

## Iodimetric Determination of 2-Thiouracils

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**Key words:** determination of 2-thiouracil and its derivatives; iodimetry; potentiometric titration

The iodimetric determination of 2-thiouracil and its derivatives in alkaline medium is presented. In the volumetric titration with the potentiometric end-point detection the determinability range is 100–1000  $\mu\text{mol}$  for 2-thiouracil, 30–700  $\mu\text{mol}$  for 6-amino-2-thiouracil, 60–500  $\mu\text{mol}$  for 5-carbomethoxy-2-thiouracil and 100–500  $\mu\text{mol}$  for 5-methyl-2-thiouracil. The relative standard deviation was below 1 % in all determinations. The elaborated method was applied to the determination of 6-propyl-2-thiouracil in drugs.

Przedstawiono jodometryczne oznaczanie 2-tiouracylu i jego pochodnych wykorzystując reakcję 2-tiouracylu z jodem w środowisku zasadowym. W miareczkowaniu objętościowym z potencjometryczną detekcją punktu końcowego można było oznaczyć 100–1000  $\mu\text{mola}$  2-tiouracylu, 30–700  $\mu\text{mola}$  6-amino-2-tiouracylu, 60–500  $\mu\text{mol}$  5-carboetoksy-2-tiouracylu i 100–500  $\mu\text{mola}$  5-metylo-2-tiouracylu. Względne odchylenie standardowe we wszystkich wykonywanych oznaczeniach wynosiło mniej niż 1%. Opracowana metoda została zastosowana do oznaczenia 6-propylo-2-tiouracylu w lekach.

2-Thiouracil and a number of related compounds inhibit the formation of thyroid hormones and are used for the treatment of hyperthyroidism [1,2]. Moreover, these substances inhibit nucleic acid metabolism [3,4].

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The structure and properties of thiouracils are applicable to various methods of determination. These compounds have been determined previously by the potentiometric titration using cerium [5,6], permanganate [7], mercuric [8–11] ions, chloramine T [12] and iodine [13,14]. Alkacymetric titrations have been also proposed [15–19]. Ion-selective membrane electrodes have been applied to the direct titration of 2-thiouracils [20] with cupric(II) [21], silver(I) [22], and mercury(II) [23] solutions. A potentiometric method for the estimation of thiouracils in perchloric acid based on their oxidation with 1-chlorobenzotriazole, has been also developed [24]. Amperometric titrations with permanganate [7] and mercuric ions [25] and thermometric [15] and conductivity [26] titrations are proposed as other methods. We have found a great number of volumetric methods titrations with the visual detection of the end-point of 2-thiouracils with mercuric [27–32], silver [33–37], permanganate [39] and dichromate [39] and cerium [6] ions, and iodine [40], bromine [41–45], iodo-chlorine [46], 1-chlorobenzotriazole [24], chloramine T [47] and alcoholate [48–51]. The determination of 2-thiouracils has been also carried out using the spectrophotometric, chromatographic, coulometric, polarographic and voltammetric methods as well as using the induced iodine-azide reaction.

The aim of the present paper was to study the influence of various substituents in the 2-thiouracil ring on the course of oxidation with iodine in alkaline medium and find the best conditions for the determination of 2-thiouracils.

## EXPERIMENTAL

### Reagents and apparatus

Water doubly distilled in glass apparatus and the following reagents of analytical grade purity: potassium iodide, sodium hydroxide, acetic acid, sodium acetate, 2-thiouracil, 5-carbethoxy-2-thiouracil, 6-amino-2-thiouracil, 5,6-diamino-2-thiouracil, 2,4-dithiouracil, 5-methyl-2-thiouracil (Aldrich) were used. Standard solutions of 2-thiouracils were obtained by dissolving a specified amount of the particular reagent in the suitable solution of sodium hydroxide. 6-Propyl-2-thiouracil was determined in the "Tyreostat II" (DR. Herbrand KG) and Propyl-Thiouracil (Lederle Laboratories Division). Iodine standard solutions of 0.1, 0.05, 0.02 and 0.01 mol l<sup>-1</sup> were prepared [52]. The standard solution of mercury(II) nitrate was prepared by adding 4.0118 g of mercury (99.99 %) (Aldrich) to 20 ml of concentrated nitric acid. The solution was heated slowly first to dissolve the mercury and then to evaporate the solution nearly to dryness. At the end 10 ml of 2 mol l<sup>-1</sup> nitric acid were added and the solution was diluted to 1000 ml. A pH-meter (type OP-206 Radelkis, Hungary), with a platinum or a silver electrode and a saturated calomel electrode, and a magnetic stirrer were used.

### Procedures

**Potentiometric titration.** Samples of 2-thiouracils were dissolved in 50 ml of suitable solution of sodium hydroxide (concentrations are given in Table 1.) and titrated with iodine, using the potentiometric detection of the end-point with a platinum indicator electrode and a saturated calomel electrode. The equivalence point of the reaction was found on the basis of the inflection point on the curve. The content of the determined substance was calculated by using the equation:

$$n = \frac{c(I) \times V}{z} \times 10^3$$

where  $n$  is content of the determined substance in  $\mu\text{mol}$ ,

$c(I)$	is concentration of the titrant in	$\text{mol l}^{-1}$ ,
$V$	is volume of the titrant in the end-point in	ml and
$z$	is number of electrons transferred	$z = 4$ , however $z = 6$ in the case of 6-amino-2-thiouracil.

**Determination of 6-propyl-2-thiouracil content in tablets.** Ten tablets were weighed and were crushed in a mortar. An amount of powder containing 250–350  $\mu\text{mol}$  of 6-propyl-2-thiouracil (in the case of Tyreostat II), or the amount equivalent to the average mass of one tablet (in the case of Propyl-Thiouracil) was dissolved in 50 ml of 2  $\text{mol l}^{-1}$  NaOH and was titrated in the same way as the pure substance [13]. The content of the tested substance in one tablet was calculated by using the equation:

$$m = \frac{c(I) \times V}{z} \times M \times \frac{m_1}{m_2}$$

where

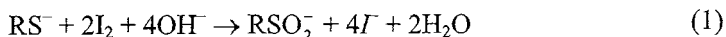
$m$	is content of the tested substance in one tablet in	mg
$c(I)$	is concentration of the titrant in	$\text{mol l}^{-1}$ ,
$V$	is volume of the titrant in the end-point in	ml,
$z$	is number of electrons transferred	( $z = 4$ ),
$M$	is formula weight for 6-propyl-2-thiouracil	(170.2),
$m_1$	is average mass of one tablet in	g, and
$m_2$	is weight of the sample in	g.

## RESULTS AND DISCUSSION

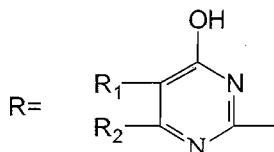
In neutral and acidic media, the reaction rate of iodine with 2-thiouracils was very low and iodimetric titration was impossible. The rates of the reactions between 2-thiouracils and iodine depend on the concentrations of NaOH and 2-thiouracils solutions. It was experimentally verified that the reaction rate of 2-thiouracils with iodine increases with an increasing concentration of sodium hydroxide. Iodine disproportionates quickly in alkaline medium to give iodide and hypiodite ions, so hypiodite is the actual oxidizing agent. As hypiodite is a stronger oxidant than iodine in neutral medium, the reaction may be fairly quantitative.

Each range of concentrations of 2-thiouracils needs a suitable concentration of NaOH to run the reaction according to equation (1 or 2). In the potentiometric titration of 100–500  $\mu\text{mol}$  of 2-thiouracil, the results were valid when the initial concentration of NaOH was in the range 3.5–4.5  $\text{mol l}^{-1}$ . But titration of 1000  $\mu\text{mol}$  of 2-thiouracil requires an initial concentration of NaOH in the range 5.5–6.5  $\text{mol l}^{-1}$ . Good results in the volumetric titration with the potentiometric end-point detection were obtained when the initial concentration of NaOH was in the range 1.5–2.5  $\text{mol l}^{-1}$  for 100–500  $\mu\text{mol}$  of 6-methyl-2-thiouracil [14], 125–500  $\mu\text{mol}$  of 6-propyl-2-thiouracil [13] and 60–500  $\mu\text{mol}$  of 5-carbomethoxy-2-thiouracil, and 2.5–3.5  $\text{mol l}^{-1}$  for

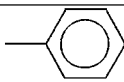
100–500  $\mu\text{mol}$  of 6-benzyl-2-thiouracil [14]. The thiols mentioned above can react with iodine according to the following equation:



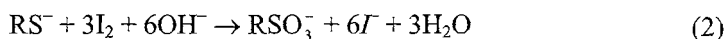
where



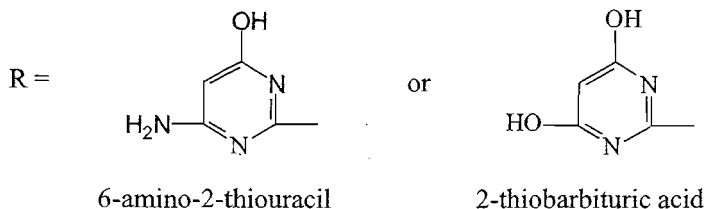
$\text{R}_1$  and  $\text{R}_2$  are listed below.

$\text{R}_1$	$\text{R}_2$	Compound
H	H	2-thiouracil
H	$-\text{CH}_2-\text{CH}_2-\text{CH}_3$	6-propyl-2-thiouracil
H		6-benzyl-2-thiouracil
H	$-\text{CH}_3$	6-methyl-2-thiouracil
$-\text{CH}_3$	H	5-methyl-2-thiouracil
$-\text{COOC}_2\text{H}_5$	H	5-carbethoxy-2-thiouracil

2-Thiouracils with 6-methyl, 6-benzyl [14], 6-propyl [13] and 5-carbethoxy groups require lower concentrations of sodium hydroxide in the titration with iodine than the titration of 2-thiouracil. The determination of 6-amino-2-thiouracil requires higher concentration of sodium hydroxide as a reaction solution. A substitution hydroxy [53] or amino groups changes the mechanism of the reaction:

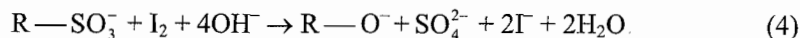
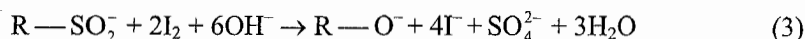


where:



Acceptable results were obtained when the initial concentration of NaOH was either in the range 4.5–5.5  $\text{mol l}^{-1}$  in the potentiometric titration of 30–700  $\mu\text{mol}$  of 6-amino-2-thiouracil, or between 2.5 and 3.5  $\text{mol l}^{-1}$  in the case of 10–350  $\mu\text{mol}$  of 2-thiobarbituric acid [53].

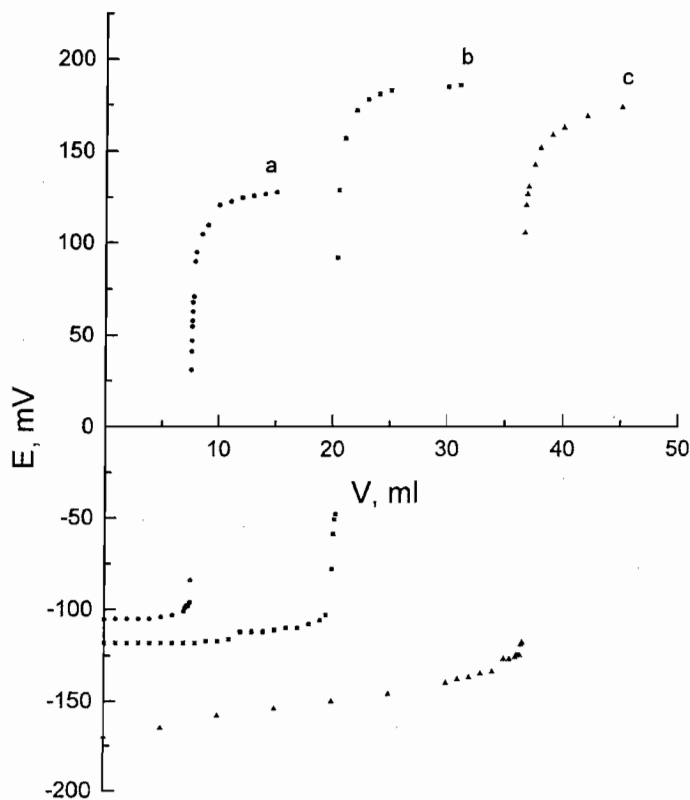
At a lower concentration of NaOH, the reactions (1 and 2) do not proceed stoichiometrically and the number of electrons transferred per mole of oxidized substance is less than 4 (or 6 in the case of 6-amino-2-thiouracil and 2-thiobarbituric acid; reaction 2). At a high concentration of NaOH the number of electrons transferred in the reaction is higher than beyond 4 or 6, because the products of reactions (1 or 2) react with iodine. Then the following reactions proceed partially according to the equations:



The results of direct iodimetric determination of 2-thiouracil and its derivatives (5-carbethoxy-5-methyl- and 6-amino-2-thiouracil) are presented in Table 1.

**Table 1.** Results of potentiometric titration of 2-thiouracils in alkaline medium;  $n = 6$

Compound	Concentration of NaOH mol l <sup>-1</sup>	Number of electrons transferred per mole of oxidized substance (z)	Taken [μmol]	Found $\bar{x} \pm t_{0.95} \cdot \frac{s}{\sqrt{n}}$ μmol	Relative standard deviation $\frac{s}{\bar{x}}$
2-thiouracil	4	4	100.0	100.8 ± 0.1	0.0010
			250.0	250.2 ± 1.1	0.0041
			500.0	498.8 ± 1.3	0.0025
	6	1000	1000 ± 2	0.0017	
5-carbethoxy-2-thiouracil	2	4	60.00	60.27 ± 0.13	0.0020
			125.0	125.7 ± 0.1	0.0009
			250.0	248.8 ± 0.7	0.0027
			500.0	502.6 ± 1.4	0.0026
5-methyl-2-thiouracil	1	4	100.0	99.71 ± 0.31	0.0029
	2		250.0	249.2 ± 1.6	0.0061
	3		500.0	497.3 ± 6.2	0.012
6-amino-2-thiouracil	5	6	30.00	29.98 ± 0.05	0.0016
			60.00	60.20 ± 0.09	0.0014
			175.0	175.3 ± 0.43	0.0026
			350.0	349.2 ± 0.9	0.0025
			700.0	702.0 ± 0.9	0.0012



**Figure 1.** Curves of potentiometric titration of: a – 100  $\mu\text{mol}$  2-thiouracil with 0.05  $\text{mol l}^{-1}$  iodine in 4  $\text{mol l}^{-1}$  sodium hydroxide; b – 500  $\mu\text{mol}$  2-thiouracil with 0.1  $\text{mol l}^{-1}$  iodine in 4  $\text{mol l}^{-1}$  sodium hydroxide; c – 1000  $\mu\text{mol}$  2-thiouracil with 0.1  $\text{mol l}^{-1}$  iodine in 6  $\text{mol l}^{-1}$  sodium hydroxide

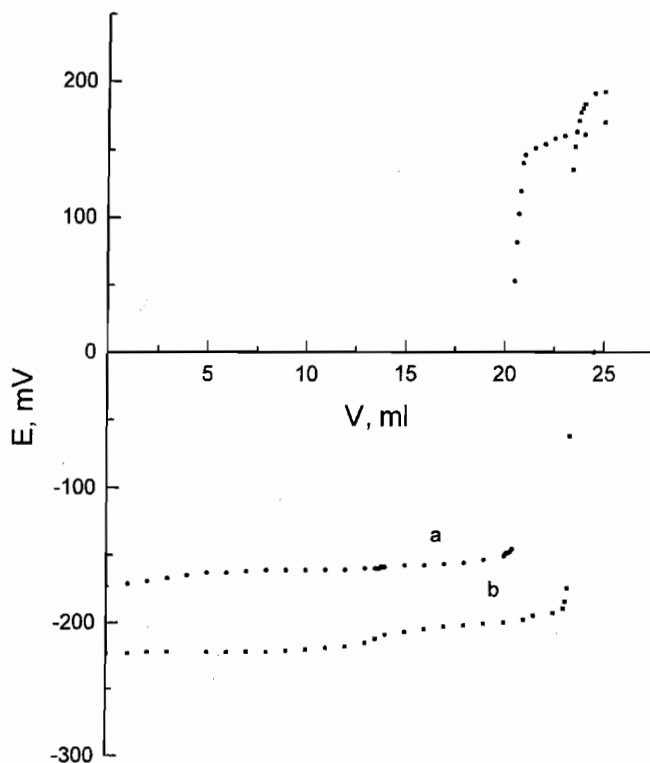
Typical potentiometric titration curves of 2-thiouracil are shown in Figure 1. The elaborated methods were applied for the determination of 6-propyl-2-thiouracils in the drugs. To establish the accuracy of the proposed procedure, a sample of drug was analysed and the results were compared with those obtained by the established procedure with mercuric ions [8, 27]. The results of the drug analysis are given in Table 2, and the potentiometric titration curves of 6-propyl-2-thiouracils (in tablets) are shown in Figure 2.

Substances reacting with iodine interfere in the determination of 2-thiouracils by the proposed method. However tablet excipients do not interfere in those specific circumstances of the proposed application.

Compared with the indirect iodimetric titration [40], the direct method considerably reduced the analysis time and the use of the potentiometric end-point detection improved the accuracy of the determination in comparison with the methods using the visual detection. The proposed method of determination provided a higher increase in the potential near the end-point (200–300 mV/0.1 ml of the titrant) than the potentiometric titration with mercury ions [8–11]. In additions there is no demand for heating.

**Table 2.** Results of determination of 6-propyl-2-thiouracils in drugs;  $n = 6$ 

Drug	Declared content mg	Manufacturer's number of drug series	Method	Found $\bar{x} \pm t_{0,95} \cdot \frac{s}{\sqrt{n}}$ mg	Relative standard deviation $\frac{s}{\bar{x}}$
Tyreostat II	25	212352	iodimetric titration	$25.08 \pm 0.16$	0.0061
			comparison method [8]	$24.89 \pm 0.11$	0.0037
Propyl-Thiouracil	50	435-322	iodimetric titration	$49.76 \pm 0.43$	0.0082
			comparison method [27]	$49.47 \pm 0.50$	0.0096



**Figure 2.** Curves of potentiometric titration of 6-propyl-2-thiouracils in drugs with  $0.05 \text{ mol l}^{-1}$  iodine. a – “Tyreostat II”, a sample containing 43 mg of 6-propyl-2-thiouracil; b – Propyl-Thiouracil, a sample containing a mass of powder equivalent to the average mass of one tablet

The uniform density of the  $\Pi$  electrons is typical. There is an electron excess on the heteroatoms and an electron deficiency at the carbon atoms in the 2, 4, and 6 positions. There is also an electron excess on the carbon atom in the position 5. The

electrophile substituent in the 5-position of 2-thiouracil decreases this excess. There is a possibility of determination of 5-carbomethoxy-2-thiouracil with iodine in alkaline medium. The electron donor substituents prevent the stoichiometric reaction with iodine in alkaline medium. However, there is a possibility of determination of 5-methyl-2-thiouracil after selecting the appropriate concentrations of NaOH for each concentration of this compound in order to run the reaction according to the equation (1). In the potentiometric titration of 100  $\mu\text{mol}$  of 5-methyl-2-thiouracil, the results were valid when the initial concentration of NaOH was in the range 0.5–1.5  $\text{mol l}^{-1}$ . 250  $\mu\text{mol}$  of 5-methyl-2-thiouracil need the initial concentration of NaOH in the range 1.5–2.5  $\text{mol l}^{-1}$ , and 500  $\mu\text{mol}$  of 5-methyl-2-thiouracil need the initial concentration of NaOH in the range 2.5–3.5  $\text{mol l}^{-1}$ .

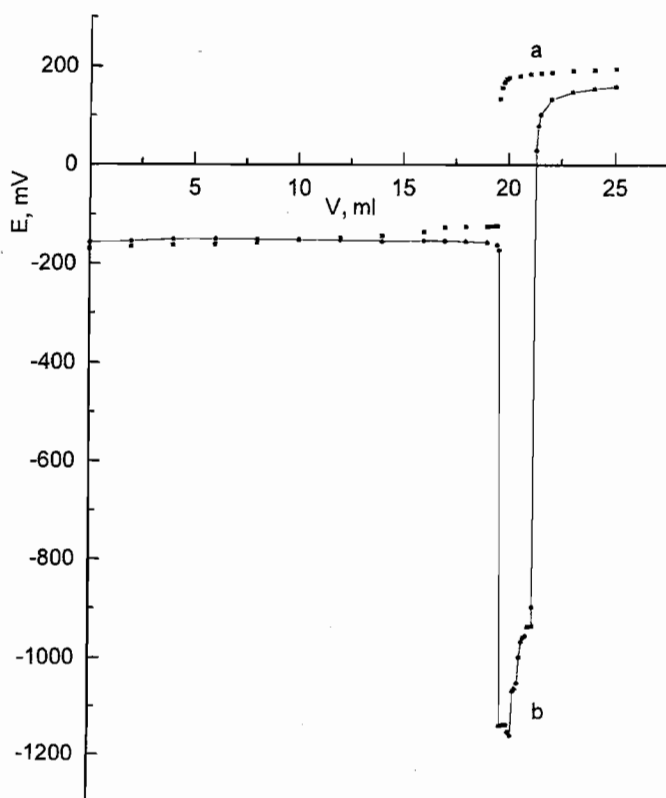
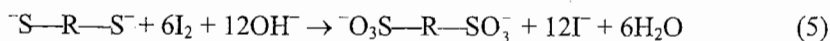


Figure 3. Curves of potentiometric titration of 250  $\mu\text{mol}$  5-carbomethoxy-2-thiouracil with 0.05  $\text{mol l}^{-1}$  iodine. a – in 2  $\text{mol l}^{-1}$  sodium hydroxide; b – in 10  $\text{mol l}^{-1}$  sodium hydroxide

It was found experimentally that the reaction rate of 2,4-dithiouracil with iodine increases with increasing concentration of sodium hydroxide. Even, in the strong alkaline medium ( $c(\text{NaOH}) = 10 \text{ mol l}^{-1}$ ) the reaction only partially proceeds according to the equation:





There was no possibility to determine 2,4-dithiouracil, because we could not find the condition where the number of electrons transferred in the reaction 2,4-dithiouracil is exactly 8, 12 or 16. It was also impossible to determine 6-amino-5-nitroso-2-thiouracil and 5,6-diamino-2-thiouracil, because the nitroso or *o*-amino groups are partially oxidised [15] and the number of electrons transferred in the reaction is greater than 8.

Titrations of 5-carbethoxy-2-thiouracil were carried out at different concentration of sodium hydroxide and the titration curves, which were obtained, are shown in Figure 3. The shape of the potentiometric titration curves is noteworthy (curve b). An introduction of iodine (oxidizer) brings about a strong potential drop in the curve. The end-point of the titration, which corresponds to the inflection point of the curve, and the shape of curve strongly depended on concentration of sodium hydroxide. Such phenomenon also occurred in the titration of thiopental [53], 2-thiocytosine [54], 2,4-dithiouracil and 5,6-diamino-2-thiouracil.

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