

SPECTROPHOTOMETRIC DETERMINATION OF
MANGANESE(VII) WITH 6-METHOXY-2-METHYLTHIO-4-PYRIMIDINECARBOXYLIC ACID
AND
SILVER(I) WITH 2-AMINO-6-METHYLTHIO-4-PYRIMIDINECARBOXYLIC ACID

by

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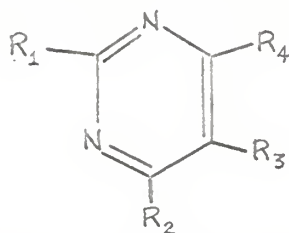
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INTRODUCTION

Pyrimidine derivatives constitute a very important class of compounds because they are components of the biologically important nucleic acids and have been shown to exert a pronounced physiological effect. Since most living systems contain metal ions which are essential for proper functioning, the question arises as to the effect of such metal ions on nucleic acid. To simplify the problem even further, one might consider the effect of metal ions on components of nucleic acids, such as the pyrimidines (1). Twenty-two possible isomers of pyrimidinethiols, aminopyrimidinethios, and hydroxypyrimidinethiols substituted at positions 2, 4, and 6 of the pyrimidine ring have now been reported (2). The syntheses of all eight structural analogs of orotic acid which contain amino, hydroxy, and thio substituents at positions 2 and 6 are reported (3). Two derivatives of alkylthiopyrimidines and six orotic acid analogs were studied here for preliminary screening to check the possibilities of forming chelates with the various metallic ions at pH values from 1 to 11. The structures of the compounds studied here are given in Table 1. The eight compounds contain two cyclic nitrogens as the base part of the molecule which has a pair of electrons to donate to the metal ions, forming a co-ordinate covalent bond and the hydroxyl, carboxylic radicals which have hydrogen atoms replaceable by metal ions forming the ionic bonds as acidic groups (4). The first compound in Table 1 does not contain an acidic hydrogen atom but it is worthwhile to attempt to discover if it forms the chelates, since it contains sulfur ($-SCH_3$) which has a pair of electrons to donate to the metal ions. The existence of coordinating groups and acidic groups shows that there are very strong possibilities for chelation. Any elucidation of metal ion effects on the pyrimidine nucleus could possibly lead to a better

Table 1. The list of pyrimidine derivatives studied



	R ₁	R ₂	R ₃	R ₄
Comp. 1	H	SCH ₃	NH ₂	SCH ₃
Comp. 2	OH	SCH ₃	H	NH ₂
Comp. 3	NH ₂	COOH	H	NH ₂
Comp. 4	SCH ₃	COOH	H	OH
Comp. 5	SCH ₃	COOH	H	OCH ₃
Comp. 6	NH ₂	COOH	H	SCH ₃
Comp. 7	SC ₂ H ₅	COOH	H	OH
Comp. 8	S-CH ₂ - 	COOH	H	OH

Comp. 1: 4,6-dimethylthio-5-amino pyrimidine.

Comp. 2: 6-amino-4-methylthio-2-pyrimidinol.

Comp. 3: 2,6-diamino-4-pyrimidinecarboxylic acid.

Comp. 4: 6-hydroxy-2-methylthio-4-pyrimidinecarboxylic acid.

Comp. 5: 6-methoxy-2-methylthio-4-pyrimidinecarboxylic acid.

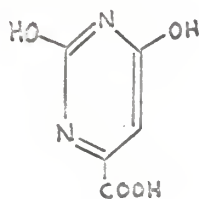
Comp. 6: 2-amino-6-methylthio-4-pyrimidinecarboxylic acid.

Comp. 7: 2-ethylthio-6-hydroxy-4-pyrimidinecarboxylic acid.

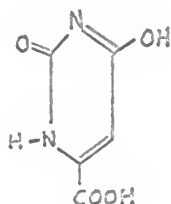
Comp. 8: 6-hydroxy-2-tolythio-4-pyrimidinecarboxylic acid.

understanding of complex biological processes occurring in living systems (1). As part of a general program on metal complexation studies involving biologically important compounds, this work was directed towards an understanding of the nature of metal binding in alkylthiopyrimidines and pyrimidine acids.

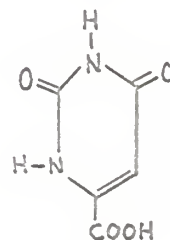
In attempting to elucidate the complexing sites of a chelating agent, the structural nature of the ligand under the conditions of complexation must first be determined. The complexity of the pyrimidine system results from the effect of pH changes and group substitution on the lactam-lactim (ketonic-enolic) isomerism.



(I)

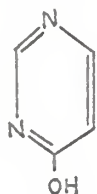


(II)

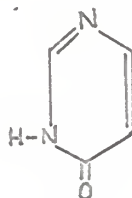


(III)

Brown and Short (5), from ultra violet and pH data, concluded that in aqueous solution the 4-hydroxypyrimidine existed predominantly in the ketonic (lactum) form (II), rather than the enolic (lactim) form (I).



(I)



(II)

Likewise, Tucci and Li (1) concluded that up to pH 12, orotic acid was still predominantly in the monoketonic (II) or diketonic form (III). Bianchi and Lomuto (6) studied the colorimetric qualitative and quantitative determination of orotic acid with FeCl_3 which quantitatively gave a red color. From ultra-

violet absorption studies, Tucci and Li (1) found that only slight complexing of orotic acid was observed with the metal ions, zinc, cobalt, and manganese but very strong complexing was observed with nickel and copper which were present in an equimolar ratio with the ligand. However, ultra-violet absorption spectra for the orotic acid ester and nickel ions verified that essentially no complexing occurred. This indicates the important role which the carboxyl group played in the complexing process. The binding sites in orotic acids are probably the carboxylate and the adjacent ring nitrogen anions. Since the ring nitrogen is charged, the orotic acid complex is very stable (7).

Issa and Sherif (8) titrated permanganate with a solution of U(IV) in 0.1 to 2N H_2SO_4 proceeding in two stages, first to MnO_2 and then to Mn^{+2} . B. Singh and S. Singh (9, 10, 11) carried out the indirect volumetric determination of $KMnO_4$ with 0.05N solution of ferrous ethylenediamine sulfate (I) in the presence of 1 to 2N H_2SO_4 and also determined $KMnO_4$ indirectly with (I) by potentiometric titration of excess of (I) (12). I. M. Issa and R. M. Issa (13) titrated potentiometrically a solution of $KMnO_4$ (0.002 to 0.02M) in 0.5 to 3.0N NaOH at $30^\circ C$ with 0.0045 to 0.18N formate giving MnO_4^{-2} as the only reduction product. Erdey, Buzás and Vigh (14) determined $KMnO_4$ indirectly by adding an excess of Fe(II) salt and titrating the Fe(III) formed with standard ascorbic acid.

A spectrophotometric method for quantitatively determining small amounts of manganese is described. It is based on the reduction of permanganate ion with 6-methoxy-2-methylthio-4-pyrimidinecarboxylic acid to form manganate ion which is very bright green in alkaline solution. The absorbance of the green solution was measured at 580 mu with a Beckman spectrophotometer. In support of this spectrophotometric determination of permanganate ion several other studies were carried out and are presented in the following order: general

characteristics of the system, the continuous studies, and the studies of Beer's law.

The spectrophotometric determination of Ag^+ was carried out in aqueous medium at pH 4.0 to 4.6 with rubeanic acid by Xavier and Rây (15). A procedure for the colorimetric determination of Ag^+ with p-dimethylamino-benzylidenerhodamine at pH 9.3 measuring the extinction at 535 m μ was given by Castagna and Chauveau (16). Miethke and Brösamle (17) and Cyrankowaska (18) determined Ag^+ with dithizone from the extinction measurements at 462 m μ . Hartkamp (19) determined Ag^+ in the presence of pyridine-2,6-dicarboxylic acid and $(\text{NH}_4)_2\text{S}_2\text{O}_8$ measuring an olive-green chelate at 578 m μ and 920 m μ . The spectrophotometric determination of trace amounts of Ag^+ by toluene-3,4-dithiol was done by Dux and Feairheller (20).

The next method proposed here depends upon the formation of the silver(I)-2-amino-6-methylthio-4-pyrimidinecarboxylic acid chelate at pH 10 ± 0.1 in aqueous solution and the measuring of the absorbance of the bright yellow solution at 375 m μ with a Beckman spectrophotometer. In support of this spectrophotometric determination of Ag^+ several studies were carried out and are presented in the following order: general characteristics of the system involving the screening test, the continuous studies, and the studies of Beer's law. This is followed by the discussion of the interfering ions and extraction studies.

The spectrophotometric determination of both Mn(VII) and Ag(I) have several advantages in that both determinations are done very rapidly, they are sensitive, and they follow Beer's law up to 5.0×10^{-4} moles per liter. The stability of the systems are one month for the Ag(I) system and at least one day for the Mn(VII) system.

EXPERIMENTAL

Chemicals

All of the organic extracting agents were distilled prior to use. Solutions of ions which were used for interference studies were prepared from analytical reagent grade compounds and deionized water. All other chemicals were reagent grades unless otherwise specified.

Apparatus

Beckman Model DB Spectrophotometer equipped with two matched 1.00 cm cells, Beckman potentiometric recorder, Beckman zeromatic pH meter, and magnetic stirrer were used.

Procedure

(1) Screening test:

A typical solution was prepared as follows:

1.5 ml of 0.01M aqueous metal ion solution

7.5 ml of 0.01M organic ligand solution

15.0 ml of distilled water

A Beckman zeromatic pH meter, standardized with Beckman buffer solution at pH 7.0, was used to determine the apparent pH. The apparent pH of the solution was adjusted by adding either dilute perchloric acid or dilute sodium hydroxide solution. Eight pyrimidine derivatives were tested at all the pH values from 1 to 11 with 59 metal ions. All experiments were carried out with continuous stirring with a magnetic stirrer.

As some metal ions possess color prior to chelation and others form either hydroxides or oxides as the pH is increased, a metal ion "blank" was

made by noting the color of the metal ion in the absence of any ligand in the pH interval 1 - 11. This was done by diluting 1.5 ml of metal ion solution with 15 ml of distilled water, adjusting the pH, and observing the solution color.

(2) Determination of optimum wavelength and pH:

A typical solution was prepared as follows:

0.6 ml of 0.01M aqueous metal ions

3.0 ml of 0.01M organic ligand solution

26.4 ml of distilled water.

A Beckman Model DB Spectrophotometer was used to measure the absorbance of the solution at the various pH's and over the wavelength range of 760 to 320 mu. The absorbance of the organic ligand solution was measured in the absence of the metal ions. The latter was subtracted from the former to give the actual absorbance of the complex system.

(3) Mn(VII) system:

A 0.01M KMnO_4 solution was prepared with distilled water. 0.01M 6-methoxy-2-methylthio-4-pyrimidinecarboxylic acid solution was prepared as follows: 0.5006 gram of the compound was dissolved in 150 ml of saturated sodium hydroxide solution and then diluted to 250 ml with distilled water. 1.5 ml of 0.01M KMnO_4 solution was added to 3.0 ml of 0.01M 6-methoxy-2-methylthio-4-pyrimidinecarboxylic acid and diluted to 25 ml with distilled water. The solution was stirred with a magnetic stirrer for at least two minutes to reach the equilibrium. The maximum spectra was obtained at 580 mu with the Beckman spectrophotometer. Under the absence of the organic solution, 1.5 ml of 0.01M KMnO_4 was diluted to 25 ml with distilled water and then the spectra of the blank was determined at 580 mu.

(4) Ag(I) system:

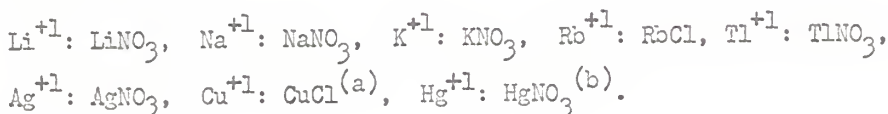
A 0.01M AgNO_3 solution was prepared in the deionized water. A 0.01M 2-amino-6-methylthio-4-pyrimidinecarboxylic acid solution was prepared as follows: 0.4630 grams of the compound was dissolved in 110 ml of dimethyl formamide and 5 drops of the saturated NaOH solution were added. It was diluted to 250 ml with the deionized water. Then 3 ml of 0.01M AgNO_3 solution was added to 2 ml of 0.01M 2-amino-6-methylthio-4-pyrimidinecarboxylic acid solution diluted to 25 ml with deionized water whose pH was adjusted to 10 ± 0.1 with dilute NaOH solution. The absorbance of the bright yellow chelate was measured at 375 m μ with a Beckman spectrophotometer. In the absence of silver ion the absorbance of 2 ml of the ligand solution, which was diluted to 25 ml with the deionized water, was measured at 375 m μ for the blank.

RESULTS AND DISCUSSIONS

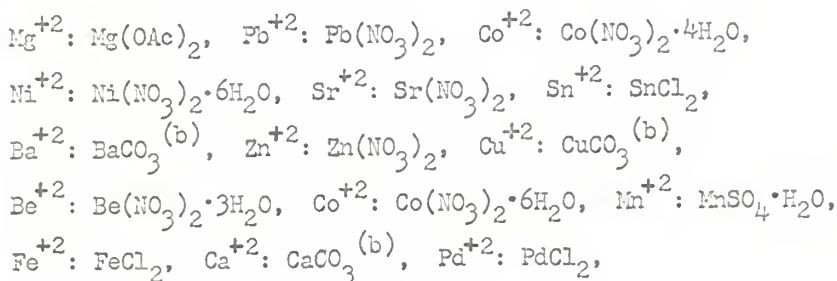
Screening

Eight pyrimidine derivatives were studied with 59 metal ions. The 59 metal ions are as follows:

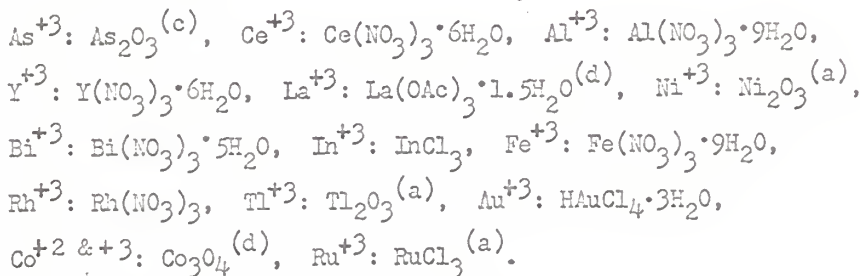
(1) Monovalent ions:



(2) Divalent ions:

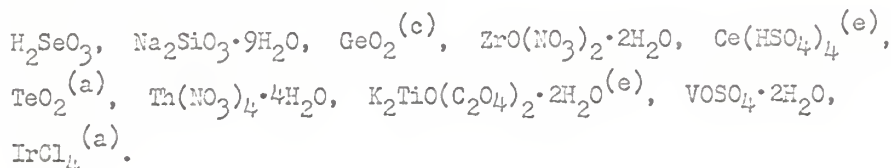


(3) Tribivalent ions:



Since it is very doubtful that the metal ions exist as separate ions, the salt of the metal used, in the proper valence state, will be listed.

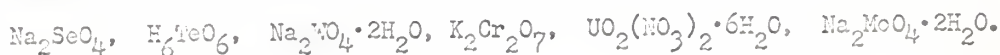
(4) Tetravalent state of the metals:



(5) Pentavalent state of the metals:



(6) Hexavalent state of the metals:



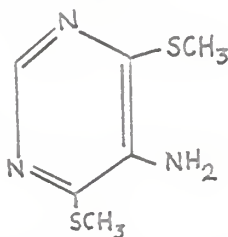
(7) Heptavalent state of the metals:



All the solutions were made with distilled water unless specified otherwise, i.e., (a) HCl, (b) HNO₃, (c) NaOH, (d) HOAc, (e) H₂SO₄ were added to make solutions.

The results of screening eight pyrimidine derivatives are as follows:

(1) 4,6-dimethylthio-5-amino pyrimidine



First 50 ml of 95% ethyl alcohol was added to dissolve the compound completely and then the solution diluted with distilled water to make 250 ml of 0.01M solution.

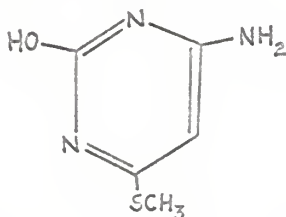
Gold(III): It gave a purplish grey precipitate with 4,6-dimethylthio-5-amino pyrimidine solution at all the pH's, whereas the metal solution gave a very pale yellow solution at all the pH's.

Palladium(II): It gave a yellow precipitate at pH 1 to 3 and then a dark orange-brown precipitate at pH's more basic than 4. The blank test gave only a yellow solution at pH 1 and 2, a reddish brown precipitate at pH's 3 to 6, and then a light orange-yellow milky solution which seemed to be almost clear at pH's more basic than 7.

Iridium(IV): It gave a very pale green-yellow solution at pH's 1 to 5,

a little darker solution at pH's 6 to 8, and then a very dark solution at pH's more basic than 9. The blank test gave a very pale yellow solution at all the pH's.

(2) 6-amino-4-methylthio-2-pyrimidinol



Enough saturated sodium hydroxide solution was added to dissolve the compound completely and then diluted to make 250 ml of 0.01M solution.

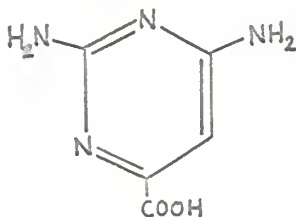
Selenium(IV): It gave a light orange solution at pH's 3 and 4, and a light orange precipitate at pH's more basic than 5. The blank gave colorless solutions at all pH values.

Selenium(VI): It gave a very light yellow solution at pH's more basic than 4. The blank gave a colorless solution at all pH values.

Copper(I): It gave a yellow precipitate at all pH values. The blank gave a blue precipitate at pH's more basic than 6.

Gold(III): It gave a pale yellow precipitate at all pH values whereas the blank gave a very pale yellow solution without precipitation.

(3) 2,6-diamino-4-pyrimidinecarboxylic acid



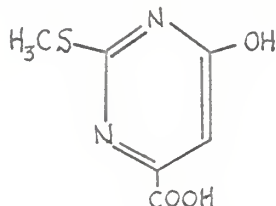
Enough saturated sodium hydroxide solution was added to dissolve the compound completely and then diluted to make 250 ml of 0.01M solution.

The solubility of this compound was so low that it precipitated out at

pH's less basic than 10, and therefore screening tests were carried out only at higher pH's.

Nickel(III): It gave a very much heavier white precipitate than the blank test gave.

(4) 6-hydroxy-2-methylthio-4-pyrimidinecarboxylic acid

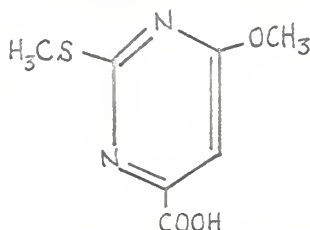


Enough saturated NaOH solution was added to dissolve the compound completely and then it was diluted to give 250 ml of 0.01M solution.

Copper(I): It gave a blue solution at pH's 5 to 11, whereas the blank test gave a light blue precipitate at pH's more basic than 7.

Silver(I): It gave a white precipitate at all pH values. The blank test gave a white precipitate at pH's 5 to 7 but a brown precipitate at pH's more basic than 8.

(5) 6-methoxy-2-methylthio-4-pyrimidinecarboxylic acid

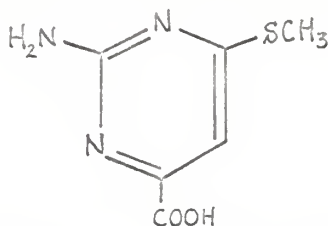


150 ml of saturated sodium hydroxide solution was added to dissolve the compound completely and then it was diluted with distilled water to make 250 ml of 0.01M solution.

Manganese(VII): It gave a very dark navy blue solution only at extremely high pH's. The navy blue color was changed to bright green on constant stirring. The apparent pH of this solution could not be determined by a

Beckman zeromatic pH meter. The blank test gave only the violet color of permanganate ion.

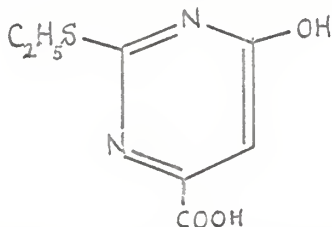
(6) 2-amino-6-methylthio-4-pyrimidinecarboxylic acid



0.463 grams of the compound was dissolved in 110 ml of dimethylformamide and then diluted to 250 ml with deionized water. The solubility of this compound was quite low and it precipitated out at temperature lower than 20°C. This difficulty was resolved by adding 5 drops of saturated sodium hydroxide solution.

Silver(I): It gave a very bright yellow solution whereas the blank gave a tan precipitate.

(7) 2-ethylthio-6-hydroxy-4-pyrimidinecarboxylic acid

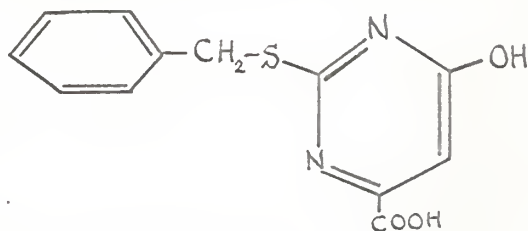


A small amount of saturated sodium hydroxide solution was added to dissolve the compound completely and then diluted with distilled water to make 250 ml of 0.01M solution.

Manganese(VII): It gave a navy blue solution at pH's higher than 11. The blank gave only the purple color of permanganate.

Gold(III): It gave a yellow precipitate at all pH values whereas the blank gave no precipitate at any pH.

(8) 6-hydroxy-2-tolylthio-4-pyrimidinecarboxylic acid



Enough saturated sodium hydroxide solution was added to dissolve the compound completely and then it was diluted with distilled water to make 250 ml of 0.01M solution. Since the solution precipitated out at pH's less basic than 8, the experiments were carried out at pH's more basic than 8.

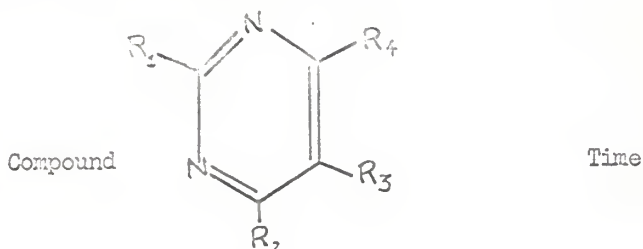
Manganese(VII): It gave a navy blue solution at pH's higher than 11. The blank test gave only the violet color of permanganate ion.


Studies on Manganese(VII) and Some Pyrimidine Derivatives

The results of the screening test show that manganese(VII) reacts with some pyrimidine derivatives to give a dark navy blue solution. Later it was found that the navy blue coloration had turned to a very bright green color on standing which was due to the green color of manganate ion in alkaline solution (21). It was found that the navy blue color came from the mixed color of purple permanganate ion and the bright green manganate ion. This coloration resulted from the reduction of permanganate to manganate ion.

The oxidation of the pyrimidine derivatives accelerated the rate of the reduction of permanganate to manganate ion. Table 2 shows the time required for permanganate to be reduced to manganate ion with the various pyrimidine derivatives and without any organic compound. All the reduction reactions of permanganate were carried out in a very strong alkaline solution. One ml of 0.01M $KMnO_4$ solution was added to 5 ml of 0.01M pyrimidine derivatives solution

Table 2. The Time Required for the Reduction of Permanganate to Manganate Ion



R_1	R_2	R_3	R_4	Navy blue	Bright green	
1	SCH ₃	COOH	H	OCH ₃	instantaneously	within 1 min.
2	S-C ₂ H ₅	COOH	H	OH	instantaneously	within 1 min.
3	S-CH ₂ - 	COOH	H	OH	instantaneously	within 1 min.
4	OH	SCH ₃	H	NH ₂	—————	instantaneously*
5	OH	COOH	H	OH	2 min.	30 sec.
6	SCH ₃	COOH	H	OH	5 min.	5 min.
7	NH ₂	COOH	H	SCH ₃	7 min.	4 min.
8	Saturated NaOH solution without any organic compound.			40 min.	10 min.	

* The green manganate ion was stable only for 4 minutes and then turned to the brown precipitate of manganese dioxide.

and the resulting solution was stirred constantly for a certain time as indicated in Table 2. For a comparison, the mixture of 5 ml of saturated sodium hydroxide solution and 1 ml of 0.01M $KMnO_4$ solution was stirred to measure the time required for the reduction of permanganate to manganate ion. Further studies were made on the manganese(VII)-6-methoxy-2-methylthio-4-pyrimidinecarboxylic acid system.

Studies on Manganese(VII) and 6-Methoxy-2-Methylthio-4-Pyrimidinecarboxylic Acid System

Reagent-to-Metal Ratio

The optimum wavelength range was found to be 565-590 mu for the navy blue-green coloration formed by 6-methoxy-2-methylthio-4-pyrimidinecarboxylic acid with permanganate ion. Fig. 1 shows the spectra for the manganese(VII) and 6-methoxy-2-methylthio-4-pyrimidinecarboxylic acid system.

Job's method of continuous variations (22) was used to determine the reagent-to-metal ratio. The experiments were carried out twice. At first solutions were diluted to 25 ml with distilled water and then the second set of solutions were diluted to 50ml. The absorbance of each solution was measured at 580 mu with a Beckman spectrophotometer. Table 3 shows the data used to obtain Fig. 2 which shows that the reagent-to-metal ratio is 1 to 2 in order to obtain the maximum absorbance. Fig. 2 shows that two moles of permanganate ions are reduced to manganate ions and at the same time 1 mole of 6-methoxy-2-methylthio-4-pyrimidinecarboxylic acid is oxidized.

Conformity to Beer's Law

The ability of the manganese(VII)-6-methoxy-2-methylthio-4-pyrimidinecarboxylic acid system to follow Beer's law in aqueous alkaline solution was

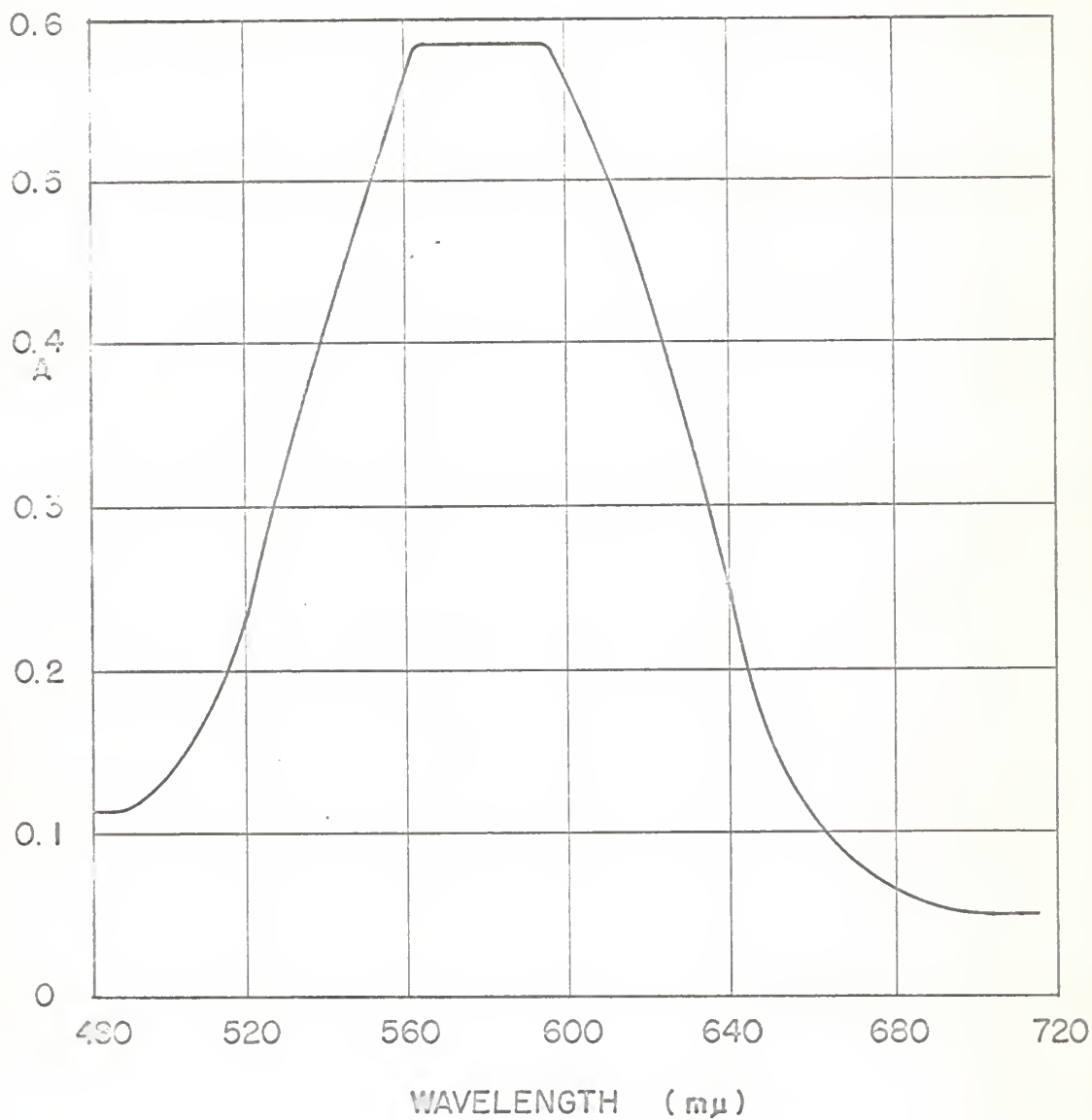


FIG. 1: SPECTRA FOR THE MANGANESE (VII) - 6-METHOXY - 2 - METHYLTHIO - 4 - PYRIMIDINECARBOXYLIC ACID SYSTEM.

Table 3 Continuous Variations Study of the Manganese(VII)-
6-methoxy-2-methylthio-4-pyrimidinecarboxylic Acid
System

Total number of moles = 5×10^{-5}

Absorbance at 580 m μ

Mole Fraction of Mn(VII)	Total Volume of Solution : 25 ml	Total Volume of Solution : 50 ml
0.1	0.296	0.142
0.2	0.559	0.279
0.3	0.849	0.432
0.4	1.130	0.545
0.5	1.450	0.595
0.6	1.600	0.655
0.7	1.580	0.639
0.8	1.200	0.530
0.9	0.620	0.280

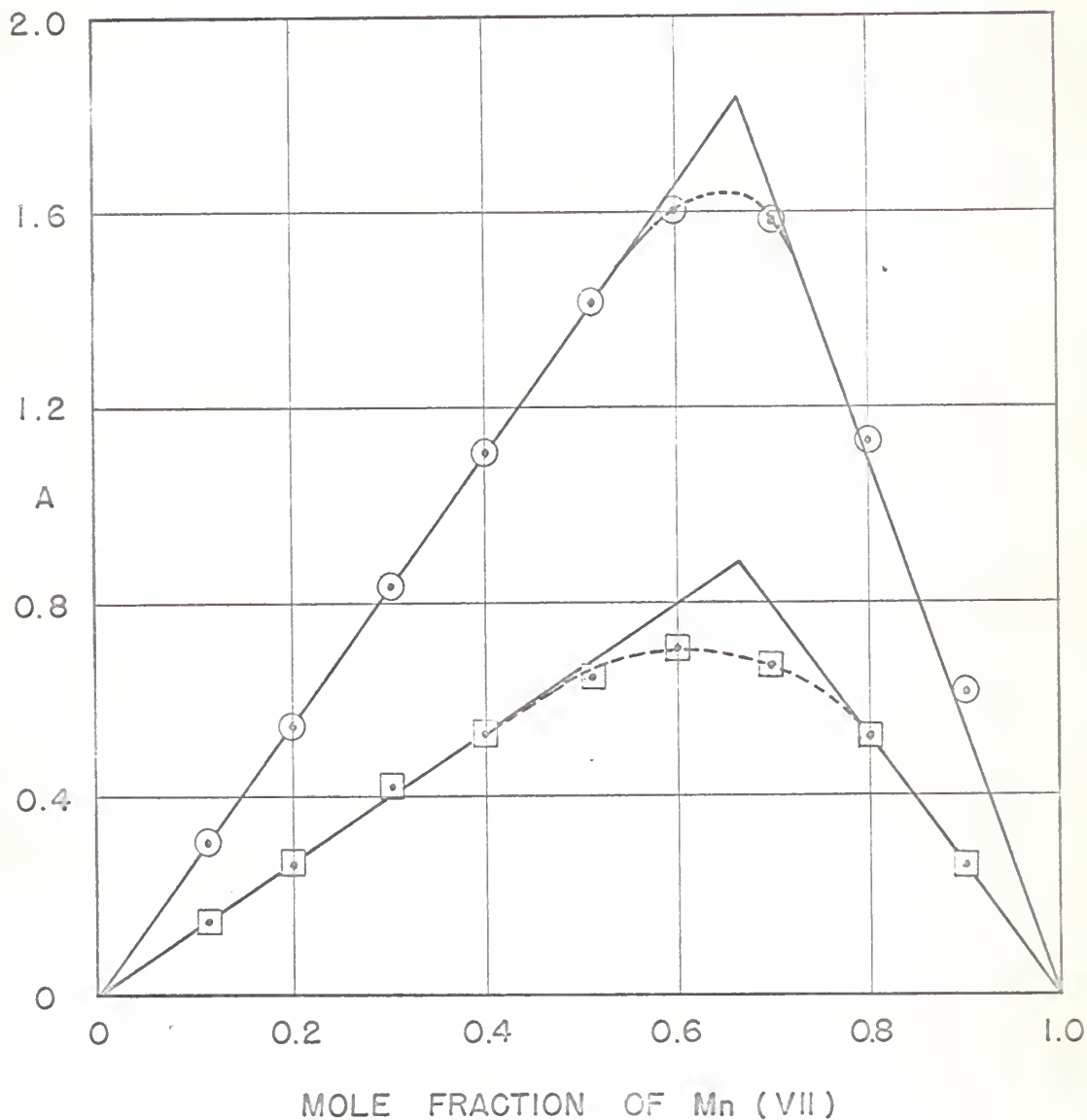


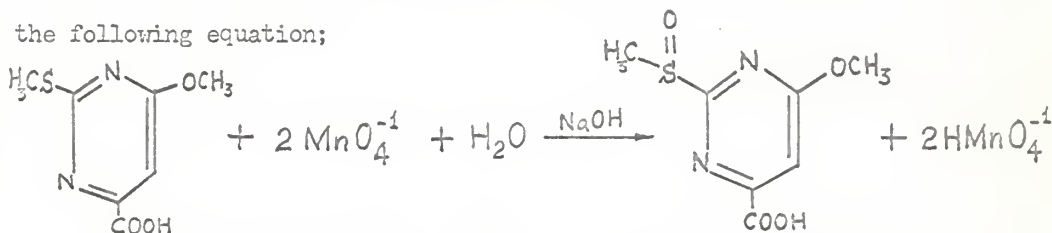
FIG.2 : CONTINUOUS VARIATION STUDY OF THE MANGANESE (VII) - 6-METHOXY-2-METHYLTHIO-4-PYRIMIDINE-CARBOXYLIC ACID SYSTEM.

- : TOTAL DILUTION VOLUME WAS 25 ml.
 □ : TOTAL DILUTION VOLUME WAS 50 ml.
 TOTAL NUMBER OF MOLES = 5×10^{-5}

studied. A graphical representation of this is shown in Fig. 3. The results reported in Fig. 3 are the average of two independent determinations. Table 4 shows the data used to obtain Fig. 3. This system obeys Beer's law very nicely from 3.3×10^{-5} to 1×10^{-3} moles per liter. The maximum molar absorptivity is 1.47×10^3 at 580 m μ .

Conclusion

Issa and Sherif (8) found that in alkaline solution MnO_4^{-1} could not be reduced quantitatively to MnO_4^{-2} with U(IV) but the reduction proceeded quantitatively to MnO_2 in 1.5 to 3N NaOH. However this work shows that MnO_4^{-1} can be reduced quantitatively to MnO_4^{-2} with 6-methoxy-2-methylthio-4-pyrimidinecarboxylic acid in very strong alkaline solution. This system can be used not only for the spectrophotometric determination of permanganate, but also possibly for titrimetric determination with the green color of manganate ion as the end point. The reagent to metal ratio is found to be 1 to 2. The alkylthioalkanes react with hydrogen peroxide to be oxidized to form alkylsulfinylalkanes (sulfoxide) (23). Probably 6-methoxy-2-methylthio-4-pyrimidinecarboxylic acid would be oxidized to form sulfoxides as shown in the following equation;



It has several advantages in that it is sensitive and precise. The system is stable for at least one day and the procedure does not require an exceedingly large excess of reagent. Since the system obeys Beer's law between 3.3×10^{-5} - 1.0×10^{-3} moles of manganese(VII), no calibration curve is required in this range.

Table 4 The Ability of the Manganese(VII)-6-Methoxy-2-Methylthio-4-Pyrimidinecarboxylic Acid System to Follow Beer's Law

Absorbance at 580 mu

Mn Concentration in the Solution	Trial No. 1	Trial No. 2	Average
3.3×10^{-5}	0.046	0.046	0.046
4.0×10^{-5}	0.054	0.056	0.055
5.0×10^{-5}	0.068	0.070	0.069
6.7×10^{-5}	0.078	0.086	0.082
1.0×10^{-4}	0.130	0.134	0.132
1.3×10^{-4}	0.200	0.196	0.198
2.0×10^{-4}	0.290	0.276	0.283
2.5×10^{-4}	0.370	0.362	0.366
3.3×10^{-4}	0.485	0.485	0.485
4.0×10^{-4}	0.585	0.580	0.583
5.0×10^{-4}	0.718	0.700	0.709
6.7×10^{-4}	0.960	0.920	0.940
1.0×10^{-3}	1.390	0.400	1.395

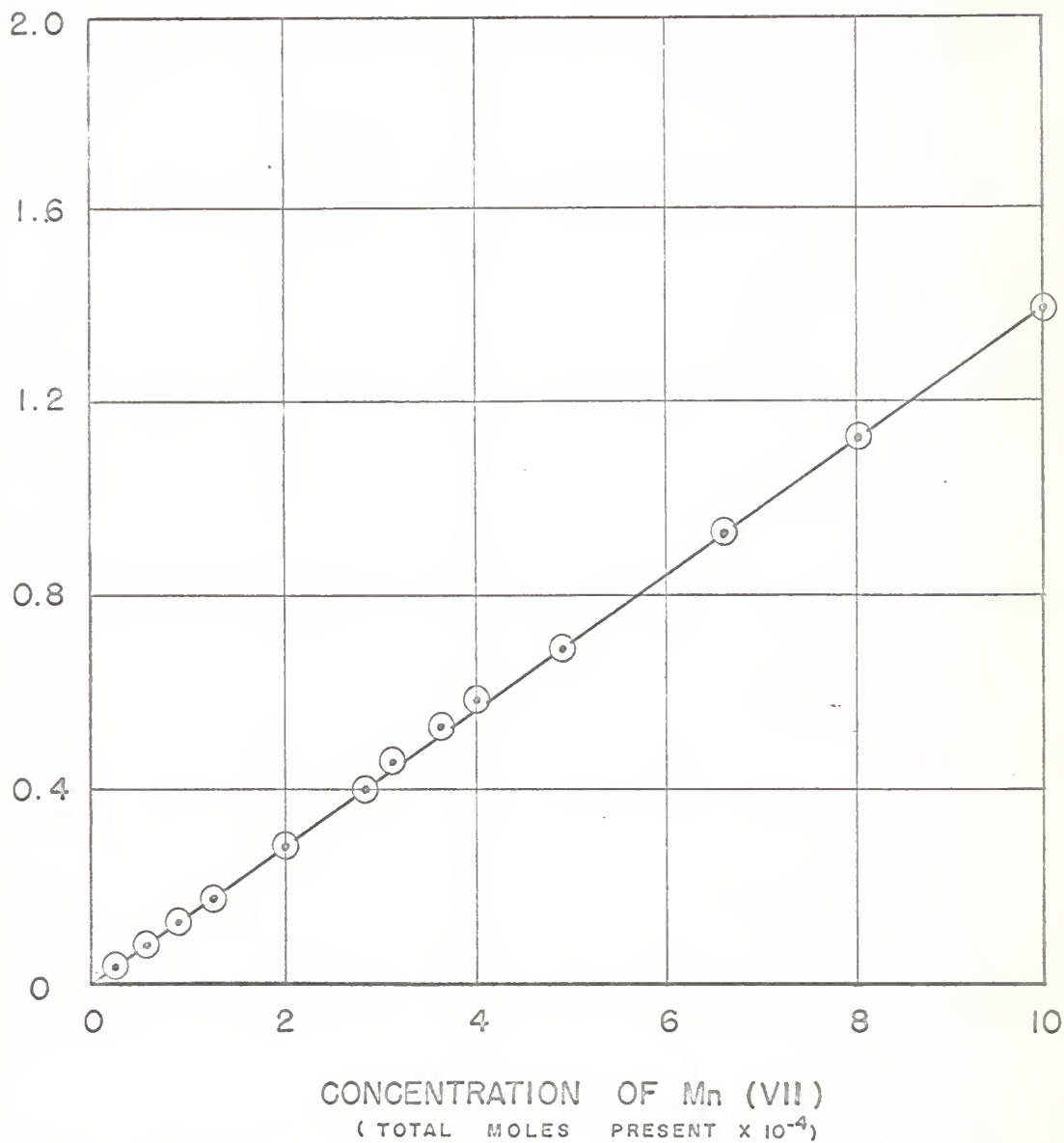


FIG. 3 CONFORMITY TO BEER'S LAW OF THE MANGANESE (VII) - 6-METHOXY-2-METHYLTHIO-4-PYRIMIDINE-CARBOXYLIC ACID SYSTEM.

Studies on the Silver(I)-2-Amino-6-Methylthio-4-Pyrimidinecarboxylic Acid Chelate

2-amino-6-methylthio-4-pyrimidinecarboxylic acid reacted with silver(I) to form a yellow species at pH's more basic than 8. The optimum wavelength is between 375 and 380 mu as is shown in Fig. 4. The formation of the colored species is dependent on pH as is shown in Fig. 5 which was obtained from Table 5. In more basic solutions than pH 11 the intensity of the color remains constant. Though it gave a maximum absorbance at pH's more basic than 11, all the experiments for the conformity of Beer's law, continuous variation studies, interfering ions studies and the extraction studies were performed at $\text{pH } 10 \pm 0.1$. The reason was that there was a strong tendency for silver ion to form a brown precipitate of silver oxide in strong alkaline solution and the precipitate adsorbed the yellow chelate species, from which separation was impossible. The maximum molar absorptivity is 2.09×10^3 at 375 mu.

Determination of Ratio of Ligand to Metal
by Job's Continuous Variations Method

The nature of the species of the silver(I)-2-amino-6-methylthio-4-pyrimidinecarboxylic acid was investigated.

The procedure was the same as the manganese(VII)-6-methoxy-2-methylthio-4-pyrimidinecarboxylic acid system. A Beckman Model DB Spectrophotometer was used to measure the absorbance of the solution at $\text{pH } 10 \pm 0.1$. The optimum wavelength was found as 375 - 380 mu, and the absorbance was measured at 375 mu. As is shown in Tables 6 and 7, each sample studied had a maximum absorbance at a mole fraction of silver(I) of 0.6 at 375 mu and 400 mu. From this result it is apparent that there is only one species formed.

Tables 6 and 7 provide the data used to obtain Fig. 6 which shows that

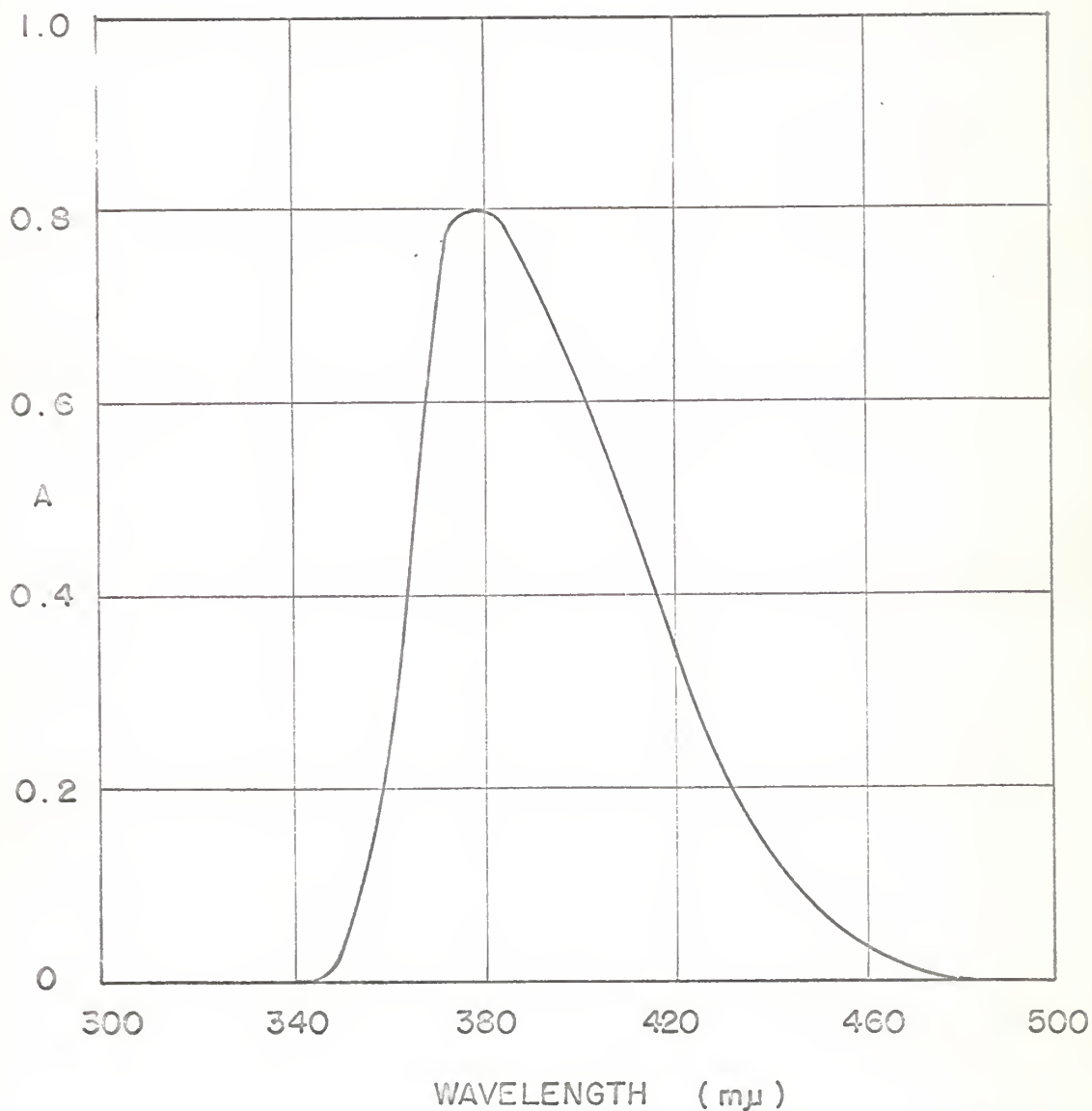


FIG. 4: SPECTRA FOR THE SILVER (I) - 2 - AMINO - 6 - METHYLTHIO - 4 - PYRIMIDINECARBOXYLIC ACID SYSTEM.

Table 5 Effect of pH on the Color of the Silver(I)-2-Amino
6-Methylthio-4-Pyrimidinecarboxylic Acid System

Wavelength, m μ	Absorbance at pH			
	8	9	10	11
350	0.013	0.041	0.087	0.098
360	0.036	0.076	0.270	0.301
370	0.061	0.125	0.347	0.456
380	0.097	0.181	0.377	0.456
390	0.114	0.222	0.347	0.432
400	0.108	0.201	0.301	0.387
420	0.066	0.114	0.187	0.229
440	0.041	0.055	0.097	0.119
480	0.018	0.018	0.018	0.018

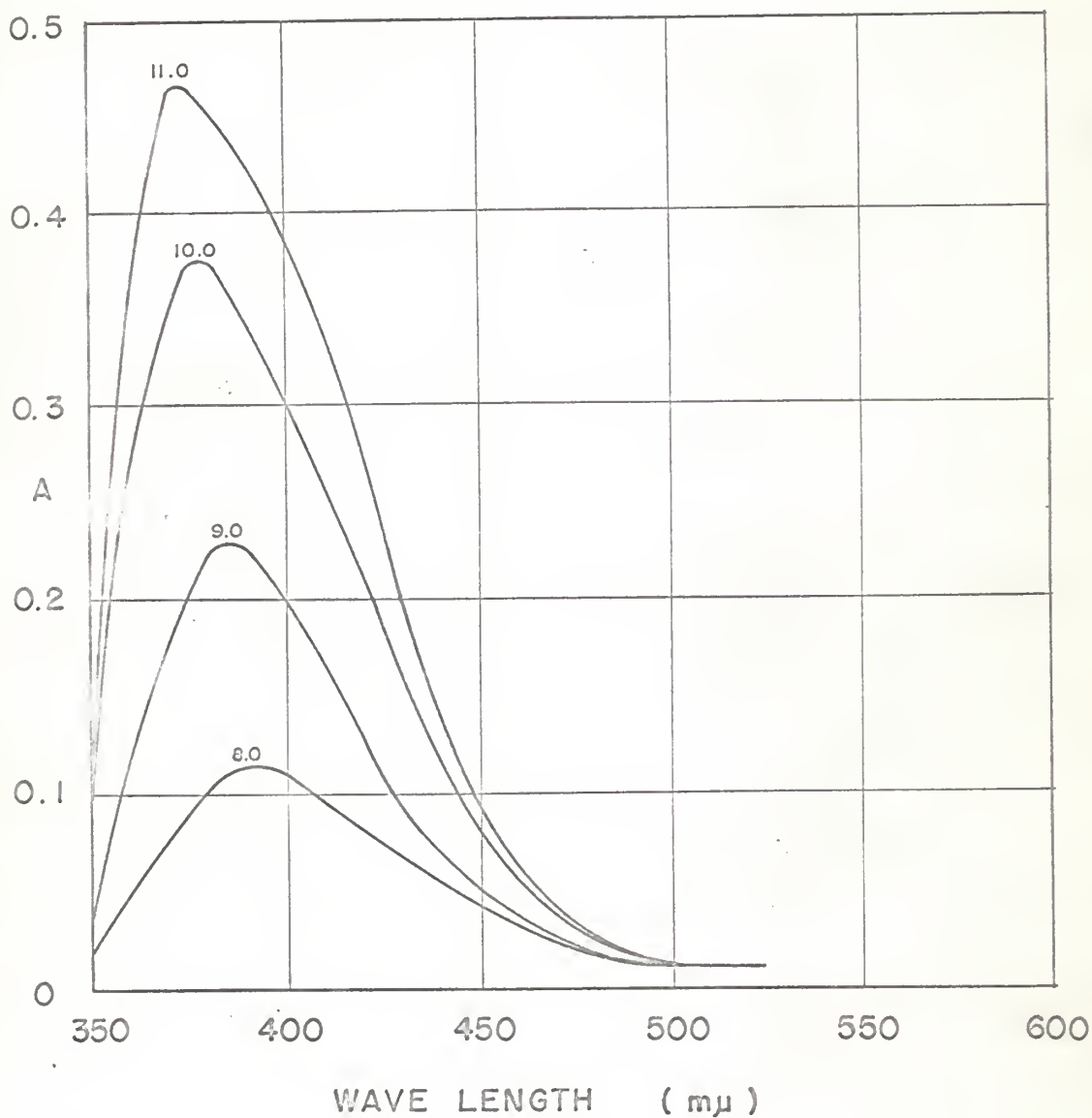


FIG. 5 : EFFECT OF pH ON THE COLOR OF THE SILVER
(1) - 2 - AMINO - 6 - METHYLTHIO - 4 - PYRIMIDINE -
CARBOXYLIC ACID SYSTEM.

Table 6 Continuous Variations Study of the Silver(I)-2-Amino-6-Methylthio-4-Pyrimidinecarboxylic Acid System at pH 10 ± 0.1

Total number of moles = 5×10^{-5}

Absorbance at 375 mu

Mole Fraction of Silver(I)	Trial No.1	Trial No.2	Average
0.1	0.155	0.156	0.156
0.2	0.365	0.374	0.370
0.3	0.480	0.469	0.475
0.4	0.611	0.629	0.620
0.5	0.765	0.793	0.779
0.6	0.818	0.854	0.836
0.7	0.674	0.682	0.678
0.8	0.438	0.444	0.441
0.9	0.288	0.282	0.285

Table 7 Continuous Variations Study of the Silver(I)-2-Amino-6-Methylthio-4-Pyrimidinecarboxylic Acid System at pH 10 ± 0.1

Total number of moles = 5×10^{-5}

Absorbance at 400 mu

Mole Fraction of Silver(I)	Trial No.1	Trial No.2	Average
0.1	0.123	0.124	0.124
0.2	0.319	0.319	0.319
0.3	0.415	0.403	0.409
0.4	0.509	0.523	0.516
0.5	0.644	0.668	0.656
0.6	0.706	0.733	0.720
0.7	0.594	0.602	0.598
0.8	0.377	0.384	0.381
0.9	0.256	0.248	0.252

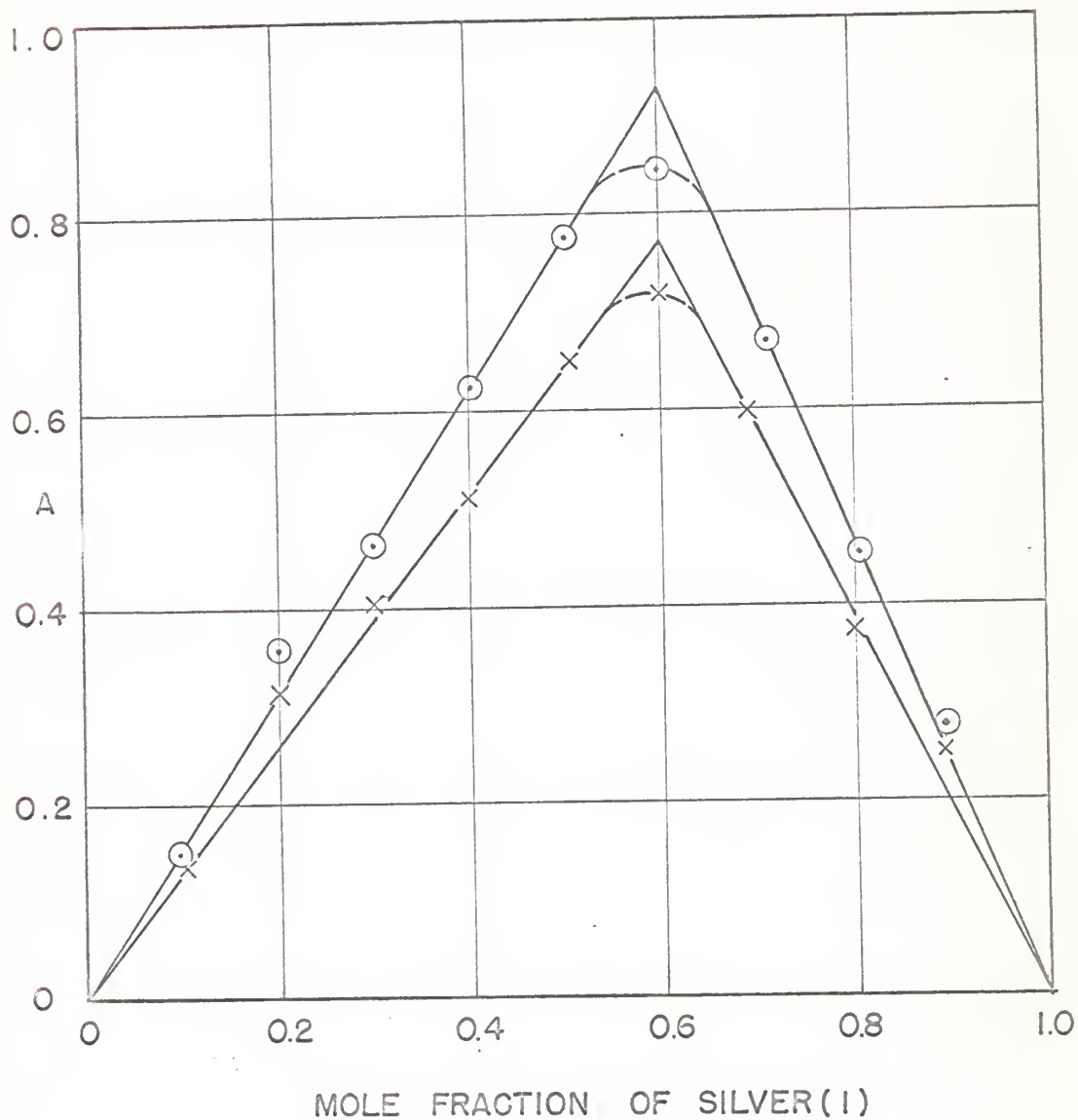


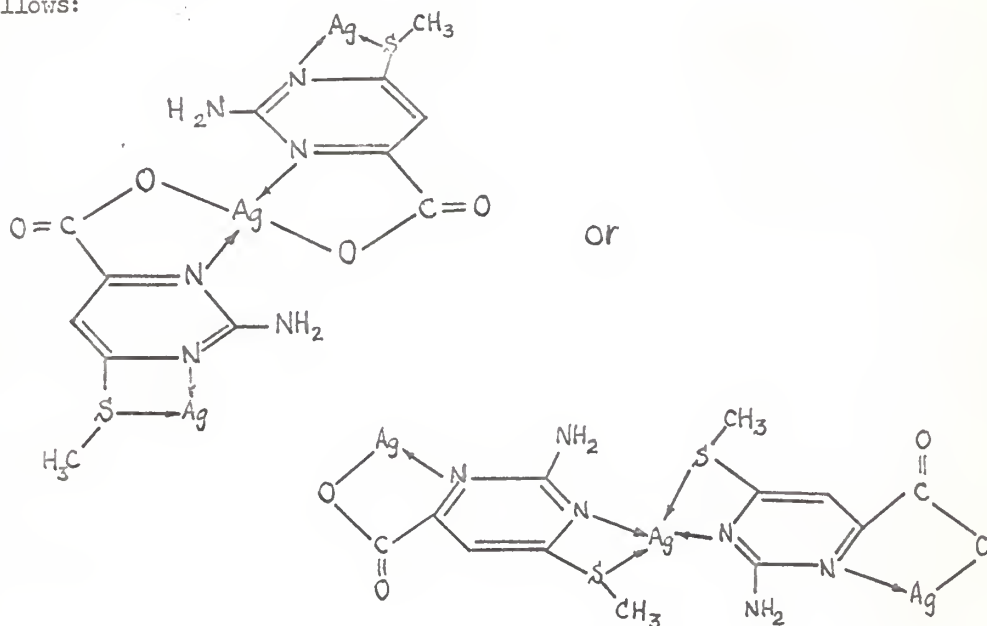
FIG. 6 : CONTINUOUS VARIATION STUDY ON THE SILVER (I) - 2 - AMINO - 6 - METHYLTHIO - 4 - PYRIMIDINE CARBOXYLIC ACID SYSTEM.

⊙ : DATA OF TABLE 1

× : DATA OF TABLE 2

TOTAL NUMBER OF MOLES = 5×10^{-5}

the ratio of ligand to metal is 2 to 3. Possible chelate structures are as follows:



Conformity to Beer's Law

The ability of the silver(I)-2-amino-6-methylthio-4-pyrimidinecarboxylic acid system to follow Beer's law in aqueous solution was studied. A graphical representation is shown in Fig. 7.

Experimental: A typical solution was prepared as follows:

1 ml of 0.01M silver nitrate solution in deionized water and 5 ml of 0.01M 2-amino-6-methylthio-4-pyrimidinecarboxylic acid. The solution was made to give the final volumes as 10 ml, 15 ml, 20ml,, 300 ml by diluting with the dilute sodium hydroxide solution whose pH was around 10 made by adding the saturated sodium hydroxide to deionized water. The pH was adjusted to 10 ± 0.1 with a Beckman zeromatic pH meter which was standardized with Beckman buffer solution at pH 7.0. All the absorbances were measured at 375 μ with a Beckman Model DB Spectrophotometer.

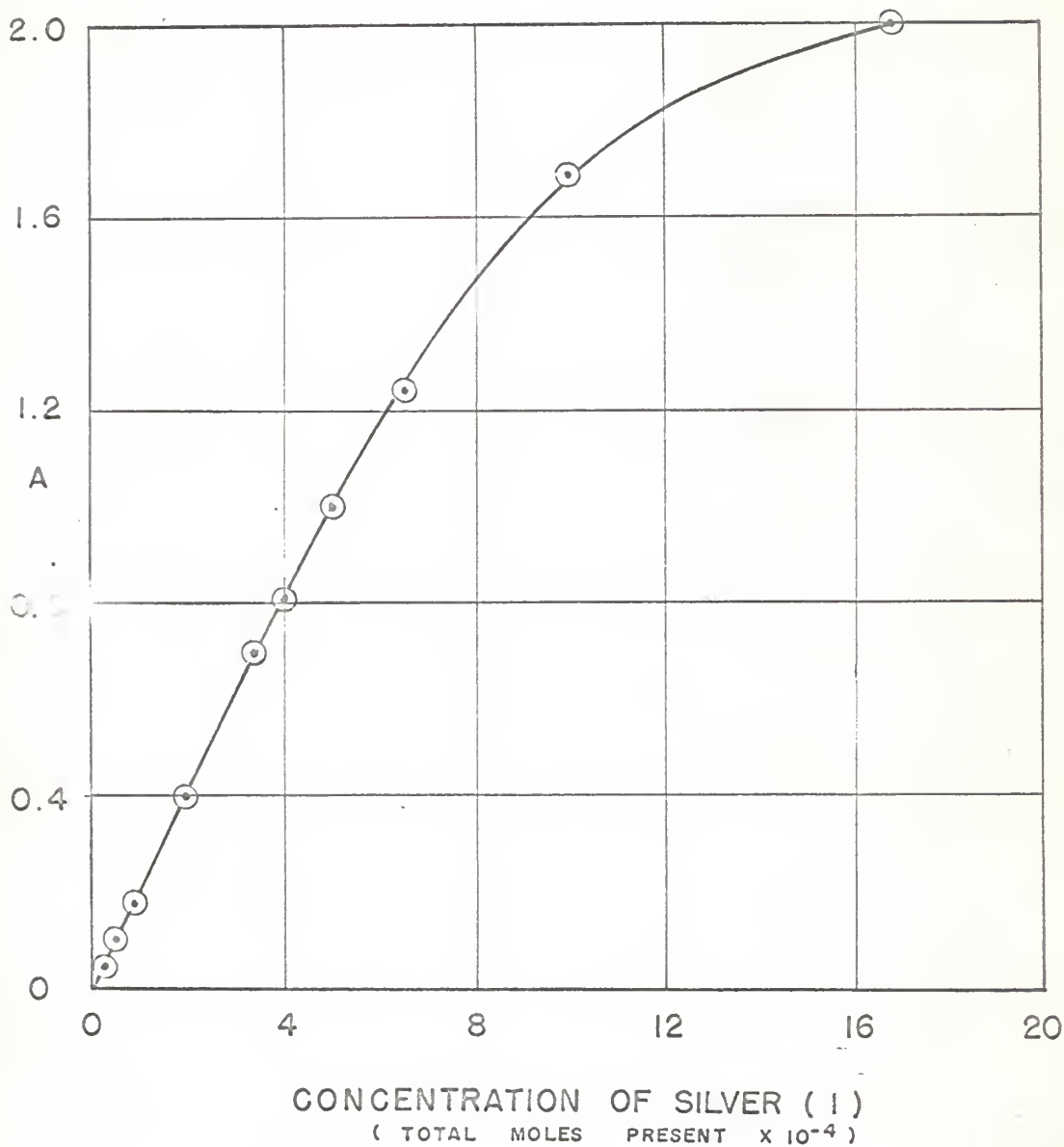


FIG. 7: COFORMITY TO BEER'S LAW OF THE SILVER (I)-
2-AMINO-6-METHYLTHIO-4-PYRIMIDINECARBOXYLIC
ACID SYSTEM.

Table 8 The Ability of the Silver(I)-2-Amino-6-Methylthio-4-Pyrimidinecarboxylic Acid System to Follow Beer's Law

Absorbance at 375 mu

Silver Conc. in the Soln.	Trial No. 1	Trial No. 2	Average
3.3×10^{-5}	0.050	0.048	0.049
4.0×10^{-5}	0.058	0.062	0.060
5.0×10^{-5}	0.078	0.076	0.077
6.7×10^{-5}	0.114	0.110	0.112
1.0×10^{-4}	0.195	0.193	0.194
1.3×10^{-4}	0.262	0.258	0.260
2.0×10^{-4}	0.401	0.405	0.403
2.5×10^{-4}	0.511	0.507	0.509
3.3×10^{-4}	0.685	0.687	0.686
4.0×10^{-4}	0.820	0.816	0.818
5.0×10^{-4}	1.050	1.042	1.046
6.7×10^{-4}	1.252	1.236	1.244
1.0×10^{-3}	1.686	1.696	1.691
1.7×10^{-3}	2.011	1.999	2.000

The results reported in Fig. 7 are the average of two independent determinations. Table 8 shows the data used to obtain Fig. 7. This system shows an apparent deviation from Beer's law at the higher concentrations. It obeys Beer's law well up to 5.0×10^{-4} moles per liter of silver(I) concentration. The maximum molar absorptivity is 2.08×10^3 at 375 mu.

Interference Studies

Forty two metal ions were studied for the determination of the interfering ions. These metal ions were obtained mostly from the nitrates and partly from the sulfates and the oxides. The chlorides and the ammonium salts could not be used because of the formation of the silver chloride precipitate which adsorbed the chelate, and the formation of the silver ammonium complex ion which interfered with the formation of the chelate. The affinity between silver ion and ammonium ion to form the complex ion was so strong that there was no free silver ion left to form the chelate with 2-amino-6-methylthio-4-pyrimidinecarboxylic acid.

Experimental: A typical solution was prepared as follows:

1 ml of 0.01M silver nitrate solution

1 ml of 0.01M ion solutions

5 ml of 0.01M ligand solution

25 ml of deionized water.

The solution was adjusted to $\text{pH } 10 \pm 0.1$ using a Beckman zeromatic pH meter. Those ions which formed either insoluble hydroxides or oxides were filtered to measure the absorbance at 480 to 320 mu.

The absorbance curves obtained at 480 to 320 mu using the various ions were compared with the one obtained with none of the ions present. From the comparisons of the curves, three groups were classified as follows:

Group I: The 14 ions which did not interfere with the chelate formed by 2-amino-6-methylthio-4-pyrimidinecarboxylic acid with silver(I).

Lithium(I): LiNO_3 ,	Sodium(I): NaNO_3
Potassium(I): KNO_3 ,	Selenium(VI): Na_2SeO_4
Silicon(IV): $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$,	Tellurium(VII): H_6TeO_6
Tungsten(VI): $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$,	Germanium(IV): GeO_2
Thallium(I): TlNO_3 ,	Aluminum(III): $\text{Al}(\text{NO}_3)_3 \cdot \text{H}_2\text{O}$
Vanadium(V): NaVO_3 ,	Beryllium(II): $\text{Be}(\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$
Uranium(VI): $\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$,	Rhenium(VII): Re_2O_7

Group II: The 10 ions which destroyed the chelate partially or completely.

Arsenic(III): As_2O_3 ,	Selenium(IV): H_2SeO_3
Chromium(VI): $\text{K}_2\text{Cr}_2\text{O}_7$,	Zirconium(IV): $\text{ZrO}(\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}^{(a)}$
Bismuth(III): $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$,	Copper(II): $\text{CuCO}_3^{(a)}$
Iron(III): $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$,	Thallium(III): Tl_2O_3
Gold(III): $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$,	Cobalt(II & III): Co_3O_4

Group III: The 18 ions which formed insoluble precipitates that adsorbed the chelate partially or completely.

Arsenic(V): As_2O_5 ,	Magnesium(II): $\text{Mg}(\text{OAc})_2 \cdot 4\text{H}_2\text{O}$
Lead(II): $\text{Pb}(\text{NO}_3)_2$,	Cadmium(II): $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$
Nickel(II): $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$,	Yttrium(III): $\text{Y}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$
Strontium(II): $\text{Sr}(\text{NO}_3)_2$,	Cerium(IV): $\text{Ce}(\text{HSO}_4)_4^{(b)}$
Barium(II): $\text{BaCO}_3^{(a)}$,	Thorium(IV): $\text{Th}(\text{NO}_3)_4 \cdot 4\text{H}_2\text{O}$
Lanthanum(III): $\text{La}(\text{OAc})_3 \cdot 1\frac{1}{2}\text{H}_2\text{O}^{(c)}$,	Titanium(IV): $\text{K}_2\text{TiO}(\text{C}_2\text{O}_4)_2 \cdot 2\text{H}_2\text{O}^{(b)}$
Indium(III): $\text{In}(\text{NO}_3)_3$,	Cobalt(II): $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$
Manganese(II): $\text{MnSO}_4 \cdot \text{H}_2\text{O}$,	Iron(II): $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$

Calcium(II): CaCO_3 , Cerium(III): $\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$

(a) : HNO_3 added, (b) : H_2SO_4 added, (c) : HOAc added

Twelve anions were studied for this purpose. These anions were obtained from the sodium or potassium salts of which cations were found not to give any interference. The experimental part was as same as in the cases of metal ions. The followings are 12 anions studied:

Acetate : NaOAc ,

Bromate : NaBrO_3

Nitrite : NaNO_2 ,

Iodate : KIO_3

Periodate : KIO_4 ,

Dihydrogen phosphate : KH_2PO_4

Sulfate : K_2SO_4 ,

Carbonate : Na_2CO_3

Sulfite : Na_2SO_3 ,

Oxalate : $\text{K}_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$

Pyrosulfite : $\text{Na}_2\text{S}_2\text{O}_5$,

Tartarate : $\text{NaKC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$

Among above 12 anions it was found that only periodate ion interfered to form a brown precipitate of AgIO_4 which adsorbed the chelate. The presence of bromate ion did not interfere but on standing for one hour it formed a white precipitate of AgBrO_3 which adsorbed the chelate.

Extraction Studies

The possibility of extracting silver(I)-2-amino-6-methylthio-4-pyrimidinecarboxylic acid chelate was studied, because in an alkaline solution a great many cations caused interference due to the formation of insoluble hydroxides or oxides which adsorbed the chelate. It was hoped that by employing an extraction some or all of these interferences could be removed.

Thirteen organic solvents were distilled, prior to use, in order to remove hydrogen peroxides which destroyed some chelates (24). 13 organic extracting agents studied are as follows:

Benzene, Hexane, Cyclohexane, n-Heptane,
Decahydronaphthalene (decalin), Benzyl alcohol, Isoamyl alcohol,
n-Hexyl alcohol, Xylene (Xylol), Diethyl ether,
Methyl ethyl ketone, Chloroform, Skelly C.

Extraction studies were made with each organic solvent and then with the mixture of two or three organic solvents with the various ratios in amount. It was found that none of above solvents could extract silver(I)-2-amino-6-methylthio-4-pyrimidinecarboxylic acid chelate.

Conclusion

Currently available methods for determining trace quantities of Ag^+ in solution are either inadequate or have serious disadvantages (20). The common turbidimetric silver chloride method (25) is not very sensitive and requires a calibration curve to be run with each batch of samples. The dithizone method requires very pure solvents and a time-consuming extraction step (26). The method presented here is very simple and direct, and it is a "one color" method. The system obeys Beer's law up to 5×10^{-4} moles of silver and therefore no calibration curve is required up to 54 ug of Ag per ml.

This method is precise and the system is stable for a month. The procedure does not require an exceedingly large excess of reagent. One limitation is that only deionized water should be used for making reagents and dilution because of the appreciable amount of chloride ion in the distilled water. The presence of chloride ion produces a white precipitate of silver chloride which adsorbs the chelate.

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SPECTROPHOTOMETRIC DETERMINATION OF
MANGANESE(VII) WITH 6-METHOXY-2-METHYLTHIO-4-PYRIMIDINECARBOXYLIC ACID
AND
SILVER(I) WITH 2-AMINO-6-METHYLTHIO-4-PYRIMIDINECARBOXYLIC ACID

by

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B.S., Ewha Womens University, Seoul, Korea, 1959

AN ABSTRACT OF A THESIS

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Eight pyrimidine derivatives are studied with 59 metal ions at pH's ranging 1 to 11 for the screening test to check the possibilities of chelate formation. Further studies are made on the Manganese(VII)-6-methoxy-2-methylthio-4-pyrimidinecarboxylic acid system and the silver(I)-2-amino-6-methylthio-4-pyrimidinecarboxylic acid chelate.

A spectrophotometric method for quantitatively determining small amounts of manganese is described. It is based on the reduction of permanganate ion with 6-methoxy-2-methylthio-4-pyrimidinecarboxylic acid to form manganate ion which is bright green in alkaline solution. The system obeys Beer's law between 3.3×10^{-5} - 1.0×10^{-3} moles per liter of manganese(VII). The maximum absorbance of the bright green color of manganate ion is obtained at 565 - 590 mu. The reagent to metal ratio is found to be one to two. The maximum molar absorptivity is 1.47×10^3 at 580 mu.

Next the quantitative determination of silver(I) is made by a spectrophotometric method. It is based on the yellow chelate which is formed when the silver ion reacts with 2-amino-6-methylthio-4-pyrimidinecarboxylic acid at pH 10 ± 0.1 . The system obeys Beer's law between 3.3×10^{-5} - 5.0×10^{-4} moles per liter of silver(I) in the aqueous solution. The effects of the following variables have been investigated: the reagent to metal ratio, the effect of pH, interference ions and extraction studies. The stoichiometry of the colored species has been determined and found to be three to two (silver to 2-amino-6-methylthio-4-pyrimidinecarboxylic acid) at pH 10 ± 0.1 . The maximum molar absorptivity is 2.08×10^3 at 375 mu.