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Stopped-Flow Determination of 6-Alkyl-2-Thiouracils in Drugs Applying Induced Iodine-Azide Reaction

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A stopped-flow method for the determination of 6-methyl-2-thiouracil and 6-propyl-2-thiouracil applying induced iodine-azide reaction has been developed. Spectrophotometric detection at 596 nm was applied to monitor the decrease in the absorbance of iodine--starch complex within 10 s. The rate of the absorbance decrease was related to the concentration of thiouracil. Linear calibration range depended on the concentration of iodine and sodium azide in the iodine-azide solution, as well as on the considered analytical signals. Under the optimum reaction conditions both thiouracils could be determined at the concentration ranging from 0.2 ppm to 3 ppm (1.4–21 μmol L^{-1} and 1.2–17 μmol L^{-1} for 6-methyl- and 6-propyl-2-thiouracil, respectively). Relative standard deviation was below 1%. The developed method was applied to the determination of both compounds in drugs.

Opracowano metodę oznaczania 6-metylo-2-tiouracylu i 6-propylo-2-tiouracylu w zatrzymanym przepływie z wykorzystaniem indukowanej reakcji jodo-azydkowej. Rejestrowano spadek absorbancji kompleksu jodo-skrobiowego w czasie 10 s. Szybkość spadku absorbancji zależała od stężenia oznaczanego tiouracylu. Zakres liniowej zmienności mierzonego sygnału analitycznego dla badanych związków zależał od stężenia reagentów w roztworze jodo-azydkowym oraz od uwzględnianego sygnału analitycznego. W optymalnych warunkach eksperymentalnych oba tiouracyle można oznaczać w zakresie od 0,2 ppm do 3 ppm (1,4–21 μmol L^{-1} dla 6-metylo- i 1,2–17 μmol L^{-1} dla 6-propylo-2-tiouracylu). Względne odchylenie standardowe było poniżej 1%. Metodę zastosowano do oznaczania obu związków w lekach.

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6-methyl-2-thiouracil and 6-propyl-2-thiouracil are extensively used in the treatment of hyperthyroism and Basedow's disease. Antithyroid activity of these compounds results from their interfering effect on iodination of thyroxine precursors.

6-methyl-2-thiouracil and 6-propyl-2-thiouracil have been determined titrimetrically with $KBrO_3$ [1], $KMnO_4$ [2], Ag(I) [3], and iodine in alkaline medium [4] as titrants. Colorimetry [5], spectrophotometry [6], potentiometric and coulometric titration [7], high performance liquid chromatography [8], gas chromatography [9, 10], and amperometry [11] have been also applied to the determination of thiouracils in tissues, after prior separation of the analytes.

Due to the presence of divalent sulfur atom in their molecules, thiouracils induce reaction of sodium azide with iodine, according to the below notation:

$$2N_3^- + I_3^- \xrightarrow{TU} 3I^- + 3N_2$$

The amount of iodine consumed in this reaction is proportional to the amount of sulfur compound present in the sample. This proportionality has been utilised in the determination of thiouracils [12].

In this paper, a stopped-flow kinetic method for the determination of thiouracils has been proposed. Determination was based on the measurement of concentration changes of the analytes in time. Immediately after the sample and the reagents were mixed, kinetic curve was recorded. Analytical results were obtained within a few seconds. The details on the stopped-flow technique and the corresponding instrumentation have been discussed by Perez-Bendito *et al.* [13,14].

To follow the progress of the induced reaction, continuous spectrophotometric detection was applied and concentration changes of iodine in the form of iodine--starch complex were monitored. Concentrations of azide and iodide ions were large compared to those of iodine; therefore, they were regarded as constant. Reaction rate was thus proportional to the concentrations of thiouracil ($c_{thiouracil}$) and iodine, according to the below equation:

$$-d[I_2]_t /dt = k\, c_{thiouracil}\, [I_2]_t$$

where k is a rate constant [15,16]. It was found that for a given concentration of iodine the rate of absorbance decrease (related to the decrease in iodine-starch complex concentration) changed linearly with thiouracil concentration, as shown below:

$$-dA/dt = k\, c_{thiouracil}$$

EXPERIMENTAL

Reagents

0.01 mol L^{-1} stock solution of iodine containing 20 g L^{-1} potassium iodide was standardised by titration with 0.01 mol L^{-1} sodium arsenate(III) solution. 0.1 mol L^{-1} stock solution of sodium arsenate(III) was prepared from standard „Titrisol" solution (Merck). 200 g L^{-1} stock solution of sodium azide was prepared. 6-methyl-2-thiouracil and 6-propyl-2-thiouracil were commercially available (Merck). Stock solutions of the examined compounds (100 ppm) were prepared by dissolving 10 mg of each thiouracil in 20 mL of 0.01 mol L^{-1} NaOH. pH of the obtained solution was neutralised with HCl and, after that, the content was diluted with water to 100 mL.

Solutions of both analytes were stable for at least 1 month, which was confirmed spectophotometrically. Working solutions were prepared daily from appropriate stock solutions.

Apparatus

A spectrophotometer equipped with a stopped-flow module was used in the measurements. A stopped-flow mixing module was constructed in the Institute of Physical and Theoretical Chemistry, Erlangen-Nuremberg University. Other units of the experimental setup included photomultiplier (Hamamatsu), monochromator, and a lamp (both from Optometrics USA, Inc.), and were purchased from and put together in the laboratory of Poznań University of Technology. The obtained kinetic data were acquisited and processed using an IBM compatible PC equipped with a Keithley-Metrabyte DAS 1600 board.

Iodine-azide reagent was prepared by mixing the appropriate amounts of sodium azide, potassium triiodide, hydrochloric acid and starch solution, and was placed in one syringe of the stopped-flow apparatus. Another syringe was filled with thiouracil solution. To monitor concentration of thiouracil, two techniques were employed:

– the maximum reaction rate method; Vmax was estimated after time corresponding to the maximum of (-dA/dt)

– the variable-time procedure, in which time t required to achieve constant absorbance was measured; the dependence of 1/t on thiouracil concentration served as the calibration plot.

The absorbance vs. time dependence was constructed for 1000 experimental points recorded in each kinetic run. Five runs were performed for each solution in the syringe and the obtained data were automatically averaged during the data acquisition process.

RESULTS AND DISCUSSION

Inducing activities of both thiouracils were examined applying batch method [12] and were found to be very similar. In order to establish appropriate conditions for the determination by the stopped-flow method, it was necessary to examine the influence of various parameters (see below in the text) on the rate of induced reaction. It was found that for both thiouracils reaction rates were similar and were similarly influenced by pH and concentrations of azide and iodine.

Figure 1 exhibits the changes of absorbance accompanying reaction progress at different concentrations of 6-methyl-2-thiouracil.

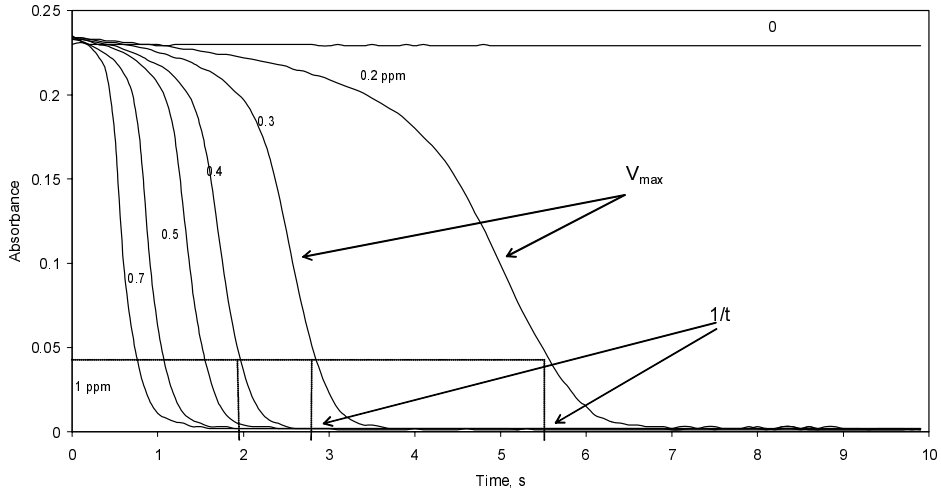

Figure 1. Absorbance changes of iodine in the reaction induced by 6-methyl-2-thiouracil present at different concentrations (0.2–1.0 ppm). Initial concentrations: I_2 5×10^{-4} mol L^{-1}, NaN_3 0.3 mol L^{-1}, pH 6.1

The influence of pH in the range from 4.8 to 8.7 was also examined. At pH higher than 7.1 the induced reaction practically did not proceed. This was a proof that fast iodometric reaction, in which thiouracil is oxidised to disulfides and higher oxidation states, indeed proceeded. A decrease in the pH slowed down the transformation rate of the intermediate product of iodometric reaction (RSI) to disulfide and also increased the induced reaction rate. Below pH = 6 an undesirable scattering of the experimental data was observed.

Table 1. The influence of pH on the reaction rate induced by 6-methyl-2-thiouracil. Concentrations: 6-methyl-2-thiouracil: 0.5 ppm, iodine: 5×10^{-4} mol L^{-1}, N_3^-: 0.3 mol L^{-1}

pH	5.5	6.1	6.5	6.8	7.1
V_{max} s^{-1}	0.394	0.372	0.240	0.107	0.003

The influence of azide concentration on the reaction rate was examined in the range 0.06–0.6 mol L^{-1} at constant ionic strength, pH, and concentrations of other reagents. An increase of azide concentration up to 0.3 mol L^{-1} reduced the time required to

generate inducing species and increased the induced reaction rate. Higher concentrations of azide did not influence the reaction rate.

Table 2. The influence of N_3^- concentration on the reaction rate induced by 6-methyl-2-thiouracil. Concentrations: 6-methyl-2-thiouracil 0.5 ppm, iodine: 5×10^{-4} mol L^{-1}; pH 6.1

N_3^- mol L^{-1}	0.03	0.06	0.15	0.3	0.6
V_{max} s^{-1}	0.032	0.226	0.297	0.313	0.323

Initial concentration of iodine in the iodine-azide solution influenced determination range of the inductor. At low concentration of iodine, thiouracil was oxidised to the non-inducing compound at a slower rate than at higher concentrations. In the latter case, thiouracil participated in the induced reaction for a longer time. Low iodine con- centration facilitated quantification of smaller amounts of the inductor, however, made the linear range of the dependences of V_{max} and 1/t on the inductor concentration nar-rower. An increase in iodine concentration allowed one to extend the linear range, as presented in Table 3.

Table 3. Linear calibration ranges for the determination of 6-methyl-2-thiouracil at different concentrations of iodine in iodine-azide solution; concentration of N_3^-: 0.3 mol L^{-1}; pH 6.1

I_2 mol $L^{-1} \times 10^{-4}$	1	2	5
V_{max} method	0.03–0.15 ppm	0.05–0.3 ppm	0.3–0.9 ppm
1/t method	0.03–0.3 ppm	0.05–0.9 ppm	0.2–3.0 ppm

To summarise, in the determination of 6-methyl-2-thiouracil and 6-propyl-2-thiouracil the following conditions were maintained: pH 6.1, azide concentration 0.3 mol L^{-1}, iodine concentration 5×10^{-4} mol L^{-1}.

Determination of thiouracils in drugs

In order to determine thiouracils in drugs, a variable-time procedure was applied. This procedure provided wider linear range of 1/t *vs* thiouracil concentration dependence and improved its correlation coefficient compared to the maximum reaction rate method (Tab. 4).

Table 4. Linear ranges and regression data for the stopped-flow determination of thiouracils

Inductor	Linear range, ppm	Slope ± SD ppm × s	Intercept ± SD, ppm × s	Correlation coefficient
$V_{max} = f(c)$				
6-CH_3-2TU	0.3–0.9	0.9614 ± 0.0095	0.0923 ± 0.0036	0.9968
6-C_3H_7-2TU	0.3–1.0	0.8072 ± 0.0066	0.0107 ± 0.0036	0.9971
$1/t = f(c)$				
6-CH_3-2TU	0.2–3.0	2.2175 ± 0.0289	–0.2071 ± 0.0169	0.9996
6-C_3H_7-2TU	0.2–3.0	1.7552 ± 0.0336	–0.2320 ± 0.0108	0.9991

The proposed method was applied to the determination of 6-methyl-2-thiouracil in Methylthiouracilum® (Plivia, Cracow), 6-propyl-2-thiouracil in Propycil® (Solvay Pharmaceuticals GmbH, Hannover), and Thyrosan® (Sun-Farm Co.Ltd, Kolbiel). Solutions of the above drugs were prepared according to the recommendations of the Polish Pharmacopoeia [17].

About 60 mL of water and 10 mL of 1 mol L^{-1} NaOH solution were placed in 100mL-in-volume flask and an accurately weighted amount of the drug was added. The contents of the flask were gently shaken for 10 min to dissolve thiouracil. Then, the solution was filtered, 10 mL of the filtrate was neutralised with HCl solution and finally diluted to 100 mL with water. An appropriate volume of the obtained solution was diluted to the concentration of thiouracil of either 0.5 or 1.0 ppm (concentrations were calculated according to the declared content of thiouracil in the analysed drugs), and then subjected to the analysis. Determination was performed immediately after preparation of the solutions, as well as 4, 8, and 24 h later. It was proved that drug solutions were stable up to 24 h. Determinations were carried out by the stopped-flow method and, simultaneously, applying absorption spectrophotometry at 275 nm for 6-methyl- and at 234 nm for 6-propyl-2-thiouracil, following Official Methods of Analysis [6]. The results of the determination of both compounds are presented in Table 5. The results of 6 independent determinations were taken to the regression analysis.

Table 5. Results of the determination of 6-methyl-thiouracil and 6 propyl-2-thiouracil in drugs

Sample analysed	Concentration in ppm		RSD %	Added ppm	Determined ppm	RSD %	Recovery %
	declared	determined					
6-methyl-2-thiouracil in methylthiouracilum							
Immediately after preparation	0.5	0.496 ± 0.0028	0.54	0.2 0.4	0.691 ± 0.004 0.905 ± 0.0056	0.55 0.59	97.50 102.25
	1.0	0.994 ± 0.0033	0.32	0.2 0.4	1.195 ± 0.0061 1.395 ± 0.0093	0.49 0.64	100.50 100.25

Table 5. (Continuation)

After 24 h from preparation	0.5	0.500 ± 0.0030	0.57	0.2 0.4	0.700 ± 0.0025 0.900 ± 0.0038	0.34 0.40	100.00 100.00
	1.0	0.999 ± 0.0037	0.35	0.2 0.4	1.198 ± 0.0039 1.388 ± 0.0059	0.31 0.41	99.50 97.25
6-propyl-2-thiouracil in Thyrosan							
Immediately after preparation	0.5	0.494 ± 0.0037	0.71	0.2 0.4	0.698 ± 0.0043 0.908 ± 0.0014	0.59 0.15	102.00 103.50
	1.0	0.998 ± 0.0035	0.33	0.2 0.4	1.198 ± 0.0077 1.396 ± 0.0056	0.61 0.38	100.00 99.50
After 24 h from preparation	0.5	0.503 ± 0.0024	0.45	0.2 0.4	0.699 ± 0.0047 0.899 ± 0.0027	0.64 0.29	98.00 99.00
	1.0	0.996 ± 0.0017	0.16	0.2 0.4	1.190 ± 0.0078 1.399 ± 0.0047	0.63 0.32	97.00 100.75
6-propyl-2-thiouracil in Propycil 50							
Immediately after preparation	0.5	0.497 ± 0.0048	0.92	0.2 0.4	0.694 ± 0.0024 0.903 ± 0.0057	0.33 0.60	98.50 101.50
	1.0	0.998 ± 0.0021	0.20	0.2 0.4	1.194 ± 0.0032 1.398 ± 0.0038	0.26 0.26	98.00 100.00
After 24 h from preparation	0.5	0.502 ± 0.0029	0.55	0.2 0.4	0.694 ± 0.0033 0.898 ± 0.0050	0.45 0.53	96.00 99.00
	1.0	0.995 ± 0.0041	0.39	0.2 0.4	1.191 ± 0.0066 1.400 ± 0.0051	0.53 0.35	98.00 101.25

CONCLUSION

The proposed method for the determination of 6-methyl- and 6-propyl-2-thiouracil can be considered an interesting alternative to other methods. Iodine-azide reaction has been already utilised in the determination of these compounds by different techniques. However, the stopped-flow method reduces sample manipulation and allows the measurements to be made immediately after mixing the components. Data acquisition and evaluation lasts only a few seconds. The proposed kinetic method is less expensive, faster, and more precise compared to the methods, in which the data are collected after the induced reaction is finished. Sensitivity of the determination is improved by about two orders of magnitude compared to the sensitivity in batch methods [1–7,11]. The proposed approach can be also applied to the determination of thiouracils in complex samples after prior TLC separation of the components [12,18].

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