

Chemistry

UNITED STATES DEPARTMENT OF THE INTERIOR
GEOLOGICAL SURVEY

A FLUORIMETRIC STUDY OF THE THORIUM-MORIN SYSTEM*

By

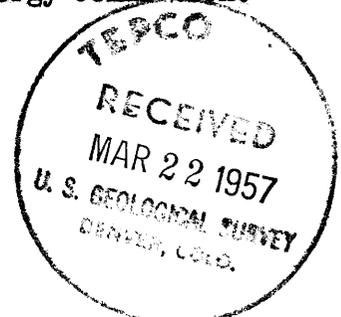
Robert G. Milkey and Mary H. Fletcher

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A FLUORIMETRIC STUDY OF THE THORIUM-MORIN SYSTEM

By Robert G. Milkey and Mary H. Fletcher

ABSTRACT

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 wavelength 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 which 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 excellent agreement with experimental curves.

INTRODUCTION

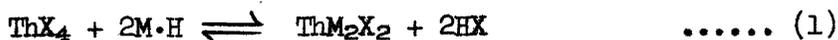
Morin reacts with thorium in weakly acid solutions (3, 4, 7, 8) to form a stable yellow complex which fluoresces yellow-green when its solutions are irradiated with ultraviolet light. Both the color and fluorescence of this complex have been investigated.

The results of the spectrophotometric study have been presented in an earlier paper (2) which evaluates the color system as a basis for the quantitative determination of trace amounts of thorium.

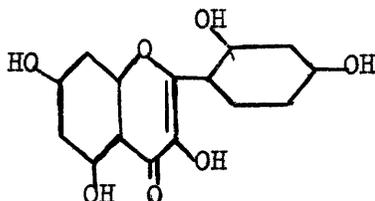
This paper evaluates the fluorescent system as a basis for the quantitative determination of trace amounts of thorium. Some of the theoretical and mathematical relationships between fluorescence and light absorption, as exhibited in the thorium-morin system, are also included. This report is part of 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



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

$$K_{\text{equil.}} = \frac{[\text{ThM}_2^{+2}][\text{H}^+]^2}{[\text{Th}^{+4}][\text{M.H}]^2} \dots\dots (2)$$

is approximately 1×10^6 (2).

Solutions of the complex fluoresce a bright green when irradiated with long wavelength ultraviolet light (3, 4, 7, 8). The wavelength band of the fluorescent light ranges from approximately 488 to 555 μ , with peak intensity between 513 and 533 μ , as determined by visual inspection with a spectroscope (personal communication, H. Jaffe, U. S. Geological Survey, and C. E. White, Univ. of Maryland).

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

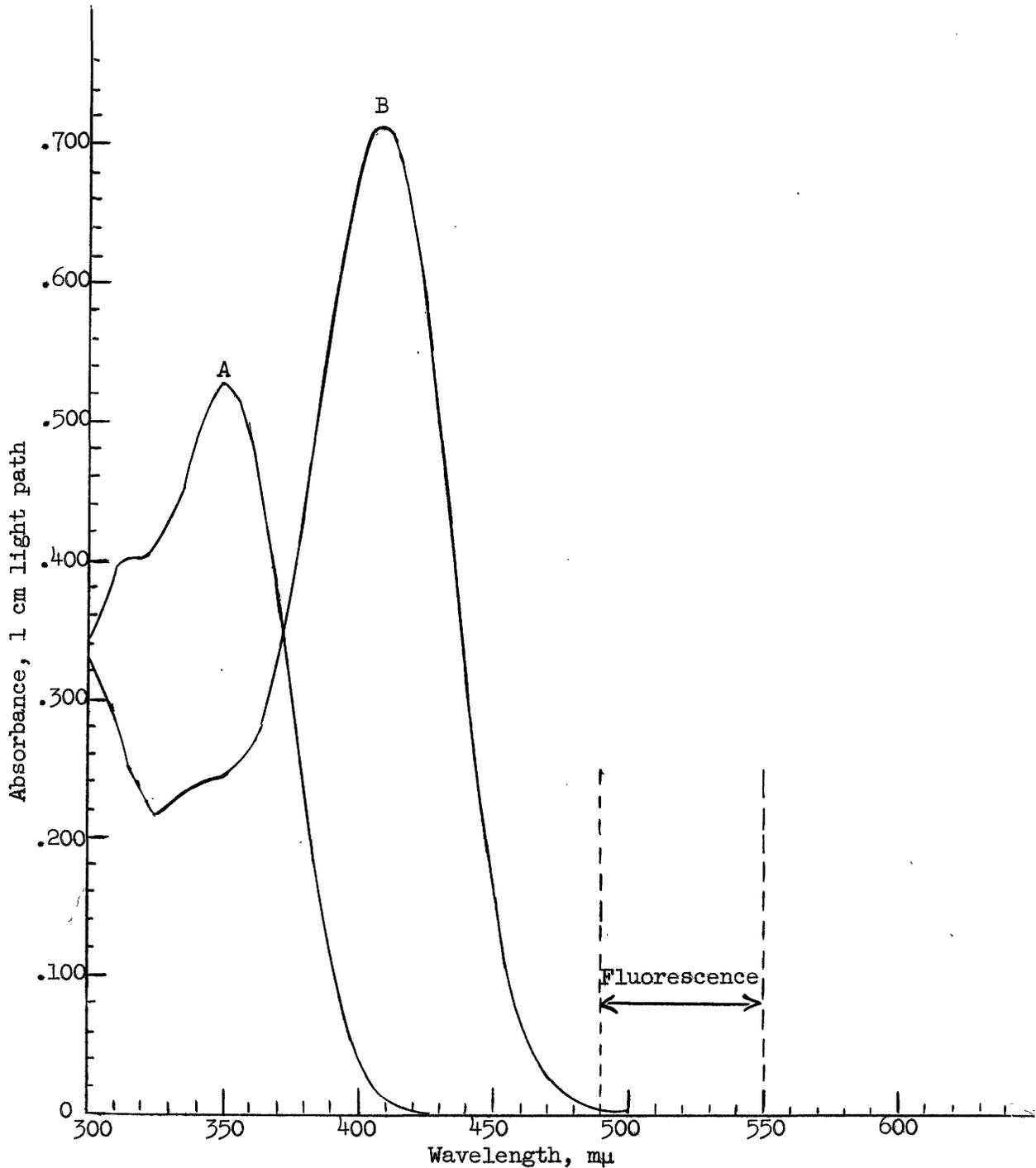


Figure 1.--Absorption spectra for pure morin (A) and thorium-morin complex (B) arrow indicates wavelength region of fluorescence. Curve A, 600 micrograms morin/50 ml of solution; curve B, 600 micrograms morin plus 2 mg ThO_2 /50 ml of solution; 2 ml alcohol in each.

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 phototube 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 inches above the top of the solution cell. (Intensity of the light supplied at wavelength 404.7 μ was approximately four times the intensity of the light at 365 μ wavelength. Personal communication, C. L. Waring, U. S. Geological Survey.)

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 μ) and 0.980 microampere for P_0 (365.0 μ), 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 wavelength 404.7 μ . 2. A combination of Corning filters no. 7380, 5874, and 5860 was used to isolate light of wavelength 365.0 μ .

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

Beckman pH meter

Morin

Morin, $C_{15}H_{10}O_7 \cdot 2H_2O$ (mol. wt. 338.26) of high purity, obtained from Dr. Theodor Schuchardt in Munich, Germany. Solutions of morin of the concentration desired were prepared by dissolving the solid material in 95 percent ethyl alcohol.

Standard thorium solutions

A stock solution of thorium chloride was prepared by dissolving the salt (reagent grade) in 3 percent 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 pH 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 pH 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 figure 2. In curve B, which represents solutions similar to those found in routine analysis, the point of maximum fluorescence occurs at pH 2, and this is also the point of maximum difference between the solutions and the blanks. Accordingly, a pH 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 figure 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 micrograms per 50 ml is shown in figures 4 and 5. Maximum fluorescence is obtained from solutions containing 100 to 200 micrograms of morin per 50 ml. This holds true for other concentrations of ThO_2 of the same order of magnitude.

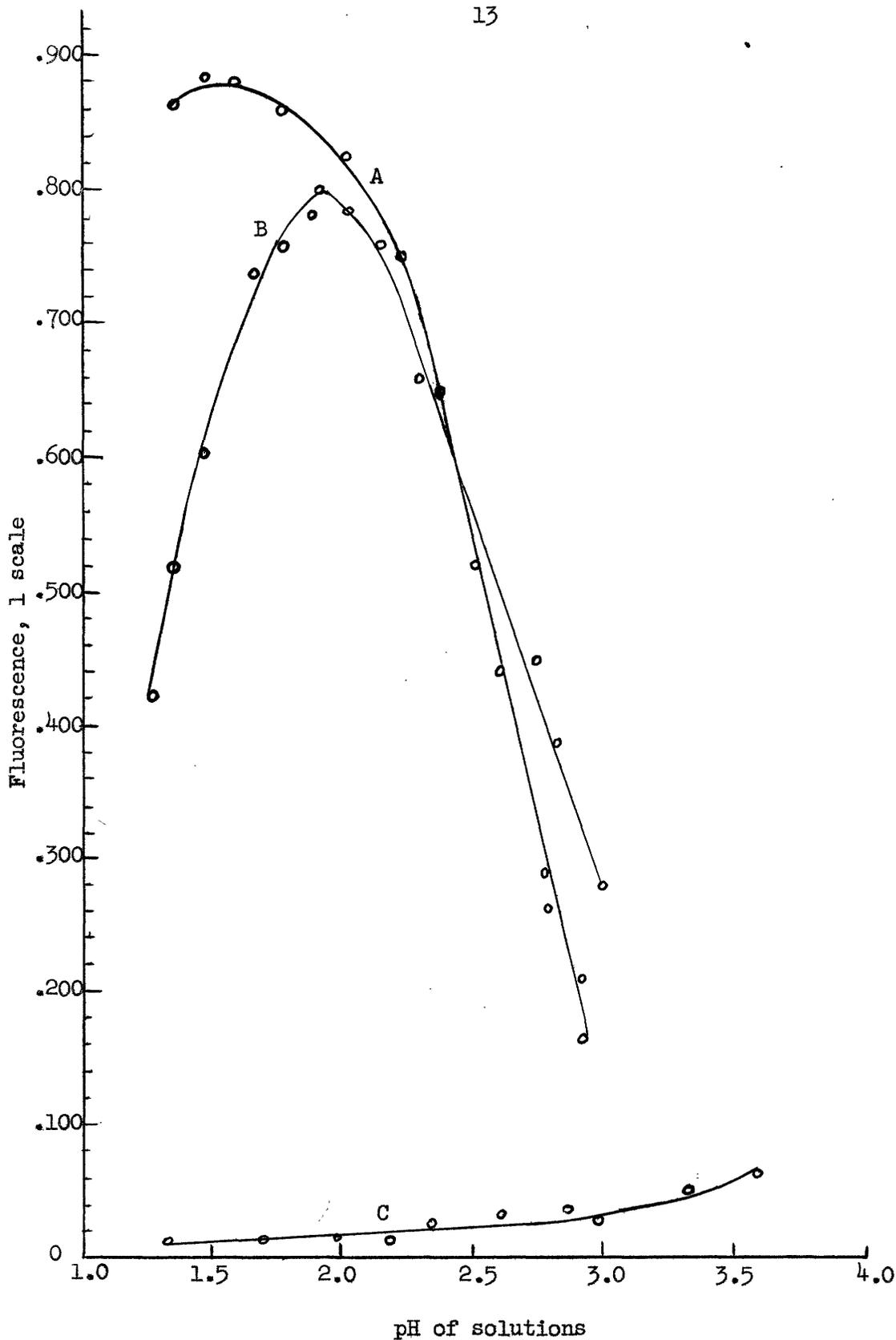


Figure 2.--Effect of pH on fluorescence. Curve A, complex (37.4 micrograms per 50 ml); curve B, mixture of complex plus uncombined morin (100.6 micrograms ThO_2 plus 165 micrograms total morin per 50 ml); curve C, morin (300 micrograms per 50 ml); 2 ml of alcohol in all solutions; P_0 (404.7 μ).

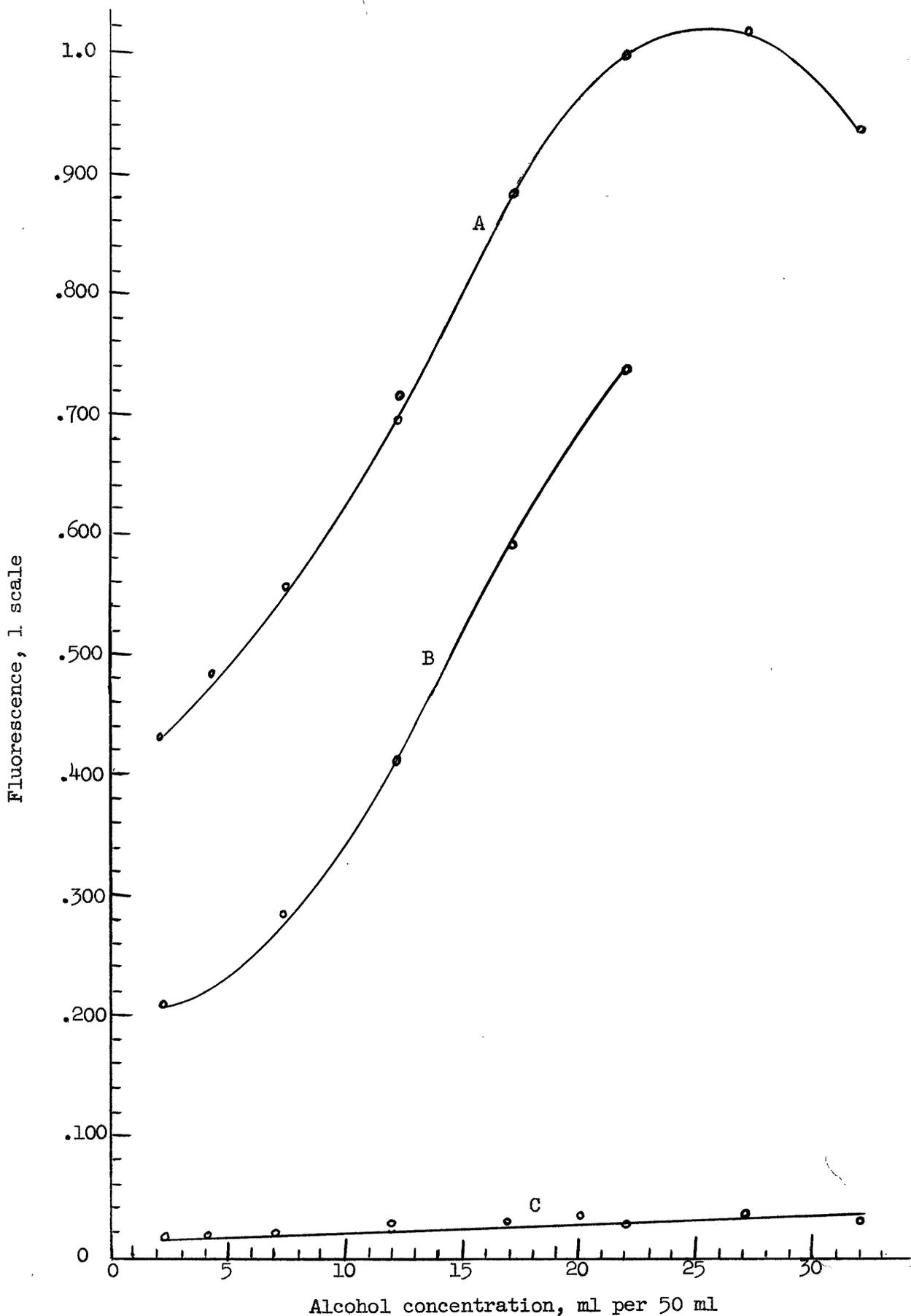


Figure 3.--Effect of alcohol concentration on the fluorescence. Curve A, complex (18.7 micrograms per 50 ml); curve B, mixture of complex plus uncombined morin (20.12 micrograms ThO_2 plus 165 micrograms total morin per 50 ml); curve C, morin (300 micrograms per 50 ml); P_0 (404.7 $\mu\mu$).

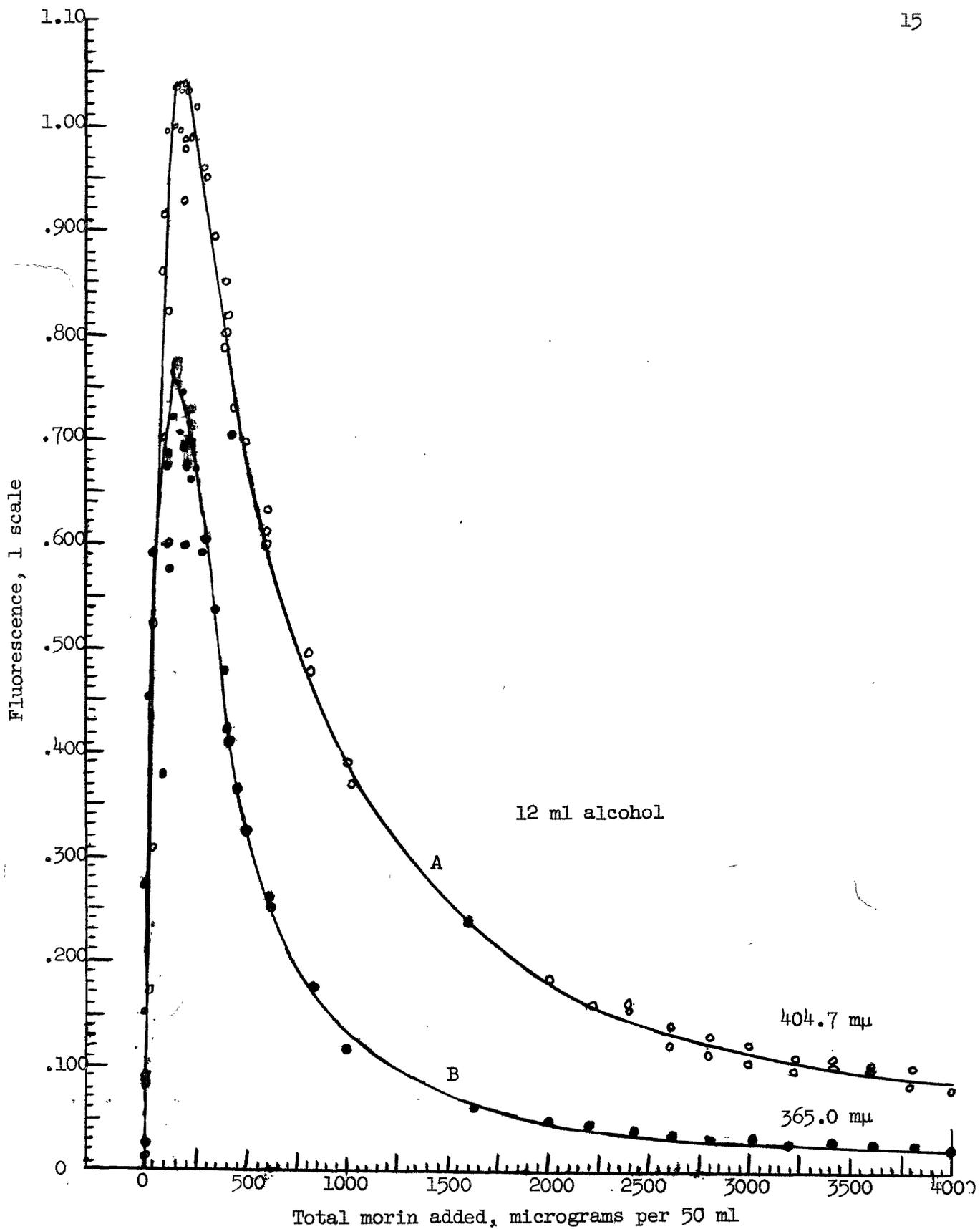


Figure 4.--Effect on fluorescence of adding increasing amounts of morin to a constant amount of ThO_2 (50.3 micrograms per 50 ml); alcohol content 12 ml per 50 ml; P_0 (404.7 m μ).

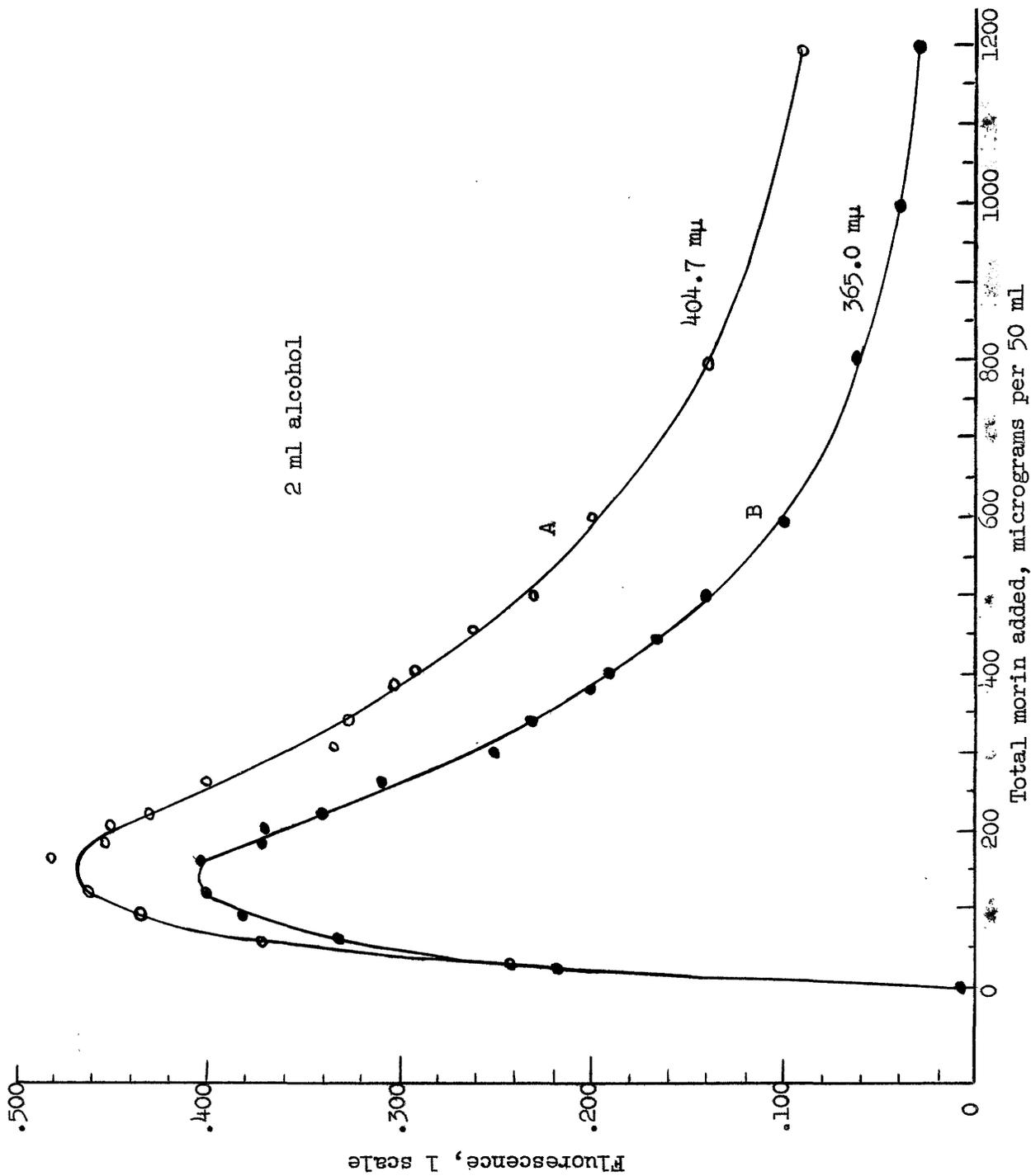


Figure 5.--Effect on fluorescence of adding increasing amounts of morin to a constant amount of thorium (50.3 micrograms ThO₂ per 50 ml); alcohol content 2 ml per 50 ml; λ_0 (404.7 m μ).

Temperature

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

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 micrograms of ThO₂ and 200 micrograms of morin per 50 ml. After the initial 30 minutes, the difference between the highest and lowest readings was less than 2 percent.

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 0.63N HCl.
2. Add thorium solution that has a pH 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 figures 6 and 7 (solid lines) and in figure 8. The curves in figures 6 and 7 indicate that thorium concentrations ranging from 10 to 150 micrograms of ThO_2 can be measured with good accuracy.

In figure 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 portion of the curve obtained with an exciting wavelength of $404.7 \text{ m}\mu$ in figure 8, a difference in fluorescence readings of 0.100 is equivalent to 1.10 micrograms 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 microgram 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 microgram of ThO_2 on the individual readings.

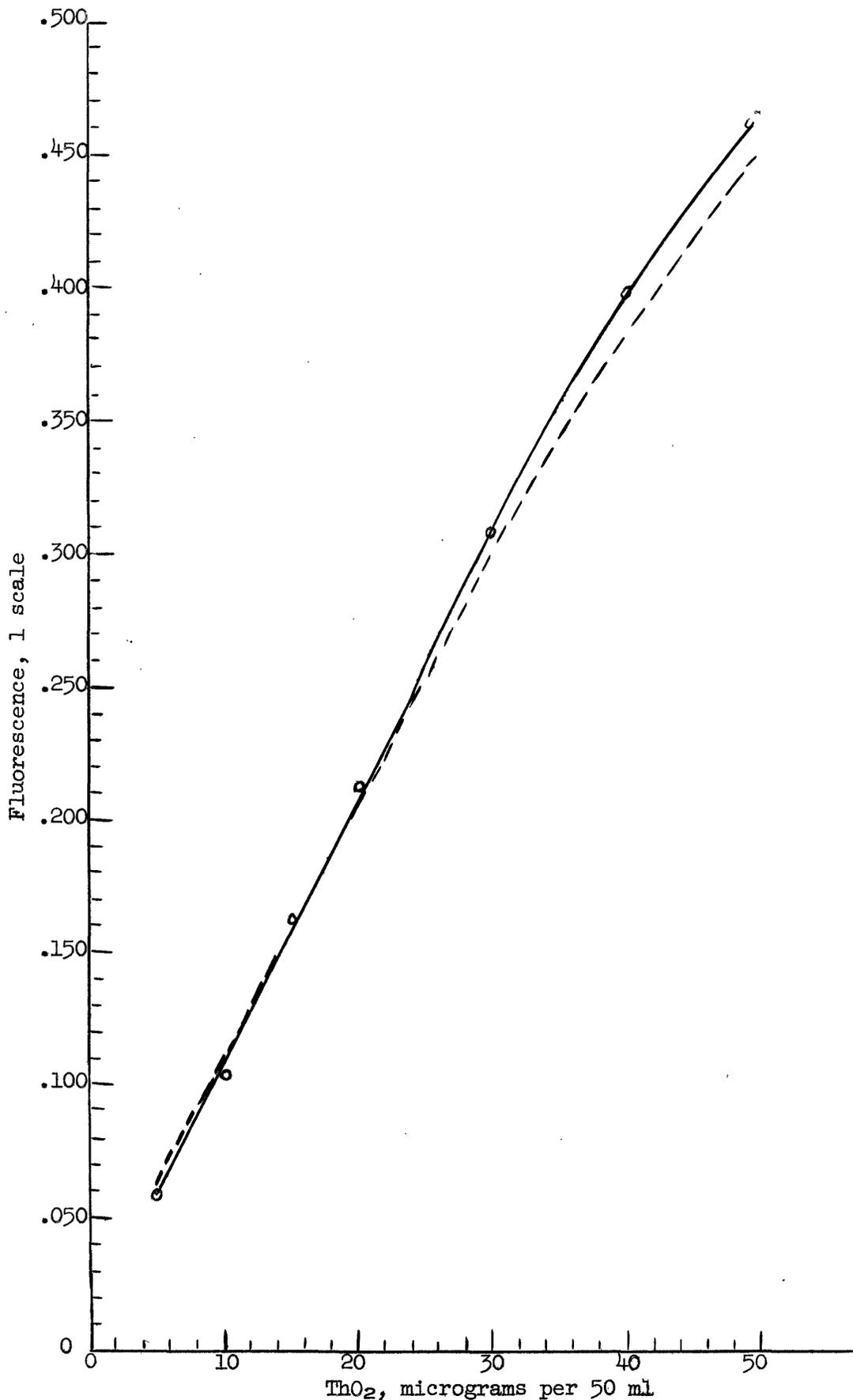


Figure 6.--Standard fluorescence curves, total morin 100 micrograms per 50 ml of alcohol; P_O (404.7 μ). Solid line, defined by points, is experimental curve; dashed line, with no points, is calculated curve.

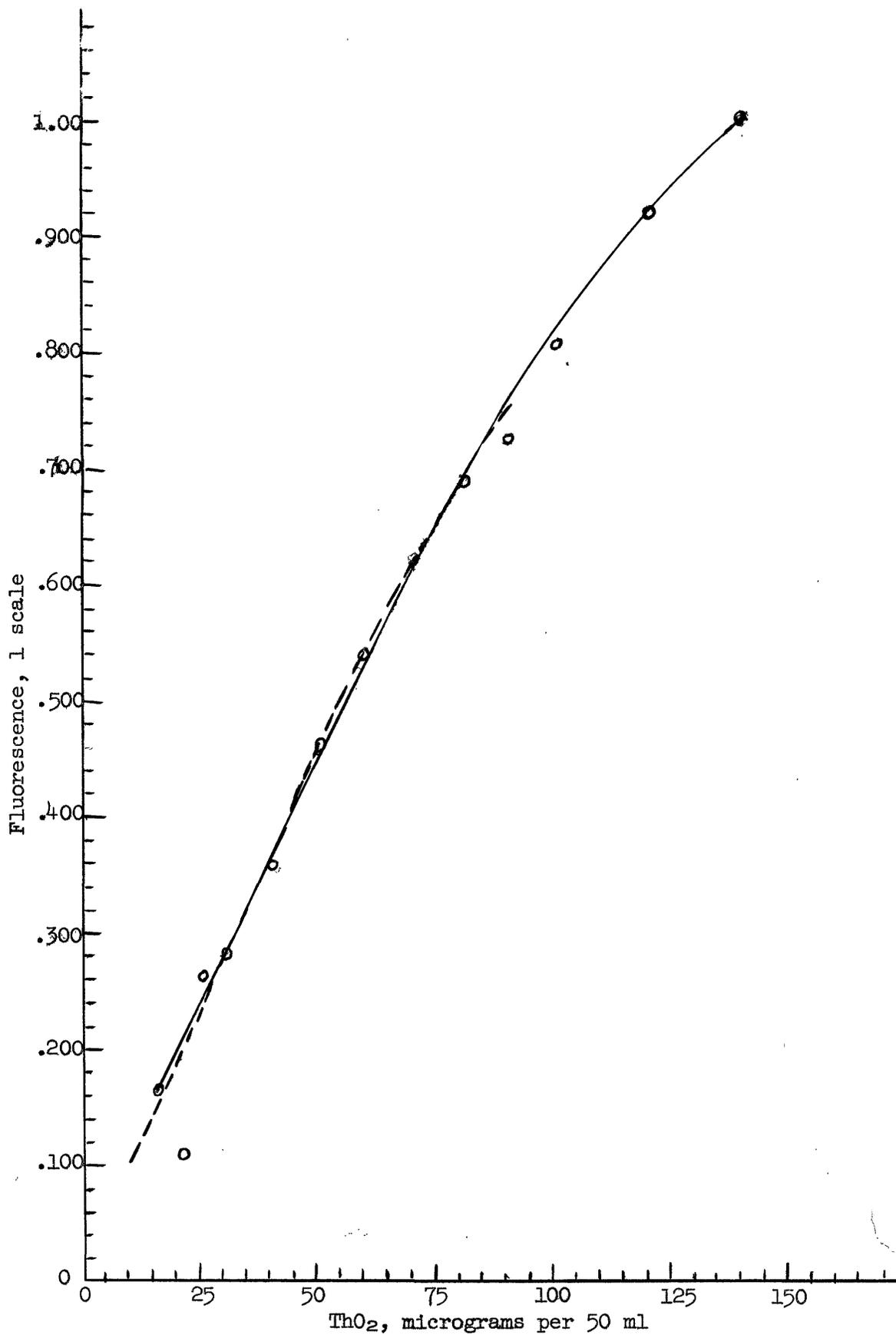


Figure 7.--Standard fluorescence curves, total morin 165 micrograms per 50 ml; 2 ml of alcohol; P_0 (404.7 $m\mu$). Solid line, defined by points, is experimental curve; dashed line, with no points, is calculated curve.

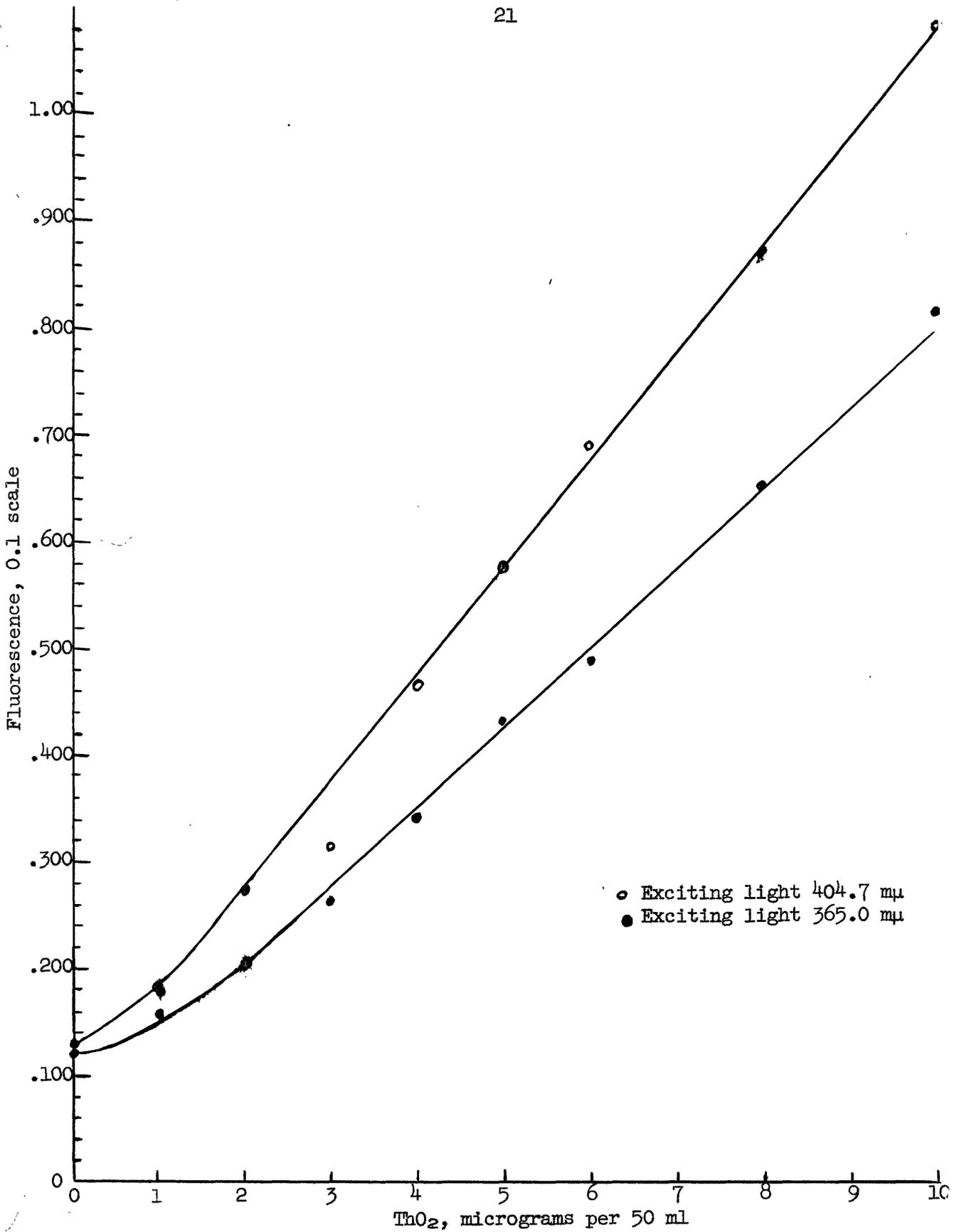


Figure 48.--Experimental standard curves. Total morin = 100 micrograms per 50 ml alcohol = 2 ml per 50 ml.

Comparison of fluorimetric and spectrophotometric methods

A sensitivity of 0.5 microgram of ThO_2 is slightly less than but comparable to that of the spectrophotometric method (2). Potentially, however, the sensitivities of the two methods are far different provided the random errors can be eliminated from the fluorimetric method. As indicated above, the limit of detection of the fluorimetric method is as low as 0.044 microgram. Although the sensitivity of the spectrophotometric method 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.

Increased fluorescence could be produced by using higher concentrations of alcohol and by increasing the intensity of the exciting light by moving the lamp closer to the sample. Additional sensitivity could be obtained instrumentally by increasing the amplification of the photocurrent. However, unless the precision were improved these expedients would result in increasing the random errors proportionately with no resultant net gain. Hence, at present, the spectrophotometric method is more advantageous for quantitative analysis.

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 micrograms of ThO_2 per 50 ml of solution. Zirconium

and aluminum were tested with exciting wavelengths of both 404.7 μ and 365.0 μ , and as the effects of these ions were the same at both wavelengths, ferric, calcium, and lanthanum ions were tested at 404.7 μ 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 1.

Table 1.--Summary of effects of other ions.

Ion	Type of interference	Sensitivity of fluorescence of interfering ion	Amount of interfering substance which causes 10% error in amount of ThO ₂ found
Zr ⁺⁴	Forms complex which fluoresces	ZrO ₂ and ThO ₂ approximately the same	ZrO ₂ = 10% of the amount of ThO ₂ present
Al ⁺³	Forms complex which fluoresces	Al ₂ O ₃ about 1/8 as sensitive as ThO ₂	Al ₂ O ₃ = amount of ThO ₂ present
Fe ⁺³	Quenches the thorium-morin fluorescence	--	15-20 micrograms Fe ₂ O 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 ₂ O ₃ per 50 ml

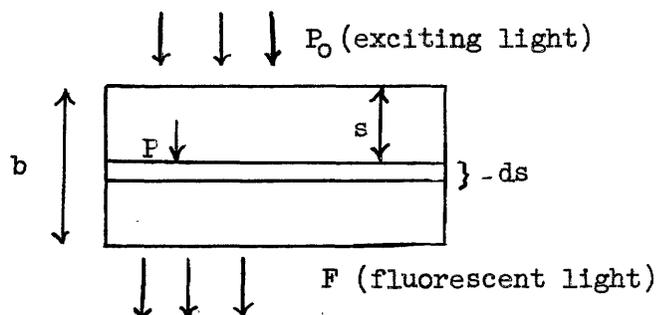
BASIC THEORY OF FLUORESCENCE: APPLICATION TO THORIUM-MORIN SYSTEM

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 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.



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, and

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

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

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 wavelengths 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 wavelength 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 :

$$dF_{M_0} = K_M \left[\frac{k_1 c_1}{k_1 c_1 + k_2 c_2 + k_3 c_3} \right] [dP]$$

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

$$dF_M = K_1 P_0 (k_1 c_1) [e^{-(k_1 c_1 + k_2 c_2 + k_3 c_3) s}] \cdot [e^{-(k_4 c_1 + k_5 c_2 + k_6 c_3)(b-s)}] ds$$

$$\text{where } K_1 = VK_M$$

$$\therefore dF_M = K_1 P_0 (k_1 c_1) [e^{-(k_4 c_1 + k_5 c_2 + k_6 c_3) b}] \cdot [e^{-[(k_1 - k_4) c_1 + (k_2 - k_5) c_2 + (k_3 - k_6) c_3] s}] ds$$

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

$$F_M = \frac{K_1 P_0 (k_1 c_1)}{(k_1 - k_4) c_1 + (k_2 - k_5) c_2 + (k_3 - k_6) c_3} \left[e^{-(k_4 c_1 + k_5 c_2 + k_6 c_3) b} - e^{-(k_1 c_1 + k_2 c_2 + k_3 c_3) b} \right] \dots (3)$$

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 waveband of fluorescence. Accordingly, the expression for the fluorescence due to the reagent in the solution which impinges on the phototube is

$$F_R = \frac{K_2 P_0 (k_2 c_2)}{(k_1 - k_7)c_1 + (k_2 - k_8)c_2 + (k_3 - k_9)c_3} [e^{-(k_7 c_1 + k_8 c_2 + k_9 c_3)b} - e^{-(k_1 c_1 + k_2 c_2 + k_3 c_3)b}] \dots\dots (4)$$

where $K_2 = VK_R$

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 waveband of the fluorescence.

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

$$F_C = \frac{K_3 P_0 (k_3 c_3)}{(k_1 - k_{10})c_1 + (k_2 - k_{11})c_2 + (k_3 - k_{12})c_3} [e^{-(k_{10}c_1 + k_{11}c_2 + k_{12}c_3)b} - e^{-(k_1c_1 + k_2c_2 + k_3c_3)b}] \dots\dots (5)$$

where $K_3 = VK_C$

Total fluorescence

In equations 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 equations 3, 4, and 5 become $0.434 k_n$. 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 $0.434 k_n$, 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:

$$\begin{aligned}
 F &= F_M + F_R + F_C \\
 &= \frac{K_1 P_0 (a_1 c_1) [10^{-(a_4 c_1 + a_5 c_2 + a_6 c_3)b} - 10^{-(a_1 c_1 + a_2 c_2 + a_3 c_3)b}]}{[(a_1 - a_4)c_1 + (a_2 - a_5)c_2 + (a_3 - a_6)c_3]} + \\
 &\quad \frac{K_2 P_0 (a_2 c_2) [10^{-(a_7 c_1 + a_8 c_2 + a_9 c_3)b} - 10^{-(a_1 c_1 + a_2 c_2 + a_3 c_3)b}]}{[(a_1 - a_7)c_1 + (a_2 - a_8)c_2 + (a_3 - a_9)c_3]} + \\
 &\quad \frac{K_3 P_0 (a_3 c_3) [10^{-(a_{10} c_1 + a_{11} c_2 + a_{12} c_3)b} - 10^{-(a_1 c_1 + a_2 c_2 + a_3 c_3)b}]}{[(a_1 - a_{10})c_1 + (a_2 - a_{11})c_2 + (a_3 - a_{12})c_3]} \dots\dots (6)
 \end{aligned}$$

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

$$F = K_3 P_0 (1 - 10^{-a_3 c_3 b}) \dots\dots (7)$$

(hereafter referred to as System I). This is the form of the equation usually found in the literature (1, 5, 6).

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

$$F = \frac{K_3 P_0 (a_3 c_3)}{(a_2 c_2 + a_3 c_3)} [1 - 10^{-(a_2 c_2 + a_3 c_3)b}] \dots\dots (8)$$

(hereafter referred to as System II). Braunsberg and Osborn (1) 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.

When the complex and metal ion both absorb exciting light, but only the complex fluoresces, and the fluorescent light is absorbed by the reagent, the equation becomes

$$F = \frac{K_3 P_0 (a_3 c_3)}{a_1 c_1 - a_{11} c_2 + a_3 c_3} [10^{-(a_{11} c_2 b)} - 10^{-(a_1 c_1 + a_3 c_3) b}] \dots\dots (9)$$

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 the process of 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 wavelength 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 wavelengths 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 wavelength of exciting light.

The absorbance data presented in figure 1 were considered in choosing the wavelengths to be used in the excitation of the fluorescence. At 410 $m\mu$ 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 $m\mu$; as a consequence, interference resulting from light absorption by uncombined morin should be at a minimum at this wavelength. By analogous reasoning, at 350 $m\mu$ interference resulting from light absorption by the uncombined morin should be at a maximum. Thus, from the standpoint of light absorption only, 410 $m\mu$ should be the most advantageous exciting wavelength and 350 $m\mu$ the least advantageous.

These two wavelengths are quite close to the wavelengths 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 $m\mu$ and 365.0 $m\mu$, and light at each of these wavelengths can be isolated by filters. Accordingly, both the 404.7 $m\mu$ and 365.0 $m\mu$ radiations of the mercury lamp were used as exciting wavelengths 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 figure 9 the fluorescence of these solutions is shown as a function of the thorium concentration.

It has been shown (2) that 600 micrograms of morin are more than 98 percent reacted to form complex when as little as 7.5 mg of ThO_2 are present in 50 ml of solution. The horizontal portion of the fluorescence

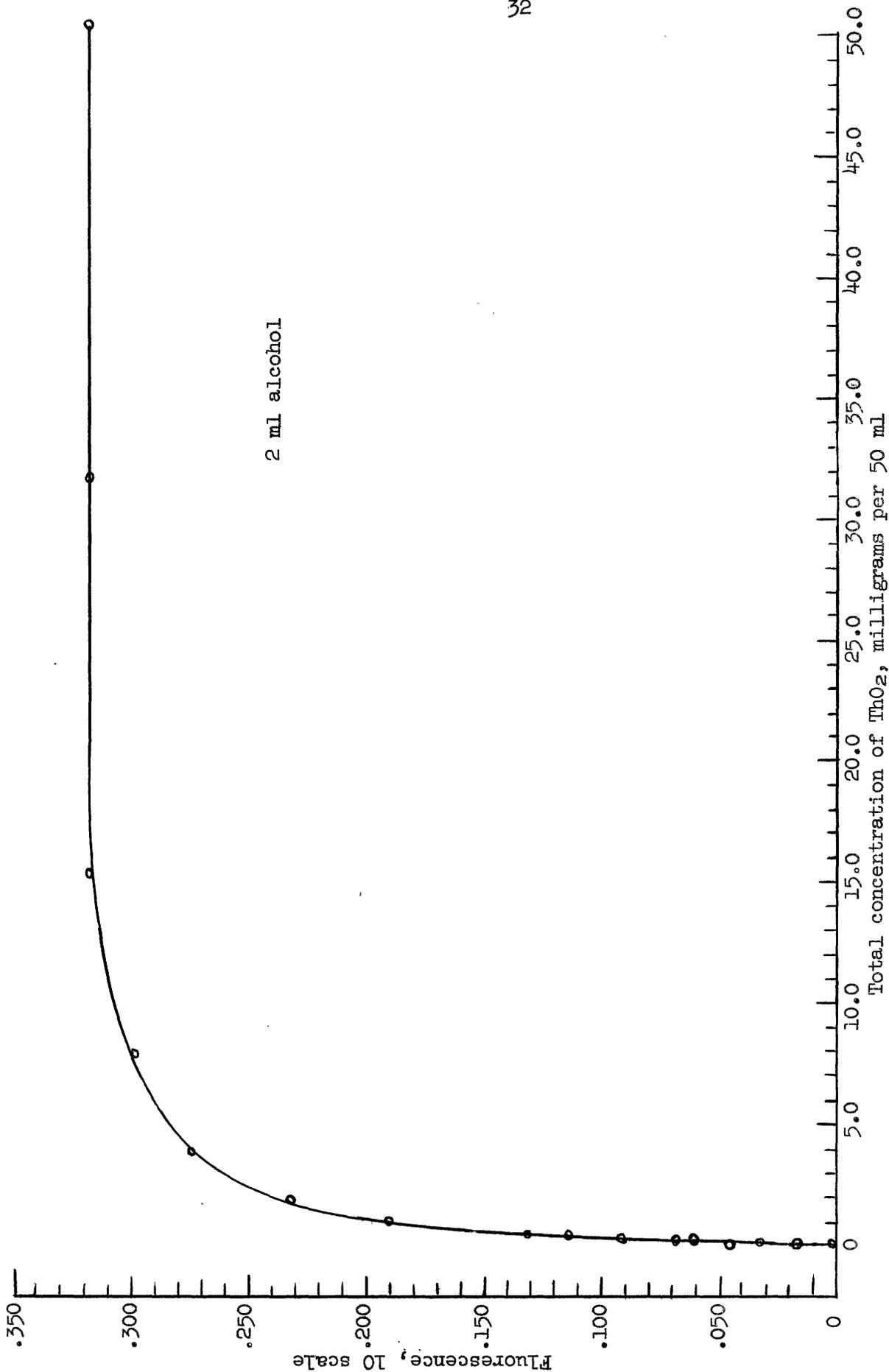


Figure 9.--Effect on fluorescence of adding increasing amounts of thorium to a constant amount of morin (600 micrograms per 50 ml; alcohol 2 ml; P₀ (404.7 mμ).

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 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 were always used in the preparation of solutions of the so-called pure complex to insure 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 micrograms were converted completely to the complex by a large excess of thorium (50 mg 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 wavelengths of 404.7 μ and 365.0 μ and a light path through the solutions of 3.241 cm. The absorbances of these solutions at these wavelengths 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 equation 7, $F = K_3P_0 (1-10^{-A})$, where $A = \text{absorbance} = a_3c_3b$. 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, figure 10, are representative of the solutions that contained 12 and 2 ml of alcohol, respectively, when the exciting light had a wavelength of 404.7 μ . The curves obtained with an exciting light of wavelength 365.0 μ are not presented because they are similar to those of figure 10.

The curves in figure 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 equation 7) is a constant, and the fluorescence of the complex obeys the basic equation.

The slopes of the curves in figure 10 are equivalent to KP_0 of the equation, and the empirical values for KP_0 calculated from the curves are given in table 2. The values obtained with exciting light of 365.0 μ are included also.

Table 2.--Empirical values for KP_0 (for fluorescence readings on 10 scale).

Wavelength of exciting light (μ)	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

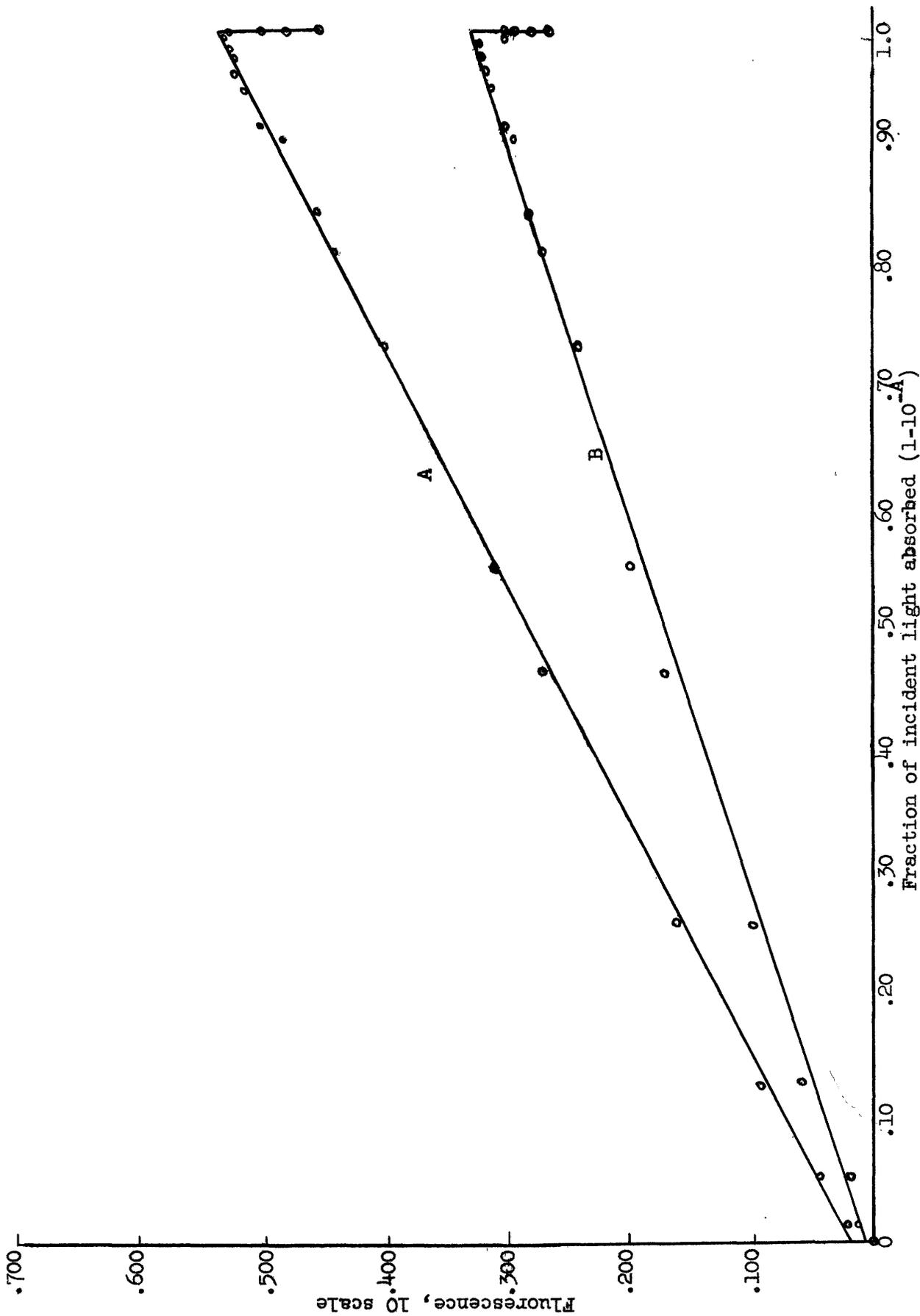


Figure 10.--Fluorescence of the complex as a function of the fraction of exciting light absorbed. Curve A, 12 ml. of alcohol per 50 ml; curve B, 2 ml of alcohol per 50 ml; P_0 (404.7 μ). For each curve the concentration of the complex varied from 0 to 4934.4 micrograms per 50 ml of solution.

As table 2 shows, the value of KP_0 is affected by alcohol content, but as P_0 is constant for each exciting wavelength used, the change in KP_0 for each wavelength 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 wavelength of 404.7 μ , K was increased by 62 percent; when the exciting light had a wavelength of 365.0 μ , K was increased by 51 percent.

The data in table 2 can also be used to obtain a rough comparison of K at the two different wavelengths. The ratio of KP_0 (404.7 μ) to KP_0 (365.0 μ) for each concentration of alcohol is roughly 1 to 2. But the ratio of P_0 (404.7 μ) to P_0 (365.0 μ) 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 μ) is about twice as large as K (365.0 μ).

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 wavelength 404.7 μ is used rather than 365.0 μ . Accordingly, for light sources of equal energy at both wavelength, sensitivity will be considerably greater when the exciting wavelength is 404.7 μ rather than 365.0 μ .

Returning to figure 10 it will be noted that a sharp break occurs in each curve when the fraction of light absorbed, $(1-10^{-A})$, equals nearly one, 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 figure 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 percent decrease in fluorescence occurred when the concentration of the complex was increased from about 500 to nearly 5000 micrograms. 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 fluorescent region and the phototube. The fluorimeter was designed to minimize the consequences of changes in the distance between fluorescence and phototube but the effect could not be entirely overcome; as a consequence fluorescence readings in the high concentration range are lower than the maximum value.

Taking this effect into account, 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 micrograms of ThO_2 , 12 ml alcohol, and amounts of morin which ranged from 0 to 4000 micrograms per 50 ml of solution; in the second series, each solution contained 50.3 micrograms of ThO_2 , only 2 ml of alcohol, and amounts of morin which ranged from 0 to 1200 micrograms per 50 ml of solution. Again the fluorescence intensity and the absorbance were measured using exciting light having wavelengths of 404.7 and 365.0 μ for each set of measurements.

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

If the uncombined morin, like the uncombined thorium, had no effect on the fluorescence, the curves in figures 4 and 5, like the curve in figure 9 should simply reflect the progress of complex formation. However, in the experimental curves, 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 micrograms of morin and 12 ml of alcohol. The thorium in this solution was completely reacted to form 158.9 micrograms 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 percent 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 (2). 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 figures 4 and 5. This was done using equation 8 of the spectrophotometric paper (2), which relates the absorbance of the solution to the amount of complex formed. The results of these calculations are shown in figure 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 micrograms, the curve is represented by the expression

$$R, \text{ fraction of total ThO}_2 \text{ reacted} = \frac{M \cdot H}{332 - 0.04 (M \cdot H)} \quad \dots (10)$$

where $M \cdot H$ = uncombined morin, micrograms/50 ml. And for amounts of uncombined morin from 110 to 200 micrograms, the expression is

$$R, \text{ fraction of total ThO}_2 \text{ reacted} = \frac{M \cdot H}{0.906 M \cdot H + 240} \quad \dots (11)$$

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 figure 11

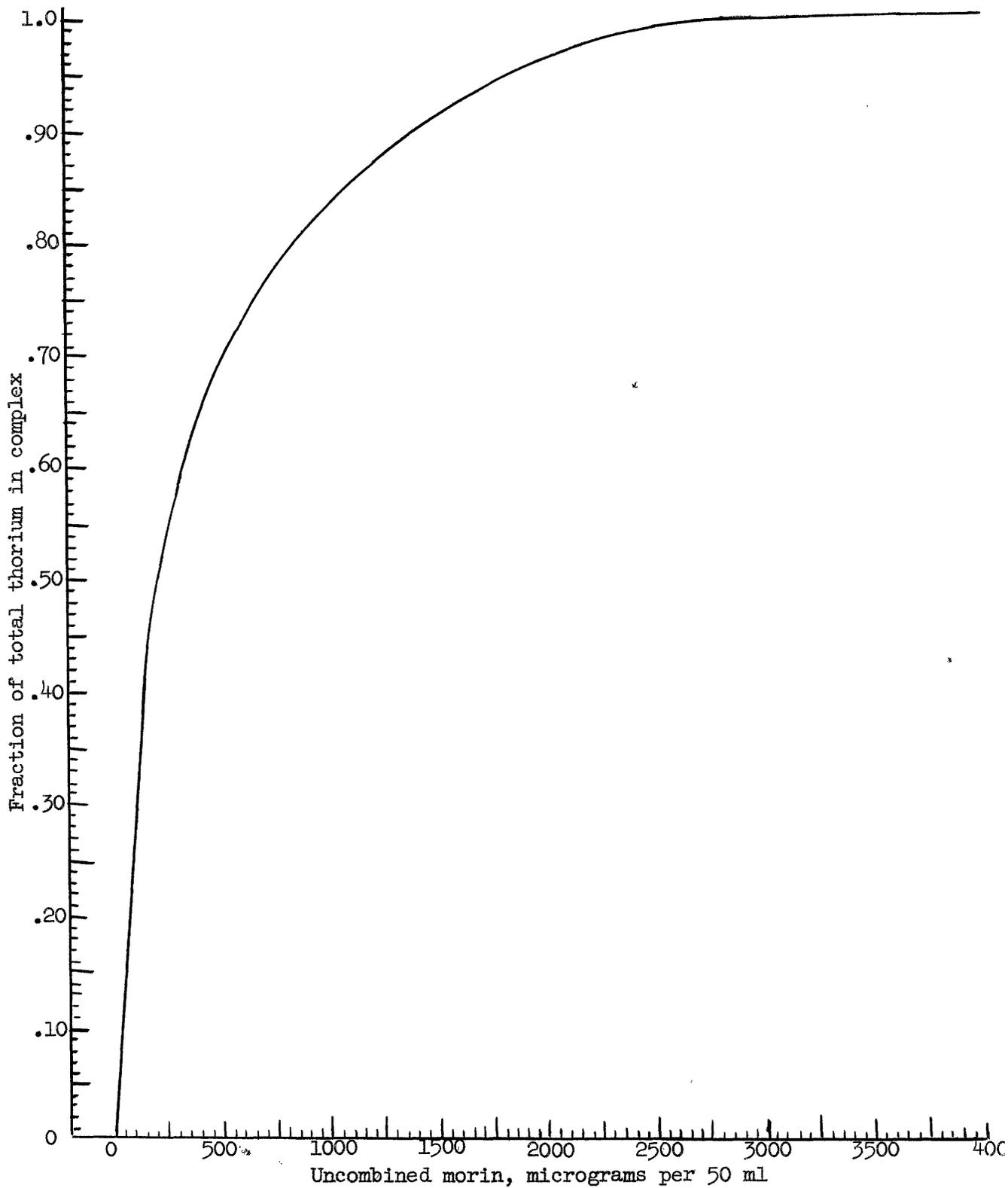


Figure 11.--Fraction of total thorium converted to complex as a function of uncombined morin in the solution. Thorium constant at 50.3 micrograms of ThO_2 per 50 ml.

represent examples of System II, (equation 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 wavelength is 404.7 μ :

$$\text{Total fluorescence} = \frac{K_3 P_0 (a_3 CR)}{a_2 M \cdot H + a_3 CR} [1 - 10^{-(a_2 M \cdot H + a_3 CR)b}]$$

where $K_3 P_0 = 3.17$ (for a total alcohol content of 2 ml)(1.0 scale)

$= 5.14$ (for a total alcohol content of 12 ml)(1.0 scale)

$a_2 = (6.179 \times 10^{-5}) =$ absorbance of 1 microgram of morin/50 ml,
1 cm light path

$a_3 = (9.404 \times 10^{-4}) =$ absorbance of 1 microgram of complex/50 ml,
1 cm light path

$M \cdot H =$ uncombined morin, micrograms/50 ml

$C =$ maximum complex possible from 50.3 micrograms ThO_2

$= 158.9$ micrograms

$R =$ fraction of total thorium reacted, represented either by equation 10 or 11.

$b = 3.241$ 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 figures 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 figure 12. Curve A, figure 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:

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

and for curve B, the expression is

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

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 figure 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 ThO₂ 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.

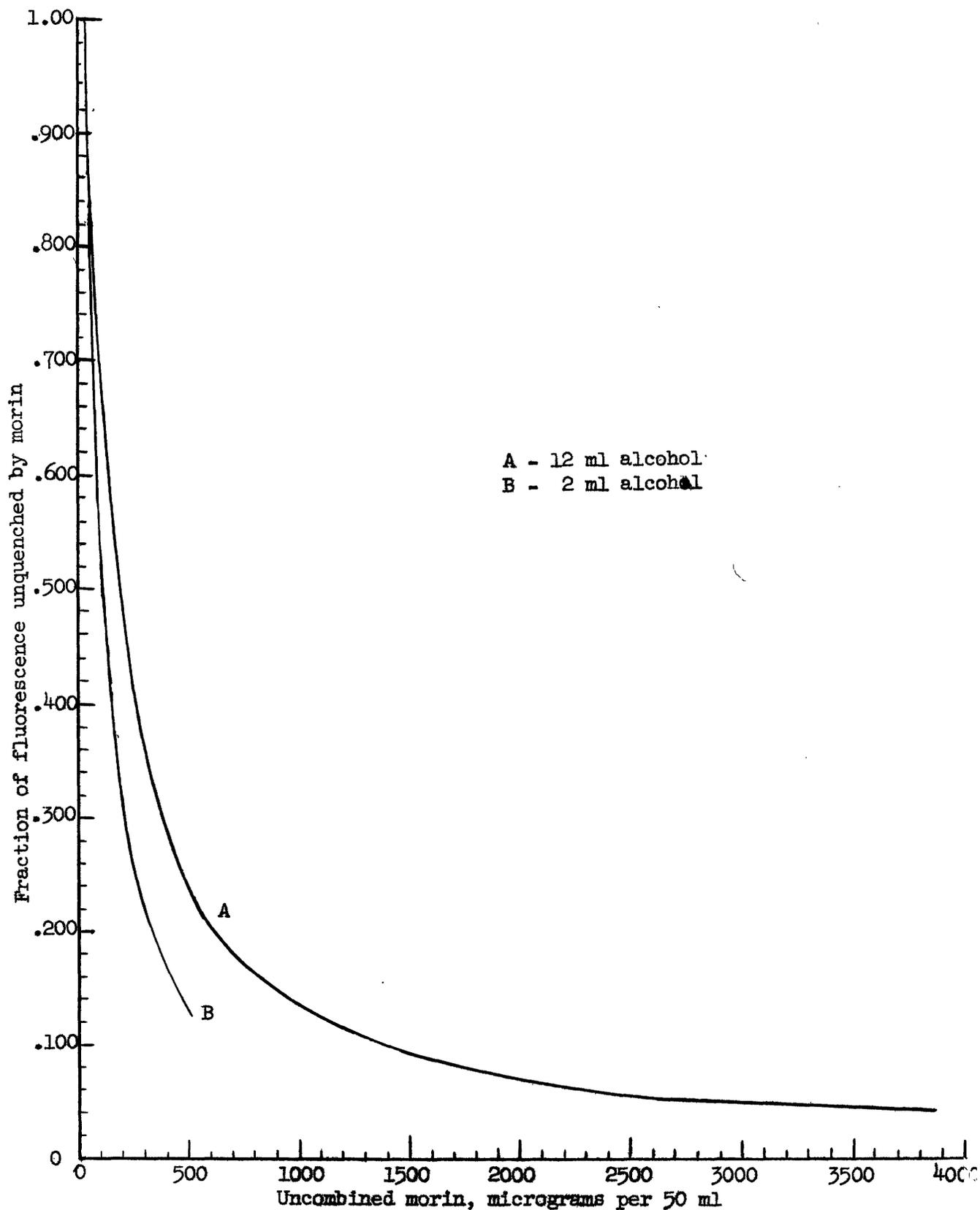


Figure 12.--Fraction of fluorescence remaining unquenched as a function of the uncombined morin. Thorium constant at 50.3 micrograms of ThO_2 per 50 ml. Curve A, 12 ml of alcohol; curve B, 2 ml of alcohol; P_0 (404.7 μ).

$$F = \left[0.861 - \frac{M \cdot H - 43.16}{61 + 1.115 M \cdot H} \right] \frac{K_3 P_0 (a_3 CR)}{a_2 M \cdot H + a_3 CR} [1 - 10^{-(a_2 M \cdot H + a_3 CR)b}] \dots (12)$$

where C = maximum complex possible from the ThO₂ present in micrograms per 50 ml of solution

R = fraction of total ThO₂ reacted to form complex

CR = complex present in solution in micrograms 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 micrograms ThO₂ per 50 ml and varying amounts of total morin; and the solutions of the other series containing a total of 15 micrograms ThO₂ per 50 ml and varying amounts of total morin.

Then, using equation 12, 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 figure 5. Figure 13 shows the portions of the calculated curves, between 50 and 200 micrograms 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 micrograms; furthermore, the maximum variation in fluorescence is less than 5 percent when morin varies from 75 to 130 micrograms.

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

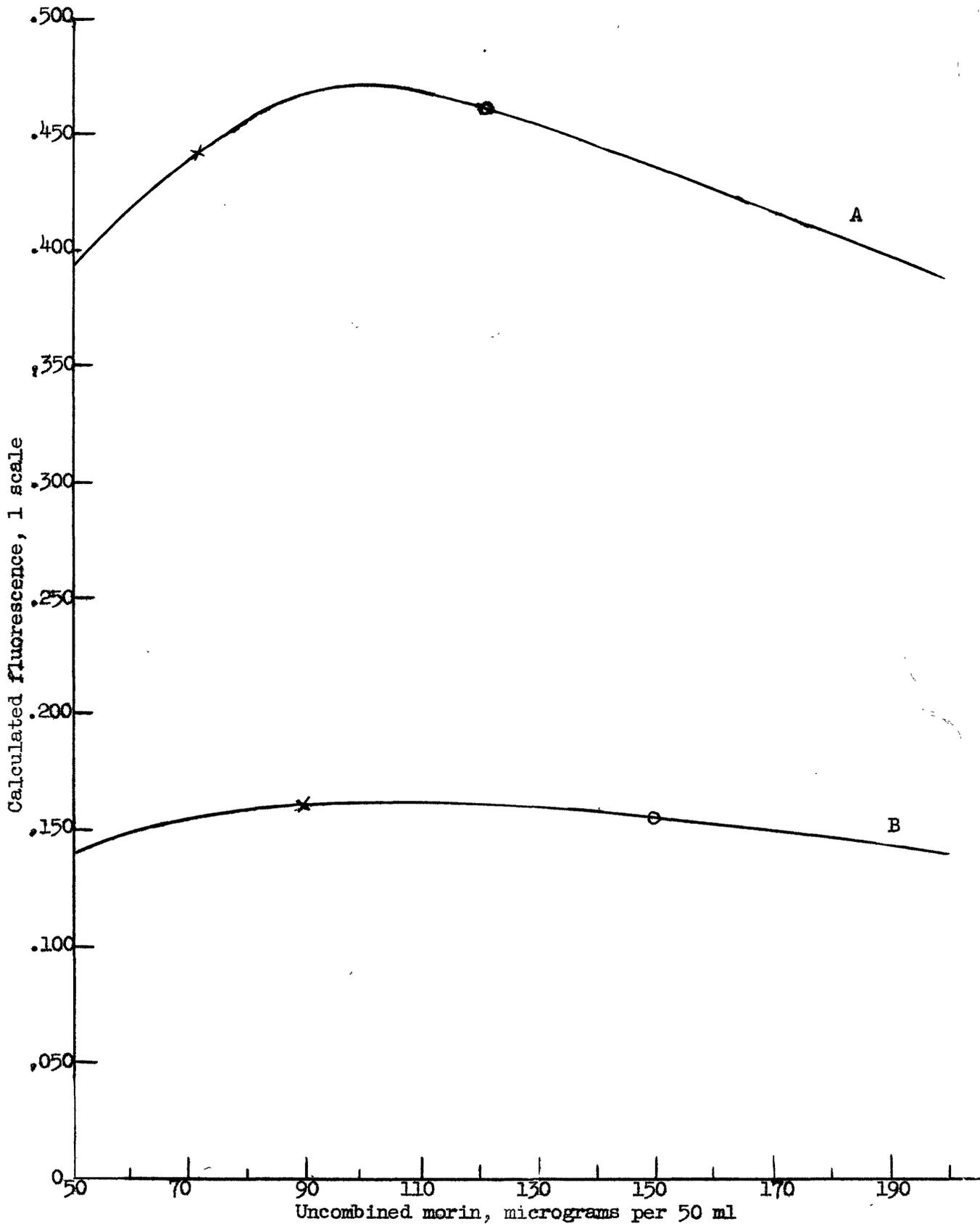


Figure 13.--Calculated fluorescence as a function of uncombined morin in the solutions. Curve A, total thorium 50.3-micrograms of ThO₂ per 50 ml; curve B, total thorium 15 micrograms of ThO₂ per 50 ml; 2 ml of alcohol; P₀ (404.7 mμ).

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 micrograms per 50 ml, then close to maximum sensitivity and linearity for the standard curve are attained. A choice of 100 micrograms total morin content results in values for uncombined morin near the lower limit of this range (see crosses in figure 13) and a choice of 165 micrograms of total morin results in uncombined morin values near the upper limit of the range (circles in figure 13).

Calculation of standard curve

Equation 12 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 15 micrograms of ThO₂. 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·H) = total morin, moles per liter
- (M·H - 2CR) = uncombined morin, moles per liter
- (ThO₂) = total thorium, moles per liter
- (ThO₂ - CR) = uncombined thorium, moles per liter
- K_E = equilibrium constant.

K_E had been determined previously (2); 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 micrograms, and 0.95×10^6 when total morin is 165 micrograms.

The calculated standard curves are shown in figures 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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APPENDIX

Tables 3 - 10

Table 3.---Absorptivity values for morin and the complex.

Units for ϵ = liter/(mole cm), i.e., ϵ = absorbance for 1 mole/liter, 1 cm light path
 Units for a = 50 ml/(microgram cm), i.e., a = absorbance for 1 microgram/50 ml, 1 cm light path.

Exciting light 404.7 m μ		Exciting light 365.0 m μ	
For solutions of the pure complex $\frac{1}{l}$			
$\epsilon_{ThM_2} = 3.924 \times 10^4$		$\epsilon_{ThM_2} = 1.580 \times 10^4$	
$a_{ThM_2} = 9.404 \times 10^{-4}$		$a_{ThM_2} = 3.786 \times 10^{-4}$	
For solutions of pure morin			
Alcohol content 2 ml	Alcohol content 12 ml	Alcohol content 2 ml	Alcohol content 12 ml
$\epsilon_{M \cdot H} = 6.849 \times 10^2$	$\epsilon_{M \cdot H} = 8.689 \times 10^2$	$\epsilon_{M \cdot H} = 1.148 \times 10^4$	$\epsilon_{M \cdot H} = 1.367 \times 10^4$
$a_{M \cdot H} = 4.500 \times 10^{-5}$	$a_{M \cdot H} = 5.138 \times 10^{-5}$	$a_{M \cdot H} = 6.79 \times 10^{-4}$	$a_{M \cdot H} = 8.08 \times 10^{-4}$
For solutions containing excess morin in the presence of complex			
	$\epsilon_{M \cdot H_1} = 1.045 \times 10^3$		
	$a_{M \cdot H_1} = 6.179 \times 10^{-5}$		

$\frac{1}{l}$ Absorptivity of pure complex the same for 2 ml and 12 ml of alcohol.

Table 4.--Fraction of total ThO_2 (50.3 micrograms) in the form of complex, when concentration of morin ranges from 0-4000 micrograms per 50 ml.

Total morin added $\gamma/50$ ml	Uncombined morin $\gamma/50$ ml	Complex formed $\gamma/50$ ml	Fraction of total possible complex formed <u>1/</u>
0	0.	0.	0.
5	3.6	1.68	0.010
10	7.2	3.36	0.021
20	14.5	6.74	0.042
40	29.0	13.5	0.084
60	43.1	20.8	0.130
80	57.5	27.7	0.174
100	71.6	34.9	0.219
120	87.5	41.3	0.260
140	102.	47.4	0.298
160	117.	53.2	0.334
180	132.4	58.6	0.368
200	149.	63.5	0.399
220	165.	68.1	0.428
240	182.	72.1	0.453
260	198.	76.0	0.478
280	216.	79.3	0.498
300	234.	81.7	0.514
340	269.	87.5	0.550
380	305.	93.1	0.585
400	323.	95.2	0.599
450	369.	99.9	0.628
500	416.	104.1	0.655
600	510.	111.2	0.700
800	701.	121.6	0.765
1000	895.	129.2	0.813
1200	1091.	135.0	0.850
1400	1287.	139.8	0.880
1600	1483.	144.6	0.909
1800	1680.	148.4	0.934
2000	1877.	151.3	0.952
2200	2075.	153.9	0.968
2400	2276.	155.9	0.981
2600	2473.	157.2	0.989
3000	2871.	158.6	0.998
3200	3071.	158.6	0.998
3400	3271.	158.6	0.998
3600	3471.	158.6	0.998
4000	3871.	158.6	0.998

1/ Total possible complex = 158.9 micrograms.

Table 5.--Fraction of total fluorescence remaining unquenched when 50.3 micrograms ThO_2 is mixed with amounts of morin ranging from 0-600 micrograms per 50 ml (2 ml of alcohol per 50 ml of solution).

Total morin $\gamma/50$ ml	Uncombined morin $\gamma/50$ ml	Total possible fluorescence (1. scale) $\frac{1}{}$	Fluorescence measured (1. scale)	Fraction of fluorescence unquenched
0	0.	0.	0.	
5	3.64	0.0382	0.042	1.10
10	7.27	0.0729	0.072	0.987
20	14.5	0.147	0.152	1.03
40	29.1	0.284	0.292	1.03
60	43.2	0.426	0.367	0.861
80	57.5	0.550	0.418	0.761
100	71.7	0.678	0.437	0.645
120	87.5	0.780	0.454	0.582
140	102.	0.876	0.458	0.523
160	117.	0.965	0.459	0.476
180	133.	1.03	0.454	0.439
200	149.	1.10	0.438	0.397
220	165.	1.15	0.423	0.365
240	182.	1.20	0.403	0.334
260	198.	1.25	0.383	0.306
280	216.	1.28	0.367	0.286
300	234.	1.30	0.350	0.269
340	269.	1.36	0.319	0.234
380	305.	1.42	0.290	0.204
400	323.	1.44	0.280	0.194
450	369.	1.46	0.251	0.172
500	416.	1.46	0.227	0.156
600	510.	1.47	0.187	0.127

$$\frac{1}{\text{Total possible fluorescence}} = \left(\frac{a_3 c_3}{a_2 c_2 + a_3 c_3} \right) (KP_0) (1-10^{-A})$$

$KP_0 = 3.17$ (1. scale).

Table 6.--Fraction of total fluorescence remaining unquenched when 50.3 micrograms ThO_2 is mixed with amounts of morin ranging from 0-4000 micrograms per 50 ml of solution (12 ml of alcohol per 50 ml of solution).

Total morin $\gamma/50$ ml	Uncombined morin $\gamma/50$ ml	Total possible fluorescence (1. scale) $\frac{1}{/}$	Fluorescence measured (corrected for blank) (1. scale)	Fraction of fluorescence unquenched
0				
5	3.64			
10	7.27			
20	14.5	0.235	0.232	0.987
40	29.1	0.462	0.452	0.979
60	43.2	0.690	0.562	0.814
80	57.5	0.892	0.682	0.764
100	71.7	1.10	0.852	0.775
120	87.5	1.28	0.957	0.750
140	102.	1.42	1.00	0.703
160	117.	1.56	1.03	0.658
180	133.	1.69	1.03	0.608
200	149.	1.79	1.03	0.575
220	165.	1.90	1.03	0.542
240	182.	1.97	0.992	0.503
260	198.	2.05	0.972	0.474
280	216.	2.11	0.942	0.447
300	234.	2.14	0.920	0.431
340	269.	2.24	0.874	0.391
380	305.	2.32	0.822	0.354
400	323.	2.36	0.794	0.336
450	369.	2.44	0.739	0.304
500	416.	2.45	0.677	0.276
600	510.	2.51	0.585	0.233
800	701.	2.59	0.463	0.179
1000	895.	2.57	0.378	0.147
1200	1091.	2.56	0.315	0.123
1400	1287.	2.54	0.267	0.105
1600	1483.	2.51	0.228	0.091
1800	1680.	2.46	0.196	0.080
2000	1877.	2.42	0.171	0.071
2200	2075.	2.38	0.151	0.063
2400	2276.	2.32	0.135	0.058
2600	2473.	2.26	0.122	0.054
3000	2871.	2.14	0.103	0.048
3200	3071.	2.08	0.096	0.046
3400	3271.	2.02	0.089	0.044
3600	3471.	1.97	0.084	0.043
4000	3871.	1.86	0.075	0.040

$$K_{P_0} = \frac{1}{5.14} \text{ (1. scale).}$$

$$\text{Total possible fluorescence} = \left(\frac{a_3 C_3}{a_2 C_2 + a_3 C_3} \right) (K_{P_0}) (1 \cdot 10^{-A})$$

Table 7.--Calculated fluorescence for solutions containing a total of 50.3 micrograms ThO_2 , and amounts of morin ranging from 50-200 micrograms per 50 ml (2 ml of alcohol per 50 ml of solution).

Total ThO_2 $\gamma/50$ ml	Uncombined morin $\gamma/50$ ml	Calculated fluorescence (1. scale)
50.3	50	0.394
50.3	60	0.421
50.3	70	0.436
50.3	80	0.457
50.3	90	0.466
50.3	100	0.473
50.3	110	0.468
50.3	120	0.462
50.3	130	0.452
50.3	140	0.444
50.3	150	0.436
50.3	160	0.424
50.3	170	0.411
50.3	180	0.406
50.3	190	0.395
50.3	200	0.388

Table 8.--Calculated fluorescence for solutions containing a total of 15 micrograms of ThO_2 and amounts of morin ranging from 50-200 micrograms of morin per 50 ml (2 ml of alcohol per 50 ml of solution).

Total ThO_2 $\gamma/50$ ml	Uncombined morin $\gamma/50$ ml	Calculated fluorescence (1. scale)
15	50	0.1403
15	70	0.1532
15	75	0.1560
15	80	0.1586
15	85	0.1599
15	90	0.1611
15	100	0.1616
15	110	0.1618
15	120	0.1614
15	125	0.1596
15	130	0.1574
15	140	0.1572
15	150	0.1550
15	170	0.1482
15	200	0.1412

Table 9.--Calculations of complex formed (micrograms per 50 ml) for solutions containing, (a) a total of 100 micrograms morin and ThO₂ ranging from 10 to 60 micrograms, (b) a total of 165 micrograms morin and ThO₂ ranging from 10 to 90 micrograms.

	Total morin γ/50 ml	Total ThO ₂ γ/50 ml	K _F used in calculations	Complex formed γ/50 ml
(a)	100	10	1.39 x 10 ⁺⁶	9.0
	100	15	1.41 x 10 ⁺⁶	13.5
	100	20	1.44 x 10 ⁺⁶	17.2
	100	30	1.48 x 10 ⁺⁶	24.3
	100	40	1.524 x 10 ⁺⁶	29.3
	100	50	1.57 x 10 ⁺⁶	34.9
	100	60	1.61 x 10 ⁺⁶	39.3
(b)	165	10	0.850 x 10 ⁺⁶	12.8
	165	15	0.875 x 10 ⁺⁶	18.9
	165	20	0.90 x 10 ⁺⁶	24.1
	165	30	0.950 x 10 ⁺⁶	36.3
	165	40	1.0 x 10 ⁺⁶	46.0
	165	45	1.025 x 10 ⁺⁶	51.2
	165	50	1.05 x 10 ⁺⁶	54.8
	165	60	1.10 x 10 ⁺⁶	63.0
	165	70	1.15 x 10 ⁺⁶	70.9
	165	80	1.20 x 10 ⁺⁶	78.2
	165	90	1.25 x 10 ⁺⁶	84.1

Table 10.--Calculated fluorescence for standard curves--100-165 micrograms of morin per 50 ml (2 ml of alcohol per 50 ml of solution).

Total morin γ/50 ml	Total ThO ₂ γ/50 ml	Calculated fluorescence (l. scale)
100	10	0.106
100	15	0.161
100	20	0.208
100	30	0.300
100	40	0.372
100	50	0.445
100	60	0.511
165	10	0.102
165	15	0.153
165	20	0.197
165	30	0.292
165	40	0.372
165	45	0.424
165	50	0.459
165	60	0.533
165	70	0.612
165	80	0.686
165	90	0.751