or peak; [b] values corresponding to the second wave or peak; [c] values corresponding to a maximum on the measured curves; [d] TAST curves that could not be evaluated.

TABLE II

Parameters of calibration curves and detection limits (a/Sa slope and its standard deviation; b/Sb intersection and its standard deviation; $S_{i,c}$ the deviation of experimental points from calculated straight line; r correlation coefficient; dl detection limit)

Method	c mol l^{-1}	a/Sa mA mol^{-1} l	b/Sb nA	$S_{i,c}$ nA	r	dl mol l^{-1}
		6-mercaptopurine				
TAST	$(1-10).10^{-6}$	24/1·4	19/10	9	0·9965	1.10^{-6}
DPP	$(1-10).10^{-7}$	60/3·0	1/2	2	0·9962	8.10^{-8}
FSDPV[a]	$(1-10).10^{-7}$	26/1·7	−2/1	0·9	0·9957	6.10^{-8}
FSDPV[b]	$(1-10).10^{-8}$	68/1·3	−0·4/0·1	0·1	0·9989	2.10^{-8}
		6-thioguanine				
TAST	$(1-10).10^{-6}$	35/0·7	7/4	4	0·9995	9.10^{-7}
DPP	$(1-10).10^{-7}$	60/3·0	−3/12	2	0·9962	8.10^{-8}
FSDPV[a]	$(1-10).10^{-7}$	71·1/4·1	15/3	3	0·9950	6.10^{-8}
FSDPV[b]	$(1-10).10^{-8}$	188/8·8	−4/0·7	0·4	0·9978	2.10^{-8}

[a] Without accumulation; [b] accumulation 90 s.

= −990 mV) with a height corresponding to the height of the first wave of 6-mercaptopurine. Another wave with a strong maximum appears at lower 6-thioguanine concentrations.

The concentration dependence of the height of the first wave was linear in the range $10^{-4}-10^{-6}$ mol l^{-1} for both substances. The calibration curve parameters calculated by the linear regression method and the detection limit calculated according to Skogerboe and Grant[14], as already described[15], are given in Table II. This table indicates that the use of TAST polarography increases the sensitivity by one order of magnitude compared to DC polarography.

The limiting current for the first wave was diffusion-controlled as follows from the linear dependence on the concentration and on the square root of the mercury reservoir height in DC polarography. The logarithmic analysis of the first wave indicated the irreversibility of the reduction process. This was also confirmed by cyclic voltammetry of 10^{-3} mol l^{-1} depolarizers at a hanging mercury drop at a polarization rate of 10−100 mV s^{-1}.

Differential Pulse Polarography at a Dropping Mercury Electrode

At pH 2 both substances yielded two peaks coalescing with increasing pH and shifting towards negative potentials. The peak height was maximal at pH 3−4 (see Table I) but simultaneously less reproducible and was not linearly dependent on the concentration. The best developed and reproducible peaks were again obtained in 0·1 mol l^{-1} H_2SO_4. The more positive peak (E_p = −970 mV for 6-mercaptopurine and −980 mV for 6-thioguanine) with a height linearly dependent on the concentration in the range $10^{-5}-10^{-7}$ mol l^{-1} is analytically usable. A deviation from linearity occurs at higher concentrations. The parameters of the dependence of the peak height on the depolarizer concentration and calculated detection limits are given in Table II, from which it can be seen that DPP at a DME is approximately ten times more sensitive than TAST polarography.

Fast Scan Differential Pulse Voltammetry at a Hanging Mercury Drop Electrode

In 0·1 mol l^{-1} H_2SO_4 the peak height for both substances increased with the drop size, polarization rate and value of the modulation amplitude. Peaks with the best resolution were obtained at a polarization rate of 20 mV s^{-1} at the maximum drop size controlled by opening the valve for 160 ms. Under these conditions, a linear concentration dependence of 6-mercaptopurine peak at −875 mV and 6-thioguanine peak at −910 mV was obtained in the range $10^{-5}-10^{-7}$ mol l^{-1}. The data are given in Table II. The dependence deviated from linearity at concentrations above 10^{-5} mol . l^{-1}.

It has been found that the peak height increases with the time elapsed between the drop formation and recording of the curve, which can be explained by adsorptive accumulation of the studied substance at the working electrode surface. Fig. 1 depicts the dependence of the peak current on the time of accumulation for two different concentrations of 6-thioguanine. At a concentration of $2 . 10^{-7}$ mol l^{-1}, the electrode surface is fully covered after 5 min; however, an optimum reproducibility is attained with a 90 s accumulation. A decrease in the peak current observed at a concentration of $2 . 10^{-6}$ mol l^{-1} for an accumulation time longer than 3 min can be explained by the formation of a mercury compound that blocks the electrode surface that is visible in blackening of the drop surface. The current increase was independent of the accumulation potential. An increase in the accumulation rate attained by stirring the solution did not prove useful because of lower reproducibility of the results. The behaviour of 6-mercaptopurine was analogous but the electrode surface was saturated within 90 s. The means of evaluation of the FSDPV curves of extremely low concentration solutions is given at Fig. 2, where the dashed line corresponds to the base line from which the peak height is measured. The calibration curve parameters and the calculated detection limit are given in Table II. The mean relative deviation of $4 . 10^{-8}$ mol l^{-1} for 6-mercaptopurine and 6-thioguanine was 5·9 and 5·5%, respectively.

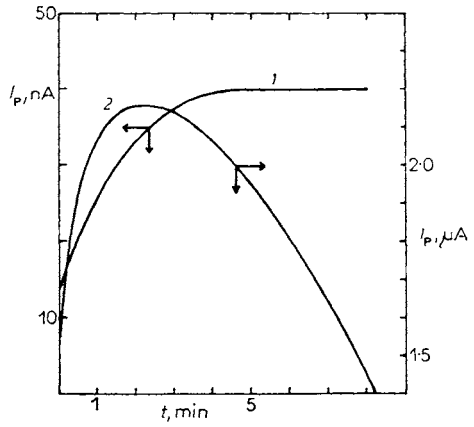

FIG. 1

The effect of the time of accumulation on the peak height for FSDPV of 6-thioguanine. The depolarizer concentration (μmol l^{-1}): *1* 0·2; *2* 2

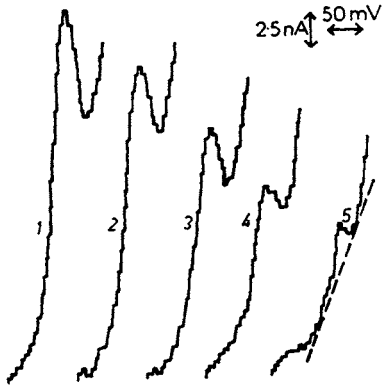

FIG. 2

Determination of 6-mercaptopurine using FSDPV at a HMDE with 90 s accumulation. The depolarizer concentration (μmol l^{-1}): *1* 0·2; *2* 0·16; *3* 0·12; *4* 0·08; *5* 0·04. Initial potential −500 mV, supporting electrolyte 0·1 mol l^{-1} H_2SO_4

FSDPV measurements in $10^{-5}-10^{-7}$ mol l^{-1} solutions of both substances can be performed in the presence of oxygen which substantially shortens the time of analysis. The slope of the linear calibration dependence decreased by approximately 30% but the obtained curves remained reproducible and readily evaluable. Oxygen must be removed in the concentration range $10^{-7}-10^{-8}$ mol l^{-1} as the curves are difficult to evaluate in its presence. The depolarizer solutions exhibited low stability when in contact with mercury. In a mercury-free medium, no concentration change was observed for 10^{-3} mol l^{-1} solutions after 14 days and for 10^{-6} mol l^{-1} solutions over 24 hours.

The study of these solutions by DPP at a DME in a Novák polarographic vessel with a mercury pool revealed that the initial concentration of 10^{-5} mol l^{-1} decreased by 0·2% after 15 min and by 16% after 120 min for 6-mercaptopurine and by 1% and 24% respectively, for 6-thioguanine. At an initial concentration of $2 . . 10^{-7}$ mol l^{-1} the decrease was 10 and 14% after 15 min and 80 and 90% after 120 min for 6-mercaptopurine and 6-thioguanine, respectively. In the analysis of dilute solutions, prolonged contact with mercury should be therefore avoided. From this point of view, it is advantageous to use FSDPV at a HMDE where a mercury pool is not formed. Furthermore, in the analysis of dilute solutions, the auxiliary platinum electrode should be separated from the analyzed solution by a salt bridge to prevent loss of analyzed —SH-containing compounds through electrooxidation to disulphides.

The sensitivity of the newly developed methods allows combination with preliminary thin layer chromatographic separation for determination of the studied substances in more complex matrices.

While the newly developed methods are less sensitive than fluorimetry[16] and HPLC[17,18] they can yield reliable results (especially FSDPV at a HMDE for concentrations up to 10^{-7} mol l^{-1}) within several minutes, and are thus a welcome extension of methods for the determination of these substances[19].

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