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**Application of 2-Thiouracil and 2,4-Dithiouracil for the Determination
of Metal Ions. Part I. Spectrophotometric Determination
of Copper and Silver**

Zastosowanie 2-tiouracylu i 2,4-ditiouracylu do oznaczania jonów metali.
Część I. Spektrofotometryczne oznaczanie miedzi i srebra

Применение 2-тиоурацила и 2,4-дитиоурацила для обозначения ионов металлов.
Часть I. Спектрофотометрическое обозначение меди и серебра

2-Thiouracil and its derivatives did not find until now a wide employment in analytical chemistry. The structures of 2-thiouracil complexes with Cu^{2+} , Ni^{2+} , Co^{2+} , Fe^{3+} , Ru^{3+} , Pd^{2+} and Pt^{4+} ions were investigated by IR spectra, NMR and elementary analysis (7, 8); IR-spectra of cupric complexes of 2-thiouracil, 6-methyl-2-thiouracil and 6-propyl-2-thiouracil were used for recognition of binding atoms in ligands (13). Also stability constants of 2-thiouracil complexes with Cu^{2+} , Al^{3+} and Fe^{3+} were determined (4), but in respect of their biological activity and not analytical usefulness. Examinations of the reaction of 2-thiouracil, 6-alkylthiouracils and 5,6-diamino-2-thiouracil with several metal ions by Weiss and Hein (16) or Weber and Garret (15) have a rather theoretical value, and similarly kinetic studies of Lieberman (9).

However, 2-thiouracil and 6-methyl-2-thiouracil have been employed for spectrophotometric assay of palladium (3, 10). Thiourotic acid (6-hydroxy-2-thiouracil) may be used for detection of Ag^+ , Hg^{2+} , Cu^{2+} , Pd^{2+} and Ru^{3+} because it gives coloured soluble compounds or precipitates with these metals (1, 2). Colorimetric determinations of Pd^{2+} , Co^{2+} and Cu^{2+} with 4-amino-5-nitroso-2-thiouracil (14), and Os^{8+} and Ru^{3+} with 4,5-diamino-2-thiouracil (12) have been published. Conditions for quantitative precipitation of Ba^{2+} , Se^{4+} , Co^{2+} and Pd^{2+} complexes with 5,6-diamino-2,4-dithiouracil have been elaborated by Izquierdo and Prat (6).

Since it was stated that 2-thiouracil and 2,4-dithiouracil react quantitatively forming the complexes with Cu^{2+} and Ag^+ ions, an intent of this work was to examine the named organic compounds as reagents for the spectrophotometric determination of both metal ions.

EXPERIMENTAL

Reagents and apparatus

2-Thiouracil, a.r. — Fluka (Buchs, Switzerland).

2,4-Dithiouracil was synthesized after Wheeler and Liddle (17), by warming 2-thiouracil with phosphorus pentachloride and then converting the obtained 2,4-dichloropyrimidine into dithiouracil by the reaction with potassium hydrosulphide; the product was twice recrystallized from hot water, dried in vacuum and analyzed (by elementary analysis of C, H, N, S and by titration with iodine).

Buffer solutions: *pH* 3.30—6.55 — acetate after Walpole, *pH* 7.20—11.85 — borate after Michaelis.

All reagents used were analytical grade, prod. by POCh (Gliwice, Poland).

Spectrophotometer VSU2-P — Zeiss (Jena, GDR).

Potentiometer (for *pH* control) type PHM-22 — Radiometer (Copenhagen, Denmark) with electrodes: glas G-202 C, calomel K-401.

All measurements were carried out at 20°C ±1°.

Preliminary investigations

2-Thiouracil is insoluble in water, diluted mineral acids, ethanol, acetone, chloroform, ethyl ether, soluble in boiling methanol. 2,4-Dithiouracil is insoluble in acids, chloroform and ethyl ether, soluble in hot (ca. 60°C) water, ethanol, acetone and methanol; both are soluble in a sodium or potassium hydroxide solution and ammonia water (salt formation).

Reactions of aqueous solutions of 2-thiouracil and 2,4-dithiouracil (0.02 mole/dm³) with metal ions (conc. 0.02 mole/dm³) were examined in buffer solutions in the range *pH* 3.30—11.85, approx. every one *pH* unit. 2-Thiouracil, dissolved in the necessary amount of ammonia water and diluted, gave with Ag⁺ ions white precipitate in the whole *pH* range (similar precipitate was formed with Hg²⁺ ions); with Cu²⁺ ions brown-green soluble complex was formed in this range. An analogous solution of 2,4-dithiouracil gave with Ag⁺ ions at *pH* 3.30—11.85 pale yellow precipitate (with Hg²⁺ — white, but pale yellow with Pb²⁺ and Bi³⁺); with Cu²⁺ — brown-green precipitate.

A sufficiently diluted (0.5—5 · 10⁻⁴ mole/dm³) buffered solution of cupric and silver complexes of 2-thiouracil and 2,4-dithiouracil, when examined spectrophotometrically in U.V. and visible region in respect to their stability, revealed suitable *pH* and analytical wave length at which each complex could be tested; that is for Ag-thiouracil: *pH* 5.25 and λ = 340 nm, for Cu(II)-thiouracil: *pH* 6.65 and λ = 346 nm, for both Ag- and Cu(II)-dithiouracils: *pH* 6.25 and λ = 390 nm. The reactions of complex formation were then investigated by a continuous variation (Job) method

and by a spectrophotometric titration (Y o e - J o n e s) method and at the above given conditions the molar ratio 1:1 (metal:ligand) was stated for all complexes. Stability constants of 1:1 complexes were calculated after S c h a e p p i and T r e a d w e l l (11) and after H a g e n m u l l e r (5) and were found to be: Ag-thiouracil $\log K=5.75$, Cu(II)-thiouracil $\log K=6.31$, Cu(II)-dithiouracil $\log K=6.57$.

Spectrophotometric determination using 2-thiouracil

Silver. To obtain a calibration graph, standard solutions of silver nitrate in volumes containing 64—350 μg Ag were pipetted into 50 cm^3 volumetric flasks, 20 cm^3 2-thiouracil solution ($2 \cdot 10^{-4}$ mole/ dm^3 in acetate buffer pH 5.25) was added to each flask, which was filled up to the mark with water. Absorbance was measured after 20 min at 340 nm, in a 10 mm quartz cell, against adequate 2-thiouracil buffered solution as reference.

A series of silver determinations (samples of AgNO_3 and Ag_2SO_4 weighed in amounts of 85—300 μg Ag) were carried out similarly for statistical evaluation of the method. The results are presented in collective Table 1.

Copper (II). Volumes of standard cupric sulfate solution containing 64—640 μg Cu(II) were pipetted into 50 cm^3 volumetric flasks, 20 cm^3 2-thiouracil solution ($5 \cdot 10^{-4}$ mole/ dm^3 in acetate buffer pH 6.65) was added to each flask which was filled up with water and absorbance was measured after 1 hour, at 346 nm, in a 10 mm quartz cell. The readings were used for drawing the calibration curve.

Samples of cupric sulfate and cupric acetate (64—445 μg Cu) were determined in the like manner and used for statistical evaluation; the results are given in Table 1.

Table 1. Statistical evaluation of spectrophotometric determinations of silver and copper (II)

Determination of	with	Number of variables /n/	Standard deviation %	Standard deviation of the mean %	Confidence interval /prob. level 95%/ %
Ag ⁺	2-thiouracil	8	0.78	0.29	100.83 \pm 0.63
Cu ²⁺	2-thiouracil	9	0.79	0.23	100.45 \pm 0.49
Ag ⁺	2,4-dithiouracil	7	0.93	0.32	101.02 \pm 1.11
Cu ²⁺	2,4-dithiouracil	9	0.57	0.22	99.17 \pm 0.91

Spectrophotometric determination using 2,4-dithiouracil

Silver. Calibration graph was plotted by employment of spectrophotometric data from measurements of solutions containing in the volume of 50 cm³ (volumetric flask) 32.3—180 µg Ag and 20 cm³ solution 1 · 10⁻⁴ mole/dm³ 2,4-dithiouracil in acetate buffer pH 6.25, read at 390 nm in a 50 mm glass cell 20 min. after mixing the reactants.

Precision of the method was tested by the amounts of 32.3—140 µg Ag and the results are cited in Table 1.

Copper (II). Calibration graph was prepared for the range 25—95 µg of Cu, complexed with an identical — as for Ag — 2,4-dithiouracil solution in 50 cm³ of total volume, and measured in a 50 mm cell at 390 nm, after equilibration within 1.5 hour.

Examination of the procedure was made with 25.4—81.7 µg quantities of Cu and calculated with the results given in Table 1.

I n t e r f e r e n c e s

Determinations of silver and copper were carried out with 2-thiouracil or 2,4-dithiouracil reagents as given above, but now in the presence of the following ions: Hg²⁺, Pb²⁺, Cd²⁺, Co²⁺, Ni²⁺, Fe³⁺, Mn²⁺, Zn²⁺, CN⁻, SCN⁻, S₂O₃²⁻, oxalate, tartrate, versenate.

It appeared that mercuric and ferric ions, already in concentrations equal to those of Ag⁺ or Cu²⁺ (to be determined), interfere with an assay when using both 2-thiouracil and 2,4-dithiouracil. Versenate ions (up to 1 · 10⁻⁴ mole/dm³) do not interfere with the determinations of silver by the use of 2,4-dithiouracil, whereas they should be absent when both reagents for copper determination are employed. Besides Hg²⁺ and Fe³⁺, all other named accompanying ions do not interfere, with an exception of oxalates, tartrates and thiosulfates which disturb an assay if present in conc. higher than 3 · 10⁻⁴ mole/dm³ when using 2-thiouracil for Ag⁺ or Cu²⁺ determinations.

D I S C U S S I O N O F R E S U L T S

Explicit results of different methods for molar ratio determination of cupric complexes of 2-thiouracil and 2,4-dithiouracil (elementary analysis for C, H, N, S and AA Spectrophotometric assay of Cu in solid state, as well as procedures of continuous variation and spectrophotometric

titration in solutions) proved that 1:1 complexes are formed. Both reagents form with Ag^+ ions also 1:1 complexes, but probably 2:1 (metal:ligand) complexes are formed too, since both sides of Job curves are a little unsymmetrical, more in the case of 2,4-dithiouracil (and that's why stability constant of this complex could not be determined by Schaeppi-Treadwell or Hagenmuller calculation methods). However, in medium proposed here for spectrophotometric determinations (pH 5.25—6.25) silver complexes of 1:1 ratio are formed and revealed (at 340 nm or 390 nm, respectively) in significant prevalence and thus another molar ratio ought not to be taken into account.

Investigation of ultraviolet absorption spectra of formed complexes and also separately their components, indicated that Ag^+ and Cu^{2+} ions could not be determined quantitatively at maximal absorption values (λ_{max}) of the complexes, as at these wave lengths the absorbance of both 2-thiouracil and 2,4-dithiouracil is high. Therefore analytical wave length was found for each complex, — a point at which absorptivity of the reagent is low and it may be well surveyed by reference solution.

As seen in Table 1 — the precision and reproducibility of the method is good. The samples should contain indicated amounts of Ag or Cu in final solution. The exceeding of the upper limit results in turbidity or even precipitation of the complex, especially in the case of silver complexes.

2,4-Dithiouracil is more selective of both reagents, though some of the existing differences for the disadvantage of 2-thiouracil may be easily removed by prior decomposition of possibly present oxalates, tartrates or thiosulfates.

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STRESZCZENIE

Jony Cu^{2+} i Ag^+ tworzą z 2-tiouracylem i 2,4-ditiouracylem kompleksy, które mogą być wykorzystane do oznaczenia tych jonów metali; zbadano skład i oznaczono stałe trwałości kompleksów. Opracowano spektrofotometryczną metodę oznaczania miedzi(II) i srebra. Przy użyciu 2-tiouracylu jony Cu^{2+} oznacza się przy długości fali 346 nm i w środowisku o pH 6,65, a stosując 2,4-ditiouracyl przy $\lambda = 390$ nm i pH 6,25; Ag oznaczono 2-tiouracylem przy $\lambda = 340$ nm i pH 5,25, zaś 2,4-ditiouracylem — przy $\lambda = 390$ nm i pH 6,25. Dla mikrogramowych ilości srebra i miedzi uzyskano dobrą precyzję oznaczeń. Jony Hg^{2+} i Fe^{3+} przeszkadzają oznaczeniom, natomiast przy zastosowaniu 2-tiouracylu przeszkadzają również większe niż trzykrotne (w stosunku do oznaczanego metalu) stężenia winianów, szczawianów i tiosiarczanów.

Р Э З Ю М Е

Ионы Cu^{2+} и Ag^+ дают с 2-тиоурацилом и 2,4-дитиоурацилом комплексы, которые могут быть использованы для обозначения ионов этих металлов; исследовано состав и обозначено постоянные прочности комплексов. Обработано спектрофотометрический метод обозначения меди(II) и серебра. При использовании 2-тиоурацила ионы Cu^{2+} обозначаются при длине волны 346 nm и в среде с pH 6,65, а применяя 2,4-дитиоурацил при $\lambda=390$ nm и при 6,25; Ag обозначено 2-тиоурацилом при $\lambda=340$ nm и pH 5,25, а 2,4-дитиоурацилом при $\lambda=390$ nm и pH 6,25. Для микрограммовых количеств серебра и меди получено с большой точностью обозначения. Ионы Hg^{2+} и Fe^{3+} мешают обозначениям, но при использовании 2-тиоурацила мешают тоже, более чем трехкратно (по отношению к обозначенному металлу) концентрации винянов, оксалатов и тиосульфатов.

