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3,3′-Bipyridazine Derivatives as Spectrophotometric Reagents for Iron. II[1]

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3,3′-Bipyridazine derivatives containing the ferroin group react with iron (II) in a weakly acidic medium to form highly colored red complexes.

The spectrophotometric determination method for iron (II), based on this reaction, is moderately selective, the sensitivity being 1.8—2.8×10^{-3} μg Fe(II)/cm^2 for log $I_0/I=0.001$ at 520 mμ. The optimum concentration range is 0.5—2.5×10^{-7} moles of Fe(II)/ml. The complexes are stable at room temperature for 24 hr and contain Fe(II) and the reagents at the ratio of 1:3. 3,3′-Bipyridazine derivatives tested in this examination proved to be superior to 2,2′-bipyridine.

Compounds containing the ferroin type group $(=N-\overset{|}{C}-\overset{|}{C}=N)$ in a cyclic structure form highly colored water soluble stable iron (II) chelate complexes. This reaction, frequently called the ferroin reaction, was first reported by Blau,[3] who described the 2,2′-bipyridine, 1,10-phenanthroline and 2,2′-bipiperidine complexes. A considerable number of reagents containing the ferroin group have been prepared ever since and used most often as sensitive colorimetric reagents for iron (II).[4]

In the course of the studies on pyridazines, Igeta and his co-workers prepared 3,3′-bipyridazine derivatives from 3-halogenopyridazine derivatives.[5] It was found that these compounds formed highly colored red complexes with iron (II). The present paper describes fundamental conditions for applying the reaction to the spectrophotometric determination of iron (II). Recently, Schilt, *et al.*[6] reported in his comprehensive studies on ferroin type reagents that 3,3′-bipyridazine reacted with iron (II) to form a red complex having lower intensity (ε: 6350) at 517 mμ than that of 2,2′-bipyridine, but under the conditions proposed in this paper, the sensitivities of 3,3′-bipyridazine derivatives are higher than that of 2,2′-bipyridine as described posteriorly.

Experimental

Apparatus——The spectra of the reagents and their iron (II) complexes were measured with a Hitachi EPS-3 type recording spectrophotometer, while the absorbance was measured with a Hitachi 101 type spectrophotometer, using 1 cm cells. The pH value was measured with a Toa-Dempa glass electrode pH meter, Model HM-5A.

Iron (II) Standard Solution——An iron (II) standard solution was prepared by dissolving ferrous ammonium sulfate (G.R. grade, Tokyo-Kasei Co., Ltd.) in redistilled water. The solution contained 5×10^{-7} moles of iron (II) per ml.

Buffer Solutions——Buffer solutions of various pH values were prepared by mixing 0.05M acetic acid solution and 0.05M sodium acetate solution in different ratios.

Reagents and Reagent Solutions——The following five 3,3′-bipyridazine derivatives were used, but reagent III was excluded from the investigations of the applicabilities as the reagent, because of its low

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1) This forms Part XVI of, "Studies on Pyridazines," by H. Igeta, Part XV: H. Igeta, T. Tsuchiya, C. Okuda, and H. Yokogawa, *Chem. Pharm. Bull.* (Tokyo), **18**, 1347 (1970).

2) Location: *No. 8-5-1, Hatanodai, Sinagawa-ku, Tokyo.*

3) F. Blau, *Monatsh.*, **19**, 647 (1898).

4) T. Imamura, *Kagaku*, **17**, 424 (1962).

5) H. Igeta, T. Tsuchiya, M. Nakajima, and H. Yokogawa, *Tetrahedron Letters*, **1969** 2359. *Chem, Pharm. Bull.* (Tokyo),

6) A.A. Schilt and W.E. Dunber, *Talanta*, **16**, 519 (1969).

solubility. 3,3′-Bipyridazine (I), 6,6′-dimethyl-3,3′-bipyridazine (II), 6,6′-dimethoxy-3,3′-bipyridazine (III), 5,5′-dimethyl-3,3′-bipyridazine (IV), and 6,6′-diamino-3,3′-bipyridazine (V).

Reagent solutions were prepared by dissolving them in methanol respectively.

Other Reagents——All other reagents were of reagent grade.

Standard Procedure for Determination of Iron (II) with Bipyridazines——To 0.5 ml of a sample solution containing from 0.5 to 2.5×10^{-7} moles of iron (II) in 0.05M acetate buffer (pH:4.4), 0.5 ml of a reagent solution (0.05 or 0.1% solution) was added. After standing for 15 minutes, 4.0 ml of 50% methanol was added. The absorbance at 520 mμ was measured against a reagent blank solution treated in a similar manner.

Result and Discussion

Absorption Spectra

Table I shows the absorption maximum wavelengths and the molar extinction coefficients of 3,3′-bipyridazines used here in neutral, acidic and alkaline 50% methanol solution. None of them shows any absorption in visible region. Fig. 1 shows the absorption spectra of the solutions prepared so as to have the same amounts of iron (II). The maximum absorption wavelengths are found to be between 513 mμ and 521 mμ, and the spectra of the complexes indicate similar shape to that of 2,2′-bipyridine.

TABLE I. Absorption Maximum and Molar Extinction Coefficients of Various Bipyridazines in 50% (v/v)Methanol

Reagent	Neutral	Acidic (0.1N HCl)	Basic (0.1N NaOH)
I	230.5 mμ (14080)	234 mμ (12340)	227 mμ (13290)
	258 sh. (7590)	256 sh. (8400)	258 sh. (6560)
II	238 (17860)	241 (17290)	237 (20100)
	264 sh. (9580)	—	263 sh. (11090)
IV	237 (10520)	—	238 (8450)
	260 sh. (5260)	261 sh. (4320)	260 sh. (3570)
V	277 (29270)	268 (34980)	276 (26900)
	310 (12600)	—	310 sh. (10820)

( ) : molar extinction coefficient
sh. : shoulder

Effect of the Reagent Concentration

The absorption of a colored solution was measured by varying the concentrations of the reagent solution. As is shown in Fig. 2, the maximum absorbance is obtained by adding more than 0.05% solution of IV or V and 0.1% solution of I or II. The color intensity is constant over 0.05 or 0.1% respectively.

Effect of pH

The effect of pH on the color development of the complexes was investigated by using 0.05M acetate buffer solutions varying pH from 3.00 to 6.20. As is shown in Fig. 3, there is scarcely any variation in the absorbance of the color within the range of pH from 4.0 to 5.0

Effect of the Concentration of Buffer Solution

Though the figure was not shown, the effect of the concentration of buffer solution was examined. The absorbance of the colored solution was measured by varying the concentration of the buffer solution of pH 4.4. In consequence, the nearly constant intensity of color was obtained over 0.05M.

Effect of the Amount of Methanol

3,3′-Bipyridazines used in this experiment are very slightly soluble in water but soluble in methanol except Reagent III. The effect of the amount of methanol in the reaction solu-

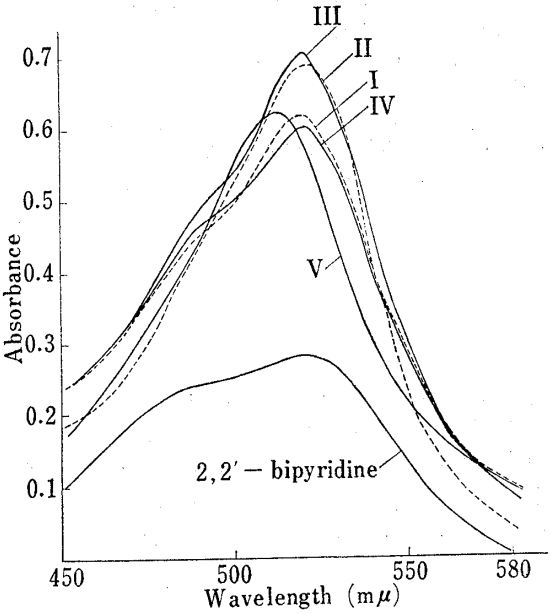

Fig. 1. Absorption Spectra of Various Fe(II)-3,3′-Bipyridazines and 2,2′- Bipyridine

Fe(II) solution : 2.0×10^{-7} mole/0.5 ml of 0.05M acetate bueffer solution (pH4.4), reagent solution : I and II; 0.1%, IV and V; 0.05%, III; saturated solution, 2,2′-bipyridine; 0.1%, reference: reagent blank

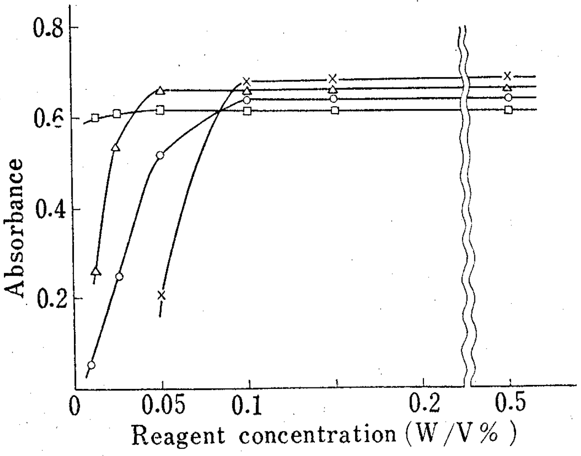

Fig. 2. Effect of Reagent Concentration on Color Intensity

Fe (II) solution: 2.0×10^{-7} mole/0.5 ml
reference: reagent blank
—○— : I —×— : II —△—: IV —□— : V

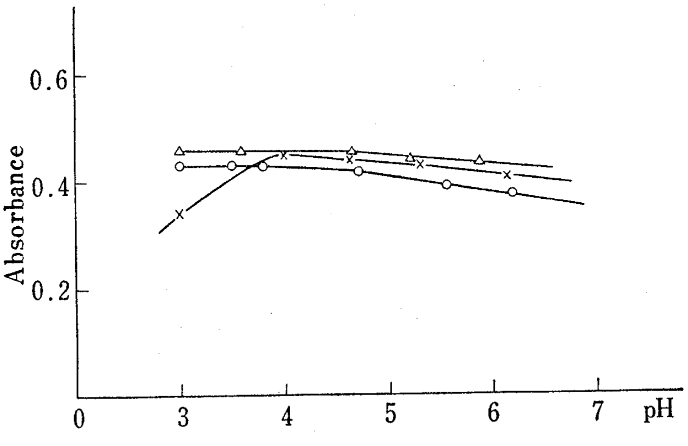

Fig. 3. Effect of pH on Color Development

Fe(II) solution: 1.15×10^{-7} mole/0.5 ml
reference: reagent blank
—○— : I and V —×— : II —△— : IV

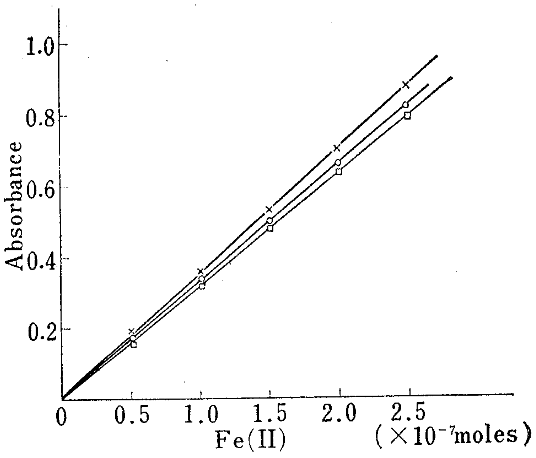

Fig. 4. Calibration Curvess

reference : reagent blank
—○— : I and IV —×— : II —□— : V

tion was examined by varying volume ratio of methanol to water. The nearly constant absorbance was obtained by using from 10 v/v % to 90 v/v % of methanol in reagent I, IV and V. Reagent II gave the maximum coloration by using 50 v/v % methanol. In this series of experiments, 50 v/v % methanol was always used as diluent.

Effect of the Reaction Time and the Stability of the Color Intensity

Though not illustrated in the figure, the color intensity reached maximum within a few minutes after mixing, therefore the reaction time had no significant effect on the color development. The absorbance of the diluted final solution with 50 v/v% methanol after reaction remained practically constant for at least 24 hr at room temperature.

Calibration Curves

Calibration curves were drawn under the directions of standard procedure. The results are shown in Fig. 4. Linear relationships exist between the absorbance and the iron (II) concentration over the range investigated. Under the conditions of the spectrophotometric determination, the wavelengths of absorption maxima, the apparent molar extinction coeffecients and the sensitivities defined by Sandell⁷⁾ are summarized in Table II. These values are about twice higher than that of 2,2′-bipyridine measured under the same conditions. Recently, Schilt and his co-workers⁶⁾ have studied much of ferroin type reagents and reported that the apparent molar extinction coefficient of 3,3′-bipyridazine was about 6350 at 517 mμ. This value is lower than that obtained by our method as shown in Table II.

Composition of the Complexes

Composition of the complexes were determined by two methods, *i.e.*, continuous variation method and molar ratio method. As shown in Fig. 5, the results indicate the formation of 1:3 complexes with all reagents,.

This can be generally expressed as:

$$\mathrm{Fe(II)} + 3\mathrm{L} \rightleftarrows \mathrm{Fe(II)L_3}$$

The formation constant, K, is then calculated from the curves of the molar ratio method on the basis of the following relationships, expressed by Harvey and Manning.⁸⁾

$$K = C(1-\alpha)/(\alpha \cdot C)(3\alpha C)^3$$
$$\alpha = (E_m - E_s)/E_s$$

where C is the total concentration of the complex assuming no dissociation; α, degree of dissociation of the complex; E_m, maximum absorbance of the complex when it does not dissociate into metal ions and free ligands; and E_s, actual absorbance of the complex. E_m and E_s are

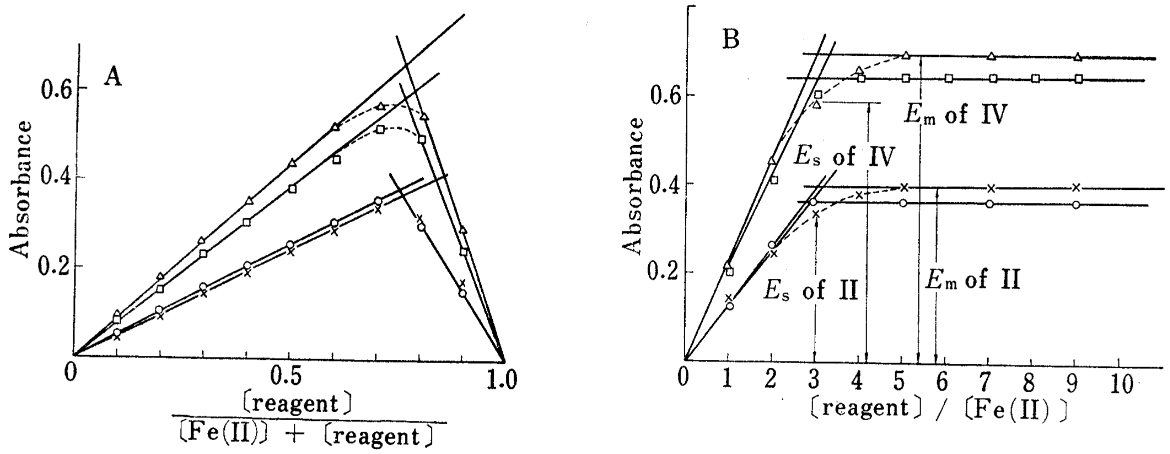

Fig. 5. Composition of Various Fe(II)-Bipyridazine Complexes

A : continuous variation method
$[\mathrm{Fe(II)}]+[\mathrm{Reagent}]=2.30\times10^{-5}$M for I and II
$[\mathrm{Fe(II)}]+[\mathrm{Reagent}]=4.0\times10^{-5}$M for IV and V
B : molar ratio method
Fe(II) concentration$=2.30\times10^{-5}$M for I and II
4.0×10^{-5}M for IV and V
Reagent concentration varied.
—○— : I —×— : II —△— : IV —□— : V

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7) E.B. Sandell, "Colorimetric Determination of Traces of Metals," 3rd ed., Interscience Publishers Inc., New York, 1959, p. 80.

8) A.E. Harvey and D.L. Manning, *J. Am. Chem. Soc.*, 72, 4488 (1950).

Table II. Iron (II) Complexes of Bipyridazines

Reagent	λ_{max}(mμ)	ε[a)]	Sensitivity[b)]	L:Fe(II)	Stability constant, K[c)]
I	520	16500	2.2×10^{-3}	3:1	1.5×10^{21}
II	521	17500	1.8×10^{-3}	3:1	1.7×10^{22}
IV	520	16400	2.1×10^{-3}	3:1	7.9×10^{21}
V	513	15900	2.8×10^{-3}	3:1	2.3×10^{21}
2,2′-Bipyridine	520	8500	—	3:1	2.8×10^{17}

a) Apparent molar extinction coefficient based on [Fe(II)].

d) sensitivity: μg/cm^2 for log $I_0/I=0.001$

c) $K=[Fe(II)\cdot L_3]/[Fe(II)][L]^3$

obtained from the curves shown in Fig. 5. The formation constants of the iron (II)-bipyridazine complexes at pH 4.4 are thus calculated to be 1.5×10^{21}—1.7×10^{22}, as shown in Table II.

Influence of Other Ions

The influence of several cations on the determination of iron (II) was studied under the conditions described in the standard procedure. Each solution contained 1×10^{-7} moles of iron (II) plus various amounts of other cations per 0.5 ml. The results are shown in Table III. Serious interferences are shown in Pb(II), Bi(III), Sn(II), Sb(III) which form precipitate with all reagents. Fe(III) and Mn(II) do not interfere, even if they are present at a concentration level of fourty times that of Fe(II). Reagent I, II and IV are not interfered by Cu(II), Ni(II) and Co(II), at a concentration level of four or ten times that of Fe(II), while Reagent V is seriously interfered by them. This may be due to formation of complexes between they and V.

Table III. Influence of Other Cations

Reagent	Added as	Amount added (mole)	Absorbance	%
I	—	—	0.590	—
	$Cu(NO_3)_2\cdot3H_2O$	8×10^{-7}	0.590	100.0
		20×10^{-7}	0.510	86.4
	$Ni(NO_3)_2\cdot6H_2O$	8×10^{-7}	0.588	99.7
		20×10^{-7}	0.211	35.7
	$Co(NO_3)_2\cdot6H_2O$	20×10^{-7}	0.622	105.4
II	—	—	0.600	—
	$Cu(NO_3)_2\cdot3H_2O$	2×10^{-7}	0.599	93.2
		8×10^{-7}	0.401	66.8
	$Ni(NO_3)_2\cdot3H_2O$	8×10^{-7}	0.603	100.5
		20×10^{-7}	0.059	9.8
	$Co(NO_3)_2\cdot6H_2O$	80×10^{-7}	0.553	93.7
IV	—	—	0.632	—
	$Cu(NO_3)_2\cdot3H_2O$	8×10^{-7}	0.625	98.9
		20×10^{-7}	0.485	76.8
	$Ni(NO_3)_2\cdot6H_2O$	2×10^{-7}	0.634	100.3
		8×10^{-7}	0.478	75.6
	$Co(NO_3)_2\cdot6H_2O$	20×10^{-7}	0.634	100.3
		80×10^{-7}	0.554	87.7
V	—	—	0.613	—
	$Cu(NO_3)_2\cdot3H_2O$	2×10^{-7}	0.370	60.4
	$Ni(NO_3)_2\cdot6H_2O$	2×10^{-7}	0.138	22.5
	$Co(NO_3)_2\cdot6H_2O$	2×10^{-7}	0.288	46.9
	$Pb(NO_3)_2$	2×10^{-7}	0.613	100.0
		8×10^{-7}	0.698	113.8
	$Zn(CH_3COO)_2 2H_2O$	20×10^{-7}	0.610	99.5
		40×10^{-7}	0.559	91.2

$FeSO_4\cdot(NH_4)\cdot SO_4\cdot6H_2O$: 2×10^{-7} moles