Metal Complexes of Thiouracils II: Solubility Analyses and Spectrophotometric Investigations

EDWARD R. GARRETT and DENNIS J. WEBER

Abstract □ Complexes of Cu^{+2}, Cd^{+2}, and Pb^{+2} with 2-thiouracil, 6-*n*-propyl-2-thiouracil, 6-methyl-2-thiouracil, 5-methyl-2-thiouracil, and 5,6-dimethyl-2-thiouracil have lower absorptivities than the parent thiouracils. On the premise that all metal-ion complexes of thiouracils have similar absorptivities, cupric ion is a much more highly complexing metal (4–10×) than cadmium and lead. The lack of spectral aberration on the addition of Fe^{+3} and Fe^{+2} to solutions of these thiouracils shows that no significant complexation occurs with these metal ions. None of the studied metals forms significantly spectrophotometrically observable complexes with 2-ethylmercapto-4-hydroxypyrimidine and 6-methyl-*N,N'*-diethyl-2-thiouracil. This fact implies that only those tautomerizable thiouracils that can form ionizable sulfhydryl groups are capable of metal complexation. Solubility analysis methods were devised for the determination of stability constants and solubilities of 1:1, 1:2, and mixed ligand complexes. However, thiouracil solubility was not significantly different in the absence and presence of metal ions. Also, metal-ion concentrations were not significant in saturated thiouracil solutions and demonstrated that the solubilities of the precipitating complexes were less than the accuracies of the spectrophotometric thiouracil and polarographic metal analyses. A previous literature proposal that a cuprous-2-thiouracil disulfide complex is the major result in mixtures of cupric ion and 2-thiouracil was denied by the demonstration that thiouracil is not oxidized by cupric ion in acidic solutions, that 2-thiouracil disulfide readily disproportionates in acid to the sulfinic acid and 2-thiouracil, and that the rate of such disproportionation is independent of cupric-ion concentration.

Keyphrases □ Thiouracil–metal complexes—solubility analyses, spectrophotometric investigation □ Complex formation—thiouracils–cupric, cadmium, ferric ions □ Solubility analyses—thiouracil–metal complexes □ Potentiometric titration—stability constant analysis, complexes □ UV spectrophotometry—stability constant analysis, complexes

It was shown (1) that the divalent metals (M^{+2}): Pb^{+2}, Cd^{+2}, Ni^{+2}, and Zn^{+2}, complex with the 5- and/or 6-alkyl-substituted thiouracil monanions, U^- (II), as shown in Scheme I. Significant concentrations of MU^+ (III) and MU_2 (IV) complexes, with bonding of metal ion to the sulfur anion, permitted estimation of the respective K_1 and K_2 constants by potentiometric titrations in homogeneous solutions. Precipitated complexes MU_2 and M_2U_2 (VII) were isolated and characterized for the more stable Pb^{+2} and Cd^{+2} complexes, where M_2U_2 is most probably the cyclic dimer, bis-(thiouracil–metal), formed by mutual head-tail bonding of the positively charged divalent metal ion complexed with the sulfide anion and the anionic oxygen of the dissociated 4-hydroxyl of the enolized pyrimidine ring.

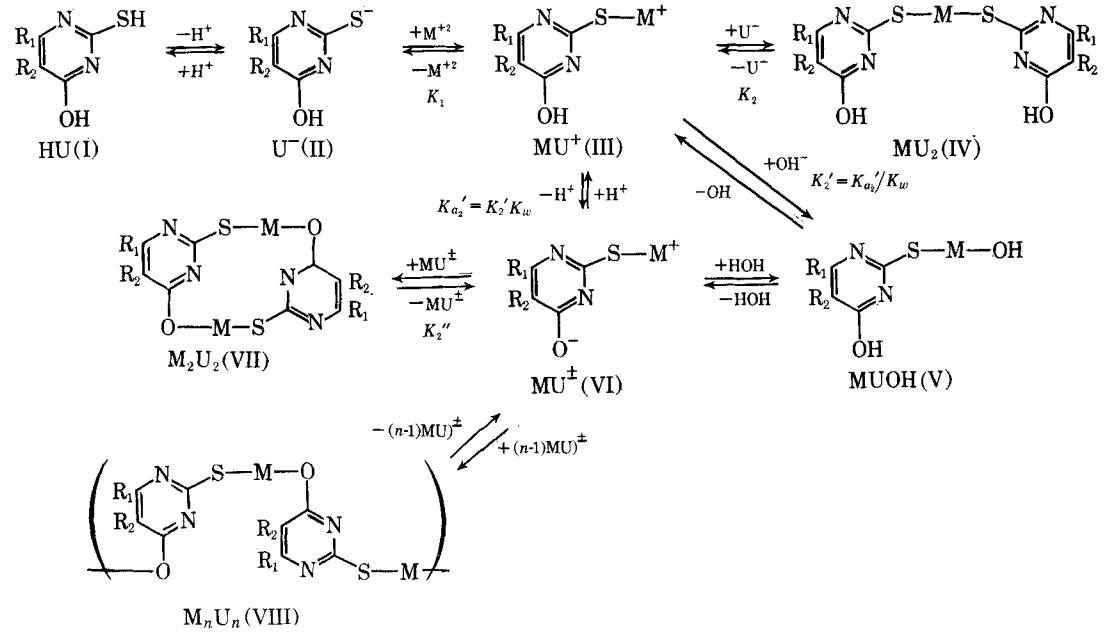
These subsequent studies were made to utilize spectrophotometric techniques to provide evidence for complex formation of these metal ions with the variously substituted thiouracils. A major purpose was to investigate the stability of the cupric-ion complexes by such methods since their high insolubility made potentiometric titration techniques impractical. Potentiometric titration techniques for the calculation of stability constants are limited in that the complexes formed must stay in solution and, in general, must be constituted with homogeneous or unmixed ligands. Another purpose of these studies was to evaluate the ability of solubility analyses under controlled conditions to identify the presence and stoichiometry of formed complexes and to calculate the solubilities and stability constants of such possible complexes as $MU^{\pm}$, MUOH, M_2U_2, and M_nU_n (Scheme I) which could not be determined potentiometrically (1).

Moreover, it had been proposed that the result of interaction of thiouracil and cupric copper was oxidation to the 2-thiouracil disulfide to form a stable complex with the simultaneously formed cuprous ion (2). This does not seem reasonable since 2-thiouracil disulfide is known to disproportionate readily to thio-

Table I—Absorbance Decrease from Additive Absorbances at $\lambda_{max.}$[a] of Thiouracil–Metal-Ion Mixtures

Compound	Absorbance at $\lambda_{max.}$[a] of 1.00×10^{-4} *M* TU	Metal, M^{+n}							
		Cu^{+2}		Cd^{+2}		Pb^{+2}		Fe^{+3}	Fe^{+2}
		10^4 $[M^{+n}]$							
		1.00	7.00	1.00	7.00	1.00	7.00	1.00[b]	1.00[c]
2-Thiouracil	1.328	0.208	0.341	0.051	0.086	0.050	0.120	−0.020	0.015
6-*n*-Propyl-2-thiouracil[d]	1.520	0.460	0.464	0.012	0.150	0.028	0.069	−0.012	−0.013
6-Methyl-2-thiouracil	1.512	0.512	0.567	0.011	0.085	0.025	0.065	0.006	−0.019
5-Methyl-2-thiouracil[e]	1.535	0.478	0.463	0.039	0.123	0.048	0.085	0.024	0.008
5,6-Dimethyl-2-thiouracil[d]	1.763	0.293	0.438	0.030	0.035	0.027	0.059	0.002	−0.003
2-Ethylmercapto-4-hydroxypyrimidine	0.550	−0.011	−0.020	−0.005	−0.013	0.003	0.006	0.024	0.000
6-Methyl-*N,N'*-diethyl-2-thiouracil	1.320	−0.001	−0.012	−0.001	−0.021	0.026	0.033	—	—

[a] $\lambda_{max.}$ for 2-ethylmercapto-4-hydroxypyrimidine was 284 nm., whereas the values for the other compounds lay between 272 and 278 nm. [b] A significant absorbance of about 0.210 was due to 1.00×10^{-4} *M* Fe^{+3}; in the noniron cases, the metals gave no absorbances at the $\lambda_{max.}$. This absorbance made reasonable differences unobtainable at 7.00×10^{-4} *M* Fe^{+3}. [c] The ready oxidation of ferrous ion to ferric ion by air at high concentrations gave anomalous interferences at 7.00×10^{-4} *M* Fe^{+2}. [d] Precipitates formed when these compounds were mixed with cupric ion so that the values under Cu^{+2} of absorbance decrease from additive absorbances for these compounds may not be considered as exact for complex formation in homogeneous solutions. No obvious precipitate was observed on mixing of the other thiouracils and Cu^{+2}. If precipitation had occurred in these cases, a relatively significant and wavelength-independent absorbance would have appeared at values above 310 nm. This was not observed (Fig. 1). [e] When the solvent was 0.001 *M* $HClO_4$ rather than water, there were negligible decreases in absorbance at the $\lambda_{max.}$ for mixtures of 10^{-4} *M* 5-methyl-2-thiouracil and 1.00×10^{-4} *M* and 7.00×10^{-4} *M* Cu^{+2} where the apparent absorbance decreases were 0.001 and 0.007, respectively.



Scheme I

uracil and the corresponding sulfinic acid (3, 4), and cuprous ion is known to readily disproportionate to cupric ion and metallic copper in solution (5). Spectral monitoring of these phenomena could give insight into the validity of this proposal.

EXPERIMENTAL[1]

Materials—The purification and characterization of the various thiouracils studied: 2-thiouracil, 6-*n*-propyl-2-thiouracil, 6-methyl-2-thiouracil, 5-methyl-2-thiouracil, 5,6-dimethyl-2-thiouracil, 2-ethylmercapto-4-hydroxypyrimidine, and 6-methyl-*N*,*N*′-diethyl-2-thiouracil, and the metals used were reported previously (1).

Spectra of Thiouracil–Metal-Ion Mixtures—The UV spectra of aqueous solutions, 1.00×10^{-4} *M* in the thiouracils and 0.00, 1.00, or 7.00×10^{-4} *M* in cupric, cadmium, lead, ferric, or ferrous nitrates, were recorded on the Cary model 15 dual-beam UV recording spectrophotometer against a water blank (Table I). The general procedure was to dilute stock solutions appropriately so as to record the spectra of the metal nitrate and thiouracil alone and then in combination (Figs. 1–3) at the stated molarities. Since the only metal ion studied with a large UV absorption was ferric ion (Fig. 3), spectrophotometric studies of solutions of mixtures, 1.0×10^{-4} *M* in thiouracil and 7.0×10^{-4} *M* in ferric or ferrous ion, were impractical. In the ferrous-ion case, this was due to the ready oxidation of such a large concentration to the absorbing and interfering ferric ion.

Slight precipitates were observed when cupric ion was mixed with 6-*n*-propyl-2-thiouracil and 5,6-dimethyl-2-thiouracil, so the resultant absorbance values of such mixtures (Table I) are probably below those that would have resulted from complexation in homogeneous solution. No precipitate was observed when cupric ion was added to the solutions of 2-thiouracil, 6-methyl-2-thiouracil, 5-methyl-2-thiouracil, 2-ethylmercapto-4-hydroxypyrimidine, or 6-methyl-*N*,*N*′-diethyl-2-thiouracil when the concentrations were as stated. If precipitation had occurred in these cases, it would be anticipated that absorbance due to turbidity would be independent of wavelength and would have been observed at wavelengths greater than 310 nm. This was not observed (Fig. 1).

The UV spectra of cupric-2-thiouracil mixtures were recorded at five hydrogen-ion concentrations that ranged from 1.0 to 1×10^{-4} *M* in $HClO_4$. The cupric-ion concentration was 2×10^{-4} *M* and the 2-thiouracil concentration was 10^{-4} *M* in each mixture. The spectra were read against blank solutions with appropriate $HClO_4$ concentrations. Spectra of mixtures that were 9×10^{-5} *M* in 2-thiouracil, 0.05 *M* in $HClO_4$, and 9 or 0.43×10^{-4} *M* in Cu^{+2} were also obtained.

Method of Continuous Variations (6) Applied to 2-Thiouracil and Cupric Ions—Varying aliquots of 1.00×10^{-4} *M* 2-thiouracil solu-

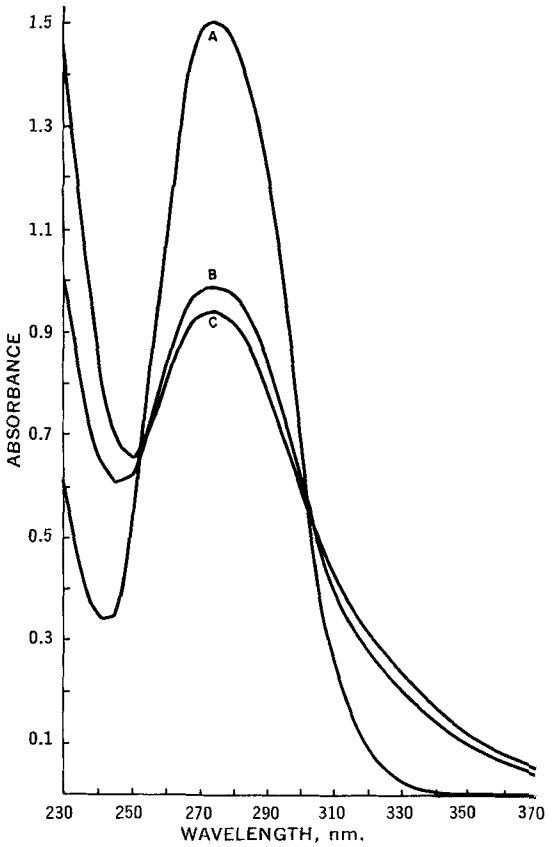


Figure 1—*UV spectra of mixtures of cupric nitrate and 6-methyl-2-thiouracil in water at 25.0°, where 1.00×10^{-4} M Cu^{+2} has no absorbance. Key: Curve A, 1.00×10^{-4} M 6-methyl-2-thiouracil; Curve B, 1.00×10^{-4} M $Cu(NO_3)_2$ and 1.00×10^{-4} M 6-methyl-2-thiouracil; and Curve C, 7.00×10^{-4} M $Cu(NO_3)_2$ and 1.00×10^{-4} M 6-methyl-2-thiouracil.*

[1] The elemental analyses and molecular weight and weight loss measurements in these investigations were performed by Huffman Laboratories, Inc., Wheatridge, Colo.

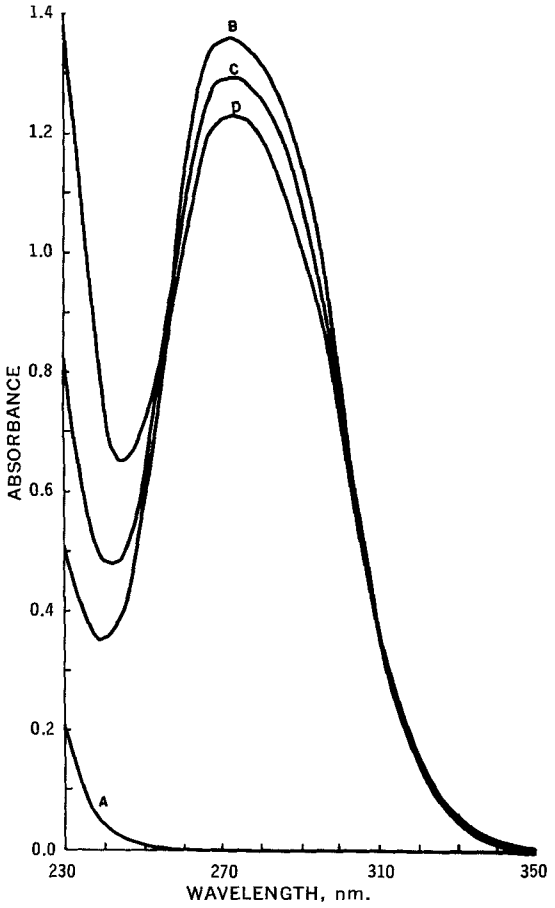


Figure 2—*UV spectra of mixtures of lead nitrate and 2-thiouracil in water at 25.0°. Key: Curve A, 1.00 × 10⁻⁴ M Pb⁺²; Curve B, 1.00 × 10⁻⁴ M 2-thiouracil; Curve C, 1.00 × 10⁻⁴ M 2-thiouracil and 1.00 × 10⁻⁴ M Pb⁺²; and Curve D, 1.00 × 10⁻⁴ M 2-thiouracil and 7.00 × 10⁻⁴ M Pb⁺².*

tion were added to aliquots of 1.00×10^{-4} *M* cupric nitrate solution so that the total number of moles of 2-thiouracil plus cupric ion was kept constant at 1.00×10^{-4}. The UV spectrum of each solution was recorded against a water blank.

Comparison of Total Thiouracil Solubilities at Various pH Values in the Absence and Presence of Cadmium Ion—Samples (200 mg.) of 2-thiouracil and 6-*n*-propyl-2-thiouracil were placed in volumetric flasks, and 40 ml. of solutions (ranging from 10^{-3} to 0.1 *M*) of varying concentrations of perchloric acid and sodium hydroxide was added. The final pH values ranged up to 6.5. The solutions with excess thiouracil were equilibrated at 25.0° in a controlled-temperature shaker bath, and the excess thiouracil was allowed to settle. Aliquots (5 ml.) of $Cd(NO_3)_2$ solutions were added slowly to some of the flasks; those that showed a precipitate on addition of the cadmium ions were discarded. The volume of each flask was made up to 50.0 ml. with the same aqueous solutions and reequilibrated at 25.0° in the shaker bath. The resultant molarity in Cd^{+2} was 0.001 *M*, although in some cases a 0.005 *M* Cd^{+2} solution was possible at lower pH values.

Filter sticks (Sargent No. S-30417) were used to remove samples from the equilibrated solutions. The first filtrate was discarded; 2.0-ml. aliquots of the filtered equilibrated solution were taken, appropriately diluted (50- or 100-fold) with 0.1 *M* $HClO_4$, and read on the Beckman DU spectrophotometer at $\lambda_{max.} = 272$ nm. against a 0.1 *M* $HClO_4$ blank solution to calculate the thiouracil in solution. Repetitive samples were taken with time to assure complete equilibration. The pH values of the equilibrated solutions were read in the presence of precipitate in a 10-ml. beaker.

Determination of Metal Content at Various pH Values in Saturated Thiouracil Solutions—Solutions (50 ml.), saturated with 2-thiouracil, 6-*n*-propyl-2-thiouracil, and 6-methyl-2-thiouracil, were prepared as described in the previous section; the final solutions were varied in $Cd(NO_3)_2$ from 1 to 15×10^{-3} *M*. Included in the 50-ml. volume were 1, 5, or 10 ml. of 0.01 *M* NaOH solutions. These solutions were equilibrated in a 25° shaker bath. At intervals, 5 ml. of the equilibrated solution was filtered through fritted filter sticks. Aliquots of these filtrates were diluted up to volume (50 ml.) with 1 *M* HNO_3, and 0.125 ml. 0.2% of a surface-active agent[2] solution was added to suppress the polarographic maxima. The solutions were purged with nitrogen and polarographically analyzed for cadmium ion, using a calibration curve made from similarly prepared solutions of known cadmium nitrate concentrations (6).

The $E_{1/2}$ in 1 *M* HNO_3 for Cd^{+2} is −0.59 v. against the saturated calomel electrode. A Sargent model XV polarograph was used. The H-type electrolysis cell was immersed in a constant-temperature bath at 25.0 ± 0.1°.

Preparation and Purification of Disodium Salt of 2-Thiouracil Disulfide Monohydrate, RSSR (X)—This material was prepared according to the procedure of Miller *et al.* (3). 2-Thiouracil (2.56 g.) was dissolved in 41 ml. of 1.0 *N* NaOH, and the solution was cooled in an acetone–dry ice bath until nearly frozen. Sodium iodide (4.57 g.) and iodine (2.54 g.) were dissolved in 20 ml. of water and also cooled in acetone–dry ice. The iodine–iodide solution was added slowly, with stirring, to the alkaline 2-thiouracil solution in an 800-ml. beaker. About 500 ml. of acetone was added, and the slow-forming white precipitate was collected. The precipitate was recrystallized from water–acetone twice and dried at 70°. Equivalent weight 156.6; calculated for $C_8H_4N_4Na_2O_2S_2 \cdot H_2O$, 158.1. UV spec-

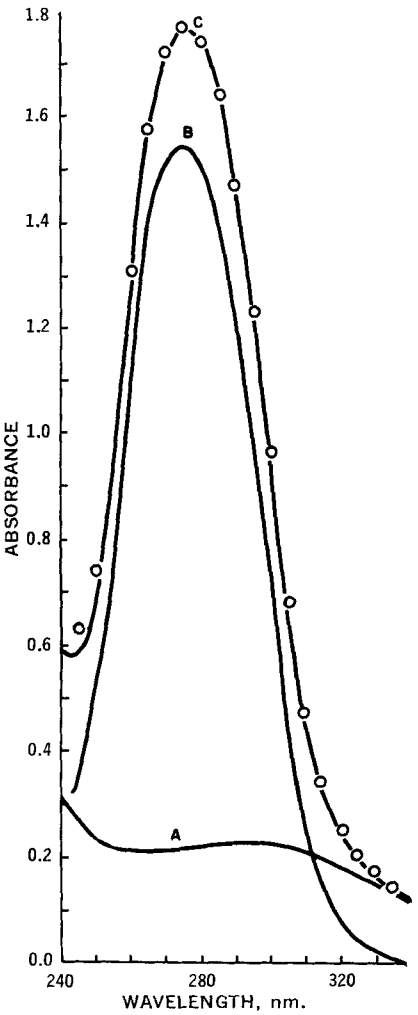


Figure 3—*UV spectra of mixtures of ferric nitrate and 6-methyl-2-thiouracil in water at 25.0°. Key: Curve A, 1.00 × 10⁻⁴ M Fe⁺³; Curve B, 1.00 × 10⁻⁴ M 6-methyl-2-thiouracil; and Curve C, 1.00 × 10⁻⁴ M 6-methyl-2-thiouracil and 1.00 × 10⁻⁴ M Fe⁺³. The plotted points are the summation of the absorbances of A and B.*

[2] Triton.

trum (3) (H_2O), $\lambda_{max.}$ 270 (ϵ 18,520), $\lambda_{max.}$ 211 (ϵ 25,485); (pH 9.56 ammonia buffer), $\lambda_{max.}$ 273 (ϵ 12,469), $\lambda_{max.}$ 213 (ϵ 31,537). IR spectrum, $\bar{\nu}$ in $cm.^{-1}$ (mineral oil mull): 3250 (OH), 1570, 1530, 1350, 1170, 1000, 828, 720. Molecular weight (ebullioscopic in water) 96; calculated for $C_8H_4N_4Na_2O_2S_2 \cdot H_2O$, 105.

Anal.—Calcd. for $C_8H_4N_4Na_2O_2S_2 \cdot H_2O$: C, 30.38; H, 1.91; N, 17.71; Na, 14.54; S, 20.27. Found: C, 29.87; H, 2.73; N, 14.38; Na, 15.15; S, 20.67.

Synthesis and Purification of Disodium Salt of 2-Sulfinyl-4-hydroxypyrimidine Trihydrate, RSO_2H (XI)—2-Thiouracil (2.56 g.) was dissolved in 26 ml. of 1.0 *N* NaOH, and 2.1 ml. of 34% hydrogen peroxide was slowly added with stirring to the acetone–dry ice chilled solution. Acetone was added to the point of precipitation, and the reaction mix was maintained cold overnight. The reaction mix was filtered and the precipitate washed with acetone; the washings were added to the filtrate, and the filtrate was put back into the refrigerator overnight. A white precipitate came down, which was collected, recrystallized twice from acetone–water, and dried at 50° in a vacuum oven. Equivalent weight 257.3; calculated for $C_4H_2N_2Na_2O_3S \cdot 3H_2O$, 256.1. UV spectrum (0.05 *M* $HClO_4$), $\lambda_{max.}$ 257 (ϵ 4924), $\lambda_{max.}$ 213 (ϵ 5885), $\lambda_{max.}$ 270 (ϵ 3510); (0.1 *M* $HClO_4$), $\lambda_{max.}$ 270 (ϵ 3631).

Anal.—Calcd. for $C_4H_2N_2Na_2O_3S$: C, 23.8; H, 1.00; N, 13.8; Na, 22.8; S, 15.87; weight loss for trihydrate, 21.15. Found: C, 23.96; H, 3.16; N, 12.38; Na, 21.03; S, 16.27; weight loss, 21.53.

For both Compounds X and XI, the consistencies of the C, S, and Na analyses of single samples were considered as sufficient evidence for the anticipated identities of the compounds since H and N analyses are notoriously difficult for the salts of such polyelemental compounds.

Degradation of 2-Thiouracil Disulfide in Absence and Presence of Cupric Ion—A solution (1.999×10^{-4} *M*) of the disodium salt of 2-thiouracil disulfide monohydrate was prepared by dissolving 6.322 mg. in 100 ml. of water. Aliquots (2.0 ml.) of the disulfide stock solution were added to: (*a*) 3.0 ml. H_2O or (*b*) 2.0 ml. H_2O and 1.0 ml. of 4.00×10^{-4} *M* cupric nitrate. Each solution was prepared when needed; then 5.00 ml. of 0.1 *M* $HClO_4$ was added very rapidly from a 5.00-ml. syringe (Hamilton) and mixed. The change in absorbance was recorded continuously at 270 nm. The UV absorption was measured using 1-cm. cells and a 0.05 *M* $HClO_4$ blank. A water-jacketed (25.0°) Beckman DU spectrophotometer, fitted with an automatic sample changer (Gilford) and connected to a strip-chart recorder (Sargent SRL), was used to record the absorbance as a function of time. The absorbances of all three solutions were recorded up to 400 min., well beyond the time when no further change occurred.

RESULTS AND DISCUSSION

Spectral Studies—The data of Table I summarize the spectral information for the solutions of mixtures of various thiouracils and metal ions obtained from the UV spectra shown in Figs. 1–3.

The premise of additivity of the absorbances attributable to the concentrations of thiouracil and metal ion should hold when there is no interaction. Only ferric ion of the studied metal ions had significant absorption at the $\lambda_{max.}$ values of the thiouracils. Since these particular studies (Figs. 1–3 and Table I) used a relatively high concentration of thiouracil (10^{-4} *M*) with high absorbances at the $\lambda_{max.}$ (about 1.5), differences between the anticipated and observed absorbances would have to exceed 0.02 to permit conclusions of significant interaction between the metal ion and the thiouracil.

On these premises, it is apparent (Table I) that Cu^{+2} (Fig. 1), Cd^{+2}, and Pb^{+2} (Fig. 2) show significant complex formation with almost all the studied thiouracils. The exceptions are the two compounds that cannot form an ionizable sulfhydryl group. These nontautomerizable compounds are 2-ethylmercapto-4-hydroxypyrimidine and 6-methyl-*N*,*N*′-diethyl-2-thiouracil. Absorbances of mixtures of the studied metals and these compounds were the same as the absorbance contributions of the compounds alone at all wavelengths.

There are no significant interactions of Fe^{+3} or Fe^{+2} with any of the listed thiouracils (Table I). This is clearly shown in the typical case of Fe^{+3} and 6-methyl-2-thiouracil (Fig. 3), where the plotted summed absorbances, points on Curve C, of the separate solutions of Fe^{+3} (Curve A) and 6-methyl-2-thiouracil (Curve B) are coincident with the recorded absorbances (Curve C) at all wavelengths of a mixture of the same concentrations.

The interaction of copper ions with the sulfhydryl-containing thiouracils, as monitored by the absorbance decrease from expected additivity, is obviously of a higher order of magnitude than such interactions with Cd^{+2} or Pb^{+2}, as can be seen in Table I and on comparison of Figs. 1 and 2 for equimolar Cu^{+2} and Pb^{+2}. The complexes in solution probably are similar in nature and have absorptivities that are not highly dependent on the nature of the complexed metal ion. It was shown previously (1) that the primary metal complexes of thiouracils formed in aqueous solution are predominantly MU^+ (III) and MU_2 (IV) (Scheme I) in the resultant homogeneous, acid solution.

The decrease in absorbance of thiouracil solutions, when the nonabsorbing metal ions are introduced, implies that Structures III and/or IV have lower absorptivities than the undissociated thiouracil I. It also follows on these premises that the greater the absorbance decrease for a given metal ion at equivalent concentrations and conditions, the greater the stability of the formed complex with thiouracil of that metal ion. Thus, the stabilities of the III and IV complexes of the thiouracils with Cu^{+2} are very much greater according to Table I than those for Cd^{+2} and Pb^{+2}. When the solvent was aqueous $HClO_4$ (0.001–1.0 *M*) rather than water alone, no significant decrease in absorbance from that expected from the thiouracil alone was observed for Cu^{+2}–5-methyl-2-thiouracil mixtures. This indicates that the equilibria of Scheme I are readily reversed by the addition of hydrogen ion and that noninteracting M^{+2} and thiouracil are in acidic solutions with minimal complexes such as III and IV.

The range of 5–10% loss of absorbance at $\lambda_{max.}$ values of about 272 nm. for the mixtures containing a sevenfold excess of Cd^{+2} and Pb^{+2} over thiouracil (10^{-4} *M*) is insufficient to permit studies of complex formation for these combinations by spectral means.

Method of Continuous Variations Applied to Cu^{+2} and 2-Thiouracil Mixtures—The spectra of Cu^{+2}–thiouracil mixtures indicate the formation of complexes by a decrease in the thiouracil absorption at the 272-nm. maximum (Fig. 1) and an increase in absorbance in the region of 345 nm. (Fig. 1). At both of these wavelengths, Cu^{+2} does not absorb.

The spectrophotometric method of continuous variations (7) can determine the apparent stoichiometry of a strong, major complex formed in solution. This method depends on the determination of the spectral absorbance of mixtures of equimolar solutions of the two complexing species. The mixtures are made in varying ratios so that the total number of moles of both species is kept constant, although the mole fraction of each species continuously varies.

The absorbances of such mixtures were obtained at 272 nm. (Curve B) and 345 nm. (Curve D) and are plotted against the mole fraction of each species in Fig. 4. The straight line (Curve A) represents the expected absorbances for all ratios if there are no interactions between the two species. Thus, the difference (Curve C) between Curves A and B represents the spectral aberration at 272 nm. attributable to complex formation. Curve D for the absorbance of the varying ratios is a similar representation *per se* since the reacting species do not absorb at 345 nm.

The method of continuous variations states that such difference plots (Curves C and D, Fig. 4) show maxima at mole fractions representative of the stoichiometry (with continuously varying mixtures of equimolar solutions) or of the stability constant (with continuously varying mixtures of nonequimolar solutions) of a single strong complex (7).

Unfortunately, the difference plots (Curves C and D, Fig. 4) are relatively small and the data have a relatively large variability; no decision can be made as to stoichiometry. This may be attributed to several factors: (*a*) the absorptivities of the metal complexes such as III and IV in Scheme I are only slightly less than that of the thiouracils; (*b*) under the conditions of study, the amount of complex formed is small; and (*c*) the conditions for a sharp maximum are destroyed when more complexes than one exist significantly in solution. Although all three of these reasons are valid, item *b* may be the most important. Complex formation is hindered in acidic solutions since addition of acid destroys the complex and reverses the equilibria of Scheme I; the "natural" pH values of these aqueous mixtures of Fig. 4 are acidic. When alkali was added to increase the concentration of the complexing thiouracil anion, II, ready precipitation of the complex occurred (1) and thus destroyed the utility of the spectral method to deduce the stoichiometry and stability constants of Cu^{+2} complexes of thiouracils in solution.

Limitations of Potentiometric Titration Methods in Stability

Constant Analysis of Thiouracil–Metal Complexes—The use of potentiometric titration data to determine the stoichiometries and stability constants of complexes demands the net substitution by one of the complexing species for a proton (or protons) on the other. It also demands the maintenance of homogeneous solutions during the simultaneous determination of pH (for the calculation of $\gamma_{\pm}[H^+]$ and/or $\gamma_{\pm}'[OH^-]$) and the amount of titer (alkaline or acid) necessary to obtain that pH for various mixtures of the complexing species, such as the doubly charged metal ion, M^{+2}, and thiouracil, HU.

In the present case, this information is sufficient to calculate the stability constants for the formation of MU^+ and MU_2 complexes (Scheme I). However, if $MU^{\pm}$ and/or MUOH complexes are formed and maintained in solution, the data from potentiometric titrations are inadequate for this purpose. As previously developed in detail (1), the following expressions define the various necessary dissociation and stability constants:

$$K_a' = \frac{\gamma_{\pm}[H^+][U^-]}{[HU]} \quad \text{(Eq. 1)}$$

where K_a' is the dissociation constant at the sulfhydryl group of the thiouracil, HU, to the thiouracil anion, U^-; and $\gamma_{\pm}$ is the activity coefficient for hydrogen ion:

$$K_1 = \frac{[MU^+]}{[M^{+2}][U^-]} \quad \text{(Eq. 2)}$$

where K_1 is the association constant for the formation of the simple complex, MU^+, due to the formation of a covalent bond between the divalent metal ion and the negatively charged sulfur of the thiouracil anion, U^-:

$$K_2 = \frac{[MU_2]}{[MU^+][U^-]} \quad \text{(Eq. 3)}$$

where K_2 is the association constant or step stability constant for the formation of the MU_2 complex due to the formation of the second covalent bond between the metal ion and the negatively charged sulfur of the second thiouracil anion so that the overall stability constant is:

$$\beta_2 = K_1K_2 = \frac{[MU_2]}{[M^{+2}][U^-]^2} \quad \text{(Eq. 4)}$$

The dissociation of the available proton from the hydroxyl in the 4-position of the MU^+ complex to form the $MU^{\pm}$ zwitterion may be defined as:

$$K_{a_2}' = \frac{[MU^{\pm}][H^+]\gamma_{\pm}}{[MU^+]} = K_2'K_w \quad \text{(Eq. 5)}$$

The association or step stability constant of a hydroxyl ion with the MU^+ complex to form a mixed ligand complex MUOH may be defined as:

$$K_2' = \frac{[MUOH]}{[MU^+][OH^-]\gamma_{\pm}'} = K_{a_2}'/K_w \quad \text{(Eq. 6)}$$

where K_w is the autoprotolytic constant for water, and $\gamma_{\pm}'$ is the activity coefficient for the hydroxyl-ion concentration. The overall stability constant for the formation of MUOH or $MU^{\pm}$ can be defined by:

$$\beta_{11} = K_1K_2'K_w = \frac{[MUOH][H^+]\gamma_{\pm}}{[M^{+2}][U^-]} = K_1K_{a_2}' = \frac{[MU^{\pm}][H^+]\gamma_{\pm}}{[M^{+2}][U^-]} \quad \text{(Eq. 7)}$$

where, in this particular case, the formation of a mixed ligand complex, MUOH, V cannot be distinguished from the formation of a zwitterion, $MU^{\pm}$, in terms of equilibrium constants.

It was shown previously (1) that the equation which relates the degree of formation, $\bar{n}$, *i.e.*, the average number of ligands bound to a metal ion, for the multiple equilibria in homogeneous solution of

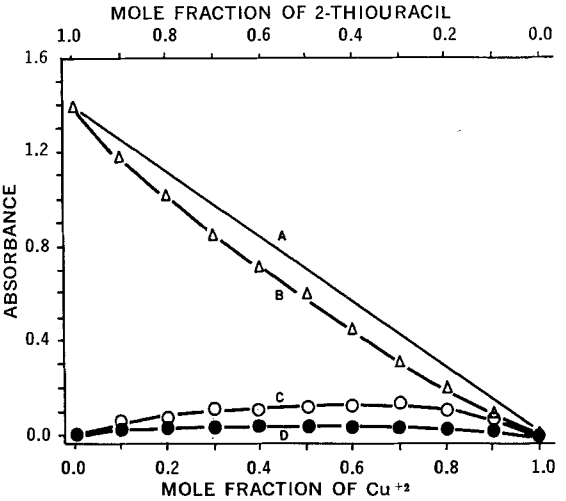


Figure 4—*Absorbances of continuously varied ratios of volumes of 10^{-4} M equimolar solutions of $Cu(NO_3)_2$ and 2-thiouracil so that the mole fraction of the solution, which is 10^{-4} M in both species, continuously varies. Curve A represents the absorbances at 272 nm. of 2-thiouracil if they are not affected by Cu^{+2}. Curve B represents the observed absorbances at 272 nm. of the mixtures of the specified molar ratios. Curve C represents the difference between Curves A and B at those molar ratios. Curve D represents the observed absorbances of the mixtures at 345 nm. where 2-thiouracil alone does not absorb.*

Scheme I to the respective dissociation and stability constants of Eqs. 1–7 is:

$$\frac{\bar{n}}{(1-\bar{n})[U^-]} = K_1 + \frac{\beta_{11}}{[H^+]\gamma_{\pm}} + \frac{(\bar{n}-2)}{(\bar{n}-1)}\beta_2[U^-] \quad \text{(Eq. 8)}$$

where $\bar{n}$ may be calculated from:

$$\bar{n} = \frac{[HU]_0 - \{[U^-] + [HU]\}}{[M^{+2}]_0} = \frac{[HU]_0 - [U^-](1 + [H^+]\gamma_{\pm}/K_a')}{[M^{+2}]_0} \quad \text{(Eq. 9)}$$

where $[HU]_0$ and $[M^{+2}]_0$ are the prepared concentrations of thiouracil and metal ion and are the sums of the respective concentrations in all forms and complexes and must refer to the amounts maintained in homogeneous solution, $[H^+]\gamma_{\pm}$ is calculable from the measured pH of the solutions, K_a' is the dissociation constant of thiouracil (Eq. 1), and the concentration of thiouracil anion may be calculated from:

$$[U^-] = \frac{[HU]_0 - [NaOH]}{[H^+]\gamma_{\pm}/K_a' - \beta_{11}[M^{+2}]/[H^+]\gamma_{\pm}} = \frac{[HU]_0 - [NaOH]}{[H^+]\gamma_{\pm}/K_a' - [MU^{\pm}]/[U^-]} = \frac{[HU]_0 - [NaOH]}{[H^+]\gamma_{\pm}/K_a' - [MUOH]/[U^-]} = \frac{[HU]_0 - [NaOH]}{[H^+]\gamma_{\pm}/K_a'} + \frac{[MU^{\pm}]}{[H^+]\gamma_{\pm}/K_a'} \quad \text{(Eq. 10)}$$

where [NaOH] is calculated on the basis that none of the added alkali to achieve the measured $\gamma_{\pm}[H^+] = 10^{-pH}$ had been consumed.

However, $[U^-]$ in Eq. 10 cannot be calculated for the multiple equilibria of Scheme I in homogeneous solution unless $\beta_{11}[M^{+2}]$ is also known, where $[M^{+2}]$ is the concentration of uncomplexed or free metal ion at any measured pH. This value of $[M^{+2}]$ cannot be obtained solely by the potentiometric titration of homogeneous solutions; its determination demands some selective analytical procedure, such as a specific metal/metal-ion electrode. The only exception would be when the potentiometric titrations could be carried out in a homogeneous solution in the presence of a large excess of metal ion and $[M^{+2}]$ could be taken as approximately equal to $[M^{+2}]_0$, where the amount of free metal ion lost to form the complex is insignificant with respect to the total metal ion originally

added. Such a case would demand small stability constants and soluble complexes. The latter is not so with these studied metal complexes of thiouracils.

When β_{11}, K_2', or $K_{a_2}' = 0$, *i.e.*, when there is no significant amount of MUOH or $MU^{\pm}$ formed or in solution (Eqs. 5–7), only then will $[U^-]$ be calculable from potentiometric titrations of homogeneous solutions since Eq. 10 then reduces to:

$$[U^-] = \frac{[HU]_0 - [NaOH]}{[H^+]\gamma_{\pm}/K_a'} \quad \text{(Eq. 11)}$$

and Eq. 8 reduces to:

$$\frac{\bar{n}}{(1 - \bar{n})[U^-]} = K_1 + \frac{(\bar{n} - 2)}{(\bar{n} - 1)}\beta_2[U^-] \quad \text{(Eq. 12)}$$

where $\bar{n}$ is calculable from Eq. 9 since $[U^-]$ is now calculable from Eq. 11. Thus, $\beta_2 = K_1K_2$ and K_1 can be obtained from the slope and intercept, respectively, of the plot of $\{\bar{n}/(1 - \bar{n})[U^-]\}$ *versus* $[U^-]$, all values obtainable from the potentiometric titrations of homogeneous solutions containing only MU^+ or MU_2 complexes (Eqs. 2–4).

At high acidities, Eqs. 11 and 12 can be used as valid approximations to obtain β_2 and K_1 since:

$$\beta_{11}[M^{+2}]/[H^+]\gamma_{\pm} = [MU^{\pm}]/[U^-] = [MUOH]/[U^-] \ll [H^+]\gamma_{\pm}/K_a' \quad \text{(Eq. 13)}$$

and Eq. 10 reduces to Eq. 11 at low pH values.

An alternate perspective is that $MU^{\pm}$ (or MUOH) formation is a result of MU^+ dissociation (or its association with $[OH^-]$, Eq. 6) and would be restricted by a high pKa_2 (or $pK_b = pK_2'$). The $[MU^{\pm}]$ should be relatively negligible at 2 pH units below pKa_2', and [MUOH] should be relatively negligible at 2 pH units below its $pK_b = -pK_2'$ (Eq. 6), where $K_b = 1/K_2'$ is its basic dissociation constant to yield hydroxyl ions and the MU^+ complex.

Possible Circumvention of Titration Method Limitations by Total Thiouracil Solubility Determination in the Presence of Complexing Metal Ions—One way to determine $[U^-]$ in the presence of an $MU^{\pm}$ (and/or MUOH) complex when Eqs. 8–10 must hold is to maintain the solutions saturated with thiouracil at various $[M^{+2}]_0$ and pH values. The concentration of undissociated thiouracil, [HU], is then fixed at its intrinsic solubility, S_i. Thus, $[U^-]$ is calculable from Eq. 1 at all known hydrogen-ion activities and is restricted to:

$$[U^-] = K_a'S_i/[H^+]\gamma_{\pm} \quad \text{(Eq. 14)}$$

The total solubility, S_0, of thiouracil as the undissociated acid and its anion in the absence of complexing metal ion on consideration of Eqs. 1 and 3 may be expressed by:

$$S_0 = [HU] + [U^-] = S_i(1 + K_a'/[H^+]\gamma_{\pm}) \quad \text{(Eq. 15)}$$

and Eq. 9, for all complexes in the equilibria of Scheme I, becomes:

$$\bar{n} = \frac{S_T - S_0}{[M^{+2}]_0} \quad \text{(Eq. 16)}$$

where $S_T = [HU]_0$, the total solubility of thiouracil as acid, anion, and all possible complexes in the presence of an initial concentration, $[M^{+2}]_0$, of metal ion and in a solution saturated with thiouracil acid, *i.e.*, $[HU] = S_i$.

Substitution of rearrangements of Eq. 14 into Eq. 8 with subsequent rearrangements can yield:

$$\frac{\bar{n}}{(1 - \bar{n})[U^-]^2} - \frac{K_1}{[U^-]} = \frac{\beta_{11}}{K_a'S_i} + \frac{(\bar{n} - 2)}{(\bar{n} - 1)}\beta_2 \quad \text{(Eq. 17)}$$

or

$$\frac{\bar{n}\gamma_{\pm}^2[H^+]^2}{(1 - \bar{n})K_a'S_i} - K_1\gamma_{\pm}[H^+] = \beta_{11} + \frac{(\bar{n} - 2)}{(\bar{n} - 1)}\beta_2K_a'S_i \quad \text{(Eq. 18)}$$

so that plots of left-hand sides of these expressions against $(\bar{n} - 2)/(\bar{n} - 1)$ will give intercept values of $\beta_{11}/K_a'S_i$ and β_{11}, respectively, and slope values of β_2 and $\beta_2K_a'S_i$, respectively. In saturated solutions of undissociated thiouracil, where $[HU] = S_i$, all values of $\bar{n}$ (Eq. 16), $[U^-]$ (Eq. 14), and $\gamma_{\pm}[H^+] = 10^{-pH}$ are calculable; K_a' and S_i are obtainable by the respective titration and solubility studies of HU, and K_1 may be well estimated from appropriate plots (Eq. 13) from the potentiometric titrations of homogeneous solutions in the more acidic regions. Thus, β_2 and β_{11} are obtainable from the slopes and intercepts of Eqs. 17 and 18 to permit the calculation of K_{a_2}' and K_2' (Eq. 7).

The concentrations of $MU^{\pm}$, its equivalent mixed ligand complex, MUOH, or the sum of both concentrations are also calculable if the amount of NaOH necessary to give a measured pH value where the total solubility, $S_T = [HU]_0$, of complexing thiouracil is measured and the intrinsic solubility, S_i, at that pH is known. This follows from the substitution of Eq. 14 into the rearranged Eq. 10, where:

$$[HU]_0 - [NaOH] = [H^+][U^-]\gamma_{\pm}/K_a' - [MU^{\pm}] = S_i - [MU^{\pm}] = S_i - [MUOH] \quad \text{(Eq. 19)}$$

and thus:

$$[MUOH] \text{ and/or } [MU^{\pm}] = S_i + S_T + [NaOH] \quad \text{(Eq. 20)}$$

where the alkali added is calculated in terms of [NaOH] in that solution to give $\gamma_{\pm}[H^+] = 10^{-pH}$.

Theoretical and Practical Limitations of Thiouracil Solubility Analysis in Stability Constant Determination of Thiouracil–Metal Complexes—The previous discussion and outlined procedures permit the determination of all stability constants—*viz.*, K_1, K_2, β_{11}, K_2', and/or K_{a_2}' for significant concentrations of the pertinent complexes in the multiple equilibria of Scheme I, provided that (*a*) the solutions of mixtures of thiouracil and metal ion are saturated with undissociated thiouracil at all equilibrated and measured pH values, and (*b*) no metal is precipitated as a thiouracil or mixed ligand complex or as its hydroxide at any equilibrated and measured pH value. If the latter is not so, the known total concentration of metal ion in the solutions, $[M^{+2}]_0$, cannot be used to estimate the sums of the concentrations of all forms (ions and complexes) containing metals. Thus, $\bar{n}$ would be unobtainable from Eq. 16.

A practical consideration of Eq. 16 is that the total experimentally determined solubility of thiouracil, S_T, as the sum of the concentrations of thiouracil in all forms as acid, ions, and complexes must significantly exceed the solubility, S_0, of thiouracil as the acid and anion at any given pH.

It can be readily shown from Eqs. 14 and 15 that:

$$\log \frac{S_0 - S_i}{S_i} = \log \frac{A - A_{H^+}}{A_{H^+}} = pH - pKa' \quad \text{(Eq. 21)}$$

where A is the spectrophotometric absorbance of an aliquot of the thermostatically filtered solution of a saturated thiouracil solution at a given pH which has been diluted 1:1 and made 0.1 N in $HClO_4$. The A_{H^+} is the absorbance of an aliquot of a saturated solution of thiouracil in 0.1 N $HClO_4$ diluted 1:1 and read in 0.1 N $HClO_4$. A typical plot of the logarithmic function of absorbances against pH is given in Fig. 5 in accordance with Eq. 21 for 2-thiouracil at 25.0°. This plot has the expected positive slope of unity, and the pKa′ is 7.52 as estimated from the intercept. Similar plots were obtained for the other thiouracils studied; the data were summarized in Table II of *Reference 1*.

When cadmium ion was added to these saturated solutions of thiouracils at various pH values, the filtrate diluted, and the absorbances measured in the manner described, no significant difference in absorbance was observed for a given pH value over those saturated solutions studied without cadmium ion. These solubility studies on 2-thiouracil and 6-*n*-propyl-2-thiouracil with cadmium ion had to be restricted to pH values less than 6 since the complex precipitation that occurred above that value (1) had to be avoided. Since the total solubility, S_T, in the presence of cadmium ion did not exceed the total solubility, S_0, in its absence for any measurable pH value, significant values of $\bar{n}$ could not be calculated from Eq. 16; this total thiouracil solubility method was not applicable in this case.

This is not unexpected, as can be shown by a specific set of calculations for 6-*n*-propyl-2-thiouracil and cadmium ion at 25.0° based on the data given in Table II of *Reference 1*, where $S_i = 7.07 \times 10^{-3}$ M/l. and $K_a' = 1.74 \times 10^{-8}$ M/l. The total solubility of all 6-*n*-propyl-2-thiouracil forms in solution with metal ions can

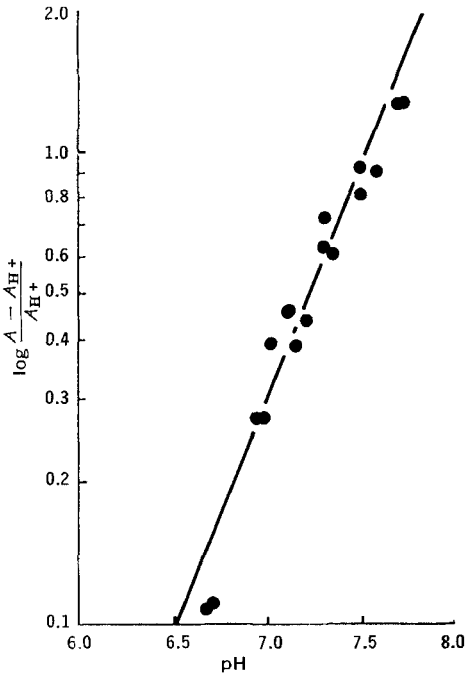


Figure 5—*Plot of log* $(A - A_{H^+})/A_{H^+}$ *against pH for aqueous, saturated 2-thiouracil solutions with and without added metal ions in solution at 25.0° according to log* $(A - A_{H^+})/A_{H^+} = pH - pKa'$, *where* A *is the absorbance of a saturated solution in the buffer region and* A_{H^+} *is the absorbance due to the intrinsic solubility as measured in 0.100* M $HClO_4$. *The slope is 1.00 and pKa' is 7.52.*

be defined as:

$$S_T = S_0 + [MU^+] + 2[MU_2] + [MU^{\pm}] + [MUOH] \quad \text{(Eq. 22)}$$

where S_0 is obtainable from Eq. 15.

If $[MU^{\pm}]$ and [MUOH] are considered to be exceedingly small and it is assumed that less than 0.1 of the total metal-ion concentration is complexed, so that $[M^{+2}] \sim [M^{+2}]_0$, then $\beta_2 = K_1K_2 = 4.49 \times 10^{-8}$ $(l./M)^2$, where $K_1 = 1.45 \times 10^{-4}$ $l./M$ and $K_2 = 3.09 \times 10^{-4}$ $l./M$ for Cd^{+2}–6-*n*-propyl-2-thiouracil complexes. Thus, $[MU^+]$ and $[MU_2]$ can be calculated from Eqs. 2 and 4, respectively, where $[U^-]$ in thiouracil-saturated solutions is known from Eq. 14 and the measured $\gamma_{\pm}[H^+] = 10^{-pH}$. The calculations of S_T (Eq. 22) for several cases gives the following results: 10^3S_T, 10^3 $[Cd^{+2}]$, and pH: 7.07, 0.00, and 4.00; 7.08, 1.00, and 4.00; 7.17, 5.00, and 4.00; 7.08, 0.00, and 5.00; and 7.30, 1.00, and 5.00. It must be concluded that the difference between S_T and S_0 up to pH 5 is not sufficiently significant for utilization of this method. It would be feasible to calculate significant differences, $S_T - S_0$, at higher alkalinities where the concentrations of complexes should be higher if their precipitation did not occur. However, complex precipitation was observed with the mixture of 2×10^{-3} *M* 6-*n*-propyl-2-thiouracil and 2×10^{-3} *M* Cd^{+2} in the region of pH 5 (1). At higher pH values, cadmium ion was observed to contaminate the excess insoluble 6-*n*-propyl-2-thiouracil, which implied that the cadmium complexes had precipitated and these methods were contraindicated.

Possible Circumvention of Titration Method Limitations by Analysis of Total Metal, M_S, in Solution—The concentration, M_S, of metal in solution prior to the precipitation of any metal–thiouracil complex is the same as the calculated total metal concentration (1), $[M^{+2}]_0$, so that a plot of M_S against $[M^{+2}]_0$ would be linear and of unit slope for any given pH value (Curves a–b, a–b′, and a–b″ of Fig. 6) until the solubility of any one complex given in the following expression (see Scheme I) is exceeded:

$$M_S = [M^{+2}] + [MU^+] + [MU^{\pm}] + [MU_2] \quad \text{(Eq. 23)}$$

For the sake of convenience in this development, $[MU^{\pm}]$ (VI) is considered as the sum of the concentrations of this complex and the mixed ligand complex, MUOH (V), which was shown in Eq. 7 to be stoichiometrically equivalent. Appropriate substitutions of rearranged Eq. 2 for $[MU^+]$, Eq. 4 for $[MU_2]$, and Eq. 7 for $[MU^{\pm}]$ in Eq. 23 result in:

$$M_S = [M^{+2}]\left\{1 + K_1[U^-] + \frac{\beta_{11}[U^-]}{[H^+]\gamma_{\pm}} + \beta_2[U^-]^2\right\} \quad \text{(Eq. 24)}$$

where $[U^-]$ of Eq. 11 may be calculated only if $[M^{+2}]$ can be obtained from direct analysis in solution.

$[MU^{\pm}]$ may be considered negligible at low pH values since the pKa_2' for the dissociation of MU^+ must be approached for β_{11} of Eq. 7 to become significant. If the conditions are such that MU_2 precipitates at these low pH values, then from Eq. 4,

$$[M^{+2}] = S_{MU_2}/\beta_2[U^-]^2 \quad \text{(Eq. 25)}$$

where S_{MU_2} is the solubility of MU_2. It follows that when the precipitation of MU_2 is just noted (at points b, b′, and b″ for various decreasing $[H^+]$ in Fig. 6), Eq. 24 becomes:

$$M_S = [M^{+2}]\{1 + K_1[U^-] + S_{MU_2}\} = S_{MU_2}\{(1/\beta_2)(1/[U^-]^2) + (K_1/\beta_2)(1/[U^-]) + 1\} \quad \text{(Eq. 26)}$$

where $[U^-]$ is calculable from Eq. 12 using the experimental values of $[HU]_0$, [NaOH], and $[H^+]$ when the $[MU^{\pm}]$ of Eq. 11 is negligible. This Eq. 26 is of the polynomial form:

$$M_S = a(1/[U^-])^2 + b(1/[U^-]) + c \quad \text{(Eq. 27)}$$

where $a = S_{MU_2}/K_1K_2 = S_{MU_2}/\beta_2$, $b = K_1S_{MU_2}/\beta_2 = S_{MU_2}/K_2$, and $c = S_{MU_2}$. Thus, a series of analyses of concentrations of metal ions in solution, M_S, at various known hydrogen-ion concentrations to permit calculations of $[U^-]$ from Eq. 12 when negligible $MU^{\pm}$ exists (see Fig. 6) can permit fitting of the constants of Eq. 27 and determination of the solubility of MU_2.

The data obtained on potentiometric titrations of metal–thiouracil mixtures (1) permitted observation of pH values at which the

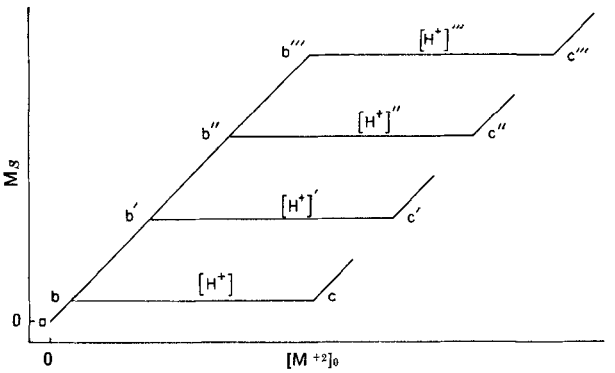


Figure 6—*Anticipated plots of concentrations of complexing metal,* M_S, *in solutions equilibrated at various hydrogen-ion concentrations* versus *the concentration of metal ion* $[M^{+2}]_0$ *that should have been present in these solutions if no complexation or interaction with thiouracil had occurred. The ordinate value of* M_S *at precipitation (b, b′, b″, or b‴) should decrease with decreased hydrogen-ion concentration,* i.e., $[H^+]''' > [H^+]'' > [H^+]' > [H^+]$. *Each plateau represents a constant pH condition for a given amount of added excess thiouracil where a higher concentration of metal would be needed to form and precipitate complexes at the lower pH values since the concentration of the complexing thiouracil anion is lessened. The abscissa lengths, bc, b′c′,* etc., *represent the concentration of metal ion consumed in the precipitation of the least soluble complex. The ordinate distance, b,b′,* etc., *either approaches the solubility of* MU_2 *if this is the preferable precipitated complex (Eq. 33) or approaches a value proportional to, but greater than, the solubility of* $MU^{\pm}$ *(or a* M_nU_n *complex) if this is the preferable precipitated complex (Eq. 36). The fact that, at the lowest and highest pH values studied, no significant values of b could be observed for such solubility studies on mixtures of* $Cd(NO_3)_2$ *with several thiouracils implies that the solubilities of such complexes are exceedingly small and more sensitive measurements of total metal in solution are needed than were available. The plot subsequent to points c, c′,* etc., *is not necessarily linear or of unit slope. For example, if* MU_2 *is precipitated first, on exhaustion of excess thiouracil it may redissolve and form* M_nU_n *complexes with the increased added metal.*

possible precipitation of complexes was initiated. If these complexes were MU_2, and if there was no significant supersaturation of MU_2, the calculated $[M^{+2}]_0$ should be just equal to M_S at this pH value. Since the $[U^-]$ values can be calculated (Eq. 11) from the known $[HU]_0 - [NaOH]$ and $[H^+]$ values, the necessary data for use of Eqs. 26 and 27 should be available if the complex whose solubility was first exceeded was MU_2.

Unfortunately, prior studies (1) indicated that the precipitating complex on potentiometric titration normally had a 1:1 stoichiometry and most probably was the cyclic dimer, M_2U_2, bis-(thiouracil–metal), obtained from the head-tail dimerization of the zwitterion, $MU^{\pm}$.

The known total metal concentrations, as prepared in the previously cited potentiometric titrations, were plotted against $1/[U^-]$, where $[U^-]$ was calculated from Eq. 11 for the $[H^+]$ at the pH of initial precipitation, in accordance with the assumptions underlying Eqs. 25–27. The resultant plots for Cd–2-thiouracil and Pb–2-thiouracil mixtures were linear and not parabolic as would be predicted by Eq. 27. There was no statistically significant intercept different than zero. The slopes were 7.3×10^{-8} and 4.57×10^{-8} M^2 for the respective mixtures of Cd–2-thiouracil, where $\log K_1 = 4.02$ and $\log K_2 = 3.49$ (1), and of Pb–2-thiouracil, where $\log K_1 = 4.74$ and $\log K_2 = 3.44$ (1). If these slope values are assigned to $b = S_{MU_2}/K_2$ of Eq. 27, then $S_{MU_2} = 2.26 \times 10^{-4}$ M for $Cd(TU)_2$ and $S_{MU_2} = 1.26 \times 10^{-4}$ M for $Pb(TU)_2$. These values are not inconsistent, within error, with the apparent zero intercepts. However, if one inserts these values for $S_{MU_2} = [MU_2]$ into Eq. 4 and uses the above ascertained values of K_1 and K_2, where $[U^-]$ is calculated from Eq. 11, an utterly impossible concentration of free metal ion, $[M^{+2}]$, is estimated. For example, in the case of the Cd–2-thiouracil mixture at a total metal concentration of $M_S = 2 \times 10^{-3}$ M,

$$[M^{+2}] = S_{MU_2}/K_1K_2[U^-]^2 = \frac{2.26 \times 10^{-4}}{(1.05 \times 10^4)(3.10 \times 10^3)}\left(\frac{1}{3.64 \times 10^{-5}}\right)^2 = 3.4 \times 10^{-3}\ M \quad \text{(Eq. 28)}$$

Since it is impossible that the free or uncomplexed metal-ion concentration, $[M^{+2}]$, can exceed the total metal concentration, M_S, the underlying assumptions of Eqs. 11 and 25–27 must be invalid. This is also consistent with the fact that MU_2 normally is not the stoichiometric precipitate in the potentiometric titration studies (1).

If $MU^{\pm}$ of Eq. 23 precipitates as M_2U_2 (1) before the concentration of MU_2 exceeds its solubility, Eq. 7 dictates that:

$$[M^{+2}] = S_{MU^{\pm}}[H^+]\gamma_{\pm}/\beta_{11}[U^-] \quad \text{(Eq. 29)}$$

where $S_{MU^{\pm}}$ is the solubility of $MU^{\pm}$.

It follows that when the precipitation of $MU^{\pm}$ is just noted (at points b, b′, and b″ for various decreasing $[H^+]$ as in Fig. 6), Eqs. 23 and 24 become the more complicated expressions:

$$M_S = [M^{+2}]\{1 + K_1[U^-] + \beta_2[U^-]^2\} + S_{MU^{\pm}} = S_{MU^{\pm}}\left\{\frac{[H^+]\gamma_{\pm}}{\beta_{11}[U^-]} + \frac{K_1[H^+]\gamma_{\pm}}{\beta_{11}} + \frac{\beta_2[U^-][H^+]\gamma_{\pm}}{\beta_{11}} + 1\right\} = \frac{S_{MU^{\pm}}}{K_{a_2}'}\left\{\frac{[H^+]\gamma_{\pm}}{[U^-]}\left(\frac{1}{K_1}\right) + [H^+]\gamma_{\pm} + K_2K_a'[HU] + K_{a_2}'\right\} \quad \text{(Eq. 30)}$$

where $[U^-]$ must be obtained from Eq. 10.

Possible Circumvention of Titration Method Limitations by Analyses of Total Metal, M_S, in Saturated Thiouracil Solutions—If the solution is saturated with thiouracil, application of Eq. 14, which considers S_i as the constant solubility of undissociated thiouracil, transforms Eq. 24 to:

$$M_S = [M^{+2}]\{1 + K_1K_a'S_i(1/[H^+]\gamma_{\pm}) + (\beta_{11}K_a'S_i + \beta_2(K_a')^2S_i^2)(1/[H^+]\gamma_{\pm})^2\} \quad \text{(Eq. 31)}$$

If MU_2 precipitates first and $[MU^{\pm}]$ is negligible, then from Eqs. 4 and 14,

$$[M^{+2}] = S_{MU_2}[H^+]^2\gamma_{\pm}^2/[\beta_2(K_a')^2S_i^2] \quad \text{(Eq. 32)}$$

where S_{MU_2} is the solubility of MU_2, and it follows that Eq. 26 becomes:

$$M_S = S_{MU_2}\{[H^+]^2\gamma_{\pm}^2/[\beta_2(K_a')^2S_i^2] + \gamma^{\pm}[H^+]/[\beta_2K_a'S_i] + 1\} \quad \text{(Eq. 33)}$$

Thus, a plot of the analyzed total metal concentration, M_S, in saturated thiouracil solution against the hydrogen-ion activity, $\gamma_{\pm}[H^+]$, should fit the expression:

$$M_S = a'\gamma_{\pm}^2[H^+]^2 + b'\gamma_{\pm}[H^+] + c' \quad \text{(Eq. 34)}$$

where $a' = S_{MU_2}/[\beta_2(K_a')^2S_i^2]$, $b' = S_{MU_2}/[\beta_2K_a'S_i]$, and $c' = S_{MU_2}$, when MU_2 is the first complex to exceed its solubility.

If $MU^{\pm}$ precipitates as the dimer M_2U_2 (1) in saturated thiouracil solutions before MU_2 exceeds its solubility, then it can be shown from Eqs. 7 and 14 that:

$$[M^{+2}] = S_{MU^{\pm}}[H^+]\gamma_{\pm}/[U^-]\beta_{11} = S_{MU^{\pm}}[H^+]^2\gamma_{\pm}^2/[\beta_{11}K_a'S_i] \quad \text{(Eq. 35)}$$

where $S_{MU^{\pm}}$ is the solubility of $MU^{\pm}$, and it follows that Eq. 30 becomes:

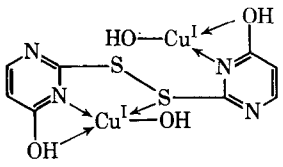
$$M_S = S_{MU^{\pm}}\{[H^+]^2\gamma_{\pm}^2/[\beta_{11}K_a'S_i] + K_1[H^+]\gamma_{\pm}/\beta_{11} + \beta_2K_a'S_i/\beta_{11} + 1\} \quad \text{(Eq. 36)}$$

Thus, a plot of the total metal concentration, M_S, in saturated thiouracil solution against the hydrogen-ion activity, $\gamma_{\pm}[H^+]$, should also fit the polynomial Eq. 34, but where the constants have a different significance, *i.e.*, $a' = S_{MU^{\pm}}/[\beta_{11}K_{a_2}'S_i]$, $b' = K_1S_{MU^{\pm}}/\beta_{11} = S_{MU^{\pm}}/K_{a_2}'$, and $c' = 1 + \beta_2K_a'S_i/\beta_{11} = 1 + K_2K_a'S_i/K_{a_2}'$, when $MU^{\pm}$ is the first complex to exceed its solubility.

The data obtained by analysis of metal concentrations, M_S, in saturated solutions of thiouracil at maintained pH values should give similar curves to those drawn in Fig. 6. The plateau values of M_S at known hydrogen-ion concentrations should permit estimation of the a', b', and c' values of Eq. 34, whether the MU_2 or the $MU^{\pm}$ complex precipitates first. No other measurements are necessary on the saturated thiouracil systems.

The metal concentration, M_S, assayed in the filtrate from the equilibrated saturated thiouracil solutions did not give the ideal plots of Fig. 6 when plotted against the concentrations of metal ion added. There was no significant increase in filtrate metal concentration with added metal until all the excess thiouracil was consumed in complex formation (point c, Fig. 5). Even then, the M_S values were widely variant and did not follow the ideal linearity for $[M^{+2}]_0$ values beyond points c, c′, and c″. This implies that: (*a*) the solubility of the precipitated complex (M_S values of idealized plateaus b–c, b′–c′, and b″–c″ of Fig. 6) was too small to be evaluated properly by this method within the error of the polarographic analysis of metals in the filtrate, and/or (*b*) metal-ion or metal–thiouracil complex is adsorbed from the solution onto the precipitated complex or onto the excess thiouracil under the conditions of the studies. Also, further proof of the difficulty of equilibration was the inability to obtain a constant pH after adding alkali over months of equilibration in the constant-temperature baths. Thus, although these equations and approaches are theoretically sound, they were not able to be put into practice in these studies.

Evaluation of Proposed Complex of Cuprous Ion and 2-Thiouracil Disulfide as a Result of Cupric Ion and 2-Thiouracil Interaction—Weiss and Venner (2) isolated precipitates after a day's presumed reaction of thiouracil with cupric ion in 1.0 *N* NaOH. They assigned the possible structure of a disulfide complex of cuprous ion, IX, on the basis of the elemental analysis of this precipitate.

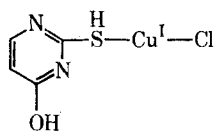


IX

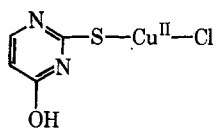
Since it has been demonstrated by the present authors that alkaline thiouracil solutions are susceptible to air oxidation, even in the absence of cupric ion, the possibility exists that the isolated complex contained cupric rather than the suggested cuprous ion.

A solution of thiouracil disulfide in water, reacted with cuprous

chloride in concentrated hydrochloric acid, produced a yellow product that Weiss and Venner (2), on the basis of nitrogen, copper, and chloride analysis, proposed to be IX except for the substitution of chlorine for hydroxyl on the copper. However, the extreme instability of thiouracil disulfide under such conditions (3, 4) casts doubt on this assertion. It is possible that the product may actually be X or even XI, if the great ease of disproportionation of cuprous to cupric ion (5) is considered.



X

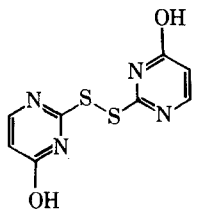


XI

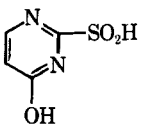
These proposals (2) imply that the complex, IX, results from the reaction of cupric ion and thiouracil as a consequence of the redox reaction:

$$2RSH + 2Cu^{+2} \rightarrow RSSR + 2Cu^{+} \rightarrow IX \quad \text{(Eq. 37)}$$

where RSH is the thiouracil, I, and RSSR is the 2-thiouracil disulfide, XII. Such statements could argue against possible assignments



RSSR, XII



RSO_2H, XIII

of the Structure III, IV, or V (Scheme I) for the copper complexes.

It is known (3, 4, 8) that the hydrolysis of the disulfide, RSSR, proceeds by disproportionation in acidic solutions to the thiouracil, RSH, and the sulfinic acid, RSO_2H (XIII) where for n moles of 2-thiouracil disulfide,

$$n\text{RSSR} + n\text{H}_2\text{O} \rightarrow (3n/2)\text{RSH} + (n/2)\text{RSO}_2\text{H} \quad \text{(Eq. 38)}$$

If n moles of cupric ion do react with the generated $3n/2$ moles of thiouracil, then,

$$(3n/2)\text{RSH} + n\text{Cu}^{+2} \rightarrow (n/2)\text{RSH} + (n/2)\text{RSSR} + n\text{Cu}^{+} \quad \text{(Eq. 39)}$$

and it follows that the resultant formed $n/2$ moles of RSSR should again disproportionate as:

$$(n/2)\text{RSSR} + (n/2)\text{H}_2\text{O} \rightarrow (3n/4)\text{RSH} + (n/4)\text{RSO}_2\text{H} \quad \text{(Eq. 40)}$$

When the sum of Eqs. 38–40 is simplified, the overall reaction of thiouracil disulfide with equimolar cupric ion, if cupric ion oxidized the thiouracil, should be:

$$n\text{RSSR} + (3n/2)\text{H}_2\text{O} + n\text{Cu}^{+2} \rightarrow (3n/4)\text{RSO}_2\text{H} + (5n/4)\text{RSH} + n\text{Cu}^{+} \quad \text{(Eq. 41)}$$

The overall reaction of 1 mole of the disulfide with 2 moles of cupric ion, if cupric ion oxidizes the thiouracil, should be:

$$n\text{RSSR} + 2n\text{H}_2\text{O} + 2n\text{Cu}^{+2} \rightarrow n\text{RSH} + 2n\text{Cu}^{+} + n\text{RSO}_2\text{H} \quad \text{(Eq. 42)}$$

The final absorbances at a given wavelength of solutions containing such 1:1 or 1:2 molar ratios of disulfide and cupric ion can be predicted from the obtained molar absorptivities for 2-thiouracil, RSH ($\epsilon_{270} = 13{,}680$), its sulfinic acid derivative, RSO_2H ($\epsilon_{270} = 3510$), and the known original concentration of the 2-thiouracil disulfide, RSSR (3.99×10^{-5} M). The predicted final absorbances of such solutions when cupric ion and thiouracil do not undergo the redox reactions of Eqs. 39, 41, and 42 should be the same as when the disproportionation of the disulfide occurs (Eqs. 38 and 40) in the absence of cupric ion, *i.e.*, 0.89. The predicted final absorbance should be 0.79 for equimolar cupric ion and 0.69 for twice equimolar cupric ion with thiouracil disulfide if the respective redox Eqs. 41 and 42 are applicable.

The observed absorbance when equimolar and twice equimolar solutions of cupric ion were equilibrated in 0.05 M $HClO_4$ with the 4.0×10^{-5} M 2-thiouracil disulfide was 0.83, which was the same absorbance observed for the disulfide alone equilibrated in 0.05 M $HClO_4$. The difference between the predicted absorbance for the disproportionation of the disulfide and the obtained final absorbances for all the solutions may be attributed to the partial oxidation of 2-thiouracil to its sulfonic acid derivative, *i.e.*, RSO_3H (8).

Thus, it can be concluded that thiouracil is *not* oxidized by cupric ion in acid solution and that the most probable copper complexes of thiouracils are with cupric ion and similar to those already postulated (1) for Pb^{+2}, Cd^{+2}, Zn^{+2}, and Ni^{+2} (Scheme I). Also, it was shown previously (1) and by the spectral evidence presented here that an apparent necessary prerequisite for metal complexation at a sulfur in these structures is a dissociable sulfhydryl group.

The apparent half-lives (6 min. at 25.0°) for the disproportionation of 4.0×10^{-5} M 2-thiouracil disulfide in 0.05 M $HClO_4$ (Eq. 38) were monitored by following absorbance changes with time at 270 nm. and were independent of the cupric-ion concentrations of 0.0, 4.0, and 8.0×10^{-5} M. This provides no evidence for cupric-ion complexation with the 2-thiouracil disulfide.

Additional evidence for the lack of cupric-ion oxidation of 2-thiouracil in 0.1 M $HClO_4$ was obtained when the absorbances, A, of 10^{-4} M thiouracil solutions containing cupric ion [0.0 M (A = 1.233), 2.15×10^{-4} M (A = 1.250), and 4.5×10^{-3} M (A = 1.305)] were determined. An absorbance of 0.995 would have been predicted if cupric ion had been the cause of sulfinic acid formation, *i.e.*, if Eqs. 39 and 40 had been valid.

The evidence denies the proposal of complexes similar to IX resulting from cupric-ion interaction with thiouracils. Thiouracil disulfide formation is most probably an artifact of sulfhydryl oxidation by atmospheric oxygen in alkaline solutions which may be cupric ion catalyzed. However, it is highly improbable that the resultant cuprous ion has sufficient stability to form a stable complex with any possible ligand in this system since chloride ion was excluded and, certainly, neither the cupric nor cuprous ion should be able to complex with the thiouracil disulfide. Metal ions should not significantly bind to the oxygen of the 4-hydroxyl groups in the thiouracil disulfide, since no complexation with metal ions was observed (1) with a compound whose dissociable proton was removed by alkylation, *i.e.*, 2-ethylmercapto-4-hydroxypyrimidine.

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