Analytical Chemistry by Means of Organic Compounds. XXV⁽¹⁾. *1-Hydroxyacridine as a New Reagent (Neo-oxine) (1)*

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It is expected that 1-hydroxyacridine will be useful in analytical chemistry, because it has the same functional group as 8-hydroxyquinoline (oxine) which is one of the most valuable organic reagents in various fields such gravimetry, volumetry, or colorimetry. Irving et al.⁽²⁾ have investigated the behavior of this compound to some metallic ions such as aluminum, chromium, iron(III), gallium, copper and zinc, concerning the study for the effects of steric hindrance of some oxine derivatives. It was found by them that 1-hydroxyacridine and 2-methyl-oxine did not form precipitate with aluminum, while forming precipitate with other metals, and the reason of the failure of these reagents to react with aluminum was attributed to steric hindrance of the groups in the 2-position of oxine. In the presence of aluminum, 2-methyl-oxine was used by Merrit⁽³⁾ as a precipitant for zinc, or by Motojima⁽⁴⁾ as not only a precipitant but also a coloring reagent for beryllium and titanium.

There is, however, no reference for use of 1-hydroxyacridine in analytical chemistry. It is presumed that this compound is more favorable in gravimetry or colorimetry with solvent extraction because of larger molecular weight than that of oxine or its derivatives. Moreover, this compound has the feature of not reacting to aluminum ion and this selective behavior will be advantageously used in determining some metals in the presence of aluminum without preliminary separation.

It is the purpose of this study to investigate the reaction between 1-hyroxyacridine and numerous cations which are used to be reactive with oxine, and to apply the results for gravimetry or colorimetry with solvent extraction.

The present paper is concerned with the results obtained by spectrophotometric method with solvent extraction. Absorption spectra were taken for the reagent in acidic, neutral and alkaline medium. Both acidic and basic ionization constants were then obtained, according to Stenstrom's method⁽⁵⁾. The basis for solvent extraction with chloroform were investigated for the chelate compounds of the reagent with some metals such as copper, cobalt, nickel and zinc. Reactions with other metals and another aspects of the study, such as formula, solubility and thermal stability of the chelate are under investigation and the results will be reported in the near future.

We propose to name this reagent as "neo-oxine".

Experimental and Results

Apparatus.—Spectrophotometric measurements were made with a Beckman quartz spectrophotometer, Model DU, with 1 cm. silica transmission cells having a tight stopper. A Beckman pH meter with glass electrode, Model H-2, was used for all pH measurements.

Materials.—*1-Hydroxyacridine.*—This compound was synthesized by the method described by Irving and his coworkers⁽²⁾. The crude product was recrystallized several times from 75% alcohol and dried in vacuo. The melting point of the dried sample was 115∼116°C, identical with that in the literature.

Anal. Found: H, 4.82; C, 79.74; N, 7.17; O, 8.36. Calcd. for $C_{13}H_9ON$, H, 4.64; C, 79.99; N, 7.18; O, 8.22%.

The pure substance obtained is brownish yellow needles and slightly soluble in water but easily soluble in organic solvent such as alcohol, or chloroform. The solution of 1-hydroxyacridine was prepared to concentration of 10^{-3} M with 95% alcohol.

Chloroform.—It was purified by distillation.

Buffer solution.—Borate, phosphate and acetate buffers were prepared using each pure acid. To 1/25 M of each acid solution proper amounts of dilute sodium hydroxide solution were added to obtain the desired pH.

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1) Paper XXIV of this series: M. Ishibashi and Y. Yamamoto, *Bulletin of the Institute for Chemical Research, Kyoto University*, **31**, 280 (1953).

2) H. Irving, E. J. Butler and M. F. Ring, *J. Chem. Soc.*, **1949**, 1489.

3) L. L. Meritt, *Record. Chem. Progr.* (*Kresge-Hooker Sci. Lib.*) **10**, 59 (1949).

4) K. Motojima, This Bulletin **29**. 29, 71, 458 (1956).

5) W. Stenstrom and N. Goldsmith, *J. Phys. Chem.*, **30**, 1683 (1926).

Metal solution.—All of the stock solutions of metals were prepared to concentration of 10^{-3} M.

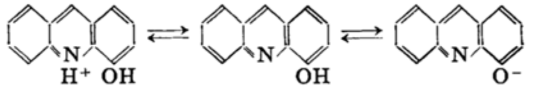
Cupric sulfate which was recrystallized from water and dried in room temperature, was dissolved and then diluted to the desired concentration. Cobaltous sulfate (Merk) was dissolved in water to approximately 10^{-1} M and after the concentration of the metal was checked by electrolysis, the solution was diluted to the desired concentration. Nickel solution was obtained by dissolving pure nickel ammonium sulfate. In the case of zinc the pure metal was dissolved in small amounts of nitric acid and after sulfuric acid was added, the solution was evaporated almost to dryness, and then diluted with water.

All other reagents as ethylenediamine tetraacetic acid, tartaric acid, cuproine (biquinolyl), dimethylglyoxime, etc., were analytical grade ones.

In the preliminary experiments, it was observed that ions such as copper, zinc, nickel, cobalt, iron, etc., were precipitated in slightly acidic (pH 5.1) or in a weak basic (pH 9.5) medium by 1-hydroxyacridine, forming red or reddish orange chelate compounds. It was also confirmed that the compound did not form a precipitate with aluminum in any medium. The filtrate which is obtained by filtrating the precipitate formed in slight excess of reagent, did not react to oxine, and it is presumed that the solubility of the metal chelate of 1-hydroxyacridine may be smaller than that of oxine chelate.

Absorption Spectrum of 1-Hydroxyacridine.—All of the solutions for absorbance measurements were prepared by diluting the required quantity of the stock solution using a buffer solution. The absorbance was measured in the range of 210～450 mμ against the respective buffer solution. Spectra obtained in acidic, neutral and alkaline mediums are shown in Fig. 1.

It is observed that many absorption bands exist in the ultraviolet region, and that the bands in neutral solution shift to longer wavelength with both the decreasing and increasing of pH. The change in absorption spectra with pH may be attributed to structural changes which are due to dissociation of 1-hydroxyacridine as follows, being similar to that of oxine.



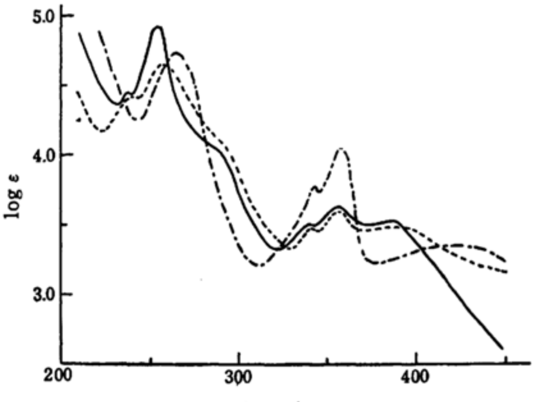


Fig. 1. Absorption spectrum of 1-hydroxyacridine.
–·–·– pH 4.6, —— pH 7.2, ------ pH 9.5

Ionization Constant.—To evaluate basic and acidic ionization constants of the reagent, absorbance was then measured for twenty-six solutions having various pH's. In almost all cases, the pH was adjusted using respective buffer solution, but dilute sodium hydroxide or hydrochloric acid solution was used when stronger alkaline or acidic medium was necessary. The absorbance values at four different wavelengths were plotted against pH, as shown in Fig. 2. The phenolic or basic ionization constant was obtained as

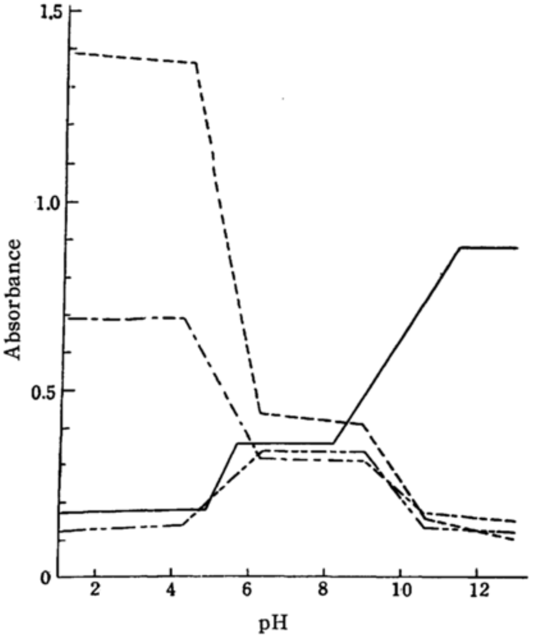


Fig. 2. pK_a and pK_b of 1-hydroxyacridine.
—— at 300 mμ, –·–·– 343 mμ, ------ 359 mμ, –··–··– 380 mμ

TABLE I. IONIZATION CONSTANT OF 1-HYDROXYACRIDINE

Wavelength (mμ)	pK_a	pK_b
300	9.85	5.27
343	9.83	5.30
359	9.83	5.31
380	9.86	5.34
Mean	9.84	5.31

pK by a graphical determination of the pH at which the absorbance was half-way between its values in neutral and alkaline or acidic solution. According to Stenstrom and Goldsmith[5], the midpoint pH=pK_a or pK_b. The values obtained are listed in Table I. The pK_a and pK_b of oxine has been reported as 9.70 and 9.08 respectively by Phillips[6]. The pK_a of 1-hydroxyacridine is thus slightly greater than that of oxine, while pK_b is considerably smaller.

Absorption Spectra of 1-Hydroxyacridine and its Chelate in Chloroform. — Procedure of extraction is conducted as follows.

Each 2 ml. of reagent and metal solution (both 10^{-3} M) was pipetted into a separatory funnel having the mark of 20 ml., in which had been contained approximately 15 ml. of the buffer solution having the desired pH and was then diluted to the mark with water. Red or reddish orange precipitate was immediately formed by mixing. After standing for 20 min. or more to digest the precipitate, 10.0 ml. of freshly distilled chloroform was added, and the vessel was vigorously shaken for 2 min. or more. The extraction seemed to be complete, when the lower chloroform layer colored, but the aqueous layer became completely colorless. The organic extract was then transferred into a little Erlenmeyer flask containing about 1 g. of anhydrous sodium sulfate. Absorbance was measured for the dried chloroform solution over a range of 300~600 mμ using chloroform as a reference solvent. The pH was measured for the aqueous phase which remained after extraction. Spectra are shown in Fig. 3, and the wavelength of maximum absorption is also listed in Table II. Quite similar experiments were made for the reagent solution, and the results are also shown in Fig. 3 and Table II. In the following experiments, all the extraction procedures are followed as above, unless otherwise indicated. It is seen that a specific absorption band for each chelate appears in the visible region at which the reagent does not show appreciable absorbance.

TABLE II. WAVELENGTH OF MAXIMUM IN CHLOROFORM (mμ)

1-Hydroxyacridine	341	358	386	
Copper chelate	353	358	368	502
Cobalt chelate	341	358	388	480
Nickel chelate	340	350	357	468
Zinc chelate	341	350 358	389	478

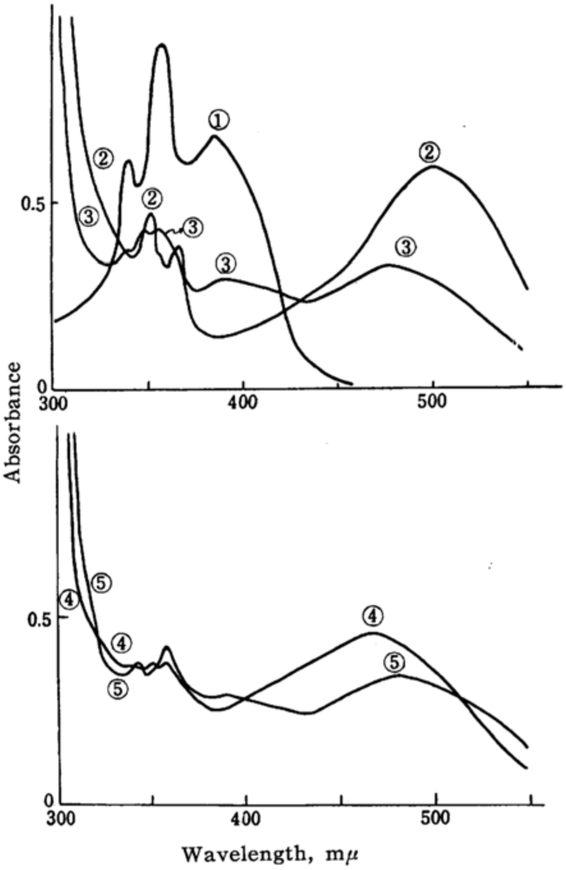


Fig. 3. Absorption spectra in chloroform.
① 1-Hydroxyacridine (extracted from pH 4.6)
② Copper chelate (〃 8.7)
③ Zinc chelate (〃 11.7)
④ Nickel chelate (〃 9.1)
⑤ Cobalt chelate (〃 11.7)

Effect of pH on Extraction.—To obtain optimum range of pH for the extraction of each element and reagent, the absorbance was measured for the organic extract from aqueous solution, the pH values of which had been adjusted to various values with respective buffer. The pH measurements were made for the aqueous phase after extraction and the absorbance was measured at wavelength of the respective peak of the metal chelate.

The results are shown in Fig. 4. The pH range of optimum extraction for the four metals is somewhat narrow, as compared with the metal chelate of oxine reported by several workers[9], contrary to the expectation. In order to obtain higher selectivity in a solvent extraction using chelate reagents, various masking reagents are extensively used. The effects of some complexing agents as cyanide, ethylenediaminetetraacetic acid and tartaric acid

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[6] J. P. Phillips and L. L. Meritt, *J. Am. Chem. Soc.*, **70**, 410 (1948).

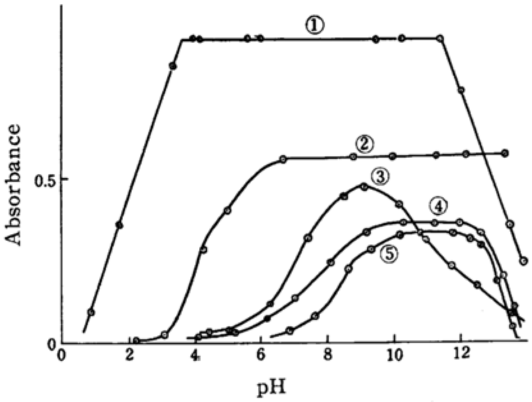

Fig. 4. Effect of pH on extraction.
① 1-Hydroxyacridine
② Copper chelate
③ Nickel chelate
④ Cobalt chelate
⑤ Zinc chelate

were thus investigated. It was found that these complexing agents inhibit the reaction of all metals with 1-hydroxyacridine. When a complexing agent is present in the concentration of 10^{-1} M, the precipitate does not form and the organic extract also does not show appreciable absorbance in the visible region.

Stability of the Metal Chelates.—The effects of time of standing were investigated for the organic extracts of the metal chelates. Standing the dried extract in the transmission cell with a stopper, the absorbance was measured at several intervals. Results are shown in Table III. The result is that the absorbance decreases slightly during the initial several minutes and then remains almost constant during 60～180 min. But when the solution was kept for 1 day, the absorbance had considerably decreased.

TABLE III. EFFECT OF STANDING TO ABSORBANCE

Time min.	Copper 502 $m\mu$	Nickel 468 $m\mu$	Cobalt 480 $m\mu$	Zinc 478 $m\mu$
0	0.554	0.448	0.330	0.305
1	.554	.447	.329	.303
2	.553	.445	.328	.303
3	.553	.443	.327	.303
5	.553	.442	.327	.302
10	.551	.439	.325	.302
20	.551	.438	.324	.300
30	.550	.437	.322	.299
60	.546	.433	.319	.299
90	.545	.433	.319	.299
120	.545	.433	.319	.299
180	.545	.433	.319	.299
1 day	.280	.231	.140	.102

Per Cent Extracted.—The per cent extracted at optimum pH in Fig. 4 was determined for the four elements. For zinc and cobalt, radioactive isotope was used as tracer, and for the other two, the amount of metal ion in aqueous or organic layer was determined by spectrophotometric method using sensitive organic reagents. The procedures are as follows and the results are listed in Table IV.

TABLE IV. PER CENT EXTRACTED

Element	%	pH
Copper	99.71	8.7
Cobalt	99.19	11.7
Nickel	99.10	9.1
Zinc	99.93	11.7

Copper.—Ten milliliters of cupric solution (10^{-3} M) and 5 ml. of the reagent solution (10^{-2} M) were pipetted into a 100 ml. volumetric flask. The solution was then diluted to the mark with the buffer solution. After standing, the red precipitate was extracted with 50 ml. of freshly distilled chloroform. The remaining aqueous solution was then evaporated to about 5 ml. on a sand bath. After cooling, the pH was readjusted to 5.5 with hydrochloric acid and about 1 g. of hydroxylamine hydrochloride was added in order to reduce cupric ion to cuprous. The cuprous ion was then extracted with 10.0 ml. of amyl alcohol containg 0.01% of cuproine7). The absorbance was measured at 540 $m\mu$ for the reddish violet organic extract. For the standard cupric solution (10^{-5} M), the same extraction procedure with amyl alcohol containing cuproine was done after reduction, and the absorbance was obtained similarly. The per cent of extraction was calculated from the two absorbance values.

Nickel.—The extraction was done under the same condition, except pH, as described above. The organic extract was destroyed by adding proper amount of concentrated perchloric acid and heating to fumes. After destroying organic matter completely, the solution was evaporated to dryness. The residue was dissolved and diluted with water and the amount of nickel ion was determined by dimethylglyoxime following the method described by Sandell8).

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7) J. Hoste and J. Gillis, *Anal. Chim. Acta*, **9**, 263 (1953).

8) E. B. Sandell, "Colorimetric Determination of Traces of Metals", Interscience Publishers, New York (1950), p. 470.

Cobalt.—Two milliliters cobalt (10^{-3} M), 1.0 ml. of the reagent (10^{-2} M) and 1.0 ml. of Co^{60} (carrier free) solution were taken and, after adjusting the pH and diluting to 20 ml., cobalt chelate was extracted as above. A 2.0 ml. of aqueous layer was then pipetted out into a small stainless dish. The solution was evaporated completely to dryness and the counts were determined using Geiger Counter. The per cent extraction was obtained by comparing the counts with that of the standard which was determined similary before extraction.

Zinc.—Zn^{65} was used as tracer. The method of determination for the per cent extracted was quite similar to that for cobalt.

It was found that per cent extracted is more than 99% in all cases under the optimum pH.

Summary

The absorption spectra of 1-hydroxyacridine were obtained both in aqueous and chloroform solution, and the pK_a and the pK_b were determined. The pK_a is slightly greater than that of oxine, and it is suggested that the stability of metal chelate with 1-hydroxyacridine will have values of the same or larger order as that of oxine chelate, because there is a direct relation between the basicity of a chelating agent, as measured pK_a values, and the stability of the chelates it forms, as cited in the literature of Morrison and Friser[9].

The various aspects of solvent extraction using chloroform were then investigated. The optimum pH range on extraction for each element was determined and it was found that the range is narrow as compared with that of the oxine chelate. The per cent extracted was determined and it was found that copper, nickel, cobalt and zinc were almost completely extracted under the conditions investigated.

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[9] G. H. Morrison and H. Freiser, "Solvent Extraction in Analytical Chemistry", John Wiley & Sons, Inc., New York (1957), p. 26.