Journal of Pharmaceutical & Biomedical Analysis
Vol. 7, No. 12, pp. 1853–1859, 1989
Printed in Great Britain

0731–7085/89 $3.00 + 0.00

Short Communication

Polarographic studies of drugs of purine derivatives — II*

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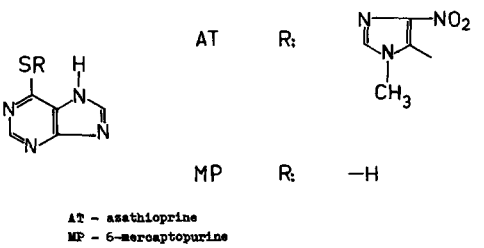
Keywords: *6-Mercaptopurine; azathioprine; differential pulse polarography; fast scan polarography; differential pulse cathodic stripping voltammetry.*

Introduction

Azathioprine, 6-(1-methyl-4-nitroimidazol-5-ilothio)purine and 6-mercaptopurine are synthetic purinic antimetabolics and belong to the group of cytostatic drugs. They are widely used in medicine, mainly as antileukemia and immunosuppressive drugs. These compounds were determined until now in pure form and in tablets by titration [1–4], spectrophotometrically [4–7] and electrochemically [8–10] and in biological material by high-performance liquid chromatography [11–15]. Direct current polarography [6, 16] and voltammetry [17–19] were also used for the determination of azathioprine (AT) and 6-mercaptopurine (MP).

Previously the authors were concerned with studies of AT and MP in pure form by direct current, alternating current, normal pulse polarography and voltammetry [20].



AT - azathioprine
MP - 6-mercaptopurine

*Presented at the "Third International Symposium on Drug Analysis", May 1989, Antwerp, Belgium.
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Further studies included electroreduction by fast scan (FSP) and differential pulse polarography (DPP) and an elaboration of a simple polarographic method of determining these depolarizers in tablets using apparatus commonly available in Poland which could be applied for routine studies. An attempt was also made to elaborate a method of determining AT and MP at nonagram concentrations in a biological material.

Experimental

Material and apparatus

AT and Azathioprine tablets a 50 mg lot no: 060686 ZChF 'Vis' (Poland); MP and Mercaptopurinum tablets a 50 mg lot no: 020787 ZChF 'Vis' (Poland). All other chemicals (from POCh Gliwice, Poland) were of analytical reagent grade and were used without additional purification.

Oscillopolarograph OP-4 (Unitra Telpod); pulse polarograph PP-04 (Unitra Telpod); xy/t recorder (ZDEMP); static mercury dropping electrode SMDE-1 (Laboratorni Přistroje); SP8-100 spectrophotometer (Pye Unicam); N-512 pH-meter (Mera Elmat); Commodore 64 personal computer.

Conditions

A three-electrode system and 20 cm^3 cells were used for polarographic studies. Mercury dropping electrode of efficiency m = 1.32 mg Hg s^{-1} (DPP, FSP) or static mercury dropping electrode (DPCSV) was the cathode. In studies by the DPP method the mercury drops were detached electromechanically with an impulse from the polarograph after the time t_{dr} = 2.0 s. A saturated calomel electrode was used for reference. A spirally coiled platinum wire was the third electrode. Oxygen was removed from the system by passing argon for 15 min. Studies were carried out at approximately 20°C.

Results and Discussion

Studies of pure substances

The polarographic reduction of AT and MP was studied by FSP and DPP over pH range 1.0–10.2 in different stock non-buffer and buffer solutions. Correct curves were obtained for AT solutions within the whole pH range studied. In acidic and neutral solutions (pH 2.7–6.9) AT underwent a three-step reduction. With an increase of pH values the peak potentials shifted towards more negative values (Table 1). In basic solutions, however, the reduction proceeded in one step and only one peak was observed. The potential of that peak at pH 8 was shifted by approximately 200 mV towards more negative values with respect to the potential of peak I in neutral solutions. The acetate buffer (AB) of pH 4.7 (0.2 mol dm^{-3}) was chosen as the best stock solution for further studies of AT. A two step polarographic reduction of MP was found in acidic and neutral solutions. The first reduction step was best outlined within pH 1–2, the second, however, was weak irrespective of the medium. Concentrations of 0.25 mol dm^{-3} $HClO_4$ and 0.2 mol dm^{-3} LiCl were chosen as stock solutions for further studies of MP (Figs 1 and 2).

The reduction of purine has been described only in acidic stock solutions [21]. The reduction and oxidation of MP over the pH range 1–9, however, was observed only on

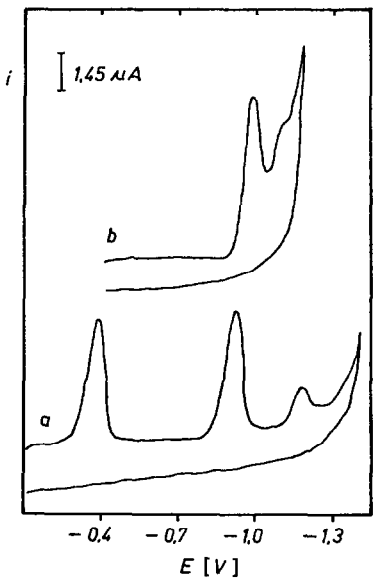

Figure 1
Fast scan polarograms recorded for the following solutions: (a) 4 μg cm^{-3} AT in 0.2 mol dm^{-3} acetate buffer pH 4.70; (b) 4 μg cm^{-3} MP in 0.2 mol dm^{-3} $HClO_4$.

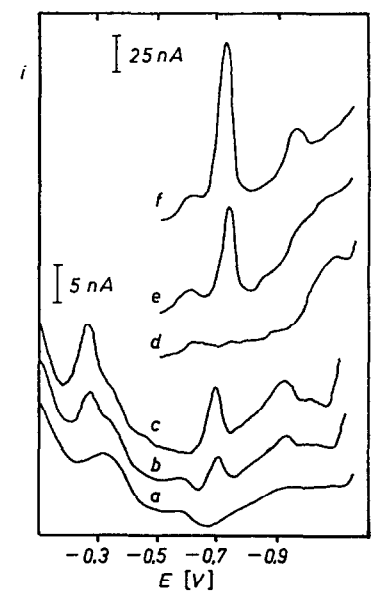

Figure 2
Differential pulse polarograms recorded for the following solutions: (a) blank–0.1 mol dm^{-3} acetate buffer pH 4.70; (b) 10 ng cm^{-3} AT; (c) 20 ng cm^{-3} AT. Blank–0.2 mol dm^{-3} acetate buffer pH 4.70 with supernatant from thymus cell culture (d) and 200 ng cm^{-3} AT after 1 h incubation (e) or without incubation (f).

stationary carbon electrodes [22]. The MP polarographic reduction in acidic solutions proceeds in two steps in a six-electron process with the addition of four protons. A four-electron reduction proceeds in the first step resulting in the formation of 1,6-dihydropurine. In the second two-electron step 1,2,3,6-tetrahydropurine is formed, which then undergoes hydrolysis to the 4-amino-imidazole form. The nitro group in the AT molecule undergoes reduction in the studied solutions in a six-electron process. An

Table 1
Polarographic characteristics of AT solution in 0.2 mol dm^{-3} acetate buffer pH 4.7 and MP solution in 0.25 mol dm^{-3} $HClO_4$

Compound	Method	Peak	E_p V	$10^4(i/C)$ $\mu A\ dm^3 mol^{-1}$	$\frac{\Delta i \cdot 100}{\Delta T \cdot i}$ %	$\Delta E/\Delta T$ mV	$\Delta E/\Delta pH$ mV
AT		I	−0.37	13.0	−0.88	0.70	54
	FSP	II	−0.88	12.5	−0.39	0.90	69
		III	−1.11	6.4	0.91	−0.30	105
		I	−0.28	1.08	0.65	0.68	57
	DPP	II	−0.74	0.92	−0.63	0.34	74
		III	−0.97	0.54	0.94	−0.20	94
MP	FSP	I	−0.98	12.5	1.57	2.33	105
	DPP	I	−0.85	1.44	1.81	0.91	
	DPCSV (0.1 mol dm^{-2} LiCl)	I	−0.29	142	—	—	—

amino group and two molecules of water are formed from that reaction with the participation of six electrons and six protons [20, 21]. The results of the present studies are in agreement with the data presented in the literature [17, 19–21].

Studies of the relationship between the AT and MP electrochemical reduction and temperature were carried out within the temperature range 10–45°C by FSP and DPP methods. The temperature coefficients of peak potentials and peak current intensities have been determined (Table 1). The influence of ionic strength of the solution on the AT and MP peak current intensities has been studied in 0.1 mol dm^{-3} AB of pH 4,7 with addition of increasing amounts of NaCl solution.

A change of the solution ionic strength in the range 0.10–1.0 caused a decrease in the peak height. The relationships between the AT and MP peak heights and potentials and the methanol content of the solution in the concentration range 0–50% was also studied by the FSP method.

Studies by the DPP method upon preconcentration on the hanging drop (DPCSV) were carried out only in MP solutions, since no preconcentration of AT on HMDE was found. A 0.1 mol dm^{-3} solution of LiCl was optimal in these studies, providing maximum sensitivity and reproducibility of the results (Fig. 3). The dependence of the MP peak height and potentials was studied on the ionic strength of solution in the 0.0025–0.20 range, stirring rate of solution, drop size in the 0.760–1.557 mm^2 range, preconcentration time in the 100–600 s range, preconcentration potential in the 0.0 to −0.20 V range and MP concentration in solution. A linear relationship between the peak heights and preconcentration time was found within the 100–300 s range at the preconcentration potential −0.05 V. The minimum detectable MP concentration was 1 ng cm^{-3} (5×10^{-9} mol dm^{-3}).

The AT and MP peak heights appeared to be proportional to their concentration in solution in the ranges: AT–FSP method from 1 to 40 $\mu g\ ml^{-1}$ I peak, from 20 to 160 $\mu g\ ml^{-1}$ II peak, from 1 to 160 $\mu g\ ml^{-1}$ III peak, DPP method from 10 to 100 ng ml^{-1} I–III peak, MP–FSP method from 5 to 100 $\mu g\ ml^{-1}$, DPP method from 40 to 120 ng ml^{-1} and DPCSV method from 2 to 25 ng ml^{-1} (preconcentration time 300 s) (Figs 2 and 3). The DPP method appeared to be most sensitive in the case of AT; it permitted determinations of concentrations from 10 ng cm^{-3} (approximately 3×10^{-8} mol dm^{-3}).

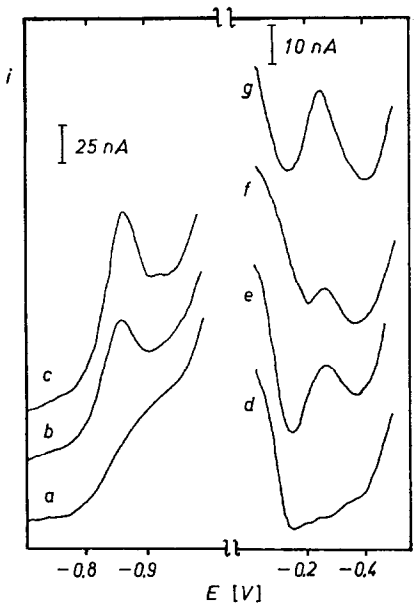

Figure 3
Polarograms recorded for solutions of MP: (a) blank–0.1 mol dm^{-3} $HClO_4$ (DPP); (b) 40 ng cm^{-3}; (c) 60 ng cm^{-3}; (d) blank–0.1 mol dm^{-3} LiCl (DPCSV); (e) 1 ng cm^{-3} (preconcentration time 600 s); (f) 2 ng cm^{-3} (preconcentration time 300 s); (g) 4 ng cm^{-3} (preconcentration time 300 s).

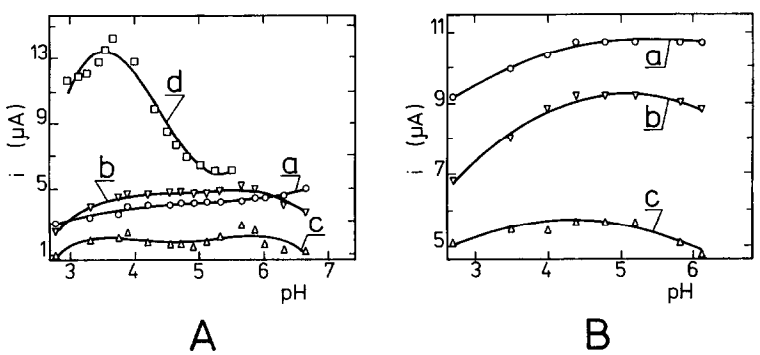

Figure 4
Dependence of the peak heights of AT and MP in 0.2 mol dm^{-3} acetate buffer on pH, (A) FSP and (B) DPP: (a) I peak AT; (b) II peak AT; (c) III peak AT; (d) I peak MP.

For MP studies, however, the DPCSV method appeared to be most sensitive permitting the determination of concentrations from 2 ng cm^{-3} (approximately 10^{-8} mol dm^{-3}).

The accuracy and precision of AT and MP determinations by FSP, DPP and DPCSV methods were calculated from 10 measurements in prepared solutions. The content of the studied depolarizers was determined from equations calculated from analytical curves by the least squares method (AT–II peak; MP–I peak). Statistical analysis of the results obtained (Table 2) shows that these methods have good accuracy and precision.

Studies of tablets

The average mass of 10 azathioprine tablets (50 mg 'Vis') and Mercaptopurinum (50 mg 'Vis') was determined. The tablets were powdered and amounts corresponding to 5 mg of the active substance were weighed into 50 cm^3 measuring flasks. Methanol (AT)

Table 2
Statistical evaluation of the results of AT and MP determinations in pure form and tablets

Form	Method	s	$s_{\bar{x}}$	w_z %	$x \pm t \cdot s_{\bar{x}}$ $n = 10$ $p = 0.95$
Pure AT	FSP (80 μg ml^{-1} solution)	0.351	0.124	0.43	80.12 ± 0.29 μg ml^{-1}
	DPP (50 ng ml^{-1} solution)	0.548	0.173	1.09	49.96 ± 0.39 ng ml^{-1}
Azathioprine 'Vis' tablets 50 mg each (49.85 mg/tablet)*	FSP	0.957	0.255	1.93	49.57 ± 0.55 mg/tablet 99.14 ± 1.10%
Pure MP	FSP (50 μg ml^{-1} solution)	0.381	0.120	0.76	49.98 ± 0.27 μg ml^{-1}
	DPP (100 ng ml^{-1} solution)	1.295	0.409	1.29	100.07 ± 0.93 ng ml^{-1}
	DPCSV (5 ng ml^{-1} solution)	0.091	0.095	6.07	4.97 ± 0.21 ng ml^{-1}
Mercaptopurinum 'Vis' tablets 50 mg each (50.88 mg/tablet*)	FSP	0.388	0.122	0.76	51.04 ± 0.27 mg/tablet 102.09 ± 0.55%

* Results of content determination by means of spectrophotometry; 10 μg ml^{-1} AT in 0.1 mol dm^{-3} HCl at the wavelength 280 nm; 10 μg ml^{-1} MP in 0.1 mol dm^{-3} NaOH at the wavelength 310 nm.

(40 cm^3) or 5 cm^3 of 0.1 mol dm^{-3} NaOH and 35 cm^3 of H_2O (MP) was added and the whole mechanically shaken for 30 min. The flasks were filled with methanol (AT) or water (MP). The solutions were filtered and the first 10 cm^3 of the filtrate removed. To 10 cm^3 measuring flasks 1.0 cm^3 of the filtrate (AT) introduced, then 2.0 cm^3 of 1 mol dm^{-3} AB of pH 4.7 was added or 1.5 cm^3 of the filtrate (MP) and 2.0 cm^3 of 1 mol dm^{-3} LiCl solution. The flasks were filled with water. The polarographic solutions then contained approximately 10 μg cm^{-3} of AT or 15 μg cm^{-3} of MP. The determinations were carried out by the FSP method.

The content of active substance was determined from the equations of previously drawn analytical curves, determined by the least squares method. In order to compare the results obtained, the extracts from the tablets were diluted with 0.1 mol dm^{-3} HCl (AT) or 0.1 mol dm^{-3} NaOH (MP) to the concentration of 10 μg cm^{-3}. The content of active substances was determined spectrophotometrically at the wavelength 280 nm (AT) or 310 nm (MP). The content of AT or MP in tablets was determined from the previously drawn analytical curves. A statistical analysis of the results (Table 2) indicates that the methods elaborated are of good accuracy and precision. The FSP method, due to its high accuracy, speed and simplicity is especially recommended for routine studies.

Studies on the biological material

Pilot experiments of the applicability of the AT determination in studies of its diffusion to leukemia cells were also carried out. Cultures of thymus cells containing 10 and 63 million cells cm^{-3} were taken for the studies. The solution used for the culture contained PbS (NaCl, KCl, $CaCl_2$, Na_2HPO_4, KH_2PO_4), 1% glucose, and 0.1% glutathione. AT was added to the cell culture in an amount to give a final concentration of 1 μg cm^{-3}; incubation was for 1 h at 37°C. The mixture (1 ml) was then introduced to each

polarographic cell followed by the addition of 1 cm^3 of AB of pH 4.7, 3 cm^3 of H_2O and 0.05 cm^3 of 1% EDTA solution. Polarograms were recorded by the DPP method (Fig. 2). The AT content was determined from the second peak by comparison with the standard (solution containing 200 ng cm^{-3} of AT and the liquid from the cell culture not submitted to incubation). It was found that about 40% of AT present in the solution underwent diffusion to the cells. This confirms the applicability of the DPP method to AT transfer studies in leukemia cells.

Acknowledgement — This work was done in the framework of research project CPBP 01.17.

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[*Received for review 29 March 1989; revised manuscript received 20 July 1989*]