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Studies of Calcichrome as a Spectrophotometric Reagent. I. A New Spectrophotometric Method for the Determination of Copper with Calcichrome and Its Basic Study

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It was found that Calcichrome, which has been recently established as a reagent for the determination of calcium, forms a stable 1-to-1 complex with copper. Basic studies were carried out, in which the acid dissociation constants of Calcichrome and the apparent formation constant of the complex were determined to be $pK_{a_7}=7.2$, $pK_{a_8}=11.5$, and $K=4.1\times10^8$ respectively. A new method for the determination of copper was also established.

Calcichrome, the self-coupling product of H-acid, was recently suggested by Close and West[1] as a selective metallochromic indicator for the complexometric titration of calcium. As the reagent has several points of excellence, for instance, an easy solubility in water and a stability for storage, it has been used for the determination of calcium in various materials by several investigators.[2-5] It has also been found that the reagent can be used as a spectrophotometric reagent for the determination of calcium.[2-4] The applicability of the reagent to the determination of elements other than calcium as a metallochromic or spectrophotometric reagent has not, however, yet been reported. The authors have recently discovered that the reagent reacts to form complexes with many other metals, such as copper(I and II), iron(II and III), aluminum(III), and titanium(IV). The present paper will describe a direct spectrophotometric method for the determination of copper with Calcichrome and the results of a basic study of it, the outline of this work has already been reported in Short Communication.[6]

Experimental

Reagents.—*Calcichrome Solution.* — A Calcichrome solution (2×10^{-3} M) was prepared by dissolving 0.9909 g. of Calcichrome in distilled water and diluting it to 500 ml. Working solutions were prepared from this solution by dilution. The Calcichrome used in this study was synthesized and purified by the method proposed by Close et al.[1] However, as it contains a small amount of chloride and sodium, furhter purification was carried out as follows for use in potentiometric titrations: Calcichrome was dissolved in distilled water, and the resulting solution was passed through a column packed with cation exchange resin, Amberlite IR-120 (H-form), and then evaporated to dryness on a steam bath, followed by drying at 105°C in an air oven. The Calcichrome thus obtained was free from chloride and sodium.

Standard Cupric Solution.—A standard cupric solution corresponding to 1×10^{-2} mol./l. of copper(II) was prepared by dissolving 2.4969 g. of cupric sulfate penta-hydrate in distilled water, by then adding small amounts of sulfuric acid to prevent any possible hydrolysis, and by diluting the mixture to 1 l. Working solutions of copper(II) were prepared from this solution by dilution.

Standard Cuprous Solution.—A standard cuprous solution corresponding to about 1×10^{-2} mol./l. of copper(I) was prepared by dissolving about 1 g. of cuprous chloride

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[1] R. A. Close and T. S. West, *Talanta*, **5**, 221 (1960).

[2] A. M. Lukin, K. A. Smirnova and G. B. Zavarikhina, *J. Anal. Chem.* (*U. S. S. R.*), **18**, 389 (1963).

[3] M. H.-Lancina and T. S. West, *Anal. Chem.*, **35**, 2131 (1963).

[4] P. Pakalns and T. M. Florence, *Anal. Chim. Acta*, **30**, 353 (1964).

[5] H. Ishii and H. Einaga, *Japan Analyst* (*Bunseki Kagaku*), **14**, 162 (1965).

[6] H. Ishii and H. Einaga, This Bulletin, **38**, 1416 (1965).

in distilled water containing small amounts of hydrochloric acid, by then adding about 1 g. of hydroxylamine hydrochloride to prevent any possible oxidation, and by diluting the mixture to 1 l. This solution was standardized by the EDTA titration method, using xylenol orange as an indicator. Working solutions of copper(I) were prepared by diluting the standard solution with distilled water.

Buffer Solutions.—Buffer solutions were prepared by mixing 1 M acetic acid and 1 M sodium acetate in the ratios required.

Apparatus. — A Hitachi automatic recording spectrophotometer, model EPS-2, and a Hitachi spectrophotometer, model 139, were used, together with 1 cm. matched cells, for obtaining absorption curves and for all the other absorbance measurements respectively. A Toa Dempa glass-electrode pH meter, model HM-5A, was used for all the pH measurements.

The Proposed Procedure for the Determination.—To a sample solution containing copper in a 50 ml. volumetric flask, 5 ml. of the Calcichrome solution (4×10^{-4} M) and 10 ml. of the buffer solution (pH 5.2) were added. After the solution had then been diluted with distilled water to the mark, the absorbance of the solution was measured at 535 or 310 mμ against a reagent blank treated in a similar manner.

Results and Discussion

Absorption Curves.—The absorption curves of the copper-Calcichrome complex are shown in Figs. 1-a, 1-b, and 1-c. By comparing Fig. 1-a with Fig. 1-b, it will be found that these two figures are exactly the same. The copper(I)-Calcichrome complex, therefore, can not be distinguished from the copper(II)-Calcichrome complex, and the

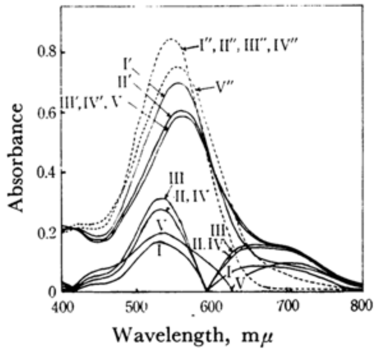

Fig. 1-a. Absorption spectra of Cu(I)-Calcichrome complex and Calcichrome.

I : pH=3.2 Cu(I)-Calcichrome complex
II : pH=4.1 Cu(I)-Calcichrome complex
III : pH=4.9 Cu(I)-Calcichrome complex
IV : pH=5.9 Cu(I)-Calcichrome complex
V : pH=7.2 Cu(I)-Calcichrome complex
Reference: reagent blank

I′ : pH=3.2 Cu(I)-Calcichrome complex
II′ : pH=4.1 Cu(I)-Calcichrome complex
III′ : pH=4.9 Cu(I)-Calcichrome complex
IV′ : pH=5.9 Cu(I)-Calcichrome complex
V′ : pH=7.2 Cu(I)-Calcichrome complex
Reference: water

I″ : pH=3.2 Calcichrome alone
II″ : pH=4.1 Calcichrome alone
III″ : pH=4.9 Calcichrome alone
IV″ : pH=5.9 Calcichrome alone
V″ : pH=7.2 Calcichrome alone
Reference: water

Calcichrome : 3.2×10^{-5} mol./l.
Cu(I) : 3.2×10^{-5} mol./l.

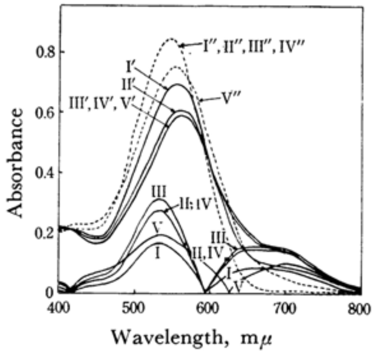

Fig. 1-b. Absorption spectra of Cu(II)-Calcichrome complex and Calcichrome.

I : pH=3.2 Cu(II)-Calcichrome complex
II : pH=4.1 Cu(II)-Calcichrome complex
III : pH=4.9 Cu(II)-Calcichrome complex
IV : pH=5.9 Cu(II)-Calcichrome complex
V : pH=7.2 Cu(II)-Calcichrome complex
Reference : reagent blank.

I′ : pH=3.2 Cu(II)-Calcichrome complex
II′ : pH=4.1 Cu(II)-Calcichrome complex
III′ : pH=4.9 Cu(II)-Calcichrome complex
IV′ : pH=5.9 Cu(II)-Calcichrome complex
V′ : pH=7.2 Cu(II)-Calcichrome complex
Reference: water

I″ : pH=3.2 Calcichrome alone
II″ : pH=4.1 Calcichrome alone
III″ : pH=4.9 Calcichrome alone
IV″ : pH=5.9 Calcichrome alone
V″ : pH=7.2 Calcichrome alone
Reference: water.

Calcichrome : 3.2×10^{-5} mol./l.
Cu(II) : 3.2×10^{-5} mol./l.

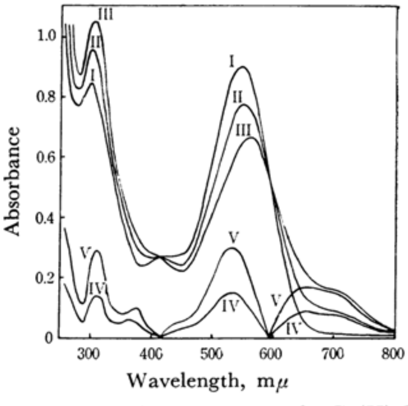

Fig. 1-c. Absorption spectra of Cu(II)-Calcichrome complex.

Calcichrome: 4×10^{-5} mol./l., pH: 5.0

I: Cu=0
II: Cu: Cch=1: 2
III: Cu: Cch=1: 1
Reference: water

IV: Cu: Cch=1: 2
V: Cu: Cch=1: 1
Reference: reagent blank

species of the complex formed under the conditions studied are one and the same because the maximum absorption wavelengths of the curves obtained against reagent blanks are identical at 535 mμ. (Although curve V in Fig. 1-a or 1-b differs from the others, this is due to the fact that the absorption curve of Calcichrome itself shifts to the long wavelength side). The ultraviolet and visible absorption curves of the copper(II)-Calcichrome complex at pH 5.0 at various ratios of copper and Calcichrome are shown in Fig. 1-c, from which it may be seen that the complex consisting of a 1-to-1 mixture has two absorption maxima in the ultraviolet (λ_{max} 307 mμ) and visible (λ_{max} 565 mμ) regions, one shoulder at about 700 mμ, and two isosbestic points at 415 and 595 mμ respectively. The curves obtained against a reagent blank have three absorption maxima (λ_{max} 310, 535, and 650 mμ). Although these three wavelengths may be used for the determination of copper(II), the sensitivities at the first two wavelengths are higher than that at the last, and approximately the same sensitivity was obtained at the former.

The ultraviolet and visible absorption curves of the copper(I)-Calcichrome complex under the same conditions were exactly the same as those of the copper(II)-Calcichrome complex; they are therefore omitted here. Judging from the results obtained above, the same considerations can be made of the complex between copper(I) and Calcichrome.

The Effect of pH on the Color Development.—The effect of pH on the color development of the copper(II)-Calcichrome complex was examined by measuring the absorbance of the complex at two wavelengths, 310 and 535 mμ, in which cases the concentrations of copper(II) and Calcichrome were made to 4×10^{-5} mol./l. The results are shown in Fig. 2, from which it can be seen that the maximum color development can be obtained in the pH ranges from 5.0 to 6.5 when measured at 310 mμ and from 5.0 to 5.5 at 535 mμ, and that approximately the same color intensity is obtained in these pH regions.

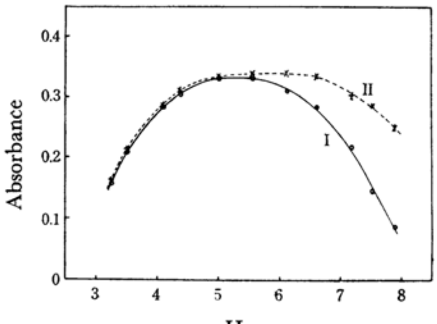

Fig. 2. Effect of pH on absorbance of the Cu(II)-Calcichrome complex.
Concn. of Cu(II): 4×10^{-5} mol./l.
Concn. of Calcichrome: 4×10^{-5} mol./l.
I: at 535 mμ II: at 310 mμ

The effect of pH on the color development of the copper(I)-Calcichrome complex was the same as on that of the copper(II)-Calcichrome complex.

The Effect of the Amount of Calcichrome.—The effect of the reagent concentration on the color development was studied with two series of colored solutions, one of which contained 31.8 μg. of copper(II) (corresponding to 1×10^{-5} mol./l.) and the other, 63.5 μg. of copper(II) (corresponding to 2×10^{-5} mol./l.). The pH values of the solutions were kept constant at 5.2, and the absorbance measurements were carried out at 535 mμ. The results are shown in Fig. 3. For both series, about a 2-fold excess of the reagent over the copper(II) concentration is necessary to obtain the maximum color intensity.

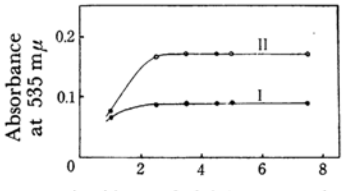

Fig. 3. Effect of amount of Calcichrome.
pH: 5.2, Reference: reagent blank
I: Cu(II), 31.8 μg. II: Cu(II), 63.5 μg.

The same results were obtained when copper(I) was used instead of copper(II). As a compromise between sensitivity and absorbance by the blank solution, 5.0 ml. of a 4×10^{-4} M reagent solution should be used with these copper concentrations.

The Stability of the Color.—The color of the copper(I or II)-Calcichrome complex develops instantaneously at room temperature and is very stable, and there was no change in absorbance over a period of 24 hr.

Adherence to Beer's Law.—The linear relationship between the absorbance and the concentration of copper at 535 mμ was found to be dependent upon the concentration of Calcichrome; Beer's law was obeyed when the molar ratio of copper(II) to Calcichrome was less than about 0.8. The molar extinction coefficient (ε) was about 8×10^3, and the sensitivity was 0.008 μg. Cu(II)/cm^2, which corresponds to log $(I_0/I)=0.001$. Similar results were obtained at 310 mμ.

With the copper(I)-Calcichrome complex, similar results (ε: 8×10^3, sensitivity: 0.008 μg. Cu(I)/cm^2) were obtained.

The Effect of Diverse Ions.—The effect of diverse ions on the determination of copper was examined at pH 5.0. The results are summarised in Table I, from which it can be concluded that aluminum(III), iron(II and III), titanium(IV), vanadium(V), and zirconium(IV) interfere markedly, even in the smallest amounts, and that nickel(II), oxalate and thiosulfate, also interfere. Among these, the interference by cations seems to be due to the complex formation with Calcichrome under the conditions examined.

TABLE I. EFFECT OF DIVERSE IONS
Cu taken: 75.5 μg.

Diverse ion, added μg.		Cu found μg.	Relative error %
Ag(I)	500	75.5	± 0.0
Al(III)	25	87.6	+16.0
	50	95.8	+26.9
	75	102.3	+35.5
Ba(II)	280	75.5	± 0.0
Ca(II)	500	75.5	± 0.0
Cd(II)	500	75.5	± 0.0
Co(II)	500	75.5	± 0.0
Cr(III)	500	75.5	± 0.0
Cr(VI)	500	75.5	± 0.0
Fe(II)	25	82.0	+ 8.6
	50	87.0	+15.2
	75	91.0	+20.5
Fe(III)	25	119.8	+58.7
Hg(II)	500	75.5	± 0.0
Mg(II)	500	74.2	− 1.7
Mn(II)	500	75.5	± 0.0
Ni(II)	50	78.4	+ 3.8
	500	79.5	+ 5.3
Pb(II)	500	75.5	± 0.0
Si(IV)	500	75.5	± 0.0
Sn(II)	500	75.5	± 0.0
Sr(II)	500	76.0	+ 0.7
Ti(IV)	30	78.5	+ 4.0
	150	85.0	+12.6
	300	87.5	+15.9
V(V)	12.5	80.0	+ 6.0
	37.5	88.5	+17.2
	125	112.5	+49.0
Zn(II)	500	75.5	± 0.0
Zr(IV)	50	78.0	+ 0.7
	250	81.0	+ 7.3
	500	84.0	+11.3
Cl^-	2500	75.5	± 0.0
NO_3^-	2500	75.5	± 0.0
PO_4^{3-}	2500	75.5	± 0.0
$C_2O_4^{2-}$	25	74.5	− 1.3
	75	73.0	− 3.3
	250	70.0	− 6.7
$S_2O_3^{2-}$	50	75.0	− 0.7
	75	72.8	− 3.6
	250	72.3	− 4.2

The Composition of the Complex.—Attempts to obtain the composition of the complex were made by two methods, the continuous variation method and the mole ratio method. These attempts were performed at pH 5.2, and the absorbance measurements were made at three wavelengths between 510 and 545 mμ. Both methods revealed that copper(I or II) forms a 1-to-1 complex with Calcichrome. These findings were further confirmed by the experiments described below. As an example, the results obtained by the application of the continuous variation method to the copper(II)-Calcichrome complex are shown in Fig. 4-a, and those obtained by the application of the mole ratio method to the copper(I)-Calcichrome complex, in Fig. 4-b.

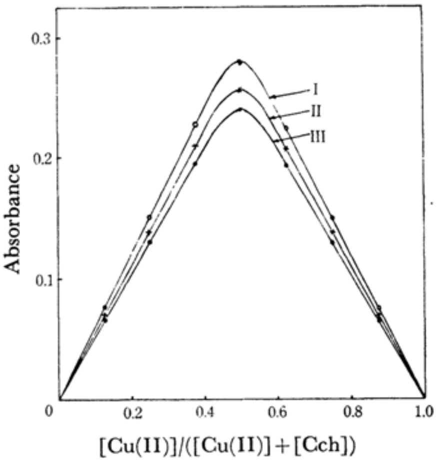

Fig. 4-a. Continuous variation method.
Copper concn. + Calcichrome concn. $=8\times10^{-5}$ mol./l.
pH: 5.2. I: 535 mμ, II: 545 mμ, III: 510 mμ

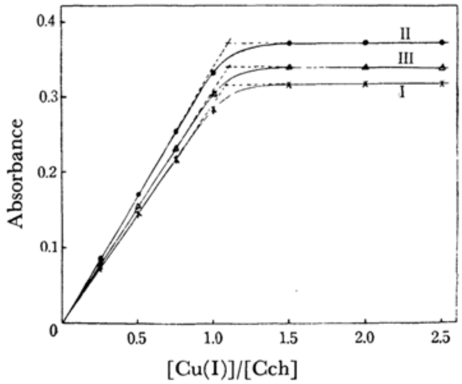

Fig. 4-b. Mole ratio method.
Concn. of Calcichrome: 4.2×10^{-5} mol./l.
I: 510 mμ, II: 535 mμ, III: 545 mμ

The Potentiometric Titration of Calcichrome and Its Copper Complex. — The potentiometric titration of Calcichrome and its copper(II) complex were carried out in order to establish the mechanism of the reaction between Calcichrome and copper, in which case the concentrations of Calcichrome and copper(II) were made to 5×10^{-4} mol./l. the ionic strength was kept constant at 0.1 using a sodium chloride solution and the titration was carried out at 25°C with a 0.3 N potassium hydroxide solution. The results are shown in Fig. 5, in which the curves I and II show the titration curves of Calcichrome and

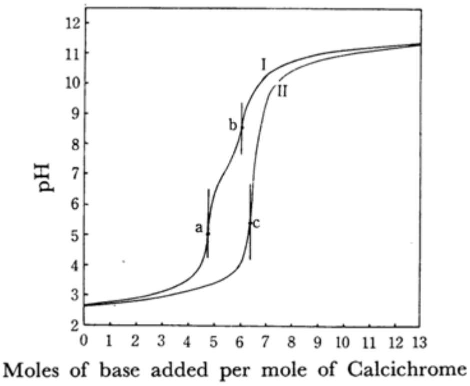

Fig. 5. Potentiometric titration curves of Calcichrome and its copper(II) complex.

Concn. of Copper(II): 5×10^{-4} mol./l.
Concn. of Calcichrome: 5×10^{-4} mol./l.
Ionic strength: 0.1
I: Calcichrome alone
II: Copper(II)-Calcichrome complex

its 1-to-1 copper(II) complex respectively. From Fig. 5 it may be noticed that Calcichrome dissociates to liberate five protons up to about pH 5 (point a) that a) sixth proton is liberated up to about pH 8.5 (point b), and that the sixth proton is also liberated in the complexation. According to the constitutional formula of Calcichrome proposed by Close et al.[1]) or Lukin et al.[2]), six sulfonic radicals are contained in one molecule of Calcichrome. Taking into consideration of the facts described above, it is considered that sulfonic radical may participate in the complexation. However, the dissociation of sulfonic radicals are generally considered to occur in the lower pH region due to their strongly acidic properties as are seen in the cases of the dissociations of Chromotropic acid and its derivatives. Therefore, there remain some doubts to be concluded directly from the results of the potentiometric titration alone that sulfonic radical may participate in the complexation and further studies should be carried out to solve the problem.

Aside from the experimental results obtained above, the authors considered here on the basis of the general consideration that the group which participate in the complexation is hydroxyl radical rather than sulfonic. Therefore, it may be considered that the dissociation of Calcichrome is shown by Eq. 1, and that copper(II) and/or copper(I) is combined with a hydroxyl radical in the Calcichrome molecule, the reaction in this case being shown by Eq. 2 or 3:

$$\mathrm{H_9Cch^0} \rightleftarrows \mathrm{H_{9-\mathit{m}}Cch^{\mathit{m}-}} + m\mathrm{H^+} \qquad (1)$$

$$\mathrm{H_3Cch^{6-}} + \mathrm{Cu^{2+}} \rightleftarrows \mathrm{CuH_2Cch^{5-}} + \mathrm{H^+} \qquad (2)$$

$$\mathrm{H_3Cch^{6-}} + \mathrm{Cu^{+}} \rightleftarrows \mathrm{CuH_2Cch^{6-}} + \mathrm{H^+} \qquad (3)$$

The Effect of pH on the Absorbance of Calcichrome.—The effect of pH on the absorbance of Calcichrome was examined. Here the concentration of Calcichrome was 4×10^{-5} mol./l., the ionic strength was kept constant at 0.25 using a sodium chloride solution, and the absorbance measurements were carried out at 630 mμ. The results are shown in Fig. 6, from which it will be found that three ionic species are present under the conditions examined. Taking account of the discussions described above, these three species are considered to correspond to $\mathrm{H_3Cch^{6-}}$, $\mathrm{H_2Cch^{7-}}$, and $\mathrm{HCch^{8-}}$ respectively.

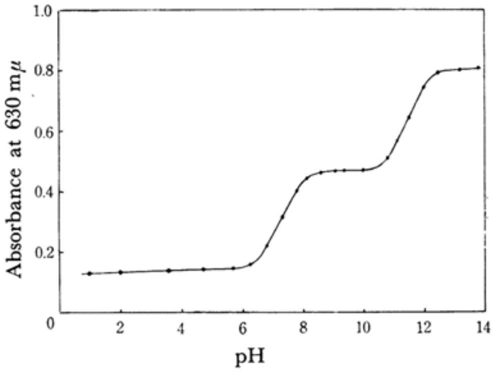

Fig. 6. Effect of pH on absorbance of Calcichrome.

Concn. of Calcichrome: 4×10^{-5} mol./l.
Ionic strength: 0.25

The Acid Dissociation Constant of Calcichrome.—As has been described already, Calcichrome is dissociated as shown by Eq. 1. The seventh and eighth acid dissociation constant, K_{a_7} and K_{a_8}, are:

$$K_{a_7} = [\mathrm{H_2Cch}]\,[\mathrm{H}]/[\mathrm{H_3Cch}] \qquad (4)^*$$

$$K_{a_8} = [\mathrm{HCch}]\,[\mathrm{H}]/[\mathrm{H_2Cch}] \qquad (5)^*$$

Therefore,

$$\mathrm{p}K_{a_7} = \mathrm{pH} - \log[\mathrm{H_2Cch}]/[\mathrm{H_3Cch}] \qquad (6)^*$$

$$\mathrm{p}K_{a_8} = \mathrm{pH} - \log[\mathrm{HCch}]/[\mathrm{H_2Cch}] \qquad (7)^*$$

As the concentrations are proportional to the absorbances, $\mathrm{p}K_{a_7}$ and $\mathrm{p}K_{a_8}$ are given by the following equations:

$$\mathrm{p}K_{a_7} = \mathrm{pH} - \log(E_1 - E)/(E - E_2) \qquad (8)$$

$$\mathrm{p}K_{a_8} = \mathrm{pH} - \log(E_2 - E')/(E' - E_3) \qquad (9)$$

in which E_1, E_2, and E_3 are the absorbances of $\mathrm{H_3Cch^{6-}}$, $\mathrm{H_2Cch^{7-}}$, and $\mathrm{HCch^{8-}}$ respectively at a constant wavelength, and E and E' are the absorbances of the solutions, consisting of $\mathrm{H_3Cch^{6-}}$ and $\mathrm{H_2Cch^{7-}}$, and $\mathrm{H_2Cch^{7-}}$ and $\mathrm{HCch^{8-}}$ respectively, at arbitrary pH values.

The actual examinations were carried out as follows: The concentration of Calcichrome was

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* Electronic charges were omitted for the sake of simplicity.

maintained at a constant value, 4×10^{-5} mol./l., and suitable amounts of a disodium hydrogen phosphate - potassium dihydrogen phosphate buffer solution (for the determination of $\mathrm{p}K_{a_7}$) or a 0.1 N sodium hydroxide solution (for the determination of $\mathrm{p}K_{a_8}$) were added to give seven arbitrary pH values between 6.60 and 8.00, or between 10.80 and 11.90, respectively. In both cases, suitable amounts of a sodium chloride solution were added to keep the ionic strength (μ) constant at 0.1. The absorbance measurements were then carried out at 630 mμ. The $\mathrm{p}K_{a_7}$ and $\mathrm{p}K_{a_8}$ values were calculated from Eqs. 8 and 9 respectively, and the average values of the seven runs are shown in Table II.

TABLE II. $\mathrm{p}K_{a_7}$ AND $\mathrm{p}K_{a_8}$ OF CALCICHROME

$\mathrm{p}K_{a_7}$	7.2	(μ=0.1, 27°C)
$\mathrm{p}K_{a_8}$**	11.5	(μ=0.1, 23°C)

The Equilibrium Constant.—From the results described above, it seems obvious that copper reacts with Calcichrome to form a 1-to-1 complex and that one proton is liberated as the result of deprotonation during the complex formation. Independent of these facts, on the other hand, the following assumptions and the related examinations were made in order to obtain the equilibrium constant, and also to confirm the facts described above. If the reaction between Calcichrome and copper is obeyed to Eq. 10,

$$\mathrm{Cu}^{2+} + \mathrm{H}_{9-n}\mathrm{Cch}^{n-} \rightleftarrows \mathrm{CuH}_{9-(n+m)}\mathrm{Cch}^{(n+m)-} + m\mathrm{H}^+ \qquad (10)$$

the total absorbance at equilibrium, D, is:

$$D = \varepsilon_1(a-x) + \varepsilon_2(b-x) + \varepsilon x \qquad (11)$$

where ε_1, ε_2, and ε are the molar extinction coefficients of copper, Calcichrome, and the complex respectively, a and b are the initial concentrations of copper and Calcichrome respectively, and x is the concentration of the complex at equilibrium. As ε_1 is equal to 0, and so may be neglected under these conditions, Eq. 11 can be rearranged to:

$$D = \varepsilon_2(b-x) + \varepsilon x \qquad (12)$$

The equilibrium constant, K', may be defined as Eq. 13:

$$K' = [\mathrm{CuH}_{9-(n+m)}\mathrm{Cch}][\mathrm{H}]^m/[\mathrm{Cu}][\mathrm{H}_{9-n}\mathrm{Cch}] = x[\mathrm{H}]^m/(a-x)(b-x) \qquad (13)^*$$

If the initial concentration of copper, a, is chosen to be equal to that of Calcichrome, Eq. 13 can be rearranged to:

$$K' = x[\mathrm{H}]^m/(b-x)^2 \qquad (14)$$

By introducing Eq. 12 into Eq. 14, Eq. 15 is obtained:

$$a/(D-D') = 1/(\varepsilon-\varepsilon_2) + \{1/K'(\varepsilon-\varepsilon_2)\}^{1/2}\{[\mathrm{H}]^m/(D-D')\}^{1/2} \qquad (15)$$

where D' is the absorbance of the reagent blank. When experiments are carried out under suitable conditions and the experimental values of $a/(D-D')$ are plotted against the values of $\{[\mathrm{H}]^m/(D-D')\}^{1/2}$, and if the assumption of the value of m is correct, the plots give straight lines with a slope of $\{1/K'(\varepsilon-\varepsilon_2)\}^{1/2}$ and an intercept of $1/(\varepsilon-\varepsilon_2)$.

The actual experiments were carried out under the following conditions. The concentrations of Calcichrome and copper were kept at the same concentration (2×10^{-5} M), the ionic strengths of the solutions were kept at 0.1 with an acetic acid - sodium acetate buffer solution which was added to each solution in order to control the pH value, and the absorbance measurements were carried out at 525, 535, and 545 mμ on six solutions of different pH values between 3.91 and 4.63. The results obtained by plotting the experimental values of

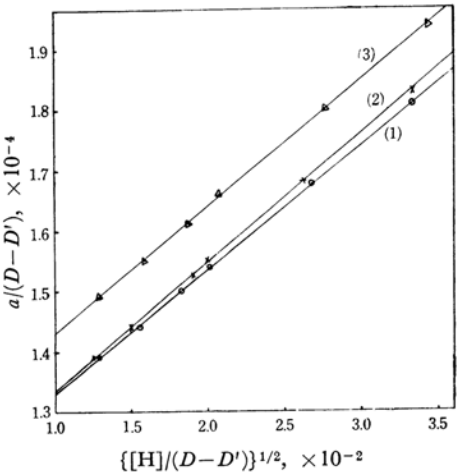

Fig. 7-a. Plots of $a/(D-D')$ vs. $\{[\mathrm{H}]/(D-D')\}^{1/2}$ at various wavelengths (in the case of $m=1$). (1): at 535 mμ, (2): at 525 mμ, (3): at 545 mμ

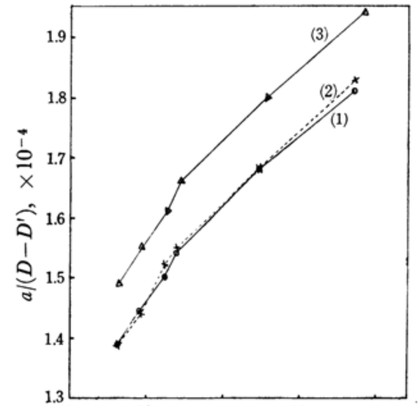

Fig. 7-b. Plots of $a/(D-D')$ vs. $[\mathrm{H}]/(D-D')^{1/2}$ at various wavelengths (in the case of $m=2$). (1): at 535 mμ, (2): at 525 mμ, (3): at 545 mμ

TABLE III. SLOPE AND INTERCEPT OF LINES AND EQUILIBRIUM CONSTANT (in the case of $m=1$)

Wavelength	at 535 mμ	at 525 mμ	at 545 mμ	Average
Slope	2.07×10^{-3}	2.12×10^{-3}	2.11×10^{-3}	2.10×10^{-3}
Intercept	1.12×10^{-4}	1.12×10^{-4}	1.22×10^{-4}	1.15×10^{-4}
K'	26.1	24.9	27.4	26.1

$a/(D-D')$ against the values of $\{[\mathrm{H}]^m/(D-D')\}^{1/2}$ are shown in Figs. 7-a and 7-b. As may be seen from Fig. 7-a, when the value of m was assumed to be unity, the plots gave straight lines with a slope of 2.10 (on the average). When another value, e. g., two, was assumed for m, however, no straight lines were obtained (shown in Fig. 7-b). Therefore, it may be concluded that one proton is liberated during the complex formation and that the copper-Calcichrome complex formed at the investigated pH value has a molar ratio of 1 to 1. The slopes and the intercepts of lines indicated in Fig. 7-a, which were calculated by the method of least squares, are shown in Table III. The equilibrium constant, K', calculated from these values is also shown in Table III.

The Apparent Formation Constant.—The apparent formation constant, K, was then calculated by Eq. 16 using the values of the acid dissociation constant, pK_{a_7}, and the equilibrium constant, K', already obtained. The value of 4.1×10^8 was obtained as the value of K:

$$K = [\mathrm{CuH_2Cch}]/[\mathrm{Cu}][\mathrm{H_2Cch}] = K'/K_{a_7} \qquad (16)^*$$

which shows a fair stability of the complex.

Summary

The reaction between copper(I or II) and Calcichrome has been studied spectrophotometrically in order to establish the optimum conditions for the determination of copper. In the pH range approximately from 3 to 8, copper(I or II) reacts with Calcichrome to form a stable bluish-violet 1-to-1 complex. The complex has three absorption maxima, at 310, 535, and approximately at 650 mμ, when measured against a reagent blank. Beer's law is obeyed when the molar ratio of copper to Calcichrome is less than about 0.8. The molar extinction coefficient of the complex has been calculated to be 8000. The acid dissociation constants of Calcichrome (pK_{a_7} and pK_{a_8}) and the apparent formation constant (K) were 7.2 and 11.5 (μ= 0.1), and 4.1×10^8 respectively. Many other metals, such as aluminum(III), iron(II and III), titanium (IV), vanadium(V) and zirconium(IV), interfere with the determination.