CATHODIC STRIPPING VOLTAMMETRY OF 2-THIOURACILS

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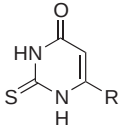
Four 6-R-2-thiouracils (R = H, methyl, propyl, benzyl) were examined by differential pulse cathodic stripping voltammetry on mercury electrode. The research led to a very sensitive analytical method that allows their determination on nanomolar level. The detection limit of the 6-propyl derivative is as low as 1.0×10^{-9} mol dm^{-3}. The procedure is very simple and utilizes only most common chemical reagents (such as acetate buffer). The buffer concentration plays an important role in the preconcentration stage, due to the adsorption processes accompanying electrode reactions. The new analytical method was tested with commercial samples of various antithyroid drugs.

Keywords: Differential pulse cathodic stripping voltammetry; Thiouracils; Antithyroid drugs; Pyrimidines; Nucleobases; Electrochemical determination; Electrochemistry.

Thiouracils are a group of substances used mainly as a thyreostatics[1]. The most common derivatives are 2-thiouracil (TU, 4-hydroxy-2-mercapto-pyrimidine), 6-methyl-2-thiouracil (MTU, 4-hydroxy-2-mercapto-6-methyl-pyrimidine), 6-propyl-2-thiouracil (PTU, 4-hydroxy-2-mercapto-6-propyl-pyrimidine) and 6-benzyl-2-thiouracil (BTU, 4-hydroxy-2-mercapto-6-benzyl-pyrimidine) (Chart 1). All these compounds suppress the formation of thyroid hormones by inhibition of thyroglobulin iodination as well as reduction of triiodothyronine and thyroxine. Besides, these thiols have a great number of other pharmaceutical and chemical applications, all of them being also used illegally as a fodder ingredient. The addition of thiouracils leads to an increase in meat weight caused by enhanced water absorption.



	R
TU	H
MTU	CH_3
PTU	$CH_3CH_2CH_2$
BTU	$PhCH_2$

CHART 1

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Since 1981 the praxis has been forbidden by the European Union (directive 86/469/EC), as the drugs can be harmful for human health[2].

Due to the applications of the above mercaptopyrimidines, there is a need for a new, sensitive determination method for these substances. The most recent, worldwide scaled survey reveals application of separation techniques such as gas[3] and liquid[4,5] chromatography.

The first documented polarographic experiments performed on thiouracils were carried out by Manoušek and Zuman[6] almost 50 years ago. Other studies concern direct current polarography[7,8] (DCP) or differential pulse polarography[9] (DPP), cyclic voltammetry[10] (CV) and square-wave voltammetry (SWV) on static mercury drop electrodes[11] and cylindrical carbon fiber microelectrodes[12]. Unfortunately, these methods do not fulfil expectations imposed by the European Community regulations. The detection limits should reach the level of 25 ppb in meat samples. Our goal has been electrochemical determination that does not require any chemical preconcentration. The research led to a new and simple procedure based on differential pulse cathodic stripping voltammetry (DP CSV) on hanging mercury drop electrode.

EXPERIMENTAL

Apparatus

All experiments were carried out with a μAutolab II voltammeter and General Purpose Electrochemical System (GPES) software (Eco Chemie B.V., The Netherlands). The working electrode was a controlled-growth mercury drop electrode (CG MDE) connected to an appropriate control unit (AGH University of Science and Technology, Krakow, Poland). The counter electrode was a platinum wire and 3 M KCl/AgCl/Ag electrode served as the reference electrode. It was separated from the cell by a saturated KNO_3 bridge, as chloride anions interfere with thiouracil signals. The pH values were determined with an Elmetron pH-meter (Poland) equipped with a Sensor glass electrode (Poland).

Reagents and Solutions

All tested mercaptopyrimidines were of pure reagent grade, MTU and PTU from Aldrich, TU from Fluka and BTU from Sigma. Other substances were of analytical grade. Triply distilled water was used in all experiments.

Stock 1.0×10^{-3} M solutions of all compounds were prepared by adding an appropriate weighed amount of the thiol to 1 ml of 1×10^{-1} M NaOH. After dissolving the substance, the solution was transferred to a 25 ml volumetric flask and diluted with water up to the mark. Working solutions were obtained by dilution with water.

Britton–Robinson buffers were prepared as recommended in the literature. Other buffers were made from suitable acids titrated with NaOH. The final pH value was controlled with a pH-meter. In the following description, “buffer concentration” is the term for the sum of the concentration of the acid and its salt.

Procedure

An appropriate volume of the buffer was transferred to the voltammetric cell. After the addition of thiouracil, the solution was diluted with water to 10 ml. The content was then deoxygenated by purging argon for 10 min. Blank samples were prepared in almost the same way.

In most measurements, the first step was electrodeposition on a freshly generated mercury drop, carried out at +200 mV for 180 s while stirring. A voltammogram was then recorded after 20 s equilibration time. The parameters of the differential pulse method were as follows: potential range from +300 to –900 mV, potential step 5 mV, pulse amplitude 20 mV. The blanks were subtracted using a computer.

RESULTS AND DISCUSSION

Preliminary Voltammetric Studies

At this stage, the thiouracils were tested at the concentration level 2.0×10^{-7} mol dm^{-3} under the conditions described above. All of them give signals in Britton–Robinson buffers (diluted ten times) in the pH range 2.6–11.0. The number and position of voltammetric peaks due to the thiouracils depend on pH. In acidic media, there are two strong and symmetric signals, which are the result of reduction of mercury(II) and mercury(I) thiolates. At pH close to 7, mechanisms of the electrode reactions change. The voltammograms show many additional signals which make interpretation of the curves impossible. In alkaline buffers, the main signals interfere and give only one maximum. However, its height is lower than the sum of the currents in acidic media. All experiments performed proved high irregularity of this peak, which leads to the conclusion that there are no reasons for the use of alkaline media.

The activity of thiouracils was also tested in more acidic and more alkaline electrolytes than Britton–Robinson buffers. The solutions of sulfuric and perchloric acids shift the peaks to more positive potentials, beyond the oxidation of the mercury electrode. In sodium hydroxide solutions, the signals of all thiouracils are weak and inaccurate for detailed studies. From the analytical point of view, slightly acidic solutions gave the best conditions for further measurements.

Before continuing the studies, the stability of all compounds was checked. The signals were almost the same in 6 h time and did not change significantly after 24 h. Both stock and working solutions were considered stable during the daily experimental period. Fresh solutions were prepared once a day.

Influence of Supporting Electrolyte

According to the results of the preliminary studies, the highest and best developed signals of thiouracils can be obtained in pH range 3.5–6.0. The activity of all compounds in different media was compared at pH close to 5.0 under the conditions employed for the preliminary measurements. The voltammograms were recorded in acetate, phosphate, citrate and phthalate buffers. Each time the curves showed additional peaks confirming the presence of thiol, but the shapes and heights of these signals were different. The citric and phthalic buffers are inappropriate for analytical uses, as the local maxima of the curves are even four times lower than in the other media. In addition, the first peak lies at potentials close to the electrooxidation of the mercury electrode and, therefore, it is strongly influenced or completely hidden by high currents of the latter process. The experiments have proven that either acetate or phosphate solutions are suitable for more detailed studies. However, the curves recorded in acetate buffers allow more straighforward interpretation.

In the previous measurements, it has been noticed that the currents strongly depend on the electrolyte concentration. This was examined more precisely in a set of samples having different pH values (stock buffers: 3.9, 4.3, 4.8, 5.2, 5.6) and different acetate concentrations (from 1×10^{-3} to 5×10^{-1} mol dm^{-3}). Figure 1 shows typical voltammograms of thiouracils in acetate buffers.

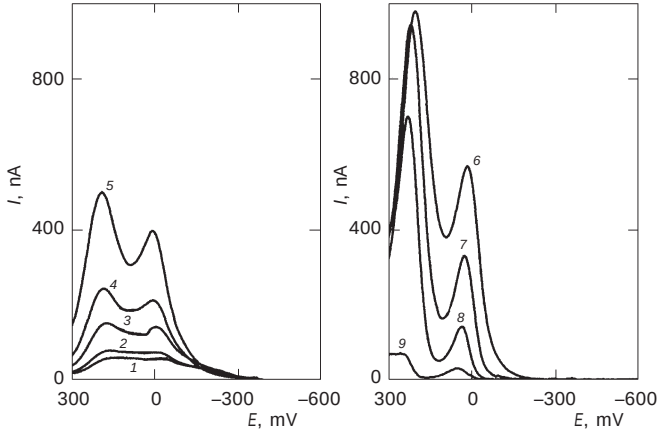

FIG. 1

Voltammograms of 2×10^{-7} M TU in acetate buffer (pH 3.9). E_{dep} = 200 mV, t_{dep} = 180 s, buffer concentration (in mol dm^{-3}): *1* 1×10^{-3}, *2* 2×10^{-3}, *3* 5×10^{-3}, *4* 1×10^{-2}, *5* 2×10^{-2}, *6* 5×10^{-2}, *7* 1×10^{-1}, *8* 2×10^{-1}, *9* 5×10^{-1}

The height of both peaks increases with the electrolyte concentration, giving a maximum at a value characteristic of the thiol. Further increase in the buffer amount lowers the signals. One of such dependences is shown in Fig. 2. Since the aim was to choose the best analytical conditions for the determination, the measurements were performed for each of the tested compounds. Similar experiments were also carried out in phosphate buffer. The most convenient media are compared in Table I.

TABLE I
Optimum experimental conditions for the determination of thiouracils

Compound	Supporting electrolyte[a] mol dm^{-3}	Linearity range[b] mol dm^{-3}	Correlation coefficient
TU	5×10^{-2} [c]	1.0×10^{-8} – 1.0×10^{-7}	0.9981
MTU	3×10^{-2} [c]	2.0×10^{-8} – 3.0×10^{-7}	0.9994
PTU	5×10^{-2} [c]	2.0×10^{-8} – 2.4×10^{-7}	0.9995
BTU	1×10^{-2} [d]	1.0×10^{-8} – 2.0×10^{-7}	0.9991

[a] Acetate. [b] E_{dep} = 200 mV, t_{dep} = 180 s. [c] pH 3.9. [d] pH 4.8.

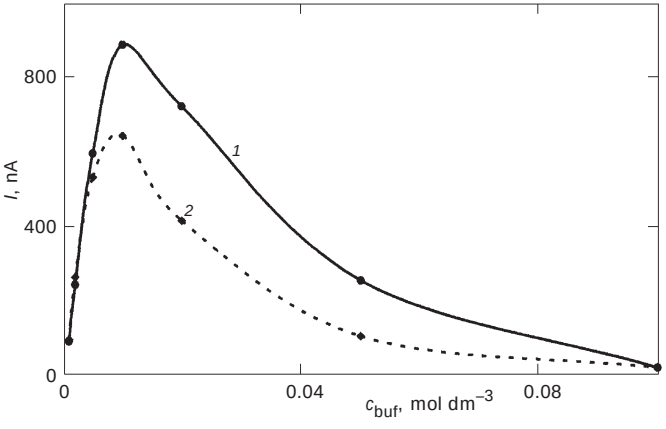

FIG. 2
Dependence of the peak current on buffer concentration. Sample: 2×10^{-7} M BTU in acetate buffer (pH 3.9), E_{dep} = 200 mV, t_{dep} = 180 s. *1* The peak at more positive potentials (Fig. 1), *2* the peak at less positive potentials (Fig. 1)

As depicted in Fig. 2, the electrolyte concentration strongly affects the current. The reason for this phenomenon is associated with adsorption of mercury thiolate. Small alteration in the solution composition leads to significant changes in the mechanisms of electrode reactions and the nature of the insoluble film. This effect was observed either in the solutions containing only the buffer, or samples with added neutral salt (KNO_3). The dependences studied at constant ionic strength are similar in shape to Fig. 2.

Preconcentration

Thiouracils show voltammetric activity after electrodeposition at the mercury electrode. This stage of measurements is essential for the final results. The potential at which preconcentration is held must be more positive (or less negative) than potentials of both peaks. The dependence of the peak height on the deposition potential is similar in all cases and for both peaks (Fig. 3). It starts at low current values, giving a rise and a plateau after reaching the precise energy for the electrode reaction. Usually, the value of +200 mV was the most appropriate for analytical purposes for all of the tested thiols.

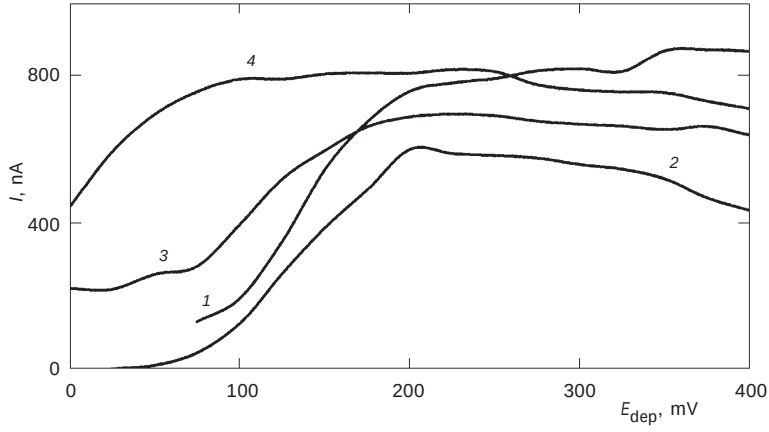

FIG. 3

Influence of the preconcentration conditions on the peak current. Conditions: Acetate buffer, t_{dep} = 180 s, the peak at more positive potentials (Fig. 1). *1* TU, pH 3.9, $c_{buff} = 5 \times 10^{-2}$ mol dm^{-3}; *2* MTU, pH 3.9, $c_{buff} = 3 \times 10^{-2}$ mol dm^{-3}; *3* PTU, pH 3.9, $c_{buff} = 5 \times 10^{-2}$ mol dm^{-3}; *4* BTU, pH 4.8, $c_{buff} = 1 \times 10^{-2}$ mol dm^{-3}

The time of electrolysis was investigated in the range 0–10 min. First, both peaks rose linearly. After deposition of the thiolate film covering the electrode surface, further increase was asymptotical. When a maximum current was reached, no significant changes, resulting from a longer preconcentration period, were observed.

Quantitative Studies

Four sets of voltammograms of the tested thiouracils were recorded at different concentration levels ranging from 0 to 1.0×10^{-6} mol dm^{-3} (Table I). For each of these compounds, the dependence of the currents on the thiol concentration starts as a straight line. As previously described, the thiolate film deposited on mercury covers the surface and inhibits the electrode reaction. The increase of the peaks is much less pronounced at higher amounts of thiouracils in the sample (Fig. 4).

The above dependence was tested statistically. Six series of voltammograms were recorded for each investigated thiol and the results were evaluated mathematically. The ranges of linearity and correlation coefficients are presented in Table I.

Variation of the deposition time gives the possibility to change the linearity range. If the first step of the measurement procedure is shortened, the thiols can be analyzed at higher concentration levels. Accordingly,

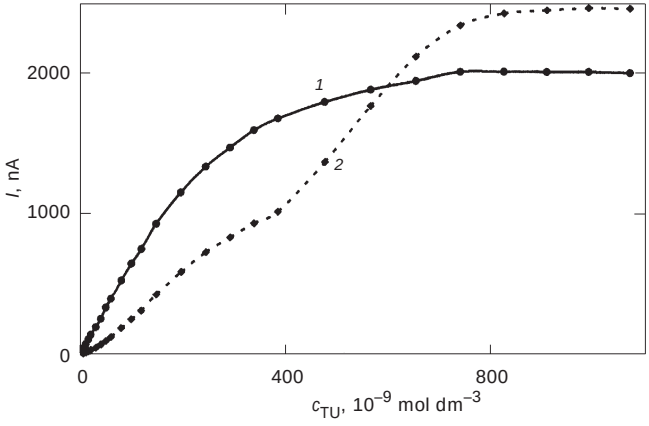

FIG. 4

Dependence of the peak current on the TU concentration. Conditions: Acetate buffer (pH 3.9), $c_{buff} = 5 \times 10^{-2}$ mol dm^{-3}, $E_{dep} = 200$ mV, $t_{dep} = 180$ s. *1* The peak at more positive potentials (Fig. 1), *2* the peak at less positive potentials (Fig. 1)

when the preconcentration is carried out for a longer period, the limits of determination are lower. This may be important for practical application of the method. For example, the linearity in the response of BTU starts at 1.0×10^{-8} mol dm^{-3} and ends at 2.0×10^{-7} mol dm^{-3} when preconcentration is held for 180 s. When shortened to 60 s, the linearity range moves to 8.0×10^{-8}–8.0×10^{-7} mol dm^{-3}.

Limits of Detection

The smallest amounts of MTU and PTU detectable by the proposed procedure were tested experimentally. To obtain a better signal-to-noise ratio, the deposition time was prolonged to ten minutes. The addition of thiols at a nanomolar level resulted in two peaks appearing at characteristic potentials.

The signals three times higher than the background current were recorded for 2.0×10^{-9} M MTU and 1.0×10^{-9} M PTU (Fig. 5).

The concentration limiting the analytical purposes can also be extrapolated from a regression line[13]. When the preconcentration was held for three minutes, the detection was possible for 4.0×10^{-9} M TU, 2.0×10^{-8} M MTU, 9.0×10^{-9} M PTU and 1.0×10^{-8} M BTU.

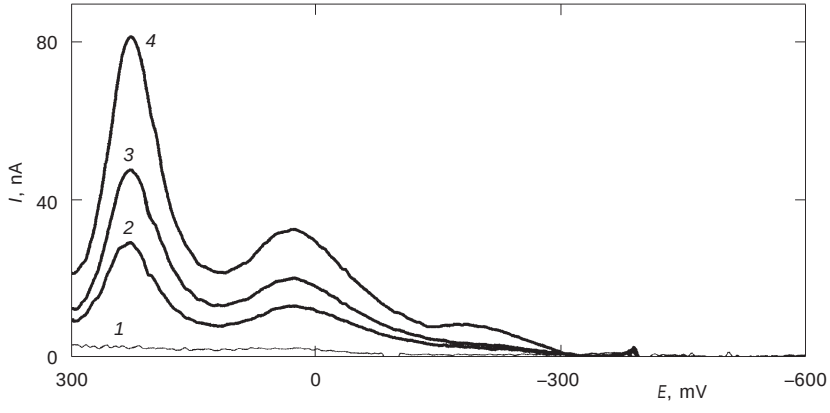

FIG. 5
Voltammograms of PTU at nanomolar level. Conditions: Acetate buffer (pH 3.9), c_{buff} = 5×10^{-2} mol dm^{-3}, E_{dep} = 200 mV, t_{dep} = 600 s. PTU concentration (in mol dm^{-3}): *1* blank, *2* 1×10^{-9}, *3* 2×10^{-9}, *4* 5×10^{-9}

Pharmaceutical Samples

The new analytical method was evaluated by determination of thiouracils in pharmaceutical samples. An appropriate amount of a thiol-containing drug was dissolved in 1×10^{-1} M NaOH and the solution in a volumetric flask was diluted with water. The solution was filtered and diluted to the level of the linearity range. The accurate value of concentration was verified by addition of a standard solution. The results are shown in Table II.

The matrixes of the drugs have no effect on the determination and all the measurements could be performed without any thiol separation from the pharmaceutical sample.

TABLE II
Determination of thyreostatics

Sample	Declared mg	Found [a] mg	n [b]	RSD [c] %	Reference method [d] mg
MTU, Methylthiouracilum [e]	100	100.2±4.8	6	4.6	100.6±0.4
PTU, Propycil® 50 [e]	50	50.8±1.6	6	3.1	49.8±0.4
BTU, Basdène® [f]	25	24.3±0.9	6	3.6	24.9±0.2
BTU, Basdène® [e]	25	24.8±1.5	6	5.6	

[a] $(m_A \pm s_A)t_{0.95}$, where m_A is average mass of thiouracil found in the samples, s_A is standard error of the mean and $t_{0.95}$ is Student's coefficient for confidence 0.95. [b] Number of samples. [c] Relative standard deviation. [d] Potentiometric titration[14,15]. [e] Standard addition method. [f] Calibration curve.

Conclusions

A new method for the determination of four thiouracils has been proposed. All of them give two well-defined peaks that can be recorded readily. The analytical signals result from dissolving the mercury thiolate film deposited in the initial phase of experiments.

The best conditions for the analysis are provided by acetate buffers. According to the character of the electrode processes, which include adsorption on the mercury surface, two parameters play a significant role in the determi-

nation method. The first one is buffer concentration. Before using the procedure for practical purposes, a full study of the influence of the supporting electrolyte has to be conducted. To eliminate errors in the standard addition method, the volumes of the injected standards should be minimum. The other parameter is deposition time.

The detection procedure is very simple, fast and requires only most common reagents. There is no need for using high-purity solvents. The sensitivity of the measurements performed after the deposition stage allows the detection of thiols at concentrations down to 10^{-8} or even 10^{-9} mol dm^{-3}. The method was successfully employed in the determination of 2-thiouracils in drugs. Before applying the procedure to biological samples, a full study of the influence of the matrix has to be made.

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