Insofar as this is true, the mechanism of the disproportionation reaction does not appear to involve either 1,10-phenanthroline or the cyanide ion.

Attempts to measure the equilibrium constants for reactions 1, 2 and 4 directly were unsuccessful. In view of the experimental difficulties involved in the study of these equilibria it seems justifiable to make use of an approximation to at least obtain the order of magnitude of their equilibrium constants. The approximation is based on the assumption that the $FePh_3^{++}$ and $FePh(CN)_4^-$ ions are the predominating ionic species produced when pure water is saturated with respect to $FePh_2(CN)_2$. In this event their concentrations are approximately equal because of electrical neutrality considerations, and K_1 and K_2 are approximately equal. Values for the various constants approximated in this manner are presented in Table IV.

The possible analytical applications of the reactions revealed by this investigation are receiving further attention and will be described at an early date.

ANN ARBOR, MICHIGAN

[CONTRIBUTION FROM U. S. GEOLOGICAL SURVEY]

A Fluorimetric Study of the Thorium-Morin System

BY ROBERT G. MILKEY AND MARY H. FLETCHER

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Thorium reacts with morin to yield a yellow complex that fluoresces when irradiated with ultraviolet light. The effect on the fluorescence of such variables as concentration of acid, alcohol, thorium, morin, and complex; time, temperature and wave length of exciting light are studied to determine experimental conditions yielding maximum fluorescence. The effects of Zr^{+4}, Al^{+3}, Fe^{+3}, Ca^{+2} and La^{+3} are discussed. The fundamental relationships between light absorption and fluorescence are expressed in a general equation that applies to a three-component system when the fluorescence is measured in a transmission-type fluorimeter. This general equation is used to obtain an expression for the fluorescence of the thorium-morin system. Equations, derived from experimental data, relate both the fraction of thorium reacted to form complex and the fraction of unquenched fluorescence to the concentration of uncombined morin. These functions, when combined with the general equation, give an expression which relates the total net fluorescence to the amount of uncombined morin in the solution. This last equation can be used to determine the one region for the concentration of uncombined morin that gives maximum sensitivity for the system. Calculated standard curves are in good agreement with experimental curves.

Introduction

Morin reacts with thorium in weakly acid solutions[1-4] to form a stable yellow complex that fluoresces yellow-green when its solutions are irradiated with ultraviolet light. Both the color and fluorescence of this complex have been investigated.

The results of the spectrophotometric study have been presented in an earlier paper[5] which evaluates the color system as a basis for the quantitative determination of trace amounts of thorium.

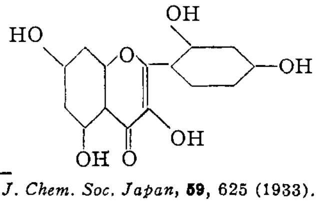
This paper evaluates the fluorescent system as a basis for the quantitative determination of trace amounts of thorium. Some of the theoretical and mathematical relationships between fluorescence and light absorption, as exhibited in the thorium–morin system, are also included. This report is part of a program conducted by the U. S. Geological Survey on behalf of the Division of Raw Materials of the U. S. Atomic Energy Commission.

Characteristics of Thorium–Morin System

The reaction between thorium and morin occurs in slightly acid solution according to the equation

$$ThX_4 + 2M{\cdot}H \rightleftarrows ThM_2X_2 + 2HX \qquad (1)$$

where M·H is morin (5,7,2′,4′-flavanol) having the structure



and X is a univalent negative ion such as Cl^- or NO_3^-. The reaction is instantaneous and results in the formation of a single, yellow, stable complex having a molar ratio of Th:M of 1:2.

The equilibrium constant for the reaction, according to the equation

$$K_{\text{equil}} = \frac{[ThM_2^{+2}][H^+]^2}{[Th^{+4}][M{\cdot}H]^2} \qquad (2)$$

is approximately 1×10^6 (5).

Solutions of the complex fluoresce a bright green when irradiated with long wave length ultraviolet light.[1-4] The wave length band of the fluorescent light ranges from approximately 488 to 555 mμ, with peak intensity between 513 and 533 mμ, as determined by visual inspection with a spectroscope (personal communication, H. Jaffe, U. S. Geological Survey, and C. E. White, Univ. of Maryland, 1952).

Absorption spectra for solutions of the complex (curve B) and for pure morin (curve A) are presented in Fig. 1. The region between the dotted lines in this figure indicates the wave length band of the fluorescent light. The fluorescence occurs in that part of the spectrum in which light is transmitted virtually completely by both the complex and morin. Moreover, solutions with thorium concentrations as large as 50 mg. of ThO_2 per 50 ml. also completely transmit all light in this spectral region. The spectra indicate that light having a wave length near 410 mμ would be advantageous for the production of fluorescence, and such light (404.7 mμ) was used to establish the experimental conditions.

Experimental Data

Apparatus and Reagents. Transmission Fluorimeter.—The transmission fluorimeter, especially built for this study, is arranged so that the light source, sample cell and photo-

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(1) H. Gôto, *J. Chem. Soc. Japan*, **59**, 625 (1933).

(2) H. Gôto, *Tohoku Imp. Univ. Sci. Repts.*, **1st ser.**, 287 (1940–1941).

(3) H. H. Willard and C. A. Horton, *Anal. Chem.*, **22**, 1190 (1950).

(4) H. H. Willard and C. A. Horton, *ibid.*, **22**, 1194 (1950).

(5) M. H. Fletcher and R. G. Milkey, *ibid.*, **28**, 1402 (1956).

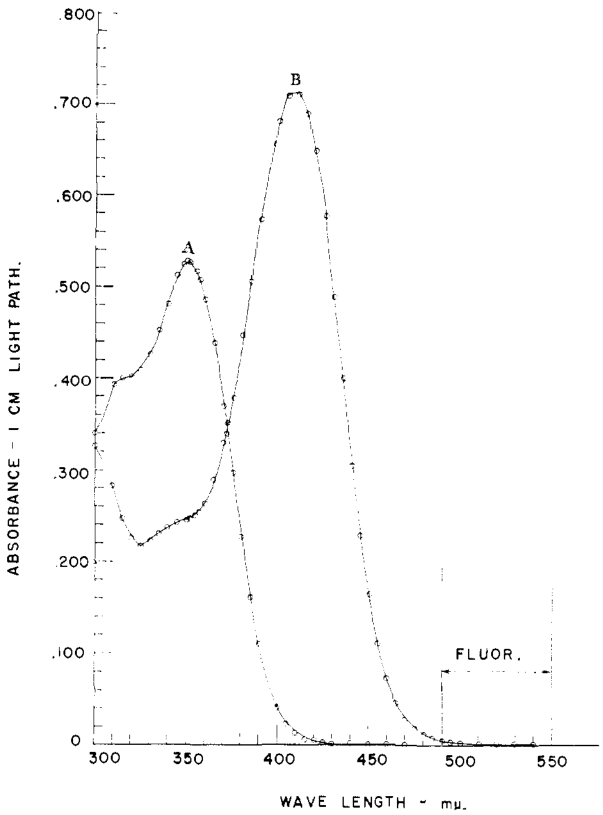


Fig. 1.—Absorption spectra for pure morin (A) and thorium-morin complex (B); arrow indicates wave length region of fluorescence: curve A, 600 μg. morin/50 ml. of solution; curve B, 600 μg. morin plus 2 mg. ThO_2/50 ml. of solution; 2 ml. alcohol in each.

tube are on a linear vertical axis, with lamp and phototube on opposite sides of the sample.

Source of Exciting Light.—An EH-4 high-pressure mercury lamp operated from a Sola no. 301,883 constant-wattage transformer and cooled with a dark-room ventilator. The lamp was 18 in. above the top of the solution cell. (Intensity of the light supplied at wave length 404.7 mμ was approximately four times the intensity of the light at 365 mμ wave length. Personal communication, C. L. Waring, U. S. Geological Survey, 1953.)

Measuring Unit.—A 1P21 multiplier phototube powered by a regulated high-voltage supply (Atomic Instrument Co., model 306) and used in conjunction with an ultrasensitive microammeter (RCA no. WV-84A). The measuring unit was used at the same sensitivity for all measurements in this study.

A potential of approximately 550 volts was always applied to the phototube. This resulted in standard deflections of 0.640 microampere for P_0 (404.7 mμ) and 0.980 microampere for P_0 (365.0 mμ) from the fluorescent uranium glass standard used for calibration.

Filters. Primary Filters.—1. A combination of Corning filters no. 3060, 4308 and 5970 was used to isolate light of wave length 404.7 mμ. 2. A combination of Corning filters no. 7380, 5874 and 5860 was used to isolate light of wave length 365.0 mμ.

Secondary Filters.—Corning no. 4010 was used to isolate the fluorescent light.

Fluorescing Standard.—Uranium glass was used to set instrument sensitivity.

Silvered Tube.—A brass tube with silvered interior connected the phototube and sample compartments of the instrument. The phototube was at a distance of about 12 inches from the bottom of the sample cell.

Beckman DU Quartz Spectrophotometer.

Morin.—Morin, $C_{15}H_{10}O_7 \cdot 2H_2O$ (mol. wt. 338.26) of high purity, was obtained from Dr. Theodor Schuchardt in

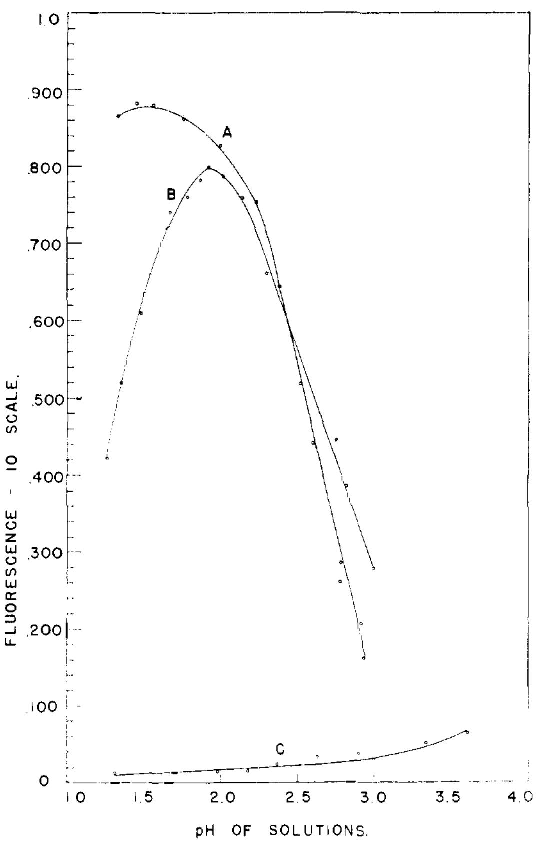


Fig. 2.—Effect of *p*H on fluorescence: curve A, complex (37.4 μg. per 50 ml.); curve B, mixture of complex plus uncombined morin (100.6 μg. ThO_2 plus 165 μg. total morin per 50 ml.); curve C, morin (300 μg. per 50 ml.); 2 ml. of alcohol in all solutions; P_0 (404.7 mμ).

Munich, Germany. Solutions of Morin in the concentration desired were prepared by dissolving the solid material in 95% ethyl alcohol.

Standard Thorium Solutions.—A stock solution of thorium chloride was prepared by dissolving the salt (reagent grade) in 3% hydrochloric acid. This solution, which contained 100 mg. of ThO_2 per ml., was standardized gravimetrically.

Working solutions were prepared from the stock solution by dilution with distilled water and, when necessary, by the addition of more acid. The acidity of all working solutions was adjusted to give a *p*H of 1.9 to 2.0.

Materials Tested as Possible Interferences.—Stock solutions of the chlorides of aluminum, calcium, iron, lanthanum and zirconium were prepared in hydrochloric acid solutions, which were diluted as required to give working solutions of the proper concentration at a *p*H of 2.0. The working solutions were used immediately after dilution.

Effect of Experimental Variables on Fluorescence. Acidity.—The effect of acidity on the fluorescent system is shown in Fig. 2. In curve B, which represents solutions similar to those found in routine analysis, the point of maximum fluorescence occurs at *p*H 2, and this is also the point of maximum difference between the solutions and the blanks. Accordingly, a *p*H of 2 was chosen for the fluorimetric study.

Alcohol.—The effect of alcohol on the fluorescence of solutions of complex, of complex and uncombined morin, and of morin alone is illustrated in Fig. 3, which shows that sensitivity increases with alcohol content until 20 to 25 ml. of alcohol are present per 50 ml. of solution.

Concentration of Morin.—The effect of morin concentration when the total ThO_2 content of each solution is 50.3 μg. per 50 ml. is shown in Figs. 4 and 5. Maximum fluorescence is obtained from solutions containing 100 to 200 μg.

TABLE I

SUMMARY OF EFFECTS OF OTHER IONS

Ion	Type of interference	Sensitivity of fluorescence of interfering ion	Amount of interfering substance that causes 10% error in amount of ThO_2 found
Zr^{+4}	Forms complex which fluoresces	ZrO_2 and ThO_2 *ca.* the same	ZrO_2 = 10% of amount of ThO_2 present
Al^{+3}	Forms complex which fluoresces	Al_2O_3 about 1/8 as sensitive as ThO_2	Al_2O_3 = amount of ThO_2 present
Fe^{+3}	Quenches the thorium-morin fluorescence	....	15-20 μg. Fe_2O_3 per 50 ml.
Ca^{+2}	Quenches the thorium-morin fluorescence	....	2 mg. CaO per 50 ml.
La^{+3}	Quenches the thorium-morin fluorescence	....	5 mg. La_2O_3 per 50 ml.

of morin per 50 ml. This holds true for other concentrations of ThO_2 of the same order of magnitude.

Temperature.—The absorbance of the thorium-morin complex is only slightly affected by temperature. However, in the temperature range from 10 to 50°, the intensity of fluorescence decreases linearly with increasing temperature. The readings on a solution that contained 25.9 μg. of complex per 50 ml. decreased 15% when the temperature was increased from 20 to 30°.

Time of Standing.—The fluorescence develops immediately and reproducible readings are obtained over a period of several hours. Typical of the reproducibility is a series of readings made at 30-minute intervals over a period of several hours on a solution that contained 46.6 μg. of ThO_2 and 200 μg. of morin per 50 ml. After the initial 30 minutes, the difference between the highest and lowest readings was less than 2%.

Method of Preparation of Solutions.—All of the solutions used in this study were prepared in 50-ml. glass-stoppered graduates or flasks. The procedure follows: (1) add 1.0 ml. of 0.63 *N* HCl; (2) add thorium solution that has a *p*H of 2.0 and contains no other metallic ions; (3) adjust volume of solution to 20 ml. with distilled water, mix; (4) add 2.0 ml. of morin solution (weight of morin added will be indicated separately for each test); (5) if total alcohol content of 12 ml. is desired, add 10 ml. of alcohol; (6) mix; (7) adjust volume to exactly 50 ml. with distilled water; (8) stopper and mix well; (9) after half an hour, measure the fluorescence of a 25-ml. aliquot.

Experimental Standard Curves.—Typical standard curves are presented in Figs. 6 and 7 (solid lines) and in Fig. 8. The curves in Figs. 6 and 7 indicate that thorium concentrations ranging from 10 to 150 μg. of ThO_2 can be measured with good accuracy.

In Fig. 8, however, which covers the thorium range most important to trace analysis and refers to a more sensitive instrument scale, the curves are not as well defined as those for the higher thorium range even though each point on the curves represents the average obtained from readings on four different solutions. In the linear part of the curve obtained with an exciting wave length of 404.7 mμ in Fig. 8, a difference in fluorescence readings of 0.100 is equivalent to 1.10 μg. of ThO_2. As the instrument can be read to a difference of 0.004, the possibility of detecting a concentration difference of 0.044 μg. of ThO_2 per 50 ml. is indicated if the 0.1 instrument scale is used. Unfortunately, it is impossible to take advantage of this sensitivity at present because random errors from unknown sources often occur; these amount to approximately ±0.5 μg. of ThO_2 on the individual readings.

Comparison of Fluorimetric and Spectrophotometric Methods.—A sensitivity of 0.5 μg. of ThO_2 is slightly less than but comparable to that of the spectrophotometric method.[5] Potentially, however, the sensitivity of the fluorimetric method is far greater than that of the spectrophotometric method, provided the random errors can be eliminated from the fluorimetric method. As indicated above, the limit of detection of the fluorimetric method is at present as low as 0.044 μg. Moreover, in contrast to the sensitivity of the spectrophotometric method, which can be improved only by such limited means as increasing the light path beyond 5 cm., the sensitivity of the fluorimetric method could be increased greatly by several different means, such as by increasing the amplitude of the photocurrent, or by increasing the intensity of the exciting light.

Effect of Other Ions.—Zirconium, aluminum and ferric ions that are serious interferences in the spectrophotometric method, and calcium and lanthanum, which might be useful as carriers in separations of thorium from other ions, were tested for their fluorescent reactions with morin. In all of these tests the solutions contained 2 ml. of alcohol and the ion was tested both alone and in the presence of 5 μg. of ThO_2 per 50 ml. of solution. Zirconium and aluminum were tested with exciting wave lengths of both 404.7 mμ and 365.0 mμ, and as the effects of these ions were the same at both wave lengths, ferric, calcium and lanthanum ions were tested at 404.7 mμ only. Both zirconium and aluminum fluoresce with morin and would cause a positive error in analytical work. Ferric ions seriously quench the fluorescence of thorium and would cause a negative error. Small amounts of calcium and lanthanum produce little or no effect, and apparently small amounts of either could be used as a carrier in separations of thorium from other ions.

A summary of these tests is given in Table I.

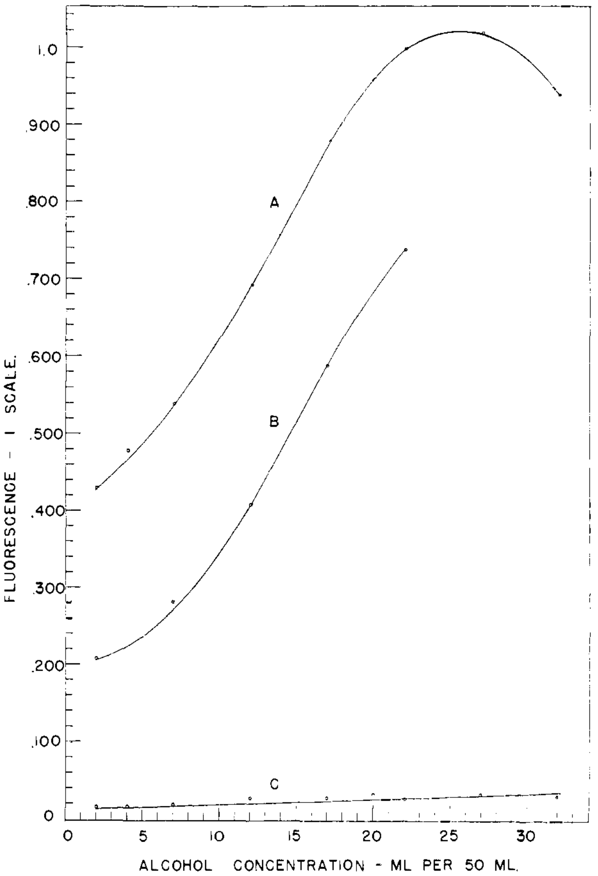


Fig. 3.—Effect of alcohol concentration on the fluorescence: curve A, complex (18.7 μg. per 50 ml.); curve B, mixture of complex plus uncombined morin (20.12 μg. ThO_2 plus 165 μg. total morin per 50 ml.); curve C, morin (300 μg. per 50 ml.); P_0 (404.7 mμ).

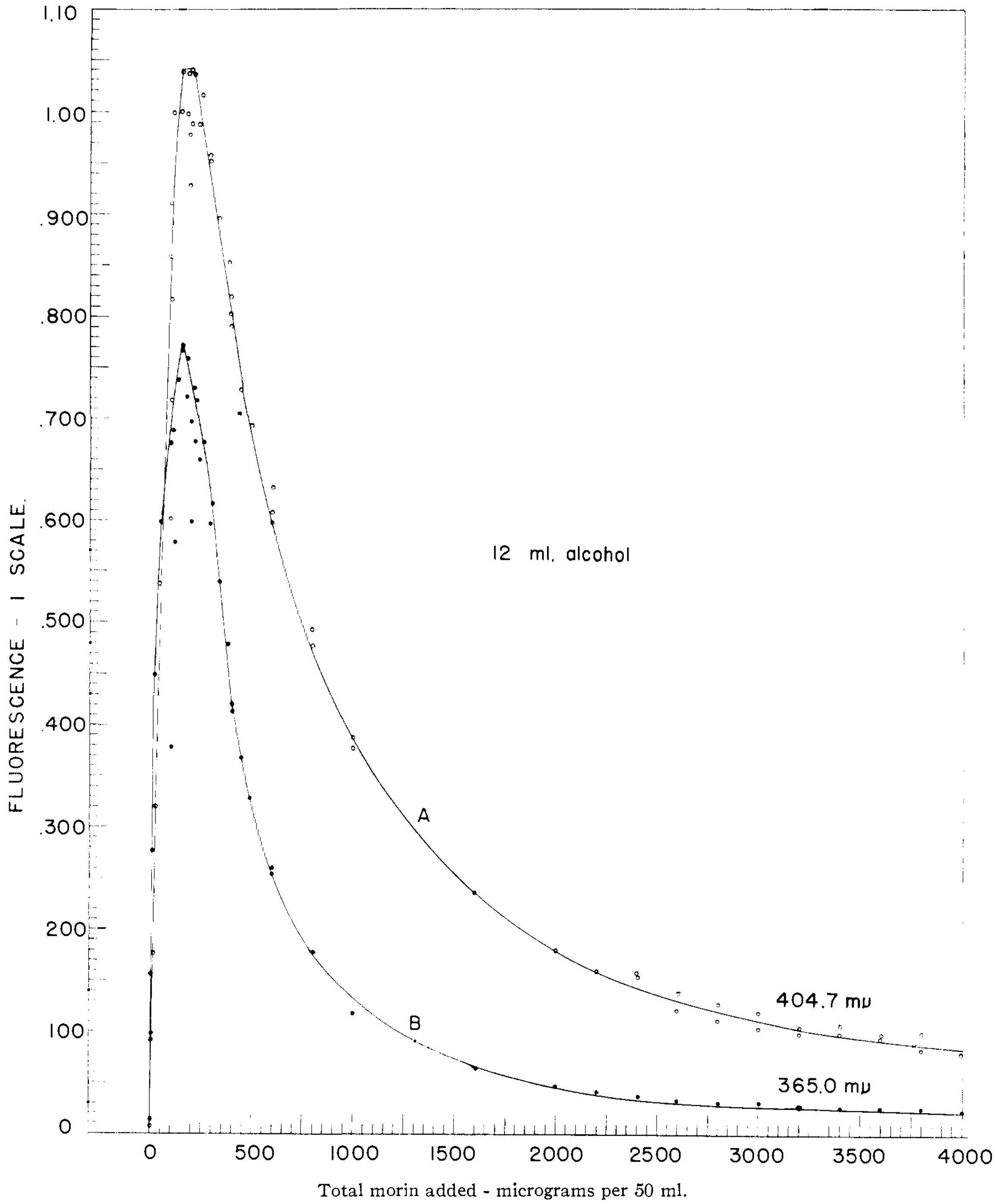


Fig. 4.—Effect on fluorescence of adding increasing amounts of morin to a constant amount of ThO_2 (50.3 μg. per 50 ml.); alcohol content 12 ml. per 50 ml.; P_0 (404.7 mμ).

Basic Theory of Fluorescence: Application to Thorium–Morin System

The theoretical factors affecting the fluorescence of the thorium–morin system are more complicated than those of the spectrophotometric system. To understand the fluorescent system better, it was studied in the following manner. A mathematical analysis of the relationship between light absorption and fluorescence was made and a general equation derived for the fluorescence produced in a three-component system assuming that all three components absorb exciting light, all three fluoresce, and all three absorb the fluorescence. This general equation reduces to simpler forms when applied to less complicated systems where many of the terms of the general equation become zero. A specific expression that applies to the thorium–morin system was obtained from one of these simpler equations by addition of a quenching factor and substitution of the proper constants. The

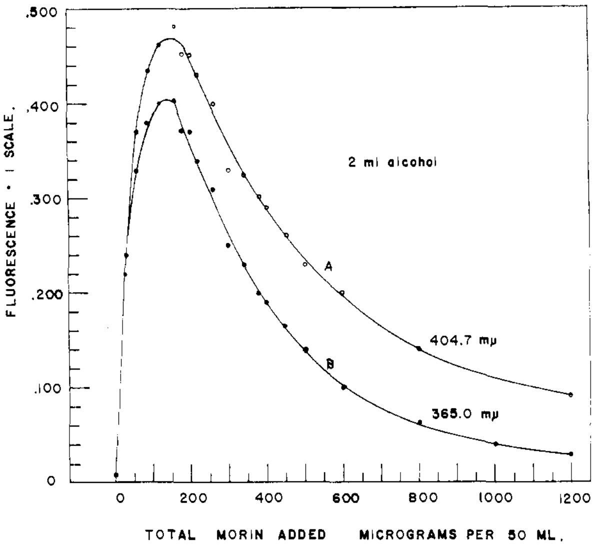


Fig. 5.—Effect on fluorescence of adding increasing amounts of morin to a constant amount of thorium (50.3 μg. ThO_2 per 50 ml.); alcohol 2 ml. per 50 ml.; P_0 (404.7 mμ).

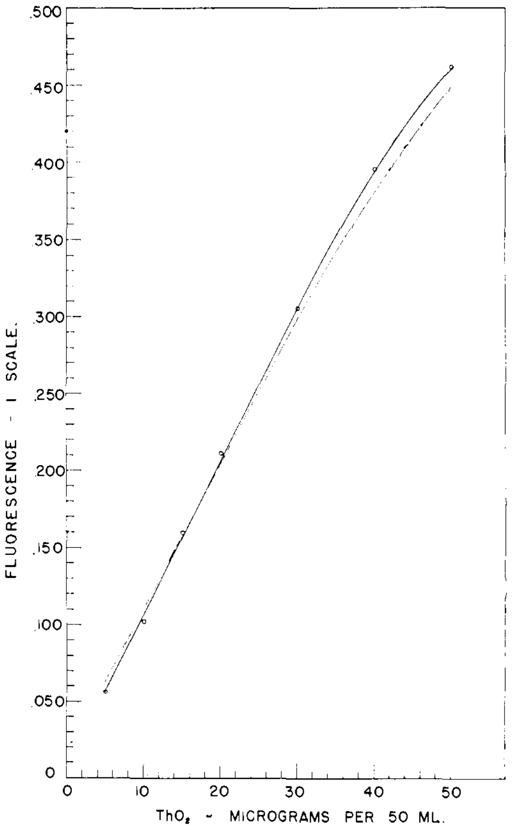


Fig. 6.—Standard fluorescence curves, total morin 100 μg. per 50 ml.; 2 ml. of alcohol; P_0 (404.7 mμ). Solid line, defined by points, is experimental curve; dashed line, with no points, is calculated curve.

validity of this expression is shown by the close agreement between calculated and experimental standard curves.

Derivation of the General Equation.—Consider a cell containing a solution with the light source above the solution and the phototube below the cell.

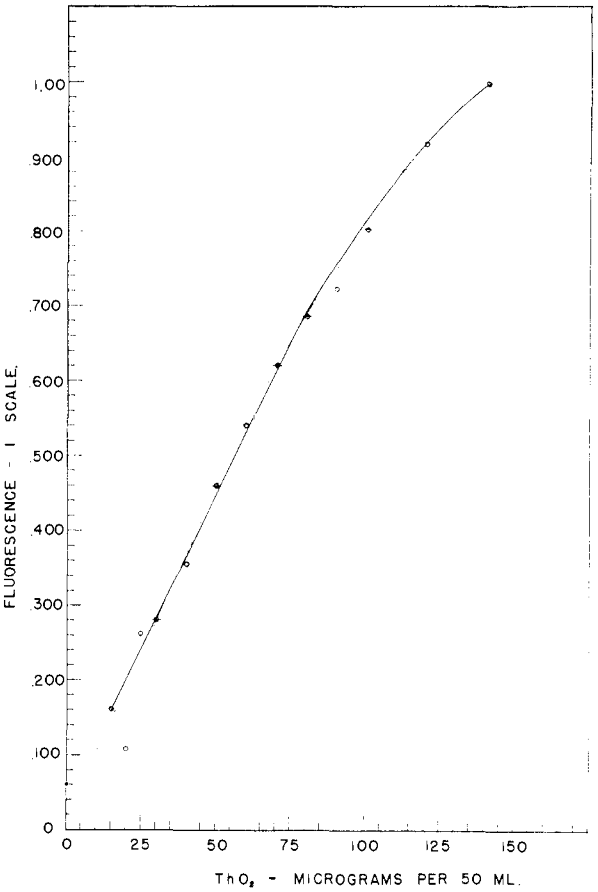
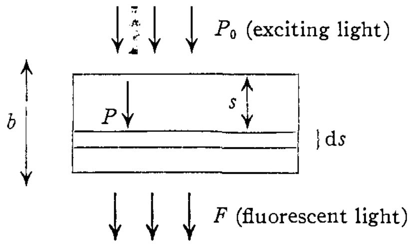


Fig. 7.—Standard fluorescence curves, total morin 165 μg. per 50 ml.; 2 ml. of alcohol; P_0 (404.7 mμ). Solid line, defined by points, is experimental curve; dashed line, with no points, is calculated curve.



Assume that the exciting light is monochromatic and that the absorption follows Beer's law

$$P = P_0 (e^{-kcs})$$

where

P is light intensity
c is concentration
k is absorptivity
s is distance from the surface of the solution to the fluorescing substance in differential layer ds

Fluorescence of Metal Ion.—If the solution contains a metal ion, the reagent and the complex, and if all three absorb exciting light, then the intensity of the exciting light (P) entering the layer ds is expressed.

$$P = P_0 e^{-(k_1c_1 + k_2c_2 + k_3c_3)s}$$

where the subscript 1 refers to the metal ion, subscript 2 refers to the reagent, and subscript 3 refers

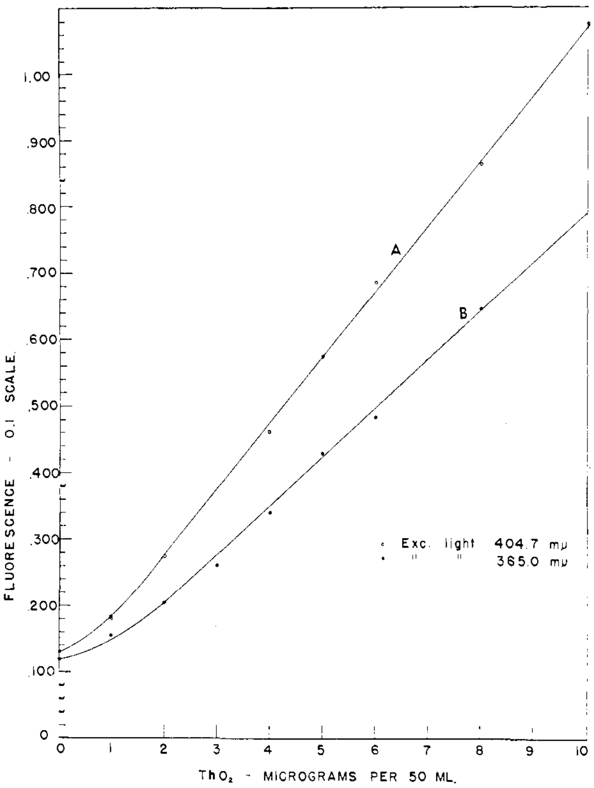


Fig. 8.—Experimental standard curves: total morin = 100 μg. per 50 ml.; alcohol = 2 ml. per 50 ml. A, exc. light = 404.7 mμ; B, exc. light = 365.0 mμ.

to the complex. Then, the light intensity absorbed in the differential layer ds is given by the expression

$$\mathrm{d}P = -P_0(k_1c_1 + k_2c_2 + k_3c_3)e^{-(k_1c_1 + k_2c_2 + k_3c_3)s}\mathrm{d}s$$

Let K_M = factor for conversion of the absorbed exciting light to fluorescent light when the metal ion is the fluorescent component. V = fraction of unabsorbed fluorescent light which impinges on the phototube, the geometrical factor. (If the distance from the solution cell to the phototube is much larger than the distance b, then V will be substantially constant for all values of s or all positions of ds.) k_4 = absorptivity of the metal ion for the fluorescent light emitted by the metal ion.

k_5 = absorptivity of the reagent for the fluorescent light emitted by the metal ion. k_6 = absorptivity of the complex for the fluorescent light emitted by the metal ion.

(As the wave lengths of the fluorescent light emitted would probably extend over a range of values, the k_4, k_5 and k_6 are understood to be average values for the wave length band.)

The fluorescent light, dF_{M_0}, arising from the metal ion in the element ds is proportional to the fraction of light absorbed by the metal ion in the element ds

$$\mathrm{d}F_{\mathrm{M}_0} = K_\mathrm{M}\left[\frac{k_1c_1}{k_1c_1 + k_2c_2 + k_3c_3}\right][\mathrm{d}P]$$

The unabsorbed fraction of this fluorescence which impinges on the phototube is expressed by the equation

$$\mathrm{d}F_\mathrm{M} = K_1P_0(k_1c_1)[e^{-(k_1c_1 + k_2c_2 + k_3c_3)s}] \times [e^{-(k_4c_1 + k_5c_2 + k_6c_3)(b - s)}]\mathrm{d}s$$

where $K_1 = VK_\mathrm{M}$

$$\mathrm{d}F_\mathrm{M} = K_1P_0(k_1c_1)[e^{-(k_4c_1 + k_5c_2 + k_6c_3)b}] \times [e^{-(k_1 - k_4)c_1 + (k_2 - k_5)c_2 + (k_3 - k_6)c_3]s}\,\mathrm{d}s$$

The fluorescent light (due to the metal ion in the entire solution) which impinges on the phototube is obtained by a summation of the differential elements ds between the limits of zero and b and results in the integrated expression

$$F_\mathrm{M} = \frac{K_1P_0(k_1c_1)}{(k_1 - k_4)c_1 + (k_2 - k_5)c_2 + (k_3 - k_6)c_3} \times [e^{-(k_4c_1 + k_5c_2 + k_6c_3)b} - e^{-(k_1c_1 + k_2c_2 + k_3c_3)b}] \quad (3)$$

Fluorescence of Reagent.—By a similar procedure, an expression can be obtained for the fluorescent light arising from the reagent in the solution.

Let K_R = conversion coefficient for exciting light to fluorescent light when the reagent is the fluorescent component. V = fraction of unabsorbed fluorescent light that impinges on the phototube. k_7 = absorptivity of the metal ion for the fluorescent light emitted by the reagent. k_8 = absorptivity of the reagent for the fluorescent light emitted by the reagent. k_9 = absorptivity of the complex for the fluorescent light emitted by the reagent.

Again, the absorptivities are assumed to be average values for the wave band of fluorescence. Accordingly, the expression for the fluorescence due to the reagent in the solution which impinges on the phototube is

$$F_\mathrm{R} = \frac{K_2P_0(k_2c_2)}{(k_1 - k_7)c_1 + (k_2 - k_8)c_2 + (k_3 - k_9)c_3} \times [e^{-(k_7c_1 + k_8c_2 + k_9c_3)b} - e^{-(k_1c_1 + k_2c_2 + k_3c_3)b}] \quad (4)$$

where $K_2 = VK_\mathrm{R}$.

Fluorescence of Complex.—Let K_C = conversion factor for exciting light to fluorescent light when the complex is the fluorescent component. V = fraction of unabsorbed fluorescent light that impinges on the phototube. k_{10} = absorptivity of the metal ion for the fluorescent light emitted by the complex. k_{11} = absorptivity of the reagent for the fluorescent light emitted by the complex. k_{12} = absorptivity of the complex for the fluorescent light emitted by the complex.

The absorptivities again are assumed to be average values for the wave band of the fluorescence.

Then the fluorescence, due to the complex in the solution, which impinges on the phototube is

$$F_\mathrm{C} = \frac{K_3P_0(k_3c_3)}{(k_1 - k_{10})c_1 + (k_2 - k_{11})c_2 + (k_3 - k_{12})c_3} \times [e^{-(k_{10}c_1 + k_{11}c_2 + k_{12}c_3)b} - e^{-(k_1c_1 + k_2c_2 + k_3c_3)b}] \quad (5)$$

where $K_3 = VK_\mathrm{C}$.

Total Fluorescence.—In eq. 3, 4 and 5, the exponential functions are expressed to the base e. In practical usage, however, the exponentials are usually expressed to the base 10, and in this case, the absorptivities k_n, of eq. 3, 4 and 5 become $0.434k_n$. Moreover, to be more specific in terminology, when the concentrations c_1, c_2 and c_3 are expressed as weight per unit volume, the absorptivity $0.434k_n$ is expressed as a_n (ϵ_n when concentration is in terms of moles per liter). Thus, expressing the exponential functions to the base 10 and the concentrations in terms of weight per unit volume, the *final equation* for total fluorescence reaching the phototube becomes

$$F = F_{\mathrm{M}} + F_{\mathrm{R}} + F_{\mathrm{C}} =$$

$$\frac{K_1P_0(a_1c_1)[10^{-(a_4c_1 + a_5c_2 + a_6c_3)b} - 10^{-(a_1c_1 + a_2c_2 + a_3c_3)b}]}{[(a_1 - a_4)c_1 + (a_2 - a_5)c_2 + (a_3 - a_6)c_3]} +$$
$$\frac{K_2P_0(a_2c_2)[10^{-(a_7c_1 + a_8c_2 + a_9c_3)b} - 10^{-(a_1c_1 + a_2c_2 + a_3c_3)b}]}{[(a_1 - a_7)c_1 + (a_2 - a_8)c_2 + (a_3 - a_9)c_3]} +$$
$$\frac{K_3P_0(a_3c_3)[10^{-(a_{10}c_1 + a_{11}c_2 + a_{12}c_3)b} - 10^{-(a_1c_1 + a_2c_2 + a_3c_3b}]}{[(a_1 - a_{10})c_1 + (a_2 - a_{11})c_2 + (a_3 - a_{12})c_3]} \quad (6)$$

Application to Simplified Systems.—Equation 6 represents the generalized case where all three of the components in the solution absorb the exciting light, all three fluoresce and all three absorb the fluorescent light. In actual practice most systems will be much simpler than this, and the equation will accordingly be much simplified. Thus, when the complex alone absorbs exciting light and fluoresces, exciting light is not absorbed by the other two components, and fluorescent light is not absorbed by any of the three components, the equation reduces to the form

$$F = K_3P_0(1 - 10^{-a_3c_3b}) \quad (7)$$

(hereafter referred to as system I). This is the form of the equation usually found in the literature.[6–8]

When the complex and reagent both absorb exciting light, but the complex alone fluoresces, and fluorescent light is not absorbed by any of the three components, the theoretical equation becomes

$$F = \frac{K_3P_0\,(a_3c_3)}{(a_2c_2 + a_3c_3)}\,[1 - 10^{-(a_2c_2 + a_3c_3)b}] \quad (8)$$

(hereafter referred to as system II). Braunsberg and Osborn[6] have derived an equation, similar to this, to show the effect on fluorescence when the exciting light is absorbed by an impurity in the solution. Other systems can be postulated and analogous equations can be derived.

The derived equations express the fluorescence theoretically possible in solutions of fluorescent substances. However, if fluorescence quenching should occur in the solutions, then the derived mathematical expressions would be multiplied by an extra term, which would express the fraction of maximum fluorescence that remained unquenched.

Study of the Fluorescence of the Thorium–Morin System.—In applying the general equation (no. 6) to a study of the thorium–morin system, it was necessary to consider each of the following variables: the concentrations of excess thorium, of complex, of excess morin, and of alcohol and also the wave length of exciting light.

In these studies the concentrations of each of the reactants and of the complex were varied individually to determine how each affects the fluorescence. Also, in many of these tests two different concentrations of alcohol, 2 and 12 ml., and two different wave lengths for the exciting light were used to study the effect on the fluorescence when it is produced under varying conditions of light absorption and also to determine whether K, the factor for conversion of exciting energy to fluorescence, varies with alcohol concentration or wave length of exciting light.

The absorbance data presented in Fig. 1 were considered in choosing the wave lengths to be used in the excitation of the fluorescence. At 410 mμ the absorbance of the complex is at its peak (curve B). Furthermore, the difference between the absorptivity of complex and the absorptivity of morin is at a maximum at 410 mμ; as a consequence, interference resulting from light absorption by uncombined morin should be at a minimum at this wave length. By analogous reasoning, at 350 mμ interference resulting from light absorption by the uncombined morin should be at a maximum. Thus, from the standpoint of light absorption only, 410 mμ should be the most advantageous exciting wave length and 350 mμ the least advantageous.

These two wave lengths are quite close to the wave lengths of light emitted by the high-pressure mercury lamp—one of the most generally used sources of ultraviolet light. This mercury lamp gives intense radiation at both 404.7 and 365.0 mμ, and light at each of these wave lengths can be isolated by filters. Accordingly, both the 404.7 and 365.0 mμ radiations of the mercury lamp were used as exciting wave lengths in this work.

Effect of the Concentration of Excess Thorium.—To test the effect of excess thorium on the fluorescence, a series of solutions were prepared in which the thorium concentration was increased to a point of great excess over that of the morin. In Fig. 9 the fluorescence of these solutions is shown as a function of the thorium concentration.

It has been shown[5] that 600 μg. of morin is more than 98% reacted to form complex when as little as 7.5 mg. of ThO_2 is present in 50 ml. of solution. The horizontal part of the fluorescence curve (Fig. 9) is representative, therefore, of solutions that are mixtures of a constant amount of complex with varying amounts of excess thorium, and it shows that amounts of thorium as large as 50 mg. of ThO_2 interfere in no discernible way with the fluorescence of the complex, as any quenching or absorption of either the exciting light or fluorescent light by the thorium would result in decreasing fluorescence as the thorium content increased and any enhancement of fluorescence due to increased thorium concentration would cause the curve to rise. Thus, insofar as the fluorescence is concerned, solutions containing a mixture of the complex and a large excess of thorium can be considered as solutions of the pure complex alone. In this study 50 mg. of ThO_2 was always used in the preparation of solutions of the so-called pure complex to ensure that all of the morin would be completely converted to the form of complex.

Effect of the Concentration of the Complex.—To determine the effect of the concentration of the complex on the fluorescence, two series of solutions were prepared in which amounts of morin ranging from 0 to 4000 μg. were converted completely to the complex by a large excess of thorium (50 mg.

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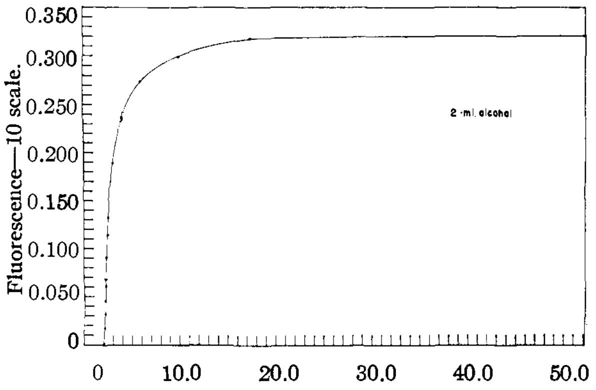

Fig. 9.—Effect on fluorescence of adding increasing amounts of thorium to a constant amount of morin (600 micrograms per 50 ml.; alcohol 2 ml.; P_0 (404.7 mμ).

ThO_2 per ml.). In one series of solutions the alcohol content was 2 ml., whereas in the other series it was 12 ml.

The fluorescences of these solutions were determined using exciting lights having wave lengths of 404.7 and 365.0 mμ and a light path through the solutions of 3.241 cm. The absorbances of these solutions at these wave lengths were calculated from data obtained with the Beckman spectrophotometer. As each of these solutions contained complex only plus a large amount of excess thorium, the complex was the only component of the solutions that absorbed the exciting light or emitted fluorescence and the system was similar to that described under Derivation as system I. Thus, the fluorescence produced in these solutions should obey eq. 7, $F = K_3P_4(1 - 10^{-A})$, where A = absorbance = a_3c_3b. Accordingly the fluorescence was plotted as a function of $(1 - 10^{-A})$ which is equivalent to the fraction of exciting light absorbed by the complex. Curves A and B, Fig. 10, are representative of the solutions that contained 12 and 2 ml. of alcohol, respectively, when the exciting light had a wave length of 404.7 mμ. The curves obtained with an exciting light of wave length 365.0 mμ are not presented because they are similar to those of Fig. 10.

The curves in Fig. 10 show that, within the limits of experimental error, the fluorescence increases linearly with the fraction of exciting light absorbed, $(1 - 10^{-A})$; thus, in this region of linearity the conversion factor K (K_3 of eq. 7) is a constant, and the fluorescence of the complex obeys the basic equation.

The slopes of the curves in Fig. 10 are equivalent to KP_0 of the equation, and the empirical values for KP_0 calculated from the curves are given in Table II. The values obtained with exciting light of 365.0 mμ are included also.

TABLE II

EMPIRICAL VALUES FOR KP_0 (FOR FLUORESCENCE READINGS ON 10 SCALE)

Wave length of exciting light (mμ)	Empirical values for KP_0 2 ml. of alcohol	12 ml. of alcohol
404.7	0.317	0.514
365.0	0.746	1.125

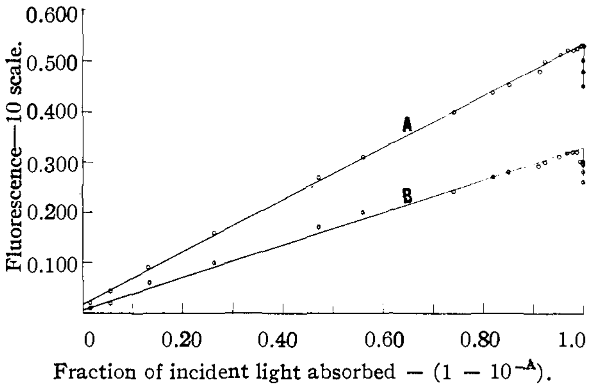

Fig. 10.—Fluorescence of the complex as a function of the fraction of exciting light absorbed. Curve A, 12 ml. of alcohol per 50 ml.; curve B, 2 ml. of alcohol per 50 ml.; P_0 (404.7 mμ). For each curve the concentration of the complex varied from 0 to 4934.4 μg. per 50 ml. of solution.

As Table II shows, the value of KP_0 is affected by alcohol content, but as P_0 is constant for each exciting wave length used, the change in KP_0 for each wave length reflects a change in the factor K only. Thus, when the alcohol was increased from 2 to 12 ml. and the exciting light had a wave length of 404.7 mμ, K was increased by 62%; when the exciting light had a wave length of 365.0 mμ, K was increased by 51%.

The data in Table II also can be used to obtain a rough comparison of K at the two different wave lengths. The ratio of KP_0 (404.7 mμ) to KP_0 (365.0 mμ) for each concentration of alcohol is roughly 1 to 2. But the ratio of P_0 (404.7 mμ) to P_0 (365.0 mμ) is about 1 to 4 for the particular light source used.

When this difference in P_0 is considered, it becomes evident that K (404.7 mμ) is about twice as large as K (365.0 mμ). Thus, not only $(1 - 10^{-A})$, the fraction of P_0 that is absorbed by the complex, but K also is greater when the exciting wave length 404.7 mμ is used rather than 365.0 mμ. Accordingly, for light sources of equal energy at both wave lengths, sensitivity will be considerably greater when the exciting wave length is 404.7 mμ rather than 365.0 mμ.

As shown in Fig. 10, a sharp break occurs in each curve when the fraction of light absorbed $(1 - 10^{-A})$, equals nearly 1, and any further increases in light absorption that result from increasing the concentration of complex are too small to result in a measurable increase in fluorescence. As a consequence the curves should terminate at this point of maximum fluorescence, which is equal to KP_0. However, at this point the curves in Fig. 10 break sharply and drop vertically, showing that increases in the concentration of the complex actually resulted in a slight decrease in the fluorescence.

A 15% decrease in fluorescence occurred when the concentration of the complex was increased from about 500 to nearly 5000 μg.

Decrease in fluorescence readings could result from absorption of fluorescence by the complex, as curve B in Fig. 1 shows that the complex may absorb slightly in the wave length region of the fluorescence. In addition, once maximum fluorescence is reached, increasing the concentration of complex results in the fluorescence becoming concentrated in an increasingly thinner layer at the surface of the solution, thus increasing the distance between fluorescence region and the phototube; decrease in fluorescence readings could result from this increase in distance. (The fluorimeter was designed to minimize the consequences of changes in the distance between fluorescence region and phototube, but the effect could not be overcome entirely.)

Taking these two effects into account, it is evident that the experimental curves over their entire range have the shape that would be expected from the basic equation with no evidence of significant self-quenching or enhancement of the fluorescence or changes in the value of K.

Effect of the Concentration of Uncombined Morin.—To determine the effect of uncombined morin on the fluorescence of the complex, two series of solutions were prepared. In one series, each solution contained 50.3 μg. of ThO_2, 12 ml. of alcohol, and amounts of morin which ranged from 0 to 4000 μg. per 50 ml. of solution; in the second series, each solution contained 50.3 μg. of ThO_2, only 2 ml. of alcohol, and amounts of morin which ranged from 0 to 1200 μg. per 50 ml. of solution. Again the fluorescence intensity and the absorbance were measured, using exciting light having wave lengths of 404.7 and 365.0 mμ for each set of measurements.

The fluorescence readings were then plotted as a function of the total morin added. Curves A and B, Fig. 4, are representative of the solutions which contained 12 ml. of alcohol when the exciting wave lengths were 404.7 and 365.0 mμ, respectively; and curves A and B, Fig. 5, are representative of the solutions which contained 2 ml. of alcohol, when the exciting wave lengths were 404.7 and 365.0 mμ, respectively.

If the uncombined morin, like the uncombined thorium, had no effect on the fluorescence, the curves in Figs. 4 and 5, like the curve in Fig. 9 should simply reflect the progress of complex formation. However, the experimental curves show that the fluorescence increases with the morin concentration until a maximum point is reached, after which the curves break sharply and the fluorescence decreases as the amount of morin increases. The sharp break, which appears well before the point of complete reaction, and the subsequent decrease in the fluorescence, are the result of two effects: absorption of exciting light by the nonfluorescent component of the solution (uncombined morin), and quenching of the fluorescence by uncombined morin.

An idea of the extent of this interference by uncombined morin can be obtained by considering the fluorescence of the solution which contained 4000 μg. of morin and 12 ml. of alcohol. The thorium in this solution was reacted completely to form 158.9 μg. of complex, and this amount of complex in the absence of free morin should have given a fluorescence reading of 0.295 on the 10 scale of the fluorimeter. But the actual reading (curve A, Fig. 4) was equivalent to 0.0092 on the 10 scale, which shows that the fluorescence had been reduced to less than 3% of its value by the presence of excess morin.

It has been shown that the complex dissociates and that most of the thorium reacts to form complex only when relatively large amounts of uncombined morin are present.[5] Thus, in addition to its absorption of exciting light, uncombined morin affects the fluorescence in two opposing ways. The fluorescence decreases with increase in morin as a result of quenching and increases with morin as a result of the law of mass action.

Calculation of Degree of Reaction.—To make a more exact analysis of the effects of uncombined morin, calculations were made to determine the components in the solutions of Figs. 4 and 5. This was done using eq. 8 of the spectrophotometric paper,[5] which relates the absorbance of the solution to the amount of complex formed. The results of these calculations are shown in Fig. 11, where the fraction of total thorium that reacts to form complex in each solution is plotted as a function of the uncombined morin in the solution. The equation of this curve was derived as a function of the uncombined morin. For amounts of uncombined morin up to 110 μg. the curve is represented by the expression

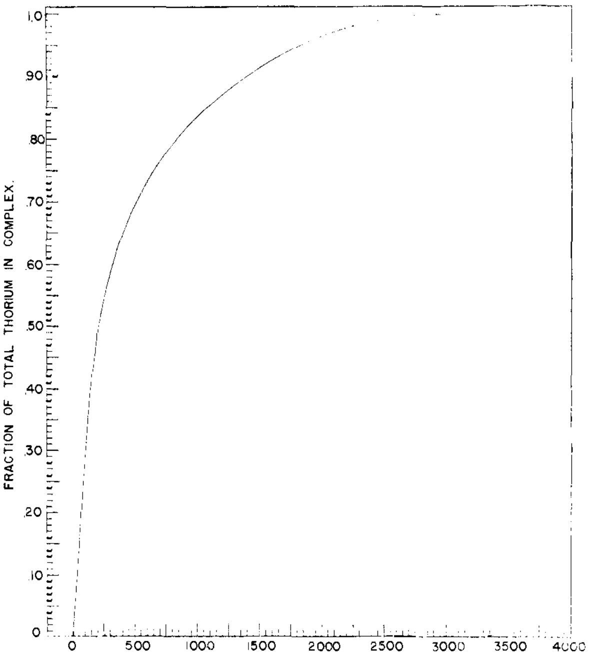

Fig. 11.—Fraction of total thorium converted to complex as a function of uncombined morin in the solution; thorium constant at 50.3 μg. of ThO_2 per 50 ml.

$$R\text{, fraction of total ThO}_2\text{ reacted} = \frac{\text{M}\cdot\text{H}}{332 - 0.04(\text{M}\cdot\text{H})} \quad (9)$$

where M·H = uncombined morin, μg./50 ml. And for amounts of uncombined morin from 110 to 200 μg., the expression is

$$R\text{, fraction of total ThO}_2\text{ reacted} = \frac{\text{M}\cdot\text{H}}{0.906\text{M}\cdot\text{H} + 240} \quad (10)$$

Determination of Quenching by Uncombined Morin.—With the aid of the above mathematical expressions, it is possible to determine the relationship between the amount of uncombined morin present and the quenching of the fluorescence. Most of the solutions whose data are plotted in Fig. 11 represent examples of system II (eq. 8), because sizable amounts of uncombined morin are present as well as complex and both absorb significant amounts of exciting light. Accordingly, the total fluorescence that is theoretically possible in these solutions is represented by the following equation when the exciting wave length is 404.7 mμ

$$\text{Total fluorescence} = \frac{K_3P_0(a_3CR)}{a_2\text{M}\cdot\text{H} + a_3CR}\,[1 - 10^{-(a_2\text{M}\cdot\text{H} + a_2CR)b}]$$

where

K_3P_0 = 3.17 (for a total alcohol content of 2 ml.)(1.0 scale)
= 5.14 (for a total alcohol content of 12 ml.)(1.0 scale)
a_2 = (6.179×10^{-5}) = absorbance of 1 μg. of morin/50 ml., 1 cm. light path
a_3 = (9.404×10^{-4}) = absorbance of 1 μg. of complex/50 ml., 1 cm. light path
M·H = uncombined morin, μg./50 ml.
C = max. complex possible from 50.3 μg. ThO_2
= 158.9 μg.
R = fraction of total thorium reacted, represented either by eq. 9 or 10
b = 3.241 cm.

Using this equation, calculations were made for the total theoretical fluorescence corresponding to the different levels of uncombined morin. Experimental values for the fluorescence of these solutions were obtained from the curves in Figs. 4 and 5 by reading the fluorescence for concentrations of total morin equivalent to the different levels of uncombined morin.

Division of the experimental value by the theoretical value for each amount of uncombined morin gave the fraction of the total possible fluorescence that remained unquenched. These values for the unquenched fluorescence, U, have been plotted in Fig. 12. Curve A, Fig. 12, represents those solutions containing a total alcohol content of 12 ml., and curve B represents those with a total alcohol content of 2 ml.

Equations for these curves were derived. For curve A, the fraction of unquenched fluorescence as a function of M·H, the uncombined morin, is expressed by the formula

$$U = 0.864 - \left[\frac{\text{M}\cdot\text{H} - 50}{154.4 + 1.142\text{M}\cdot\text{H}}\right]$$

and for curve B, the expression is

$$U = 0.861 - \left[\frac{\text{M}\cdot\text{H} - 43.16}{61 + 1.115\text{M}\cdot\text{H}}\right]$$

These expressions apply for values of M·H greater than about 50 micrograms; for smaller values of M·H the points defining the curves in Fig. 12 were erratic.

Calculation of Optimum Morin Concentration.—As the quenching is a function of the uncombined morin only, the above expressions should apply for all levels of ThO_2 concentration and should be inserted in the general equation for fluorescence as a common multiplier. Thus, the equation of system II may now be expressed as it applies specifically to the thorium–morin fluorescent system when total alcohol content of the solutions is 2 ml.

$$F = \left[0.861 - \frac{\text{M}\cdot\text{H} - 43.16}{61 + 1.115\text{M}\cdot\text{H}}\right] \times \frac{K_3P_0(a_3CR)}{a_2\text{M}\cdot\text{H} + a_3CR}\,[1 - 10^{-(a_2\text{M}\cdot\text{H} + a_3CR)b}] \quad (11)$$

where

C = max. complex possible from the ThO_2 present in μg. per 50 ml. of soln.
R = fraction of total ThO_2 reacted to form complex
CR = complex present in solution in μg. per 50 ml.

This equation is useful because the fluorescence of any solution can be calculated, provided the concentrations of complex and of uncombined morin are known. Such concentrations were calculated from experimental data for two series of solutions: the solutions of the one series containing a total of 50.3 μg. ThO_2 per 50 ml. and varying amounts of total morin; and the solutions of the other series containing a total of 15 μg. ThO_2 per 50 ml. and varying amounts of total morin.

Then, using eq. 11, the fluorescences of these solutions were calculated. Curves in which the calculated fluorescences were plotted as a function of uncombined morin were similar to curve A in Fig. 5. Figure 13 shows the portions of the calculated curves, between 50 and 200 μg. of uncombined morin, which include the peaks of the curves. The peaks of both these curves, and thus the region of greatest sensitivity, occur when the uncombined morin is near 100 μg.; furthermore, the maximum variation in fluorescence is less than 5% when morin varies from 75 to 130 μg.

The almost constant fluorescence indicates that in this limited range the effect of increased complex formation resulting from increased morin concentration is almost exactly counterbalanced by the increased effects of quenching and absorption of exciting light by morin.

This result is fortuitous for the construction of standard curves. If the total morin reagent added to the solutions results in amounts of uncombined morin between the limits of 75 and 150 μg. per 50 ml., then close to maximum sensitivity and linearity for the standard curve are attained. A choice of 100 μg. total morin content results in values for uncombined morin near the lower limit of this range (see crosses in Fig. 13) and a choice of 165 μg. of total morin results in uncombined morin values near the upper limit of the range (circles in Fig. 13).

Calculation of Standard Curve.—Equation 11 was used to compute standard curves after the term CR (amount of complex formed) was evaluated for thorium concentrations in the range of the standard curve. This evaluation is necessary because R, the fraction of total thorium reacted to form complex, was derived only for solutions having total thorium concentrations of 50.3 and

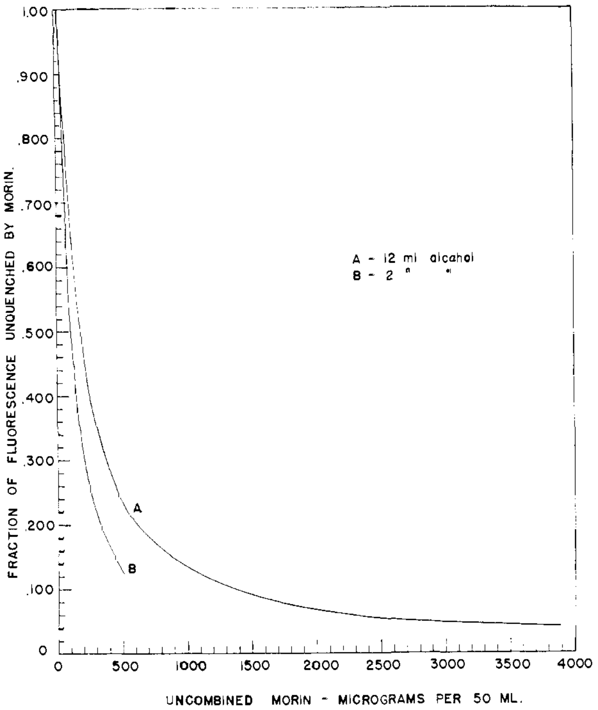

Fig. 12.—Fraction of fluorescence remaining unquenched as a function of the uncombined morin. Thorium constant at 50.3 μg. of ThO_2 per 50 ml. Curve A, 12 ml. of alcohol; curve B, 2 ml. of alcohol; P_0 (404.7 mμ).

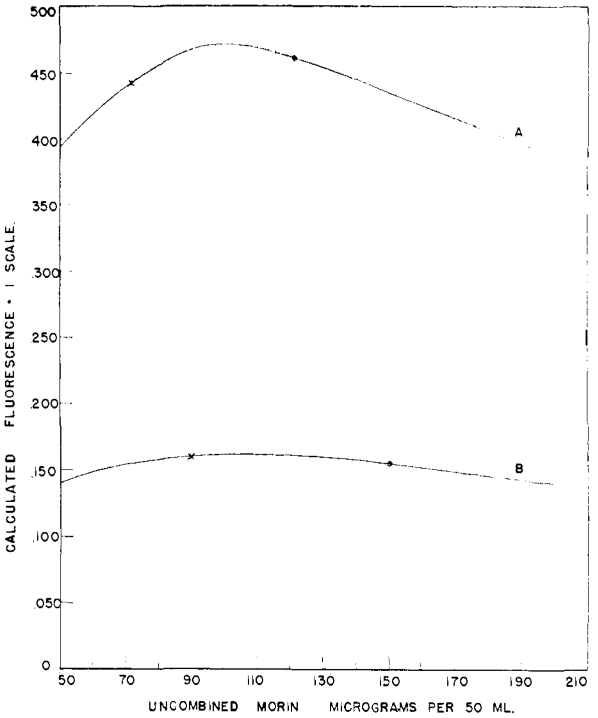

Fig. 13.—Calculated fluorescence as a function of uncombined morin in the solutions: curve A, total thorium 50.3 μg. of ThO_2 per 50 ml.; curve B, total thorium 15 μg. of ThO_2 per 50 ml.; 2 ml. of alcohol; P_0 (404.7 mμ).

15 μg. of ThO_2. *CR* was evaluated using the mass action law

$$\frac{(CR)(H^+)^2}{(ThO_2 - CR)(M{\cdot}H - 2CR)^2} = K_E$$

where

(CR) = complex formed, moles per liter
$(M{\cdot}H)$ = total morin, moles per liter
$(M{\cdot}H - 2CR)$ = uncombined morin, moles per liter
(ThO_2) = total thorium, moles per liter
$(ThO_2 - CR)$ = uncombined thorium, moles per liter
K_E = equilibrium constant

K_E had been determined previously[5]; for thorium concentrations in the range of the standard curve the average values of K_E are 1.45×10^6 when total morin is 100 μg., and 0.95×10^6 when total morin is 165 μg.

The calculated standard curves are shown in Figs. 6 and 7 as dashed lines; they coincide very closely with the experimental curves. Both the region of linearity and slope predicted by the calculated curves are corroborated by the experimental curves. It is possible, thus, to use the derived mathematical expressions for fluorescence to calculate a family of curves that show, without actual experimentation, the characteristics of the standard curves to be expected for total morin concentrations that vary over a wide range.

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[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, UNIVERSITY OF ARKANSAS]

Color Transitions in Fused Nickel(II)-Alkali Halide Systems

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Nickel(II) chloride (or sulfate) fused with alkali chlorides or bromides gives, respectively, a blue or green melt. On cooling a yellow solid is formed. The color transition is abrupt and reversible and occurs very close to the melting point.

During the course of work on molten salt systems it was observed that fusion of yellow anhydrous nickel(II) chloride and potassium chloride gives a blue liquid. On cooling, the melt becomes a blue semi-solid and finally over a short temperature interval undergoes a striking color transition to a yellow solid. Since materials which undergo distinct color changes at definite temperatures are rather rare it was decided to observe various fused salt systems to determine the generality of the phenomenon. The relationship of color transition temperatures to phase transition temperatures was also investigated.

Observations and Discussion.—The nickel chloride–potassium chloride system exhibited the phenomenon for all compositions having a nickel

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