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Thiopyrazolone Derivatives as Analytical Reagents. XVI.[1] Extraction-spectrophotometric Determination of Trace of Mercury by Ternary Complex formed from Mercury(II) and Thiopyrine in the Presence of Eosine

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When an aqueous solution containing mercury(II), thiopyrine and eosine was shaken with 1,2-dichloroethane at pH 10, an ion pair formed from cationic mercury(II)-thiopyrine complex and anionic eosine was extracted into the organic phase. Based on this reaction, a sensitive extraction-spectrophotometric determination method for trace of mercury was established by the absorbance measurement at 540 nm. This method is applicable to the determination of mercury less than 2.5 μg/ml. Various common cations and anions except for silver(I) and iodide did not interfere the determination practically. Molar ratio of mercury(II) to thiopyrine and eosine was found to be 1: 2: 1 by the continuous variation method.

Keywords——mercury(II); thiopyrine; eosine; extraction; 1,2-dichloroethane; ion pair formation; spectrophotometric determination

Some carbazones[3] including dithizone have been widely used as the reagent for the spectrophotometric determination of mercury. Recently, other sensitive methods of determination based on the formation of ion pair have been proposed. In these methods, mercury (II) in the presence of excess iodides or bromides is converted into an anionic complex, which is extracted into organic solvents as an ion pair with colored cations such as 2,2′-dipyridyl iron (II) chelate,[4] Bindschedler's green[5] and brilliant green.[6]

In the previous papers[7] of the study, it has been shown that mercury (II) reacts with thiopyrine to form a colorless, water-soluble and stable cationic complex, which is readily extractable into organic solvents such as chloroform and 1,2-dichloroethane, as an ion pair with various dyes such as halogenated xanthenes (eosine, erythrosine *etc.*), halogenated sulfophthleines (bromophenol blue, bromcresol green *etc.*) and sulfonated azo compounds (methyl orange, congo red *etc.*). This paper deals with a new extraction-spectrophotometric method for the determination of mercury by the use of eosine, which shows the highest stability and reproducibility among these dyes.

Experimental

Apparatus——The absorbance measurements were made with a Shimadzu spectrophotometer QV-50 with 1 cm glass cells. The measurements of pH were made with a Horiba Model M5 pH meter. The shaking for extraction was carried out with an Iwaki Model KM shaker.

1) Part XV: T. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **26**, 3135 (1978).
2) Location: *35–23, Nozawa 1-chome, Setagaya-ku, Tokyo.*
3) E.B. Sandell, "Colorimetric Determination of Traces of Metals," 3rd ed., Interscience Publishers, Inc., New York, 1959, p. 621.
4) K. Kotsuji, *Bull. Chem. Soc. Jpn.*, **38**, 402 (1965); Y. Yamamoto, S. Kikuchi, Y. Hayashi, and T. Kumamaru, *Bunseki Kagaku*, **16**, 931 (1967).
5) M. Tsubouchi, *Anal. Chem.*, **42**, 1087 (1970).
6) T. Sawaya, H. Ishii, and T. Odashima, *Bunseki Kagaku*, **22**, 318 (1973).
7) T. Tanaka, *Yakugaku Zasshi*, **92**, 1252 (1972); *idem, ibid.*, **92**, 1435 (1972).

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Reagents——The thiopyrine was prepared as previously described,[8] and used as a 10^{-3} M aqueous solution. A 5×10^{-4} M eosine solution was prepared by dissolving 2′,4′,5′,7′-tetrabromofluorescein disodium salt in water. A stock solution of 5×10^{-3} M mercury(II) was prepared by dissolving mercuric nitrate in water, containing sufficient nitric acid to prevent hydrolysis, and it was standardized by EDTA titration.[9] The mercury(II) solution for the experiment was prepared by diluting an aliquot of this stock solution with water. An acetate, phosphate and borate buffer solution (0.1 M, respectively) were used for the adjustment of pH, except for the use of 0.1 N sodium hydroxide for the pH value higher than 10.5. All solutions used in the experiments were prepared from analytical reagent grade chemicals and deionized water.

Recommended Procedure for Determination——A sample solution containing less than 25 μg of mercury (II), 10 ml of 10^{-3} M thiopyrine solution, 5 ml of pH 10 borate buffer solution and 1 ml of 5×10^{-4} M eosine solution were placed into a 100 ml separatory funnel. This solution was brought to 50 ml with water. After adding 10 ml of 1,2-dichloroethane into the separatory funnel, the mixture was shaken for about 10 min by a shaker. After standing for a few minutes, the absorbance of the extract was measured at 540 nm against a reagent blank.

Results and Discussion

Absorption Spectrum

In the absence of mercury (II), 1,2-dichloroethane layer was colorless, whereas a reddish orange color was observed in the organic phase in its presence. The absorption spectrum of the extract is shown in Fig. 1, in which an absorption maximum is observed at 540 nm. In the reagent blank, no significant absorption was observed in the visible region. The absorption spectrum of the complex was not affected by varying the pH of the aqueous phase or the amounts of mercury (II), thiopyrine and eosine existing in the aqueous phase on the extraction, indicating that the chemical species of extracted complex are assumed to be one sort.

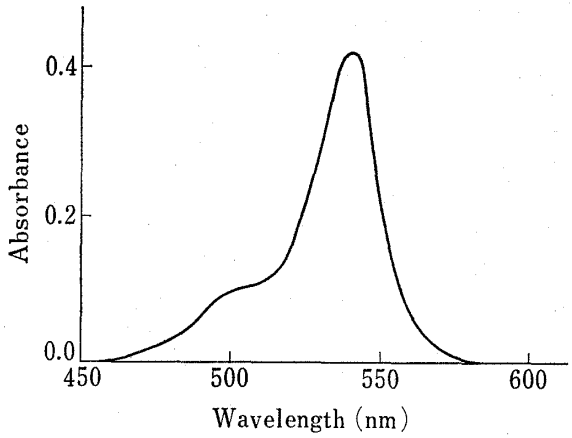

Fig. 1. Absorption Spectrum of the Associated Complex composed of Mercury (II), Thiopyrine and Eosine in 1,2-Dichloroethane

The complex was formed from 9.0 μg of mercury, according to the recommended procedure.

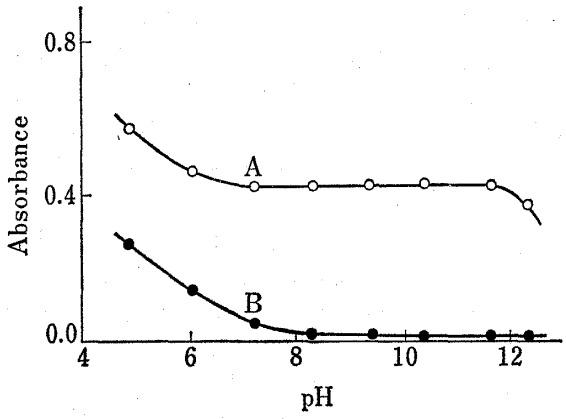

Fig. 2. Effect of pH on the Absorbance

A: 10 ml of solution containing 0.9 μg/ml of mercury(II), 10 ml of 10^{-3} M thiopyrine and 1 ml of 5×10^{-4} M eosine were mixed and the pH was adjusted with acetate, phosphate and borate buffer, and sodium hydroxide. The solutions were diluted to about 50 ml with water, and extracted with 10 ml of 1,2-dichloroethane. The blank is the reagent solution.

B: The solutions contain the same amount of thiopyrine and eosine as A without mercury(II). The blank is 1,2-dichloroethane. The absorbance was measured at 540 nm.

Effect of pH

The effect of the pH is shown in Fig. 2. The extracted complex showed almost constant absorbance in the pH range of 6.5—11, and the absorption of the reagent blank was negligible in the pH region higher than 8.

8) T. Tanaka, *Yakugaku Zasshi*, **91**, 311 (1971).
9) K. Ueno, "Chelatometric Titration," 15th ed., Nankodo, Tokyo, 1974, p. 324.

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Effect of the Amount of Thiopyrine

The absorbance of the extracts from a series of solution containing 9.0 μg of mercury (II) remained unchanged, when the amount of thiopyrine solution added was varied from 0.5 to 30 ml. Accordingly, 10 ml of thiopyrine solution was used in the recommended procedure. This amount of thiopyrine corresponds to about two hundred fold mercury in molar ratio.

Effect of the Amount of Eosine

The extractions from a series of solution containing 9.0 μg of mercury (II) with various amounts of eosine were carried out. The results are shown in Fig. 3. More than 0.3 ml of eosine solution was required to obtain maximum and constant absorbance. However, when eosine solution was added in large excess, the dye was extracted in the reagent blank. Accordingly, 1 ml of eosine solution which corresponds to about ten fold mercury in molar ratio was used in the recommended procedure.

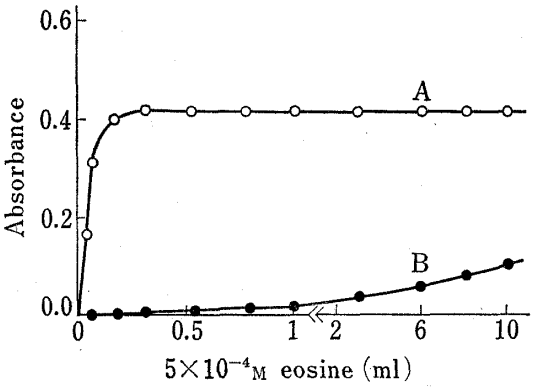

Fig. 3. Effect of the Amount of Eosine on the Absorbance

A: 0—10 ml of 5×10^{-4} M eosine was added to 10 ml of solution containing 0.9 μg/ml of mercury(II) and 10 ml of 10^{-3} M thiopyrine, and the pH was adjusted at 10 with borate buffer. The solutions were diluted to about 50 ml with water, and extracted with 10 ml of 1,2-dichloroethane. The blank is the reagent solution.

B: The solutions contain the same amount of thiopyrine and eosine as A without mercury(II). The blank is 1,2-dichloroethane. The absorbance was measured at 540 nm.

Effect of the Volume of Aqueous Phase

When the volume of 1,2-dichloroethane was kept constant at 10 ml and that of the aqueous phase was varied from 20 to 100 ml, almost the same absorbance was obtained in each case. Therefore, the volume of initial aqueous solution seems to have no effect on the extraction recovery of mercury, up to five fold volume of organic phase.

Effect of the Shaking Time

As the shaking for three minutes seemed to be enough to obtain good recovery, the shaking time was fixed at 10 minutes to ensure the complete extraction in the recommemded procedure.

Stability of the Color

The absorbance of the extract remained almost constant for an hour, and then it decreased slightly.

Percentage of Extraction

The solution containing 9.0 μg of mercury (II) was treated by the recommended procedure, and the remaining aqueous phase was subsequently added with 10 ml of thiopyrine solution and 10 ml of 1,2-dichloroethane to repeat the extraction and measure the absorbance. More than 95% of mercury was found to be extracted in the first extraction.

Calibration Curve and Reproducibility

A calibration curve which satisfies Beer's law over the range of 2—25 μg of mercury (II) was obtained by the recommended procedure. The molar absorptivity was calculated to be 9.2×10^4 l·mol^{-1}·cm^{-1} at 540 nm. In ten experiments using 9.0 μg of mercury (II), the average value of absorbance was 0.413 and the coefficient of variation was 1.5%. This method is more sensitive than dithizone method, because it is based on the formation of deeply colored associated complex with eosine.

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Table I. Effect of Foreign Ions on the Determination of Mercury

Foreign ion	Added as	Amount of ion (mg)	Mercury found (μg)	Relative error (%)
Ag(I)	$AgNO_3$	0.005	9.5	+5.5
Mg(II)	$MgSO_4 \cdot 7H_2O$	0.6	8.7	−3.3
Ca(II)	$Ca(NO_3)_2 \cdot 4H_2O$	1.0	8.8	−2.2
Ba(II)	$Ba(NO_3)_2$	3.0	8.9	−1.1
Zn(II)[a]	$ZnSO_4 \cdot 7H_2O$	1.5	9.3	+3.3
Co(II)[a]	$CoSO_4 \cdot 5H_2O$	1.3	9.1	+1.1
Ni(II)[a]	$NiSO_4 \cdot 6H_2O$	1.3	8.9	−1.1
Mn(II)[a]	$MnSO_4 \cdot 4H_2O$	1.2	8.7	−3.3
Cu(II)[a]	$CuSO_4 \cdot 5H_2O$	1.4	9.2	+2.2
Pb(II)[a]	$Pb(NO_3)_2$	4.5	8.8	−2.2
Cd(II)[a]	$CdSO_4 . 8/3H_2O$	0.08	9.4	+4.4
Al(III)[a]	$Al(NH_4)(SO_4)_2 \cdot 12H_2O$	0.6	8.8	−2.2
Cr(III)[a]	$CrK(SO_4)_2 \cdot 12H_2O$	1.2	8.7	−3.3
Fe(III)[a]	$Fe(NH_4)(SO_4)_2 \cdot 12H_2O$	1.2	8.8	−2.2
Bi(III)[a]	$Bi(NO_3)_3 \cdot 5H_2O$	1.0	8.7	−3.3
As(III)	$AsCl_3$	1.5	8.8	−2.2
Sb(III)[a]	$SbCl_3$	0.6	8.7	−3.3
Sn(IV)[a]	$SnCl_4 \cdot 4H_2O$	0.6	8.7	−3.3
Te(IV)[a]	$K_2TeO_4 \cdot 3H_2O$	0.6	8.6	−4.4
V(V)	NH_4VO_3	1.0	8.8	−2.2
Mo(VI)	$Na_2MoO_4 \cdot 2H_2O$	2.0	8.7	−3.3
Cl^-	NaCl	8.0	8.7	−3.3
Br^-	KBr	0.4	8.6	−4.4
I^-	KI	0.006	8.5	−5.5
SCN^-	KSCN	0.04	8.6	−4.4
NO_3^-	KNO_3	14.0	8.8	−2.2
SO_4^{2-}	$Na_2SO_4 \cdot 10H_2O$	22.0	8.7	−3.3
Acetate	$NaC_2H_3O_2 \cdot 3H_2O$	15.0	8.8	−2.2
Tartrate	$Na_2C_4H_4O_6 \cdot 2H_2O$	35.0	8.9	−1.1
Citrate	$Na_3C_6H_5O_7 \cdot 2H_2O$	50.0	8.9	−1.1

Mercury(II) was taken 9.0 μg.
a) Matal ions were masked previously by adding 5 ml of 0.1 M sodium citrate solution.

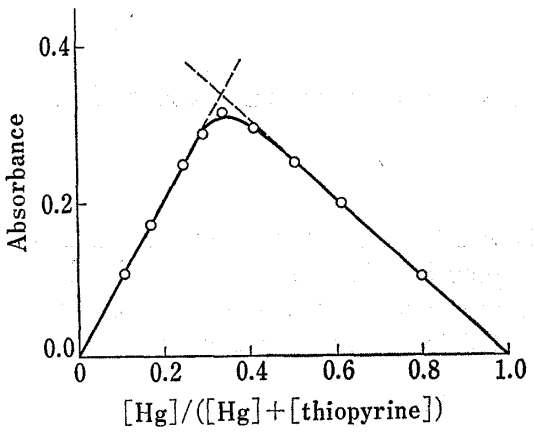

Fig. 4. Continuous Variation Method

Variation of mercury(II) and thiopyrine: 0—10 ml of 1.2×10^{-5} M mercury(II) and 10—0 ml of 1.2×10^{-5} M thiopyrine were added to 1 ml of 5×10^{-4} M eosine and 5 ml of pH 10 borate buffer. The solutions were diluted to about 50 ml with water, and extracted with 10 ml of 1,2-dichloroethane. The absorbance was measured at 540 nm against 1,2-dichloroethane.

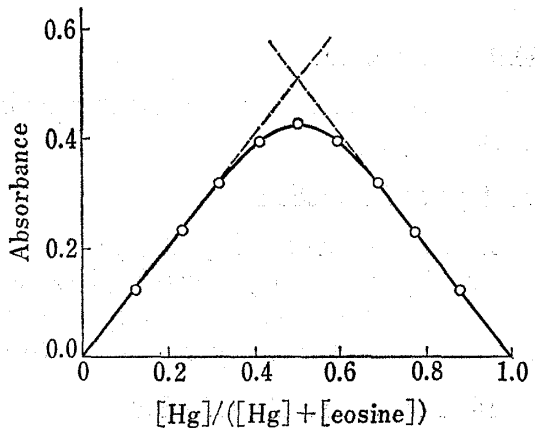

Fig. 5. Continuous Variation Method

Variation of mercury(II) and eosine: 0—10 ml of 1.2×10^{-5} M mercury(II) and 10—0 ml of 1.2×10^{-5} M eosine were added to 10 ml of 10^{-3} M thiopyrine and 5 ml of pH 10 borate buffer. The solutions were diluted to about 50 ml with water, and extracted with 10 ml of 1,2-dichloroethane. The absorbance was measured at 540 nm against 1,2-dichloroethane.

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Effect of Foreign Ions

To examine the effect of foreign ions, various ions were added to the solution containing 9.0 μg of mercury (II), which was determined by the recommended procedure. The results are shown in Table I. The presence of small amount of silver (I) or iodide interfered the determination, whereas that of cadmium (II) or thiocyanate allowed the determination within 5% relative error, when their amount did not exceed fifteen fold mercury in molar ratio. Other ions did not interfere the determination practically. Further, the addition of 5 ml of 0.1 M sodium citrate solution before the addition of the reagent was effective to mask various metal ions shown in Table I, which precipitate as the basic salts or form the cationic complex with thiopyrine. However, effective masking agent for silver (I) could not be found.

Composition of the Extracted Complex

Mercury (II) was extracted into 1,2-dichloroethane only in the presence of thiopyrine and eosine, and hence it was presumed in this method that the extraction of mercury (II) was effected by the formation of an ion pair consisting of cationic mercury (II)-thiopyrine complex and anionic eosine. Fig. 4 and 5 show the results of continuous variation method applied to the determination of the binding ratio in the ion pair. The binding ratio of mercury to thiopyrine and eosine was found to be 1:2:1. The chemical species of the complex extracted into 1,2-dichloroethane are therefore presumed to be an associated complex, $[Hg(C_{11}H_{12}N_2S)_2]^{2+}(\text{eosine})^{2-}$.

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