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ABSTRACT

A SPECTROFLUOROMETRIC AND SPECTROPHOTOMETRIC STUDY OF THE COMPLEXES OF MORIN WITH GADOLINIUM(III) AND LUTETIUM(III)

by Jack Edward Weiler

Gadolinium (III) and lutetium (III) both form fluorescent complexes with morin. The various factors which affect the fluorescence of these complexes in a dioxane-water solvent system were investigated. The nature of these complexes was also investigated.

Gadolinium and lutetium form 1:2 complexes with morin, which show maximum fluorescence intensity at pH 4.25 and 3.75, respectively. The equilibrium constants for these complexes are 14.8 and 32.9, respectively.

The fluorescence intensity peaks for both complexes are between 505 and 510 mμ and their absorption peaks are at 410 mμ. The morin absorption peak is at 355 mμ and it is nonfluorescing. Excitation radiation of 436 mμ produces somewhat more fluorescence intensity than does 365 mμ. Increasing dioxane content in the solution increases the fluorescence intensity. The fluorescence intensity and absorbance increase linearly with increasing gadolinium or lutetium concentration over a range of 0 to 100 γ in the presence of 400 γ morin per 25 ml. of 50-50 dioxane-water solution. Higher concentrations of morin result in concentration quenching.

The addition of europium, holmium, and ytterbium, which form nonfluorescing complexes with morin, produce changes in the fluorescence intensity of the complexes of gadolinium and lutetium. Initially the fluorescence produced by 365 mμ radiation increases due to the reduction

of free morin, while that produced by 436 mμ radiation remains relatively unchanged. After approximately 60 γ of nonfluorescing lanthanide ion is added, in the presence of 400 γ morin and 35 γ gadolinium or lutetium per 25 ml. the fluorescence produced by the 365 mμ radiation is relatively unaffected by further addition while that produced by the 436 mμ radiation decreases due to absorption by the additional complex formed.

Tartrate, oxalate, sulfate, and phosphate anions destroy the complexes. Chloride, bromide, iodide, carbonate, acetate, and tetraborate anions show little effect in 1.0 mg per 25 ml concentration.

Instrumental variations can produce a satisfactory linear concentration fluorescence intensity relationship in the 0-10 γ region.

A SPECTROFLUOROMETRIC AND SPECTROPHOTOMETRIC STUDY OF THE COMPLEXES OF MORIN WITH GADOLINIUM(III) AND LUTETIUM(III)

By

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INTRODUCTION

Many compounds are photoluminescent. Photoluminescence is the general term which describes the emission of radiant energy by a compound after having first absorbed radiant energy. If the time delay between the absorption and emission is short, that is to say of the order 10^{-8} seconds, the effect is more specifically termed fluorescence.

The emitted energy is usually of longer wavelength than the excitation wavelength. The excitation radiation excites the molecule to one of the many available vibrational energy levels of an excited electronic singlet state. In the excited molecule any vibrational energy above the lowest vibrational energy level of the excited electronic state is quickly lost by collisions or changes in mode of vibration leaving the molecule in the lowest vibrational energy level of the first excited electronic state. The molecule then returns to some vibrational level of the ground state with the loss of energy which results in fluorescent radiation of longer wavelength than the exciting radiation (14, 15, 35).

The equation $I_f = Q\, I_0(1-10^{-abc})$ relates the fluorescence intensity, I_f, to the incident radiation intensity, I_0, the quantum efficiency of the absorbed radiation in producing fluorescence, Q, the absorptivity, a, path length, b, and the concentration, c. For low concentrations, it may be shown that the equation simplifies to $I_f = QI_0abc$ (5, 35) and the fluorescence intensity is proportional to the concentration.

Many of the tripositive lanthanide ions in their salts fluoresce. Gadolinium is in this group. Some, however, yield no fluorescence as pure salts and lanthanum and lutetium are members of this group (21).

Only three tripositive lanthanide ions form fluorescent complexes with morin (2',4', 3, 5, 7 pentahydroxyflavone). They are lanthanum, gadolinium, and lutetium (19). The electronic structure of the other lanthanide ions prevents fluorescence by the complexes formed through intramolecular energy transfer (7, 16, 26, 36) which results in internal quenching. The three ions which yield fluorescent complexes have an empty, half-full, and full 4f shell.

It was the purpose of this investigation to extend the systematic study of the fluorescent morin complexes of the lanthanide series begun by Fleck (7). Factors effecting fluorescence such as pH of solution, solvent composition, reagent concentration, excitation conditions, and instrumental conditions were to be investigated. In addition, the nature of the complex was to be investigated through spectrophotometric measurements.

HISTORICAL

Many books, chapters of books, and articles have been written which are devoted to fluorescence and cover the field rather completely. A few books which are readily available are: "Fluorescence and Phosphorescence" by P. Prigsheim (21), "Fluorescence of Solutions" by E. Bowen (3), and "The Chemical Aspects of Light" by E. Bowen (2) give a fairly complete coverage of the subject. In addition review articles by White appear biyearly in Analytical Chemistry covering the field of organic and inorganic fluorescence and instrumental development (28, 29, 30, 31, 32, 33, 34). This series is an excellent source of references.

Fleck (7) in the initial phase of this work compiled an excellent bibliography on the fluorescence of cation complexes of morin. Morin has been tested as a reagent for the fluorometric detection of a large number of cations (1, 4, 10, 20, 22, 23, 27). Included in this group are Be(II), Y(III), Sc(III), La(III), Li(I), Th(IV), Zr(IV), Al(III), Ga(III), Ge(IV), Sn(IV), Sb(IV), Zn(II), Gd(III), and Lu(III).

Milkey and Fletcher (17, 18) conducted an investigation of the thorium(IV)-morin complex in slightly acidic ethanol-water mixtures. This investigation and the work of Fleck (7) were used as a pattern for this work in hopes that comparative data could be obtained.

EXPERIMENTAL

INSTRUMENTATION

Spectrofluorometer

Fleck (7), of this laboratory, modified a spectrofluorometer constructed in this laboratory by Thommes (24). This instrument was used throughout this investigation. A block diagram of this instrument is shown in Figure 1. The components are listed here:

Ultraviolet Source

A Hanovia S-H high pressure mercury arc with a Hanovia 110/120 volt constant voltage transformer was used as an ultraviolet source. This source provides seven mercury lines (436, 405, 365, 313, 303, 265, and 254 mμ) which are intense enough for use in fluorescence work.

Ultraviolet Monochromator

A Bausch and Lomb Model 33-86-40 grating monochromator was connected to the cell compartment with a light-tight 0-ring gasket.

Spectrofluorometer

A Beckman Model DU Spectrophotometer modified for use as a spectrofluorometer as indicated in the block diagram was used. The light-tight cell compartment was equipped with a quartz cylindrical converging lens to produce a parallel beam of ultraviolet light focused on the center of the sample cell.

Sample Cell

Two clear window silica cells 10 x 20 x 50 mm, purchased from the Farrand Optical Company were used. The cells were reproducibly positioned in the cell compartment by means of a 10 x 20 mm cell holder situated so that the ultraviolet beam struck the 10 mm side. These cells are transparent to the above listed excitation radiation.

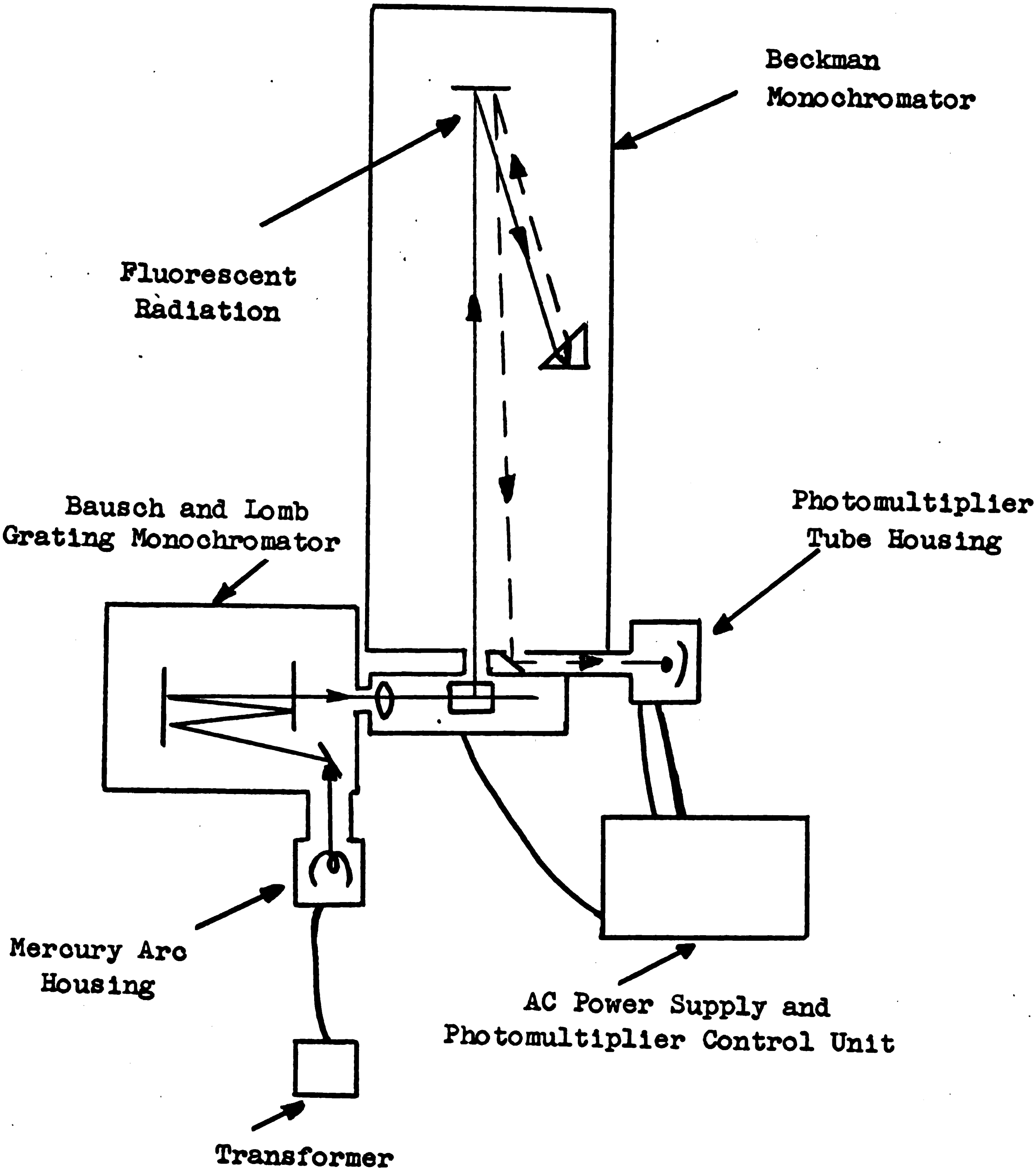

Figure 1. Block Diagram of Spectrofluorometer

Spectrophotometer

The Beckman Models DU and DK-2 spectrophotometers were used for the absorption measurements. The Model DU was used for the single wavelength measurements for the calculation of K_{eq} and the composition studies. A matched pair of 0.988 cm silica cells was used with these instruments.

Constant Temperature Bath

A Sargent constant temperature bath, equipped with a thermoregulator, No. 7530, and a heater control unit, No. S-2770, was set to maintain a temperature of $25 \pm 0.2^{\circ}$.

pH Meter

A Beckman Model G pH meter equipped with a glass-saturated calomel microelectrode pair, was standardized with Beckman standard pH 4 buffer solution.

REAGENTS

Ammonium Hydroxide

Reagent grade ammonium hydroxide was distilled and stored in a polyethylene bottle as an approximately 0.1 M solution. It was protected by an ascarite absorption bulb.

Dichlorofluorescein

Eastman Kodak White Label.

Dioxane

A modification of the procedure of Hess and Frahm (12) was used to purify commercial grade dioxane. A mixture of 4 l. of dioxane, 400 ml.

of distilled water, and 54 ml. of concentrated hydrochloric acid was refluxed for 12 hours. A slow purge of nitrogen was maintained by means of a bubbler. After cooling, potassium hydroxide pellets were slowly added, with stirring, until the solution was saturated and separated into two layers. The dioxane was decanted and treated with additional potassium hydroxide. The partially dried dioxane was then decanted into a screw-cap bottle containing anhydrous calcium chloride. After standing at least 24 hours the dioxane was filtered into a 5 l. round-bottom flask, refluxed over calcium hydride for 12 hours and distilled. A 50 ml. forecut and about 300 ml. heel were discarded. The product distilled at $100 \pm 0.5^{\circ}$ and was stored in a screw-cap brown bottle in the dark.

Europium Sesquioxide

Heavy Metals Co., Chattanooga, Tennessee. Purity unknown.

Gadolinium Sesquioxide

Michigan Chemical Corp., St. Louis, Michigan. Labeled 99.9% pure.

Holmium Sesquioxide

Michigan Chemical Corp., St. Louis, Michigan. Labeled 99.9% pure.

Hydrochloric Acid

Bakers reagent grade.

Lutetium Sesquioxide

Michigan Chemical Corp., St. Louis, Michigan. Labeled 99.9% pure.

Morin Dihydrate

Doctor Theodor Schuchardt, München, Germany.

Perchloric Acid

Bakers analyzed reagent grade, 70-72%.

Sodium Salts

The various sodium salts used to determine the effects that different anions have on the fluorescence of the complex were all reagent grade.

Water

All of the water used for the fluorescence work was distilled water that had been passed through a "Crystalab Demineralizer" ion exchange column to remove any cations present.

Ytterbium Sesquioxide

Michigan Chemical Corp., St. Louis, Michigan. Labeled 99.9% pure.

PREPARATION OF REAGENT SOLUTIONS

Ammonium Hydroxide

The ammonium hydroxide used for pH adjustment was about 0.03N and was prepared from the distilled material.

Dichlorofluorescein

A 0.4 γ per ml solution in 4% ethanol was prepared by dilution of a stock solution prepared by dissolving a weighed quantity of the reagent in 95% ethanol.

Lanthanide Sesquioxide

Portions of the various sesquioxides were ignited to constant weight in platinum crucibles at 750 ± 25°. The ignited sesquioxide was dissolved in a measured amount of perchloric acid so that the final solution had a 0.2 M perchlorate ion concentration and about 0.02 M cation concentration. Due to the purity of the reagents these solutions were not standardized. However, the hydrolysis curve of lanthanum (III) perchlorate shown by Fleck (7) and Freiser *et al.* (15) indicates a distinctive separation between the titration of the excess perchloric acid and the hydrolysis of the lanthanide perchlorate. A typical hydrolysis curve is shown in Figure 2. The concentrations based on the weight of sesquioxide used and the amount titrated are shown.

	Weight	Titration
Europium (III)	0.0197 M	0.0189 M
Gadolinium (III)	0.0243 M	0.0240 M
Holmium (III)	0.0220 M	0.0218 M
Lutetium (III)	0.0228 M	0.0227 M
Ytterbium (III)	0.0213 M	0.0209 M

Aliquots of these solutions were taken for the preparation of the working solutions of 1.0, 0.1, 0.01 and 0.001 mg. of lanthanide sesquioxide per ml.

Morin

A weighed portion of the reagent was dissolved in purified dioxane to produce a solution of 0.00148 moles per liter (0.5 mg/ml).

Perchloric Acid

The perchloric acid used for pH adjustment was about 0.04 N and was prepared by dilution of the reagent acid.

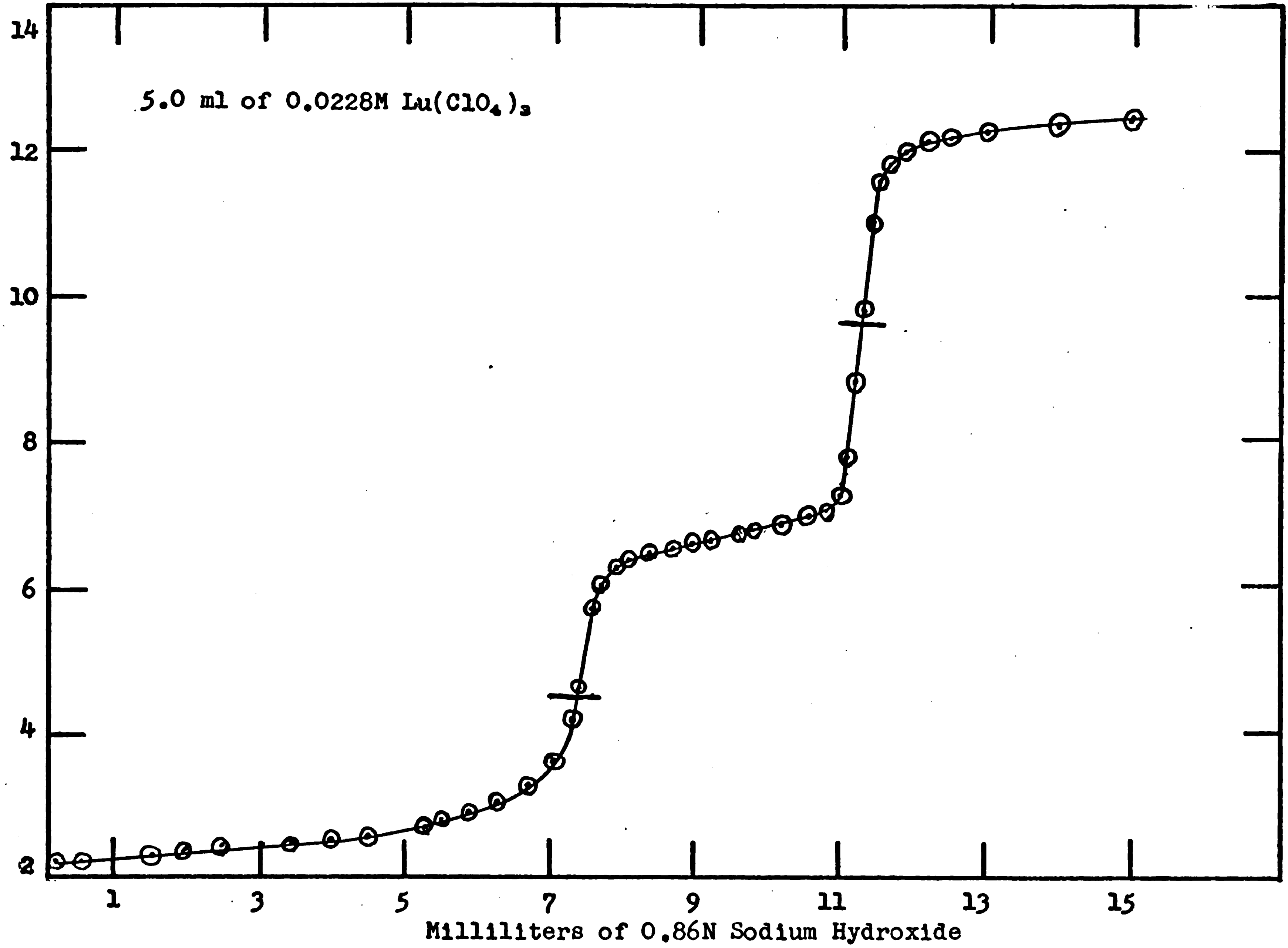

Figure 2. Hydrolysis Curve of Lutetium Perchlorate - Perchloric Acid

EXPERIMENTAL PROCEDURES

To establish the parameters affecting the fluorescence of the complexes of gadolinium and lutetium with morin, a standard approach was adopted. This approach was based on the results obtained in the investigation of lanthanum and morin by Fleck (7). The variable being investigated was altered while the other possible variables were held constant.

Method of Sample Preparation

The lanthanide perchlorate solution[1] was measured quantitatively into a 50 ml beaker and the morin solution quantitatively added to it. Pure dioxane was added to give a total dioxane volume of 12.5 ml. Demineralized water was added to give a total solution volume of approximately 21-22 ml. The pH of the solution was adjusted with dilute NH_4OH and/or $HClO_4$ to within ± 0.3 pH units of the desired pH and the solution was transferred to a 25 ml. volumetric flask.[2] After one hour in a 25° thermostated water bath the solution was diluted to volume and thoroughly mixed. The final pH adjustment was made by the addition of small increments of dilute NH_4OH and/or $HClO_4$ (less than 0.1 ml). These additions were made by using 1 ml. hypodermic needles and syringes. The solution was returned to the thermostated bath until needed for spectrofluorometric or spectrophotometric measurement.

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[1] This investigation covers the fluorescence characteristics of both the gadolinium and lutetium morin complexes. All of the work was carried out in exactly the same manner in both cases. For simplicity and ease of reading the term R_2O_3--meaning gadolinium or lutetium sesquioxide and lanthanide ion--meaning Gd(III) and Lu(III) will be used. In all cases reference to holmium, europium or ytterbium will be specific.

[2] All work in this investigation was carried out using 25 ml. volumetric flasks unless otherwise specifically stated.

Instrumental Measurements

Spectrofluorometric Measurements

All spectrofluorometric measurements were made with the instrument described in the instrumentation section and shown in Figure 1. The mercury arc, AC power supply and Beckman DU were turned on and allowed to warm up for at least thirty minutes to insure instrumental stability. The photomultiplier sensitivity control knob was set to the "full" position. The ultraviolet monochromator entrance and exit slits were set to 1.0 and 0.5 mm. respectively.

The ultraviolet monochromator was adjusted to pass the 365 mμ mercury line. The Beckman DU sensitivity knob was set three turns from the clockwise position, the selector switch to the 0.1 position, and the Beckman monochromator for 530 mμ. The percent T scale was set to 50. The 0.4 γ per ml dichlorofluorescein solution was placed in the cell, the shutter opened and the instrument balanced by adjustment of the Beckman entrance slit. Under the conditions used, the normal slit setting was 1.1 ± 0.05 mm.

After standardization, as indicated above, the Bausch and Lomb, and the Beckman monochromators were adjusted to the desired wavelength and measurement of the samples fluorescence recorded as "% T" readings.

Spectrophotometric Measurements

All spectrophotometric measurements not used for calculations of the K_{eq} for the complex by the slope-ratio method, were carried out on the Beckman DK-2 using the standard procedure and silica cells. The data for the K_{eq} and the slope-ratio method were obtained on a Beckman DU using standard procedures.

pH Measurements

All pH measurements were made with a Beckman Model G battery operated pH meter. The instrument was allowed to warm up at least one-half hour. It was adjusted using standard Beckman pH 4.0 solution. All readings were made to within ± 0.01 pH units. The instrument was equipped with micro electrodes. The sample was in the closed shielded compartment during the reading period.

Parameters Affecting Fluorescence

Effect of pH of the Solution

Solutions containing 0.1 mg of R_2O_3[1] and 0.4 mg of morin were adjusted to the desired pH readings with dilute NH_4OH and/or $HClO_4$. Twenty-eight solutions covering the range of 2.7-10.0 pH units were examined. The fluorescence of these solutions was measured at 510 mμ under an excitation of 365, 405, and 436 mμ. Several of these solutions were scanned to obtain the fluorescence spectra. The absorbance of some of these solutions was measured.

Morin Concentration

Solutions containing 0.1 mg of R_2O_3 and amounts of morin ranging from 0 to 2.0 mg were adjusted to pH 4.25 ± 0.05. The fluorescence and absorbance measurements were made. Fluorescence excitation wavelengths were 365, 405, and 436 mμ.

R_2O_3 Concentration

Solutions of 0.4 mg of morin and R_2O_3 ranging from 0.005 mg to 1.0 mg were made and the pH adjusted to 4.25 ± 0.05. Fluorescence

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[1] This notation is used for simplicity. Standard stock working solutions were made to contain 1.0, 0.1, and 0.01 mg R_2O_3 per ml. This notation avoids the use of other units which would be different for the two sesquioxides. Data shown in figures, however, are noted as micrograms of lanthanide ion per 25 ml volume for ease of comparison with data by Fleck (7) on lanthanum-morin complex.

measurements were made under excitation of 365, 405, and 436 mμ. Absorbance measurements were made.

Anion Effect

The effect of 1.0 mg of ten common anions (chloride, bromide, iodide, sulfate, carbonate, tetraborate, phosphate, acetate, oxalate, and tartrate), all added as the sodium salts, on the fluorescence of the complex was determined. Solutions of 0.4 mg morin and 0.1 mg of La_2O_3, Gd_2O_3, or Lu_2O_3 were prepared and examined under 365 mμ excitation.

Composition of the Solvent

Solvent mixtures of from 3 to 90% dioxane in water[1] were investigated. Solutions were prepared which contained 0.1 mg of R_2O_3 and 0.4 mg of morin. The pH was adjusted as usual to 4.25 ± 0.05; however, the actual pH of the solutions of greater than 50% dioxane is somewhat doubtful. Freiser (8) indicated that solutions above 50% dioxane do not exhibit true pH. Several authors have stated that nearly true pH values are obtained below 50% dioxane (6, 8, 9). Fluorescence and absorbance measurements were made.

Concentration of Nonfluorescing Lanthanide Morin Complexes

The solutions used in this portion of the investigation contained 0.04 mg R_2O_3, 0.4 mg morin, and 12.5 ml dioxane and were adjusted to a pH of 4.25 ± 0.05. In addition these solutions contained amounts of either Ho_2O_3, Yb_2O_3, or Eu_2O_3 ranging from 0.01 to 1.00 mg. The fluorescence measurements were obtained under excitation of 365, 405, and 436 mμ. In the case of the gadolinium solutions the absorbance measurements, as well, were obtained on the Beckman DK-2.

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[1] No attempt was made to correct for the volume contraction on mixing dioxane and water. In all cases the percentage refers to dioxane content per total volume. Fleck (7) and Freiser (8) indicate the error to be about 2% at 25°.

Time Stability of the Complex

The fluorescence of a solution of 0.1 mg of R_2O_3 and 0.4 mg morin was examined closely over a period of six hours and then periodically for nine days.

Wavelength of Exciting Radiation

The fluorescence of a solution of 0.1 mg of R_2O_3 and 0.5 mg morin was examined under nine different wavelengths of exciting radiation. The Bausch and Lomb monochromator was adjusted to irradiate the sample cell with the following wavelengths; 254, 265, 297, 302, 313, 365, 405, 408 and 436 mμ.

Instrumental Variation to Obtain Greater Response Per Unit Concentration

Increased Illumination

The instrument was set up and standardized normally, then the Bausch and Lomb monochromator entrance and exit slits were increased from 1.0 and 0.5 mm respectively, to 2.0 and 1.0 mm, respectively. Solutions of 0.4 mg morin and 0.005 to 0.04 mg R_2O_3 were prepared and adjusted to pH 4.25 ± 0.05. Solutions of 0.4 mg morin and 0.005 to 0.04 mg La_2O_3 were prepared and adjusted to pH 5.5. The fluorescence of these solutions under excitation of 365, 405, and 436 mμ was measured.

Increased Illumination and Changed Standardization

The Bausch and Lomb monochromator entrance and exit slits were set at 2.0 and 1.0 mm. respectively, and the wavelength at 365 mμ. The normal 0.4 γ per ml dichlorofluorescein solution was used. On the Beckman DU, the sensitivity knob was adjusted to three-quarters of a turn from the counter clockwise position, the selector switch set to the 1.0 position and the percent T set on 35. The wavelength was set at 530 mμ and with the slit open the instrument balanced by slight adjustments of the Beckman entrance slit between 1.7 and 1.8 mm.

Samples containing 0.25 mg morin and a range of 0.0002 to 0.010 mg R_2O_3 were prepared and examined under excitation radiation of 365 and 436 mμ.

The instrument was adjusted to "normal" conditions and the solutions containing 0.005 and 0.010 were examined to give comparative measurements.

Nature of the Complex

Isoabsorptive Point

To determine whether more than one complex was being formed, fourteen solutions were prepared which contained 0.4 mg morin and from 0.01 to 1.00 mg of R_2O_3. These solutions were adjusted to pH 4.25 ± 0.05, and the absorbance measured from 350 to 450 mμ using the Beckman DK-2.

Some of these solutions were then readjusted to pH of 5.30 ± 0.1 and some to pH 7.0 ± 0.1 and the absorbance again measured.

Method of Continuous Variations

The method of continuous variations suggested by Job (13) and Vosburgh and Cooper (25) was employed to prove the nature of the complexes formed by the reaction of morin and either gadolinium or lutetium. Equal molar solutions of morin, gadolinium and lutetium were prepared by dilution of the master stock solutions. These solutions were 2.70×10^{-4} M. Twelve solutions of constant total molar concentration of the two reactants were prepared. The mole ratio (R^3/R^3+morin) was varied from 0.1 to 0.9. The pH of these solutions was adjusted to 4.25 ± 0.05 and the absorbance measured using the Beckman DU at 354 and 410 mμ. The "Y" values were calculated and plotted against the mole ratio.

Since, at higher pH values, the complexes no longer fluoresce, the above solutions were adjusted to a pH of 7.05 ± 0.05 by the addition of small amounts of dilute NH_4OH. The small error introduced by the increased volume was neglected. The absorbance was again measured. Since there is a shift in the absorption peak with increase in pH these solutions were measured at 395 and 435 mμ. The "Y" values were again calculated and plotted against the mole ratio.

Solutions containing one part lanthanide ion and 1, 2, or 3 parts morin were prepared and absorbance measured on the Beckman DK-2 from 500 to 340 mμ.

Slope Ratio Method

The slope ratio method of Harvey and Manning (11) was also used to confirm the nature of the complex. To accomplish this, the following investigations were conducted.

Lanthanide Concentration Variation in the Presence of Large Excess Morin.--Sixteen solutions containing gadolinium or lutetium in the concentration range of approximately 0.6×10^{-6} M/l to 10.5×10^{-6} M/l in the presence of a large excess morin (1.157×10^{-4} M) were prepared by the standard technique. The absorbance of these solutions was determined with the Beckman DU at a wavelength of 410 mμ. The data were plotted against concentration and the slope of the line determined.

Morin Concentration Variation in the Presence of Large Excess Lanthanide.--Twelve solutions containing morin in the concentration range of 5.9×10^{-6} to 6.5×10^{-5} M/l in the presence of a large excess of gadolinium (4.84×10^{-3} M/l) or lutetium (4.56×10^{-3} M/l) were prepared by the standard technique. The absorbance of these solutions was measured at 410 mμ with the Beckman DU. The data were plotted against the concentration and the slope of the line determined.

Equilibrium Constant of the Complex

An estimation of the equilibrium constant may be obtained by employing the technique used by Milkey and Fletcher (17) in their investigation of the thorium-morin complex. The following information was obtained in order to perform the calculations of the equilibrium constant of the complex under the conditions of the investigation.

Absorptivity of Morin

The absorptivity of morin under the following conditions was determined. Solutions were prepared which contained no lanthanide ions, 12.5 ml dioxane and had a final pH of 4.25 ± 0.05. These solutions contained morin covering the range of 1.177×10^{-5} M to 2.364×10^{-4} M. The absorptivity, (a) was calculated from the measurements made on the Beckman DU using the formula $a = A/bc$ where:

a - absorptivity of morin(expressed in l/mole-cm)
A - absorbance of the solution
b - cell length
c - concentration of morin in moles/liter.

Absorptivity of the Lanthanide Morin Complex

The absorptivity of the complex was calculated from the slope ratio data obtained in the presence of a large excess of the lanthanide ion using the above formula. This large excess insured the complete reaction of all of the morin added to form the complex and therefore one-half of the morin concentration could be related directly to the concentration of the complex.

Concentration of the Components in Solution After Complex Formation

The calculation employed by Milkey and Fletcher (17) to determine the concentration of the components of a solution of the complex was carried out on the data obtained in the slope ratio investigation.

The concentration of the complex was calculated as follows:

$$X = \frac{A - a_{MH}(MH)b}{b(a_{RM_2^+} - 2a_{MH})}$$

where,

X = moles of complex per liter
A = absorbance of the solution at 410 mμ
MH = total concentration of morin in moles/liter
a_{MH} = molar absorptivity for the morin at 410 mμ
$a_{RM_2^+}$ = molar absorptivity of the complex at 410 mμ
b = cell path length in cm (0.988 cm)

and, in addition, the concentration of the uncombined morin and lanthanide ion remaining in solution may be calculated as,

$$Y = (MH) - 2(X),$$

and

$$Z = (R_T^{+3}) - (X),$$

where,

Y = moles of uncombined morin/liter
Z = moles of uncombined lanthanide ion per liter
R_T^{+3} = total moles of lanthanide ion (Gd or Lu)/liter.

Calculation of the Equilibrium Constant

The information gained through the calculation of the absorptivities and the subsequent calculation of the concentrations of the components present in solution may be utilized in the calculation of the equilibrium constant of the complex under the conditions of this investigation; namely, a solution of 12.5 ml dioxane per 25 ml total volume at a pH 4.25 ± 0.05.

The calculations were made considering the reaction to be:

$R^{+3} + 2MH === R(M)_2^+ + 2H^+$ and the equilibrium constant to be:

$$K_{eq} = \frac{[R(M)_2^+][H+]^2}{[R+^3][MH]^2}$$

where,

$[R(M)_2^+]$ = X as calculated above

$[R^{+3}]$ = Z as calculated above

$[MH]$ = Y as calculated above

$[H+]$ = The hydrogen ion concentration calculated from the pH measurement of the solution

The solutions used for this calculation were those used to determine the nature of the complex by the method of continuous variations. Calculations were also carried out for the solutions used in the slope ratio method.

DISCUSSION OF RESULTS

General

Morin (2', 4',3,5,7 pentahydroxy flavone) forms complexes with gadolinium and lutetium which fluoresce when exposed to ultraviolet radiation. The absorption spectra of morin and the two complexes are shown in Figure 3, and the corresponding fluorescence spectra are shown in Figure 4. These spectra conform to those given by Fleck (7) and Milkey and Fletcher (17, 18). Figure 3 shows that there is an absorption peak at 355 mμ for the reagent and 410 mμ for both of the complexes. Figure 4 indicates a maximum fluorescence intensity for the complex at 505-510 mμ and essentially no fluorescence exhibited by the reagent under these conditions. Since both gadolinium and lutetium were treated identically, both will be discussed and compared under the same headings used in the experimental section.

Parameters Effecting the Fluorescence and Absorbance

Effect of pH of the Solution

Figures 5 and 6 show the effect that change in pH has upon the absorbance and fluorescence of the complex. The dip in the absorption spectra may be accounted for by considering two factors: 1) the absorption maxima of both the reagent and the complex tend to shift to higher wavelength with increase in pH, and 2) the change in pH above 6 effects much less shift so the curve tends to level off.

The increase in fluorescence noted under excitation of 365 mμ radiation at the higher pH values is due to the increased fluorescence of the reagent in this region.

It is of interest to note that the maximum fluorescence intensity of the gadolinium complex is found in the pH range of 4.0-4.75 while that of the lutetium complex is in the range of 3.50-4.25. This is considerably lower than that found by Fleck (7) for the lanthanum complex.

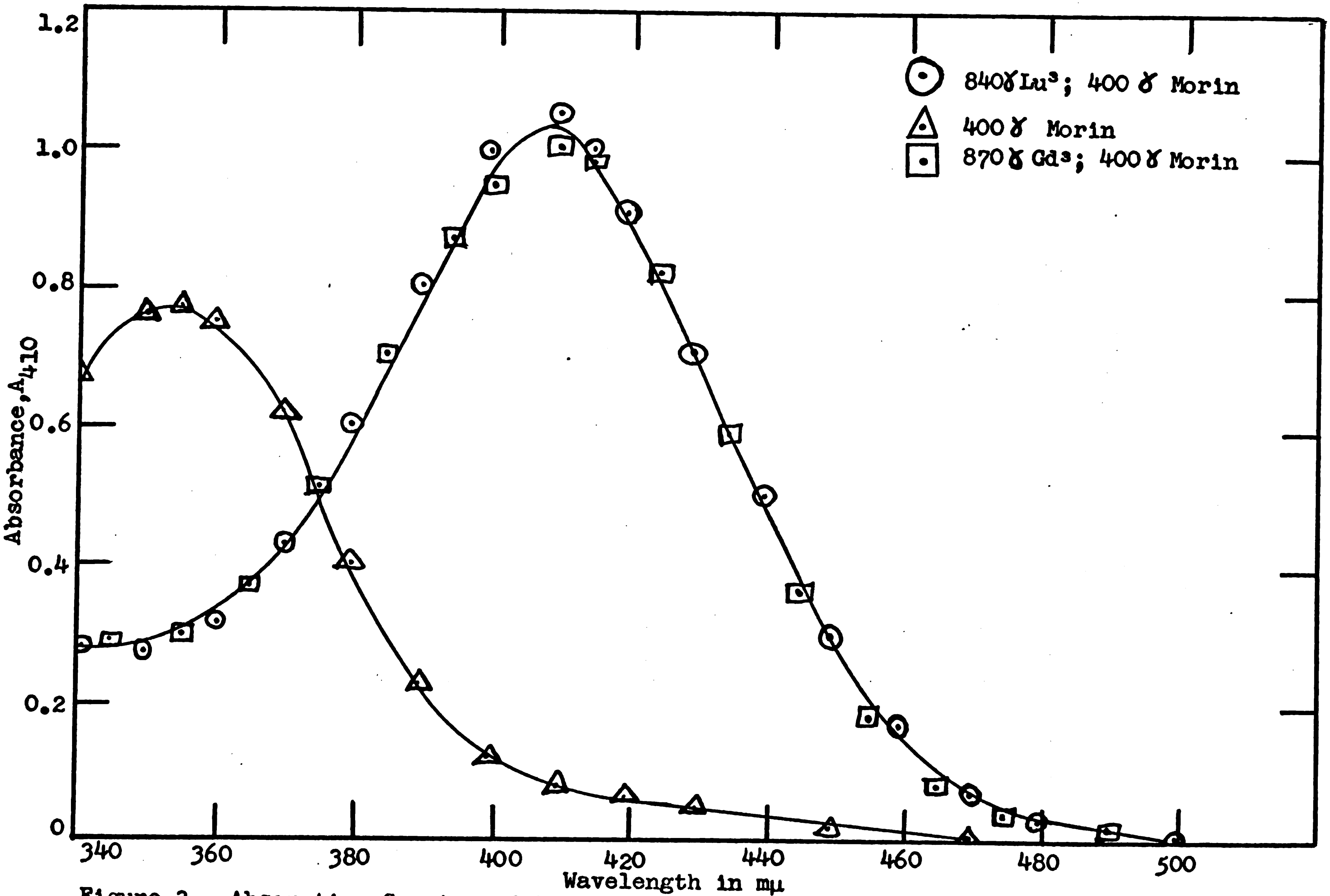


Figure 3. Absorption Spectra of Morin and the Complexes of Morin with Gadolinium(III) and Lutetium(III) in 50-50 DW at pH 4.25.

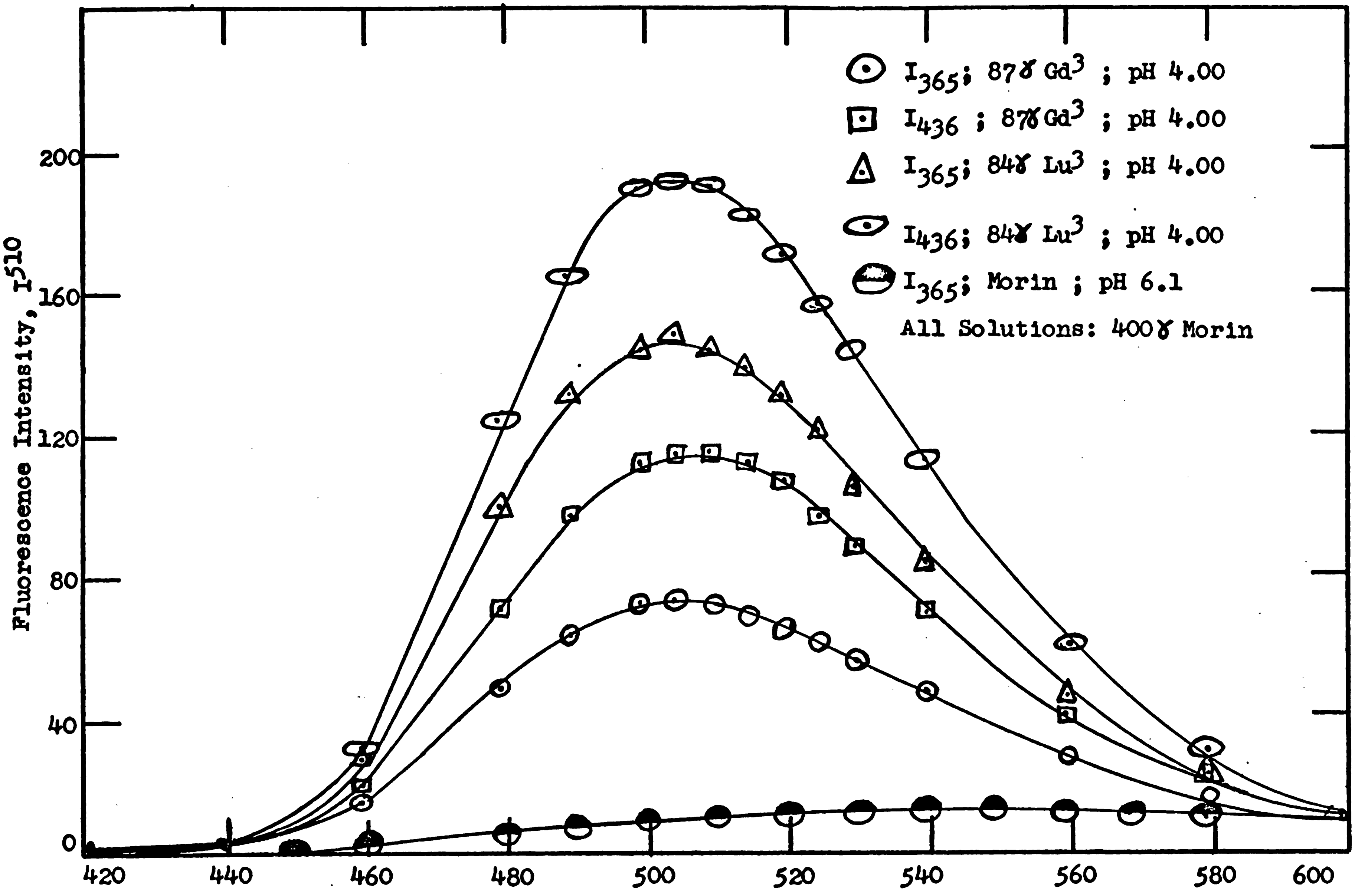


Figure 4. Fluorescence Spectra of Morin and the Complexes of Morin with Gadolinium(III) and Lutetium(III) in 50-50 DW.

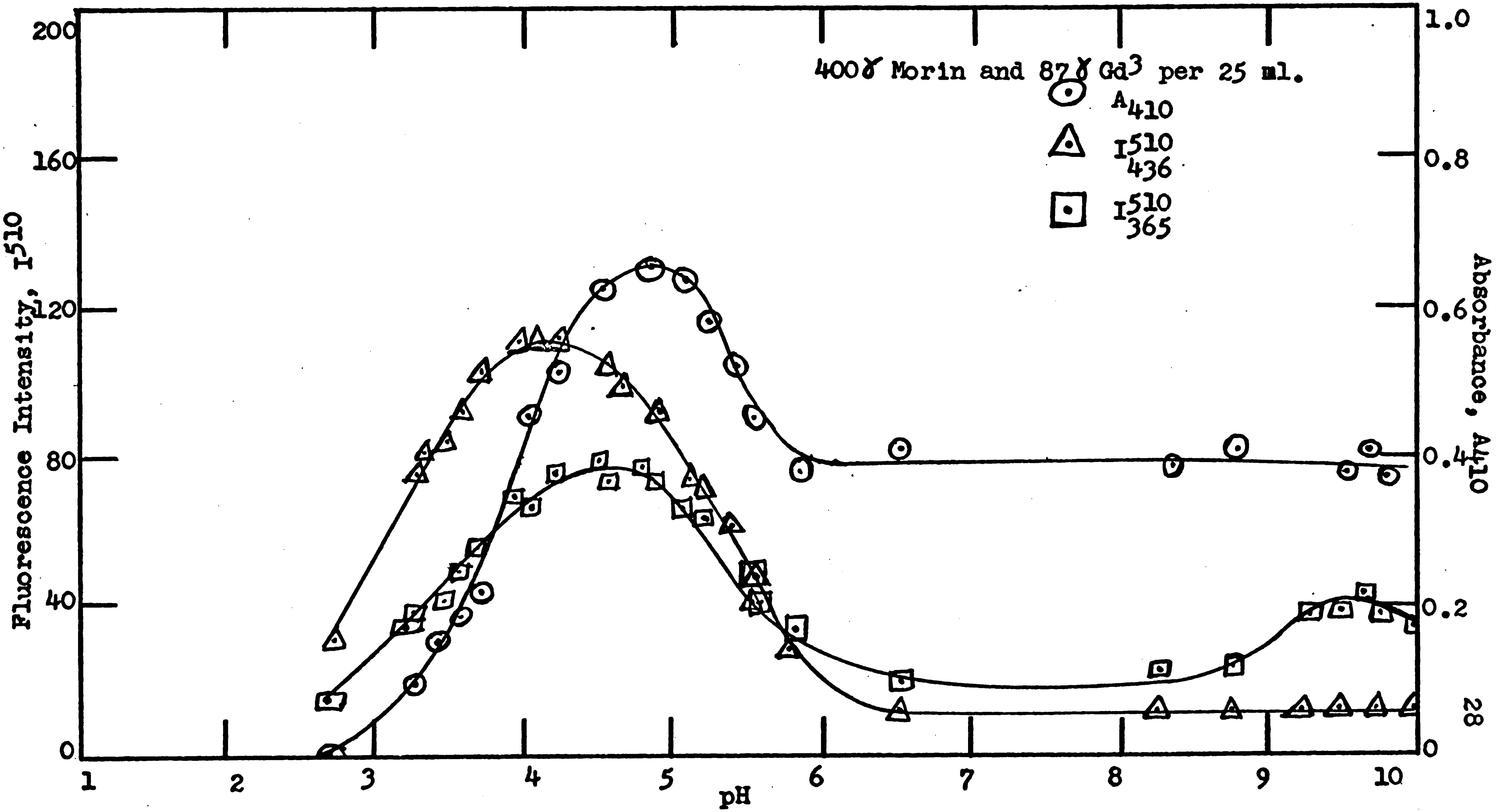


Figure 5. Effect of pH on the Fluorescence and Absorbance of the Gadolinium(III) Morin Complex in 50-50 DW.

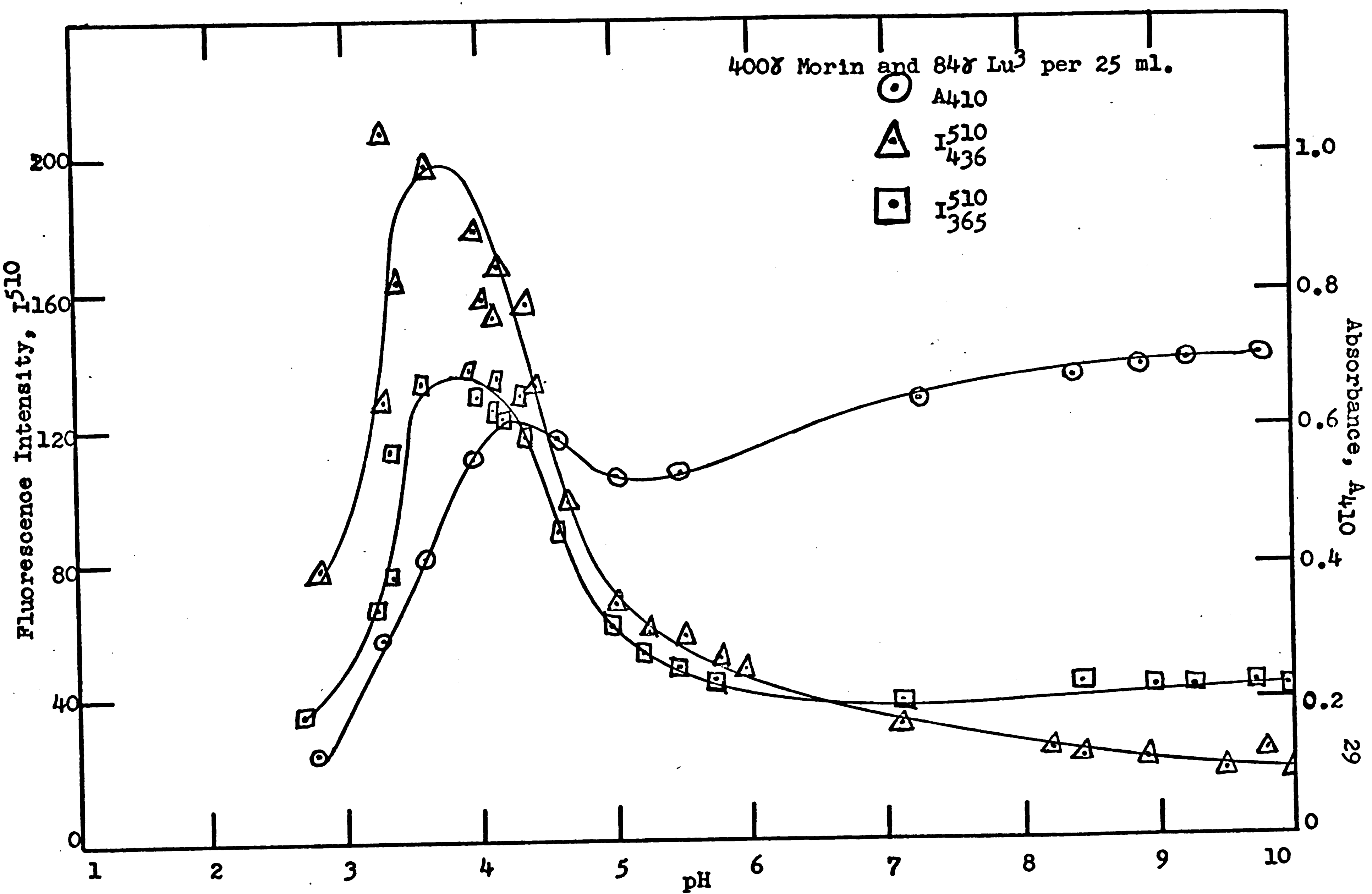


Figure 6. Effect of pH on the Fluorescence and Absorbance of the Lutetium(III) Morin Complex in 50-50 DW.

Effect of Morin Concentration

The effect of increasing the morin concentration, while holding the lanthanide ion concentration constant, is shown in Figures 7 and 8. The fluorescence intensity increases until the morin concentration is in the region of twice that of the lanthanide ion. After this point reagent concentration quenching occurs. This effect is most pronounced when the excitation wavelength is 365 mμ as this is nearest to the wavelength of maximum absorption for the reagent. The absorption of the complex itself and equilibrium considerations cause the curvature noted before the maximum is reached. The absorption spectra are shown for reference.

The maximum for the lutetium complex occurs at a lower morin concentration than that for the gadolinium. This is explained by considering the effect of pH on the equilibrium of the reaction involved, that is:

$$R^{+3} + 2MH === R(M)_2^+ + 2H^+.$$

Due to the sharpness of the pH effect on the fluorescence of the lutetium complex and also for ease of handling, all of the investigations were carried out at pH 4.25, despite the fact that this was a little above the optimum for lutetium.

Effect of the Lanthanide Concentration

Figures 9 and 10 show that both complexes exhibit a linear increase in fluorescence and absorbance over the range of 0-100 γ per 25 ml. Figures 18 and 19 confirm that the lower range (0-40 γ) is linear and could be used for analytical purposes.

Effect of Anions

The anions investigated fell into two classes: 1) those which destroyed the fluorescence of the complex completely (sulfate, oxalate, tartrate, and phosphate), and 2) those which had little or no effect (chloride, bromide,

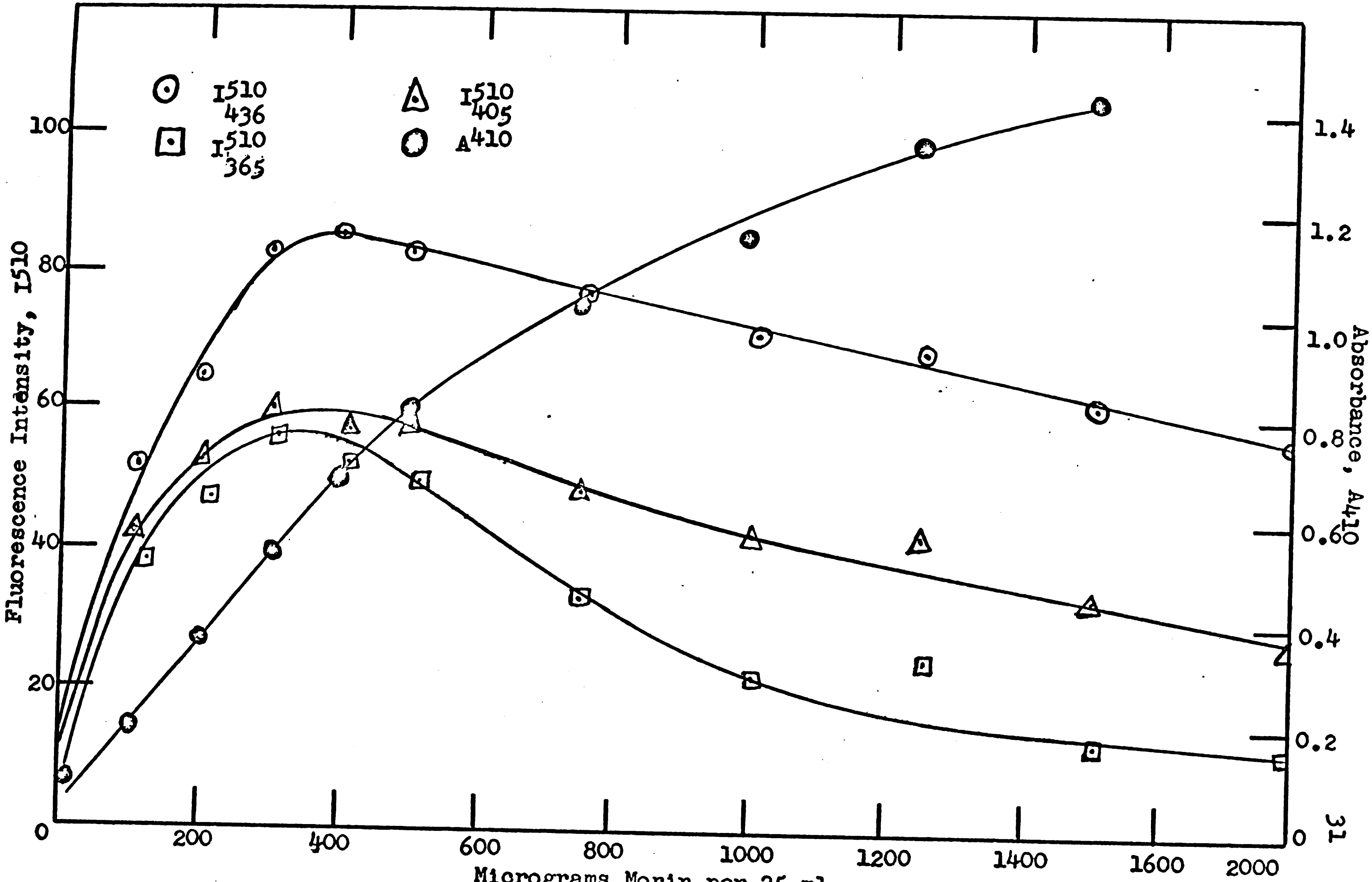


Figure 7. Effect of Morin on the Fluorescence and Absorbance of 87γ Gadolinium(III) per 25 ml. in 50-50 DW at pH 4.25.

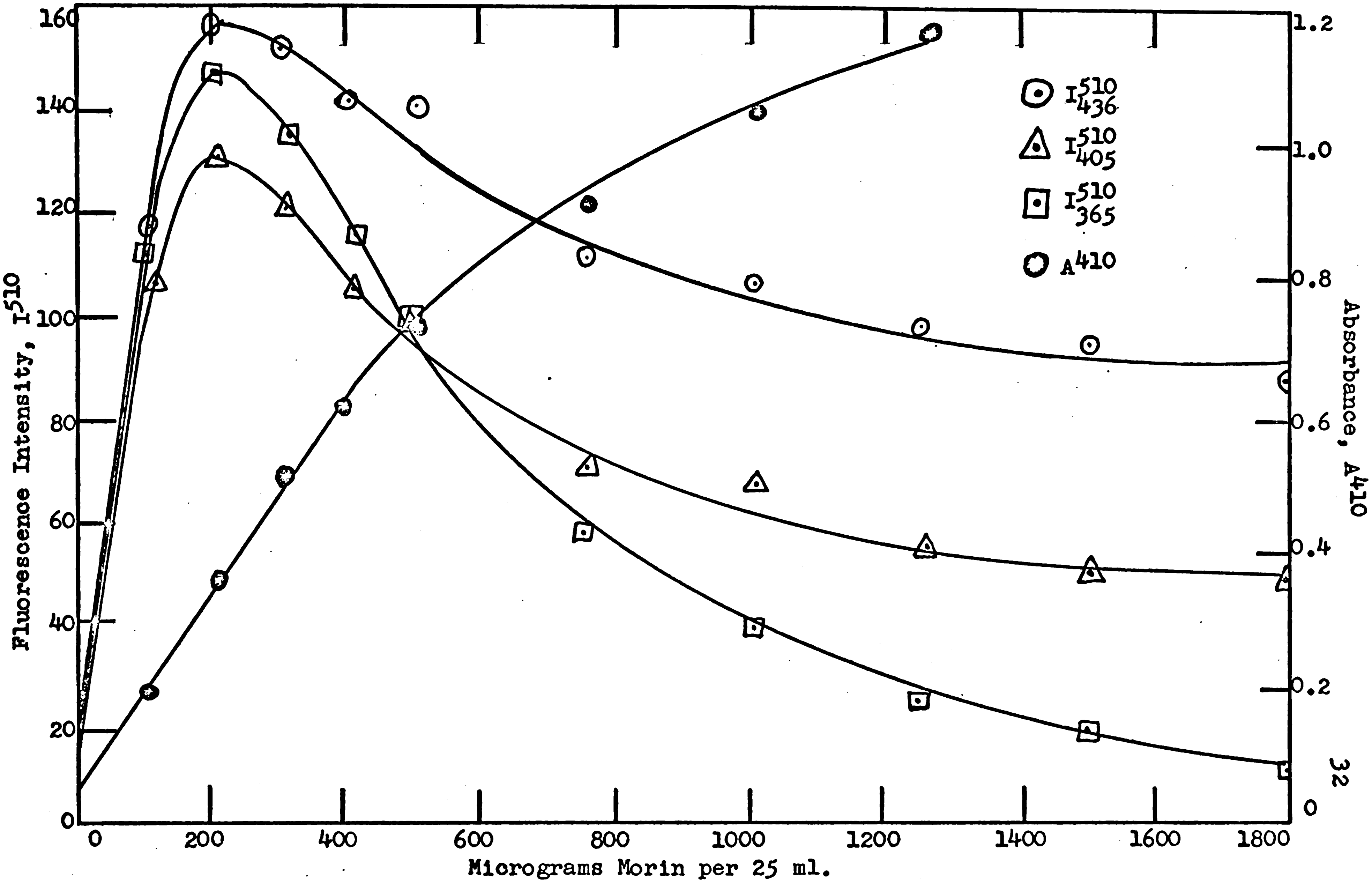


Figure 8. Effect of Morin on the Fluorescence and Absorbance of 84γ Lutetium(III) per 25 ml of 50-50 DW at pH 4.25.

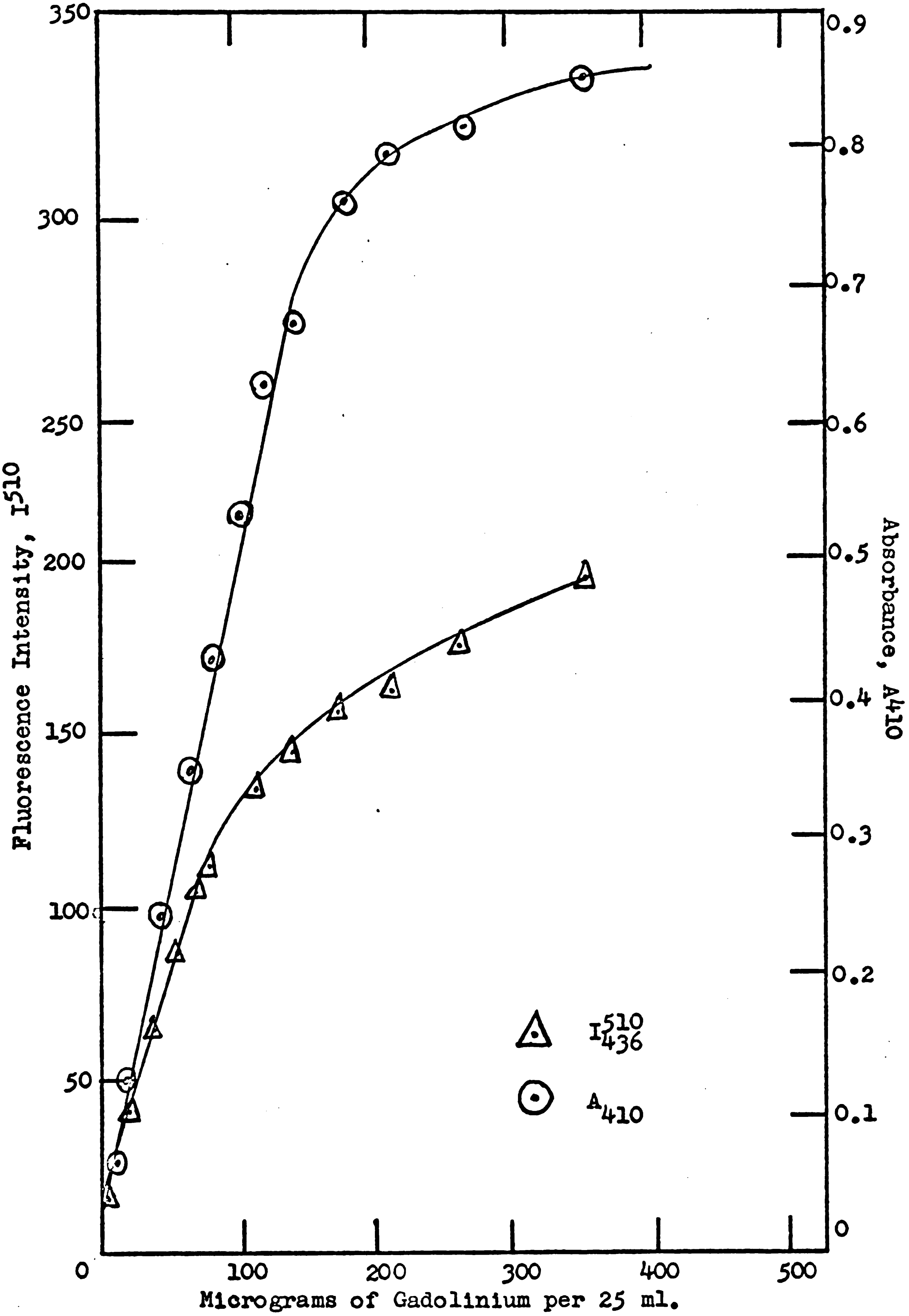


Figure 9. Effect of Gadolinium(III) on the Fluorescence and Absorbance of 400γ Morin per 25 ml of 50-50 DW at pH 4.25

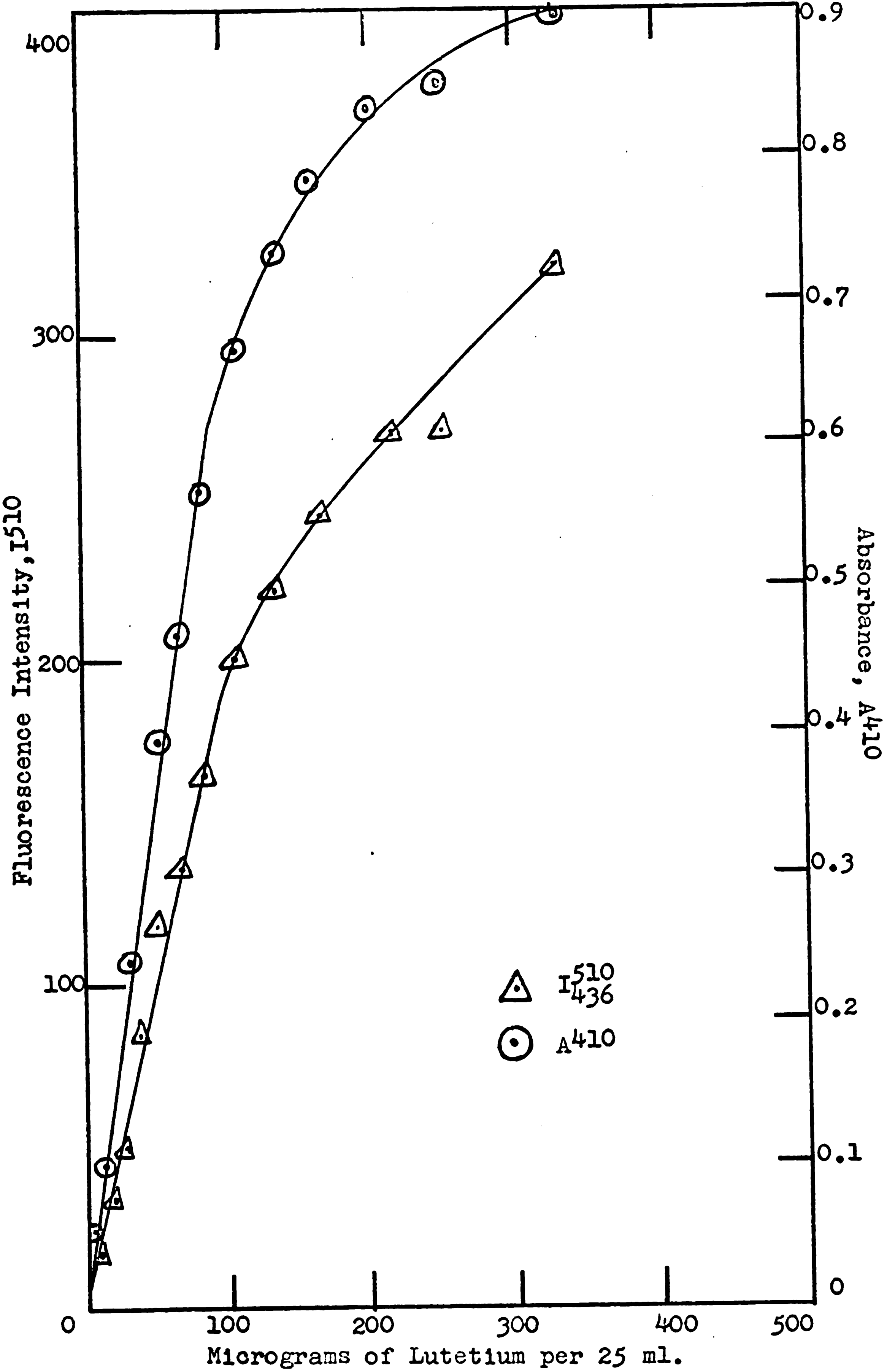


Figure 10. Effect of Lutetium(III) on the Fluorescence and Absorbance of 400γ Morin per 25 ml of 50-50 DW at pH 4.25.

iodide, carbonate, acetate, and tetraborate). The absorption spectra, not shown, indicate that this is not a matter of quenching but rather complete supression of the complex formation. These data are shown graphically in Figure 11.

Effect of the Composition of the Solvent

The fluorescence and absorbance of a given amount of complex increases with increasing dioxane concentration, as shown in Figures 12 and 13, and then falls off above 70 percent dioxane -30 percent water. However, at the higher dioxane levels the pH values are doubtful. The absorbtion spectra of these solutions, not shown, tend to confirm this as they show a shift of maxima to higher wavelength which is an indication that the solutions were really at higher pH values than the measurements indicated.

Effect of Nonfluorescing Lanthanide Complexes

To establish the formation of a complex between morin and holmium, europium or ytterbium, the absorbance of various solutions was measured. Figure 14 is a typical curve which indicates that the complex is being formed. Figures 15-17 show the effect of the above three cations on the fluorescence of the gadolinium and lutetium complexes.

Initially fluorescence produced by 365 mμ excitation increases while that produced by the 436 mμ excitation remains constant. This is due to the removal of free morin from solution through the formation of additional complex and as a result reduces the absorption of the 365 mμ excitating radiation. The fluorescence produced by the 436 mμ excitation falls off while that produced by the 365 mμ excitation remains constant. This is a result of two factors: 1) the absorption of the 436 mμ radiation by the complex formed, and 2) eventually by competition between the ions for the morin, since these complexes are relatively weak and the equilibrium constants for the complexes would be of the same order of magnitude.

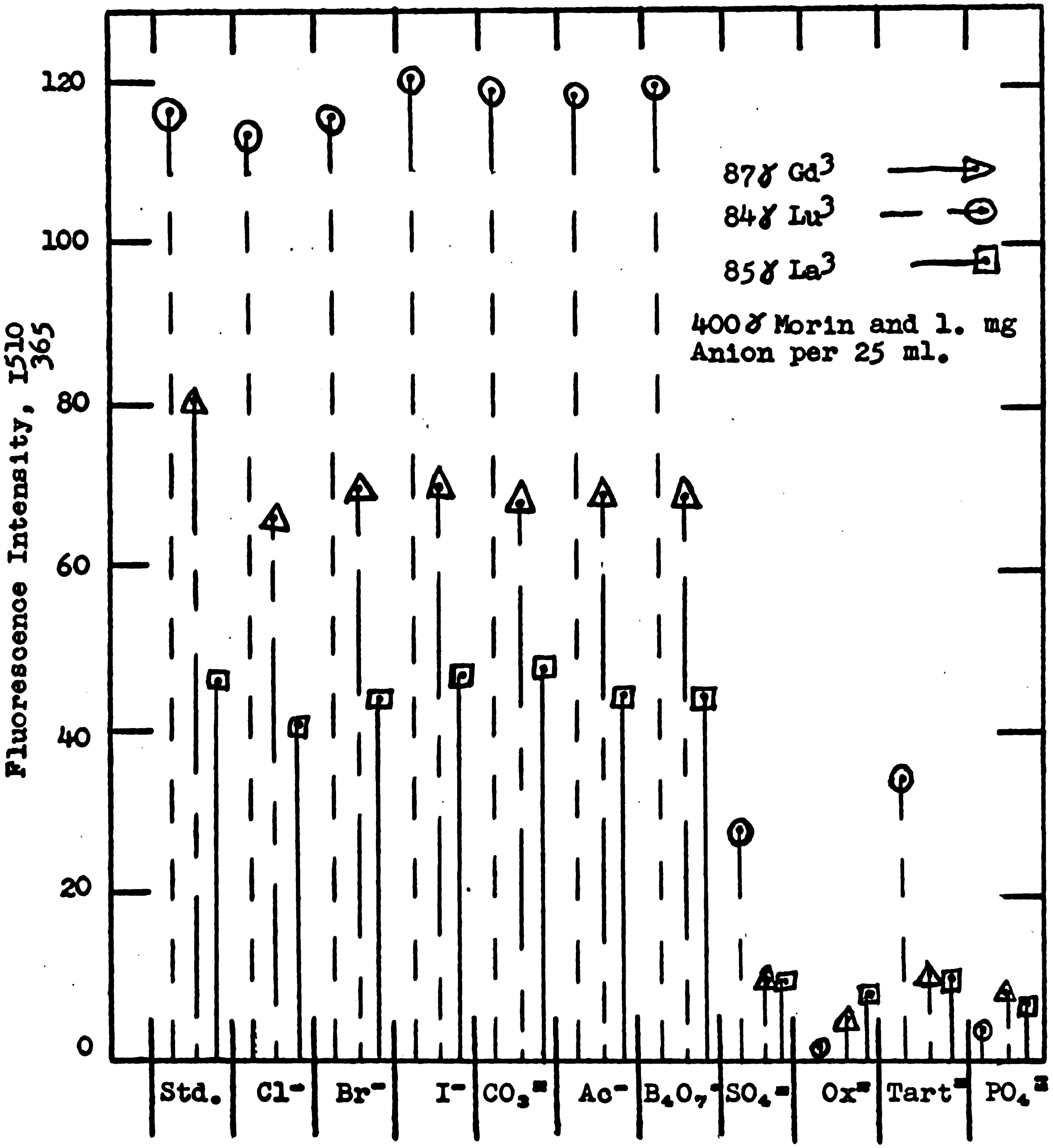


Figure 11. The Effect of Anions on the Fluorescence of the Morin Complexes of Lanthanum(III), Gadolinium(III), and Lutetium(III) at pH 4.25 in 50-50 DW.

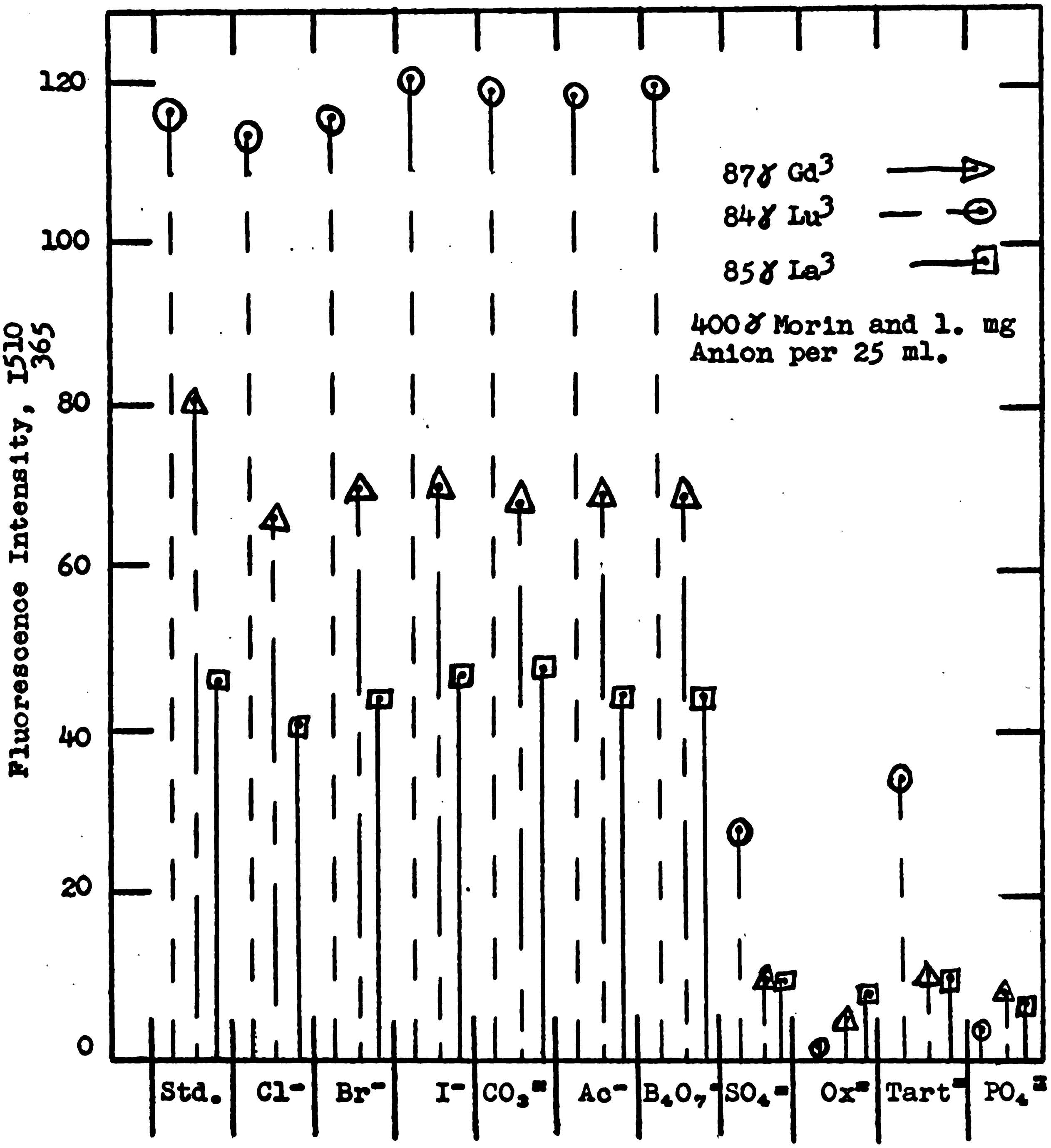


Figure 11. The Effect of Anions on the Fluorescence of the Morin Complexes of Lanthanum(III), Gadolinium(III), and Lutetium(III) at pH 4.25 in 50-50 DW.

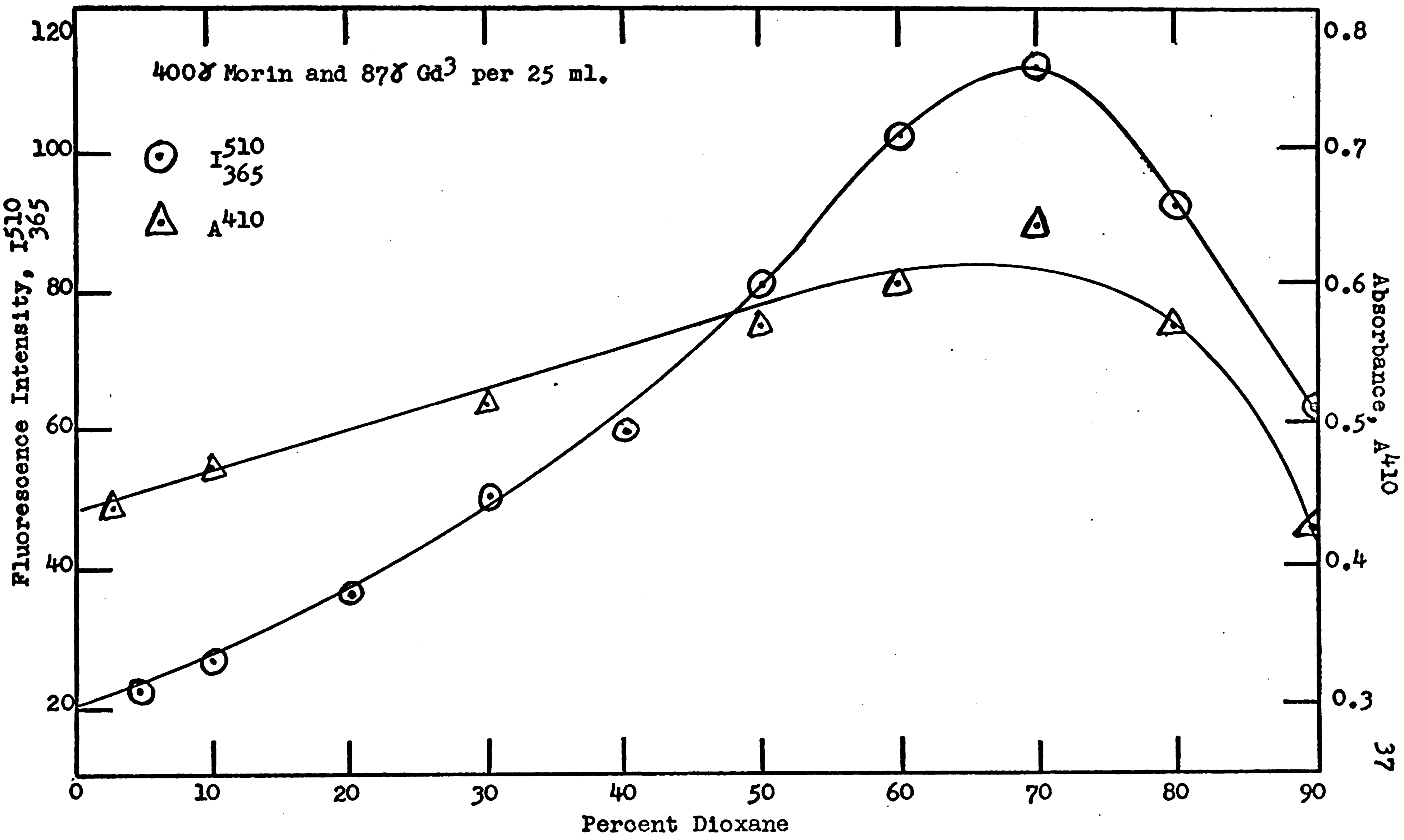


Figure 12. Effect of Dioxane Content on the Fluorescence and Absorbance of the Gadolinium(III) Morin Complex at pH 4.25.

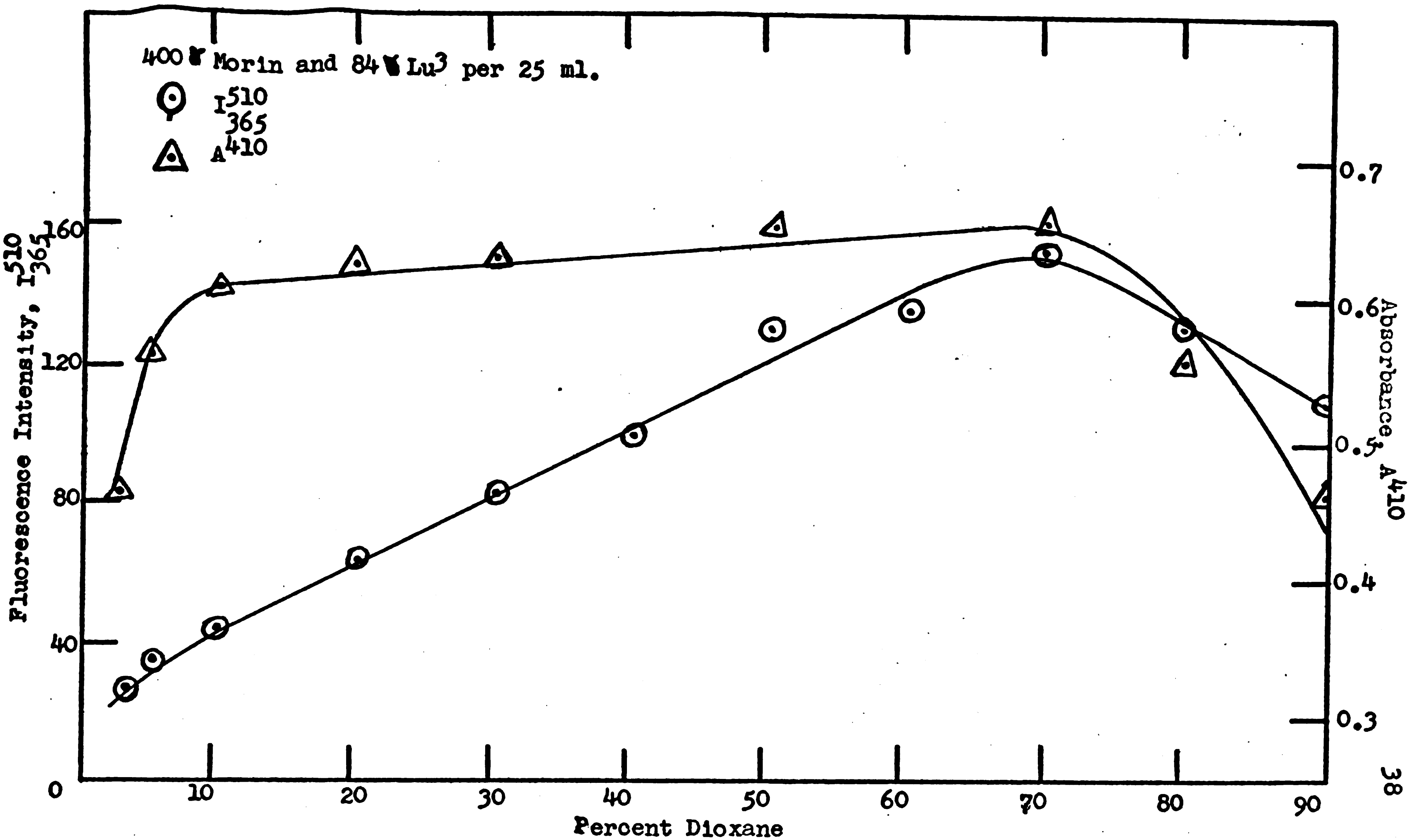


Figure 13. Effect of Dioxane Content on the Fluorescence and Absorbance of the Lutetium(III) Morin Complex at pH 4.25.

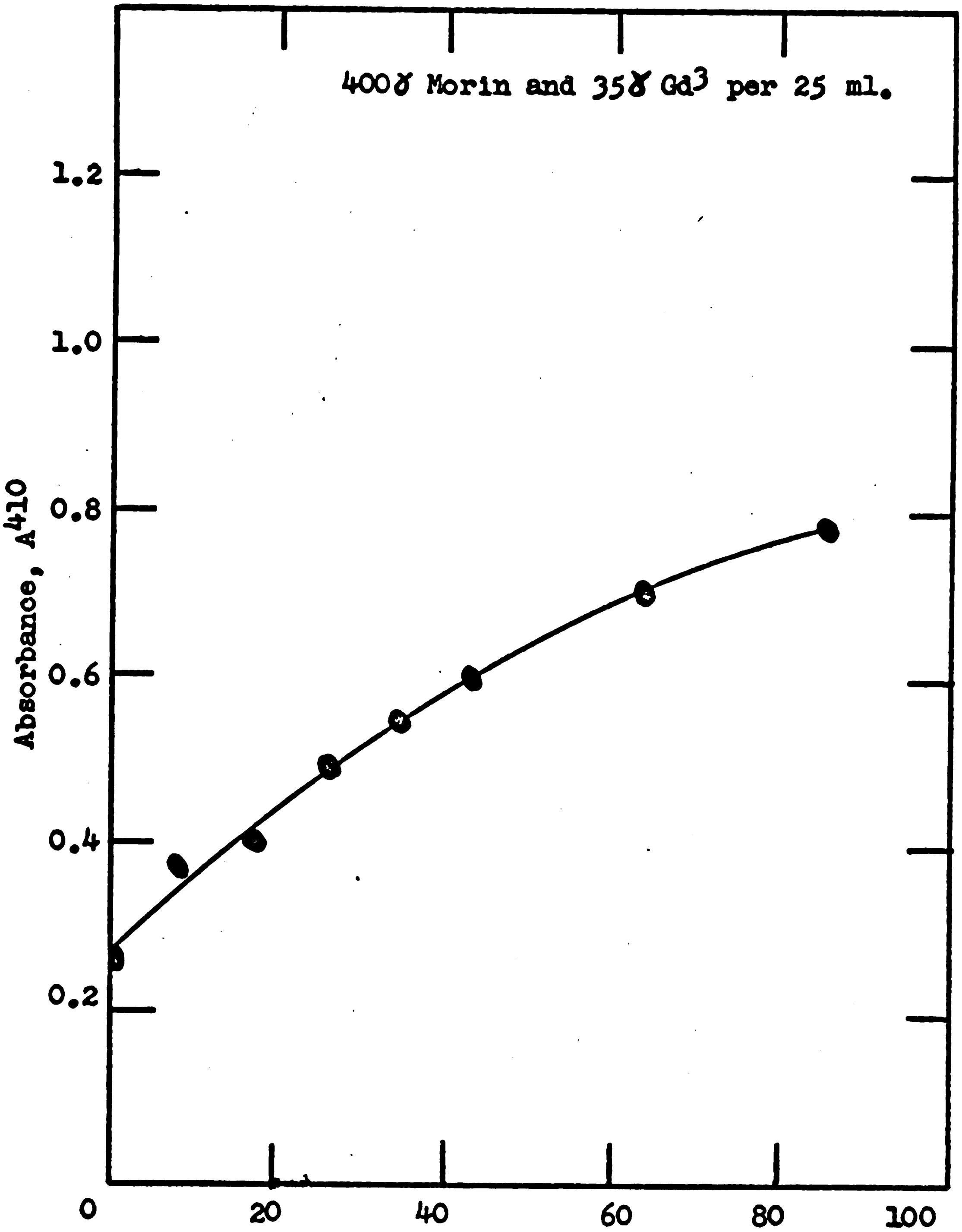


Figure 14. Effect of Holmium(III) on the Absorbance of the Gadolinium(III) Morin Complex per 25 ml of 50-50 DW at pH 4.25.

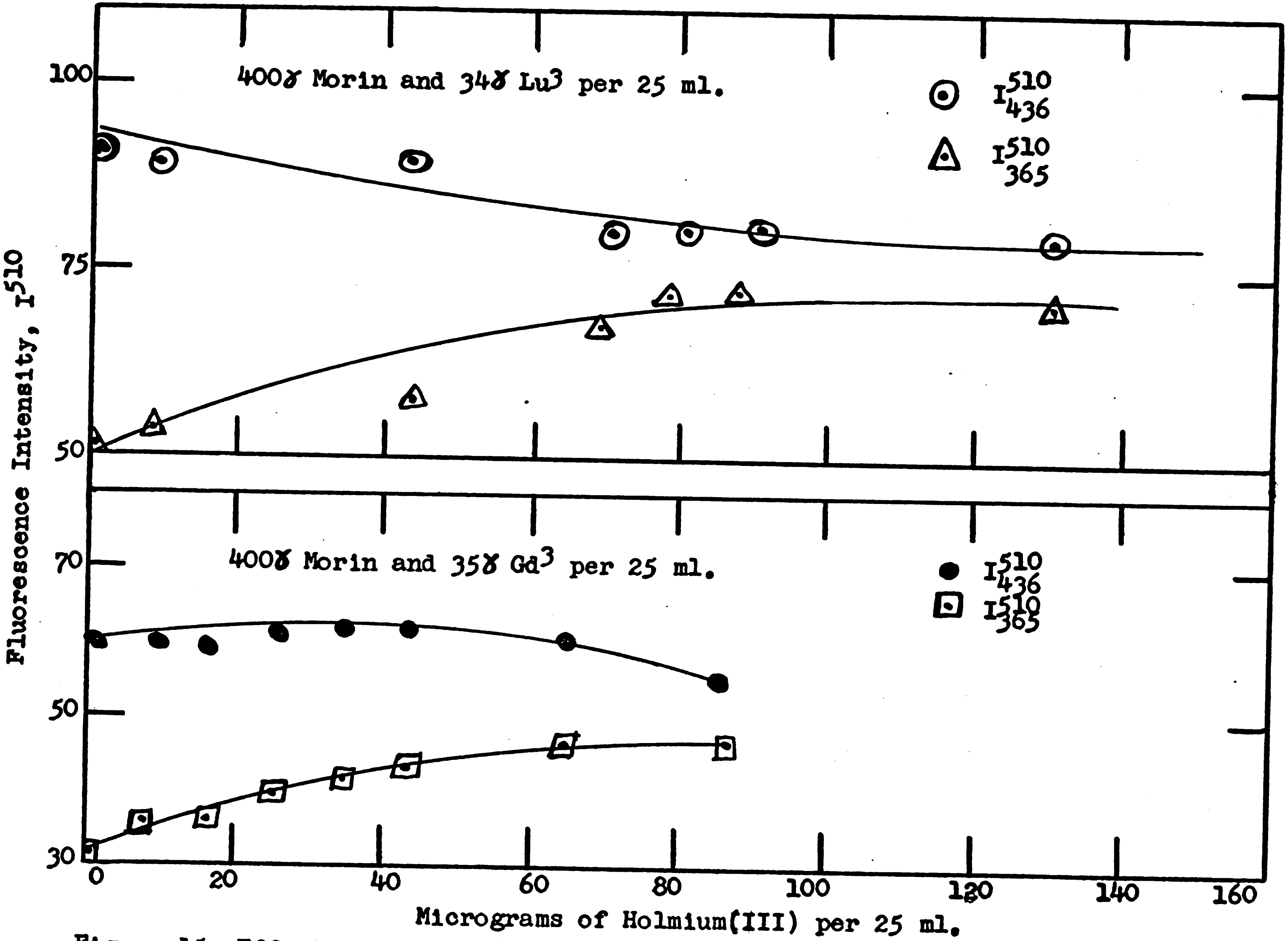


Figure 15. Effect of Holmium(III) on the Fluorescence of the Complexes of Morin with Gadolinium(III) and Lutetium(III) in 50-50 DW at pH 4.25.

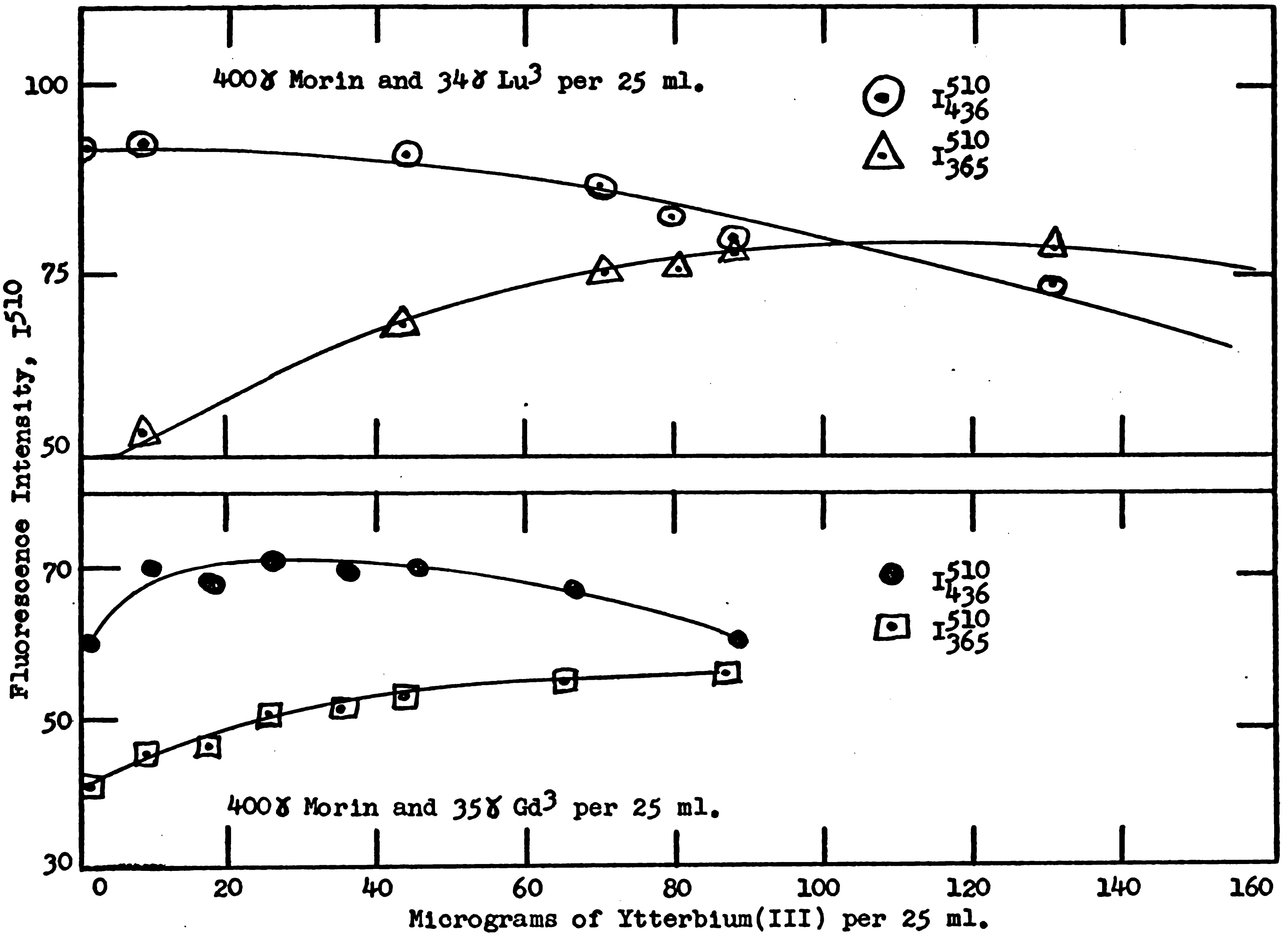


Figure 16. Effect of Ytterbium(III) on the Fluorescence of the Complexes of Morin with Gadolinium(III) and Lutetium(III) in 50-50 DW at pH 4.25.

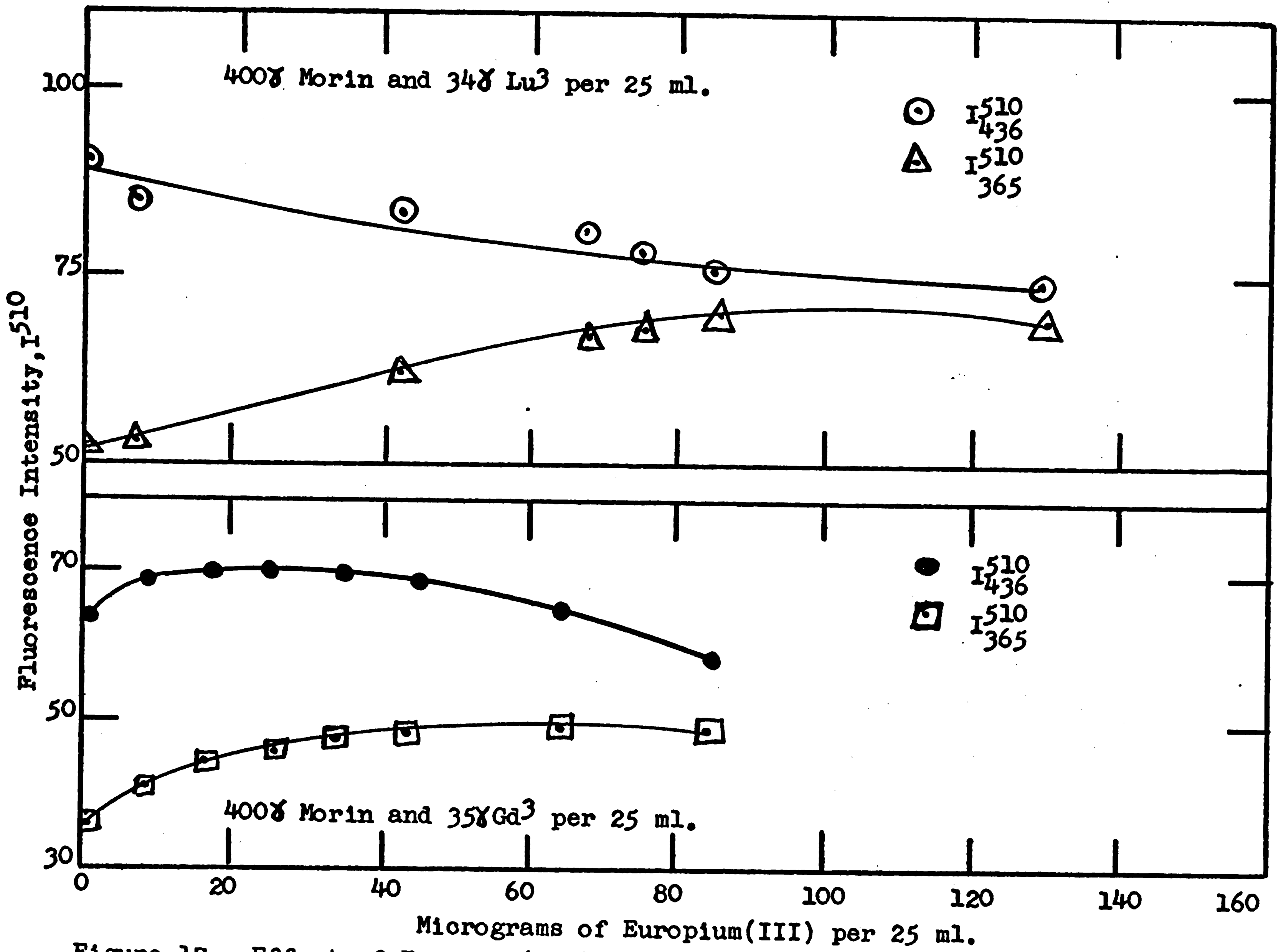


Figure 17. Effect of Europium(III) on the Fluorescence of the Complexes of Morin with Gadolinium(III) and Lutetium(III) in 50-50 DW at pH 4.25.

Time Stability of the Complex

The fluorescence of the complex remains relatively constant for a period of at least two days. After this period the fluorescence began to fall off. This drop might be partially accounted for by the fact that this investigation was carried out by making all of the measurements on the same sample and contamination during storage and handling could result in lower fluorescence.

Wavelength of Exciting Radiation

Excitation wavelengths of 436, 365, and 405 mμ produced fluorescence of sufficient intensity (in decreasing order) to be of value in the detection of the gadolinium or lutetium complex. Below 365 mμ the five lines investigated produced little or no fluorescence.

Instrumental Variation to Obtain Greater Response per Unit Concentration

Figure 18 indicates that by doubling the slit width of the ultraviolet monochromator, the fluorescence intensity recorded was almost doubled for a given amount of complex. Under these conditions readings satisfactory for analytical use were obtained over a range of 0-40 γ per 25 ml of the lanthanide ion.

By using the increased illumination in conjunction with the changes in standardization of the Beckman DU, the intensity of the fluorescence is increased six fold. Figure 19 illustrates that the 0-10 γ per 25 ml range would yield very satisfactory results if used for the determination of small amounts of gadolinium or lutetium.

The fluorescence of the lanthanum complex is shown in Figure 18 for comparison.

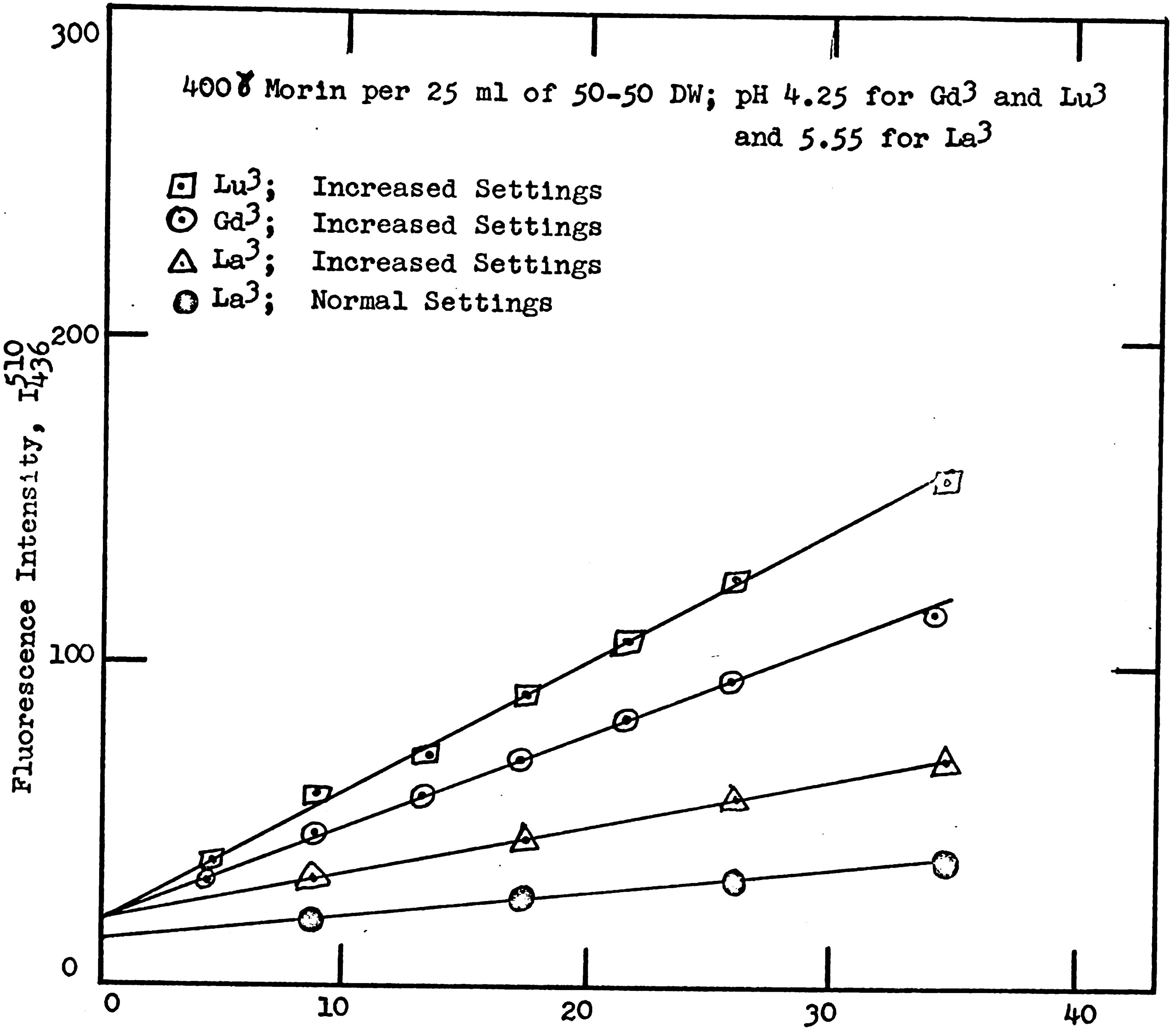


Figure 18. Instrumental Variation -- Effect of Increasing Monochromator Slit Settings on Fluorescence Readings.

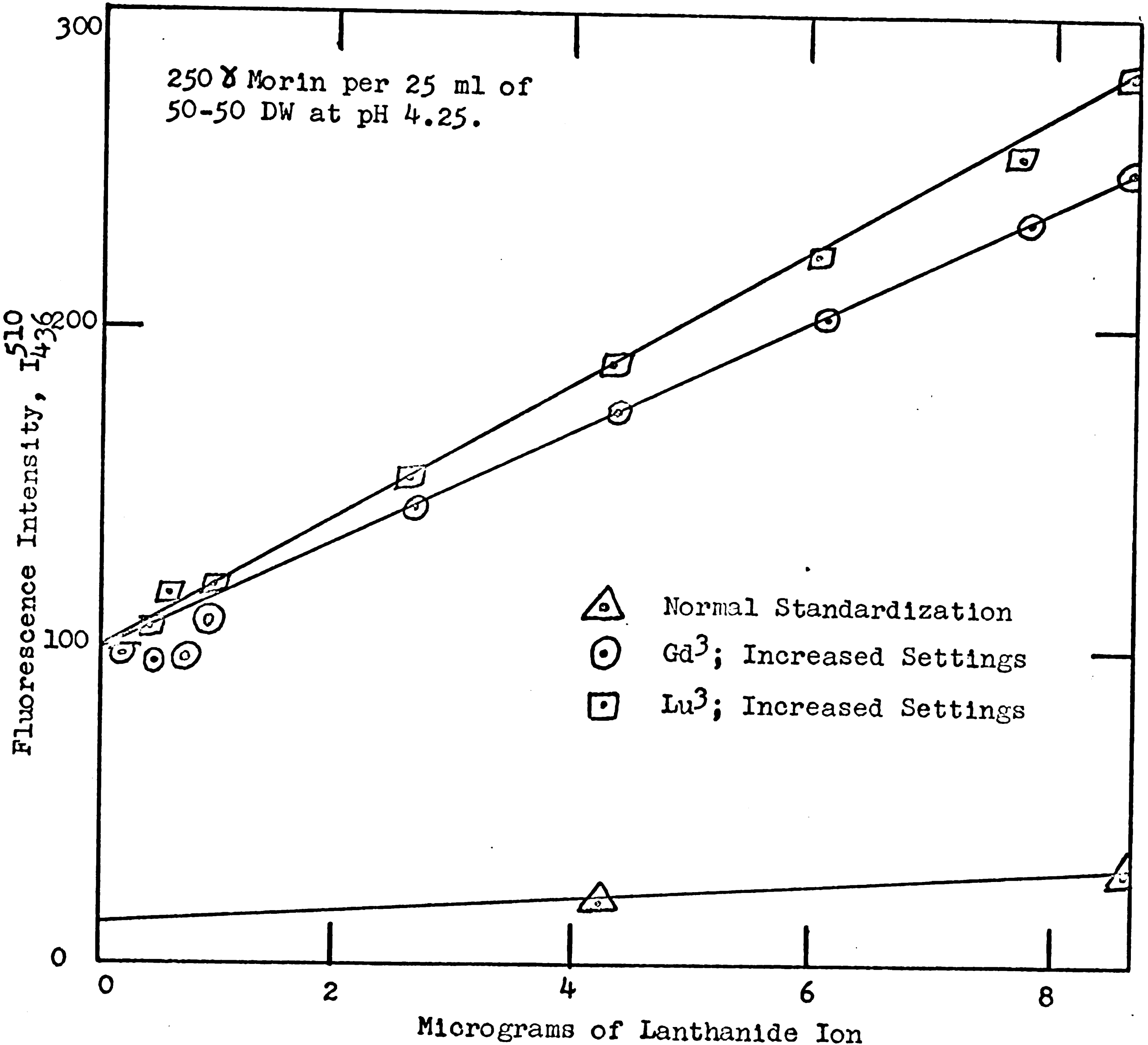


Figure 19. Instrumental Variation-- Effect of Increasing Instrumental Sensitivity on Fluorescence Readings.

Nature of the Complex

Isoabsorptive Point

Figures 20-23 show spectrophotometric isoabsorptive points for both lanthanide complexes as determined at a pH of 4.25 and 5.2-5.4. The formation of an isoabsorptive point or a point of common intersection produced by varying the concentrations of two reacting components is an indication of the occurrence of a single reaction (17) between the lanthanide ion and morin at both pH values. The loss of fluorescence with increasing pH must then result from a change in the complex itself rather than the formation of a different complex.

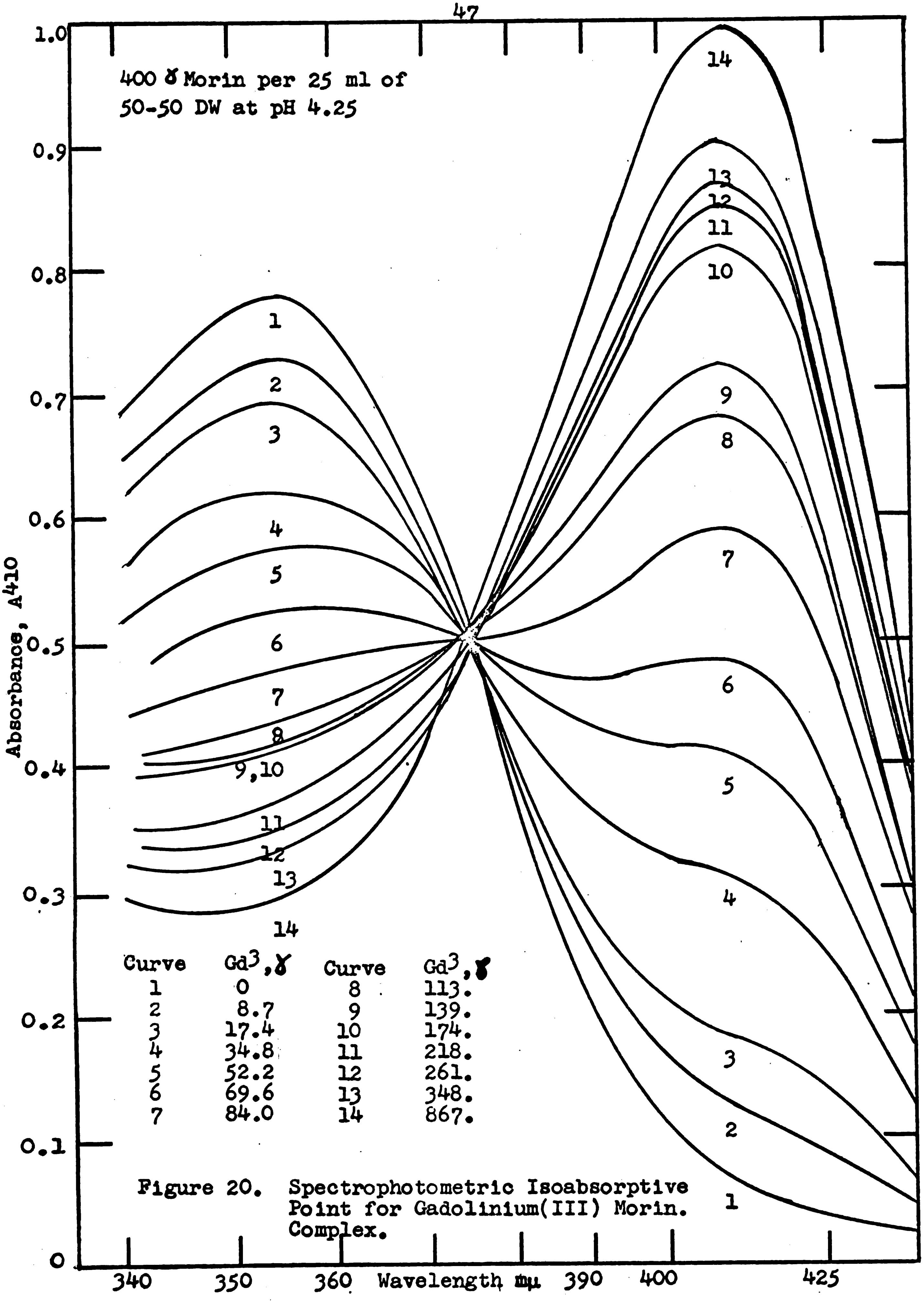
In the higher pH range the actual pH value is apparently more critical and since reproduction of a given pH from sample to sample was very difficult, no resemblance of order could be found in the absorption spectra.

Method of Continuous Variations

Figures 24-27 indicate the formation of a single complex both at pH 4.25 and 7.0. The maximum or minimum in the curves occur at the position which indicates the complex formed has the composition of one lanthanide ion to two morin ions. This agrees with the findings for thorium-morin complex (17, 18) and the lanthanum-morin complex (7).

Slope Ratio Method

The data are plotted in Figures 28 and 29. The large excess of the one component insures complete reaction, to form the complex, of the measured second component. From this the concentration of the complex is known and is related to the absorbance measurements. The following information was obtained from the plots.



Curve	Gd3, γ	Curve	Gd3, γ
1	0	8	113.
2	8.7	9	139.
3	17.4	10	174.
4	34.8	11	218.
5	52.2	12	261.
6	69.6	13	348.
7	84.0	14	867.

Figure 20. Spectrophotometric Isoabsorptive Point for Gadolinium(III) Morin. Complex.

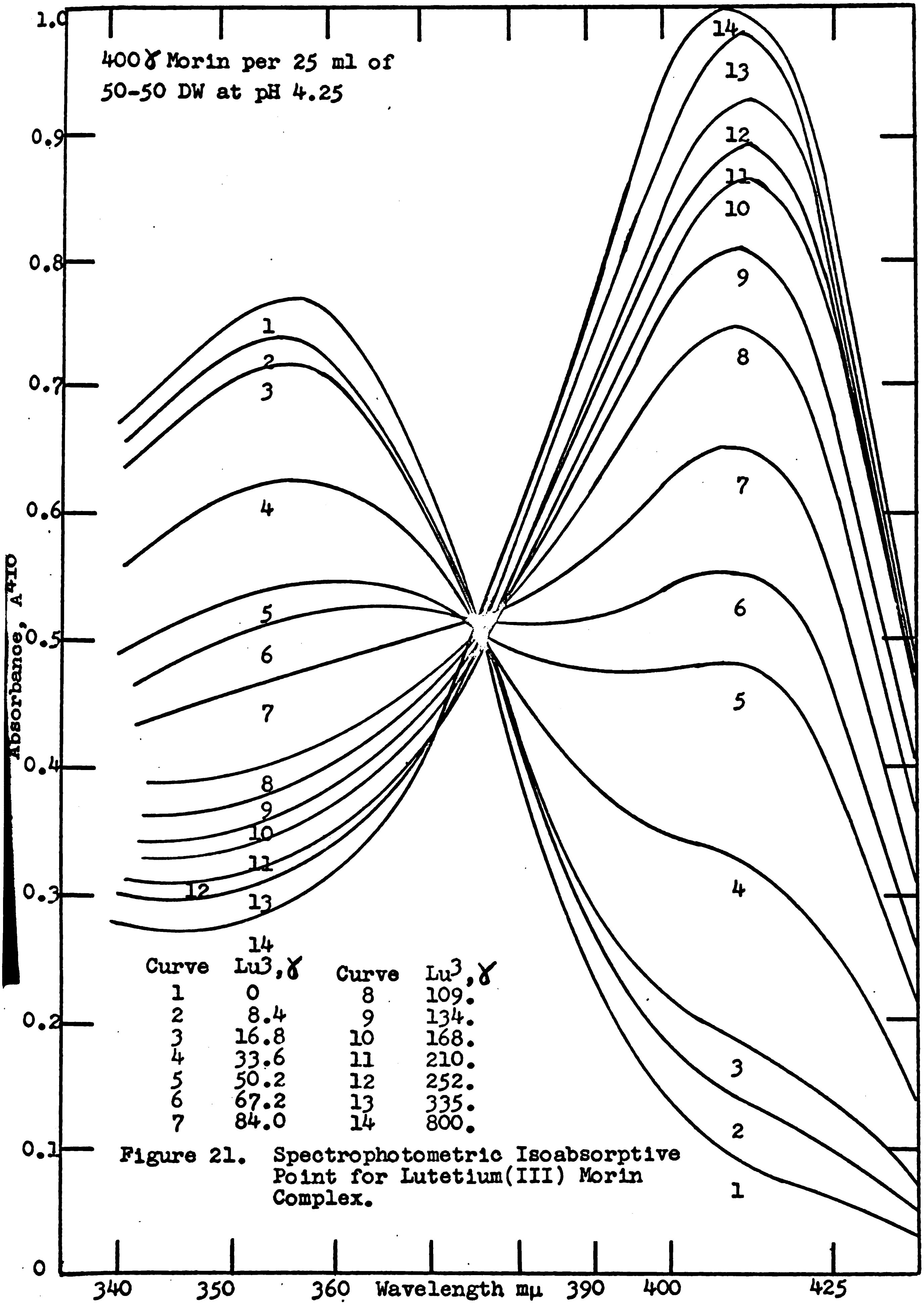


Figure 21. Spectrophotometric Isoabsorptive Point for Lutetium(III) Morin Complex.

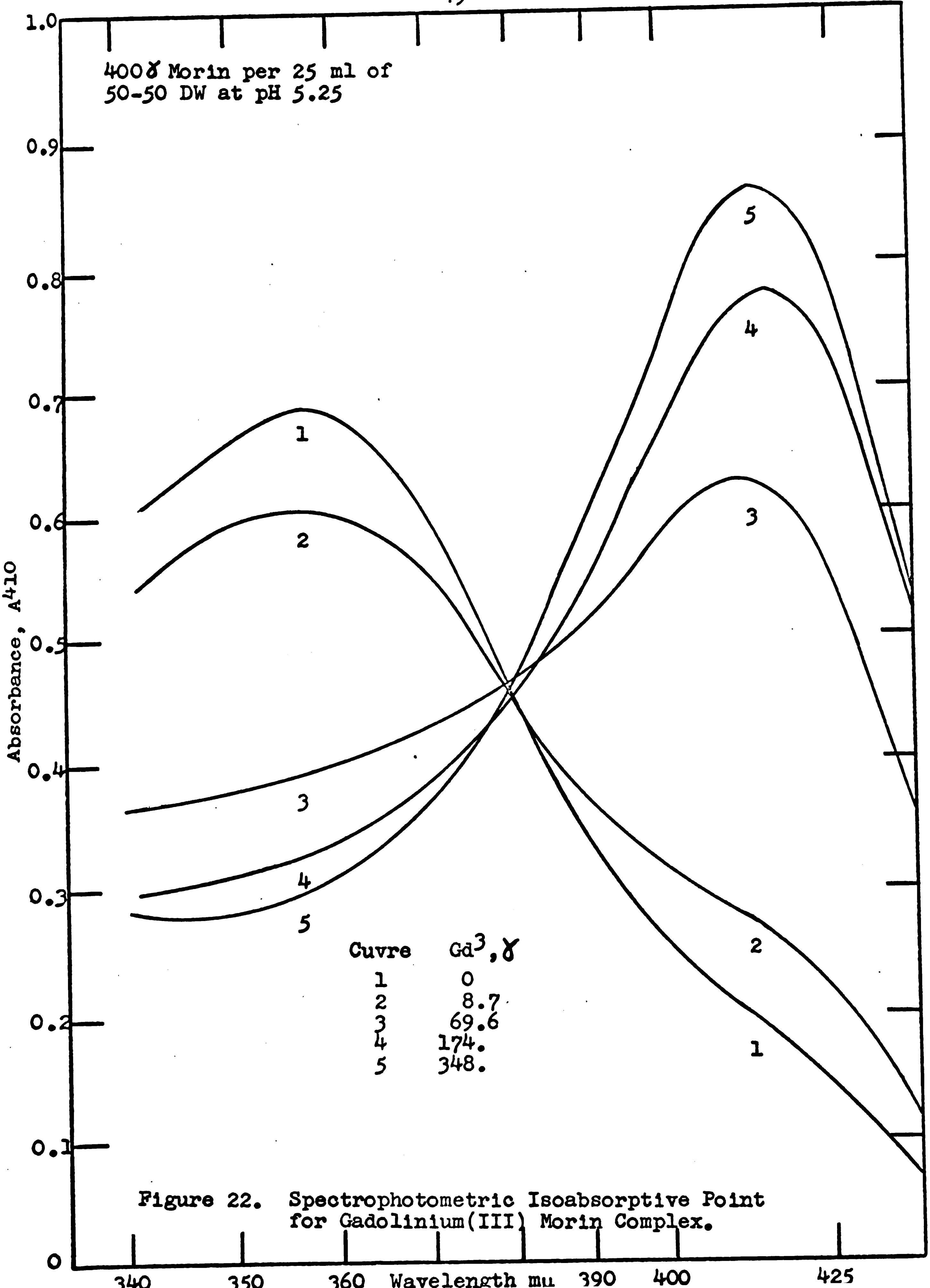


Figure 22. Spectrophotometric Isoabsorptive Point for Gadolinium(III) Morin Complex.

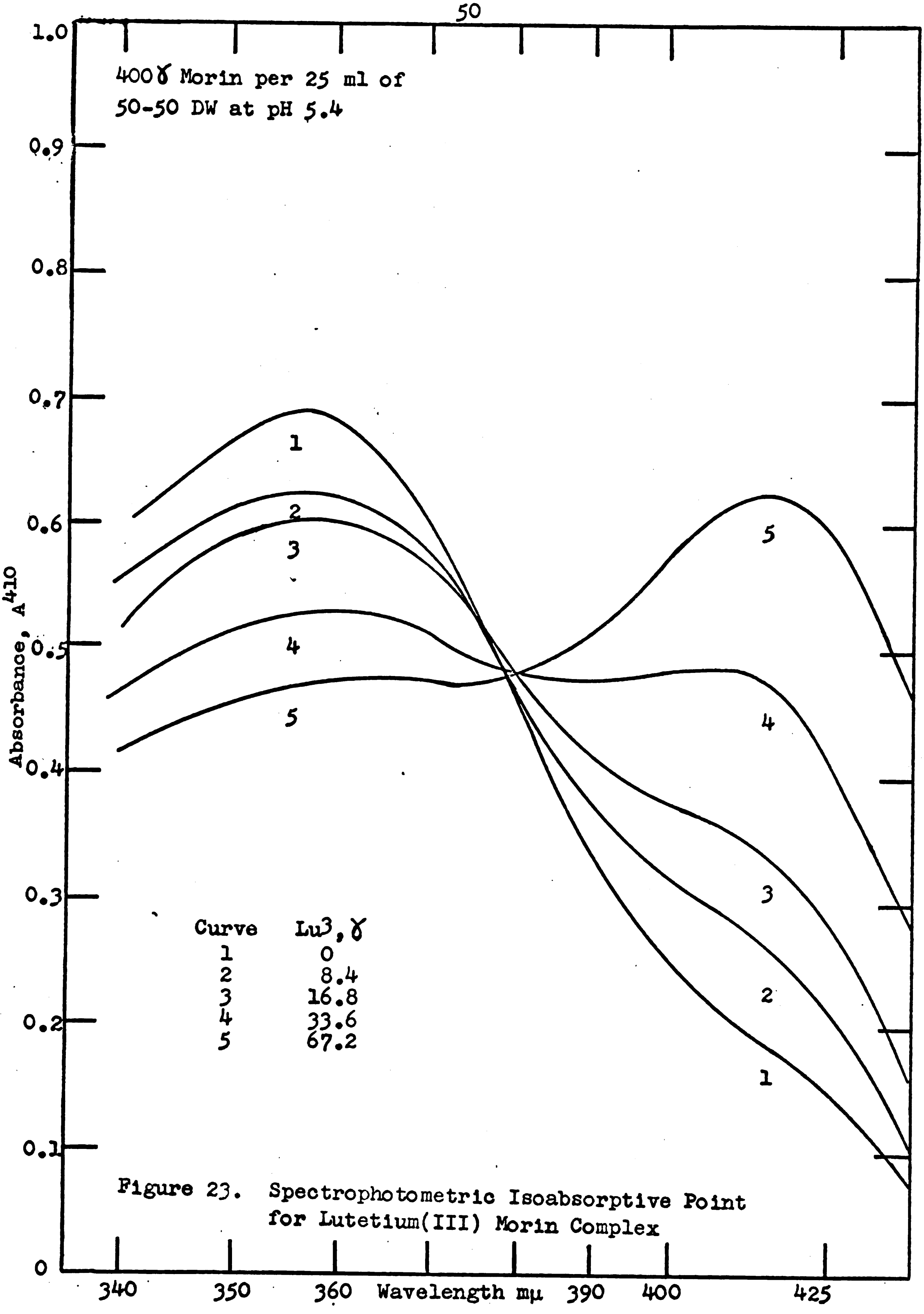


Figure 23. Spectrophotometric Isoabsorptive Point for Lutetium(III) Morin Complex

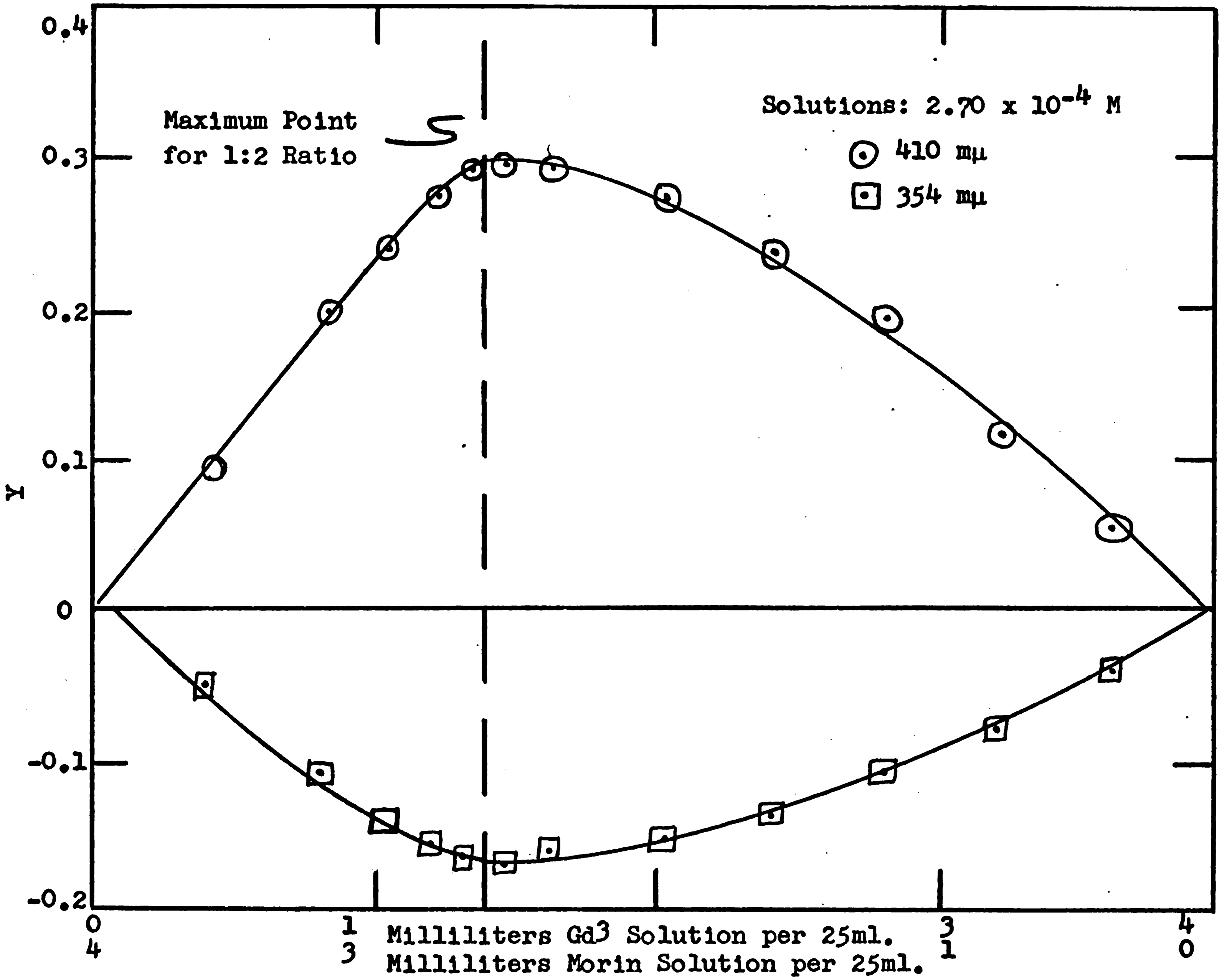


Figure 24. Determination of Composition of Gadolinium(III) Morin Complex in 50-50 DW at pH 4.25 by Method of Continuous Variations.

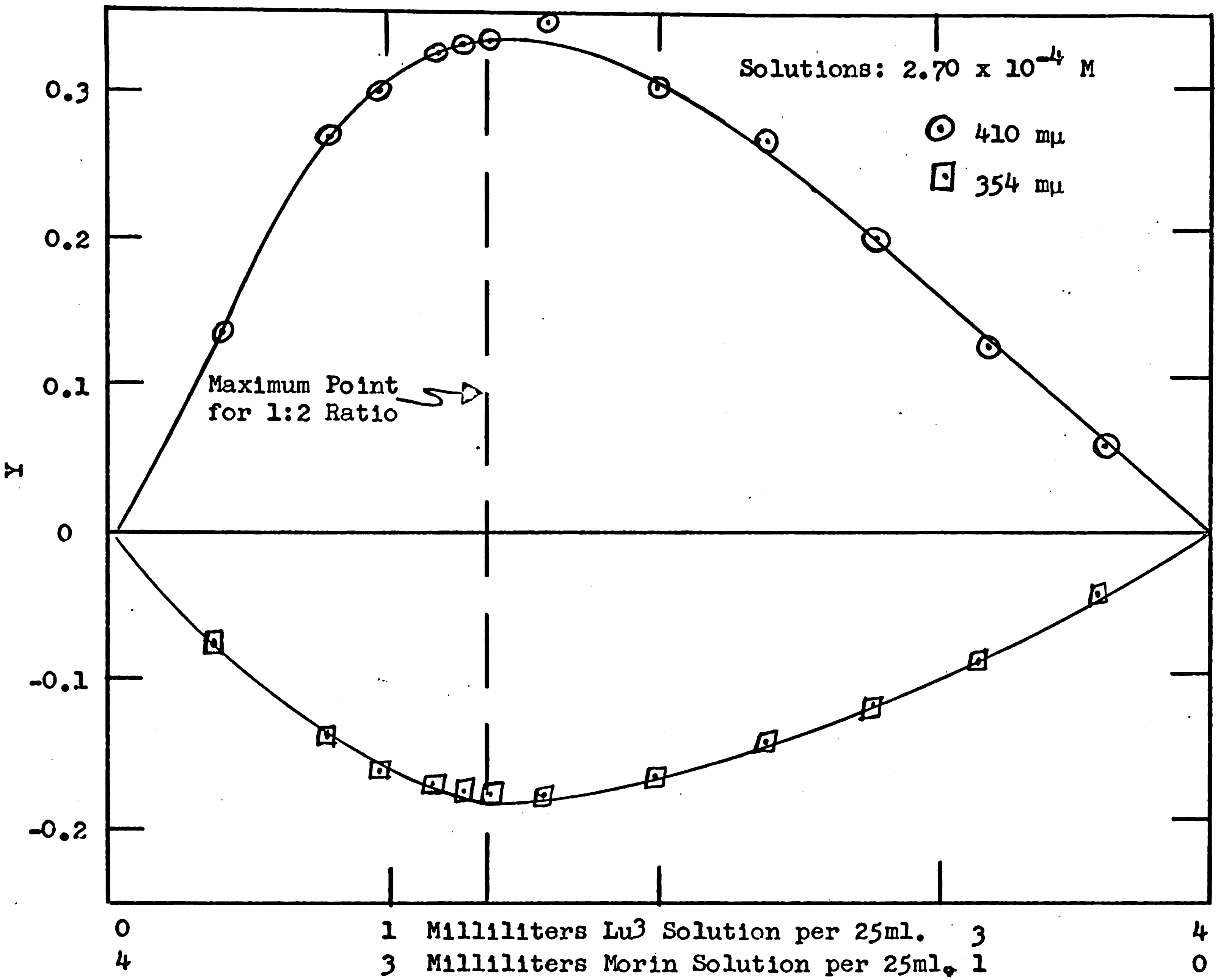


Figure 25. Determination of Composition of the Lutetium(III) Morin Complex in 50-50 DW at pH4.25 by Method of Continuous Variations.

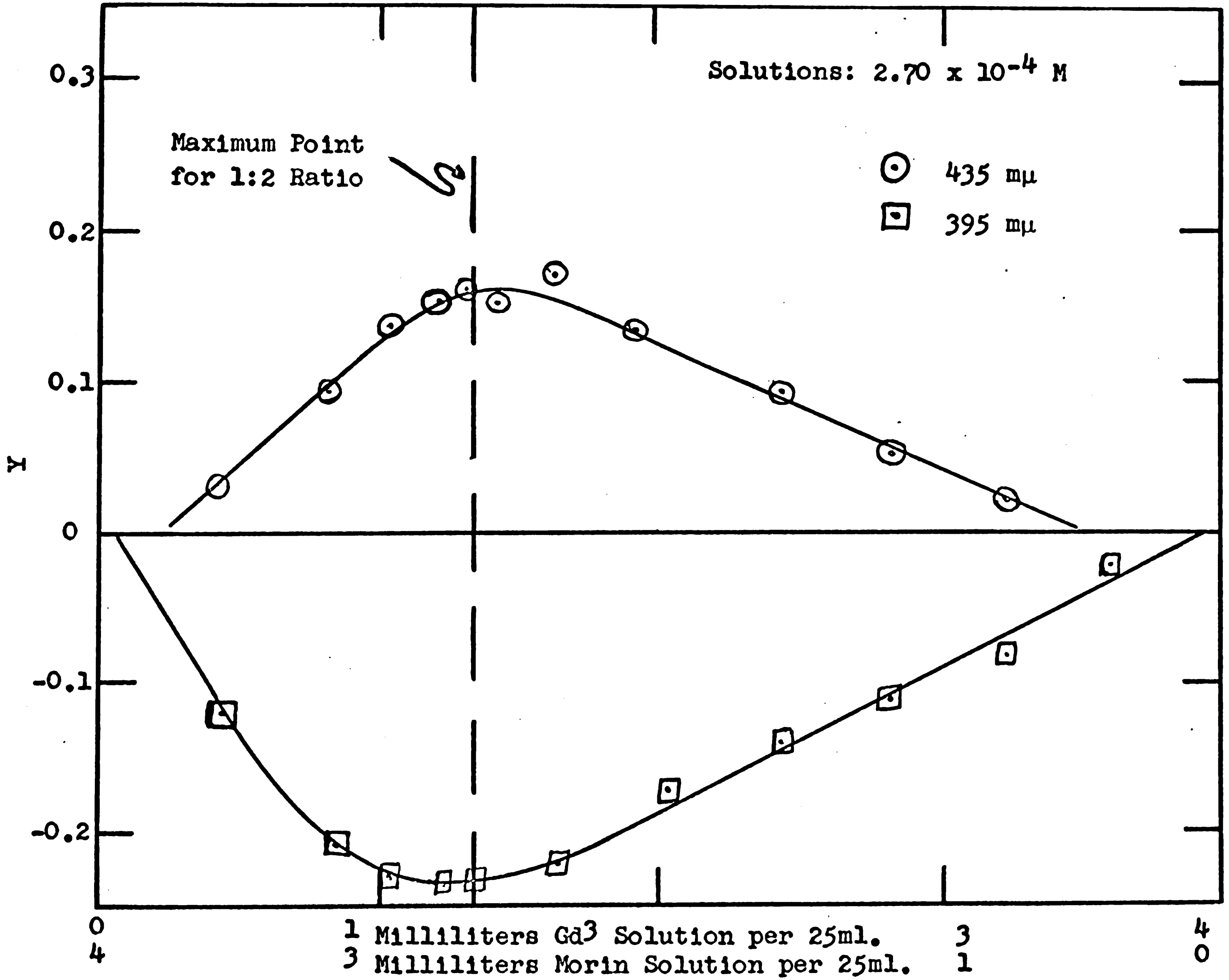


Figure 26. Determination of Composition of Gadolinium(III) Morin Complex in 50-50 DW at pH 7.05 by Method of Continuous Variations.

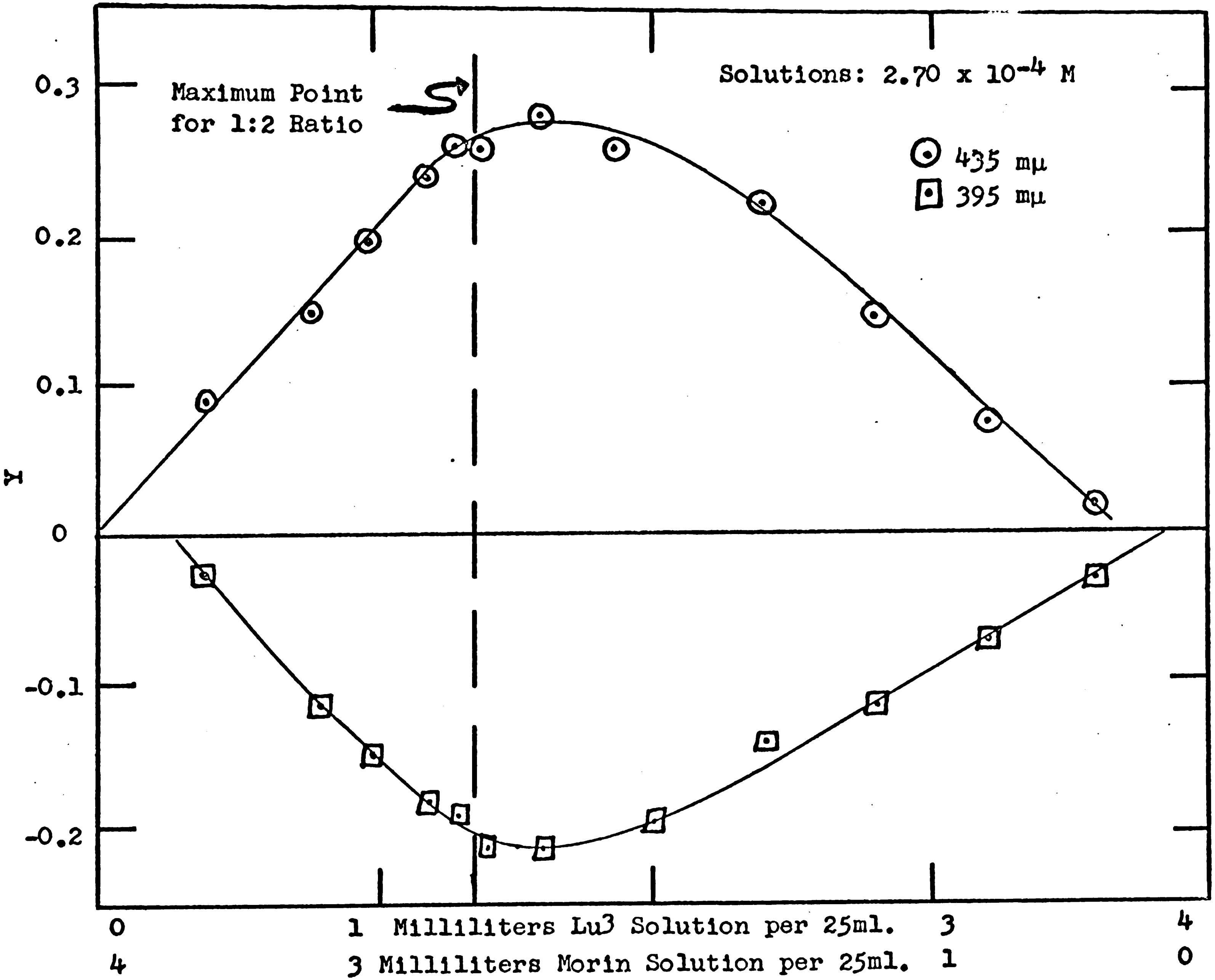


Figure 27. Determination of Composition of the Lutetium(III) Morin Complex in 50-50 DW at pH 7.25 by Method of Continuous Variations.

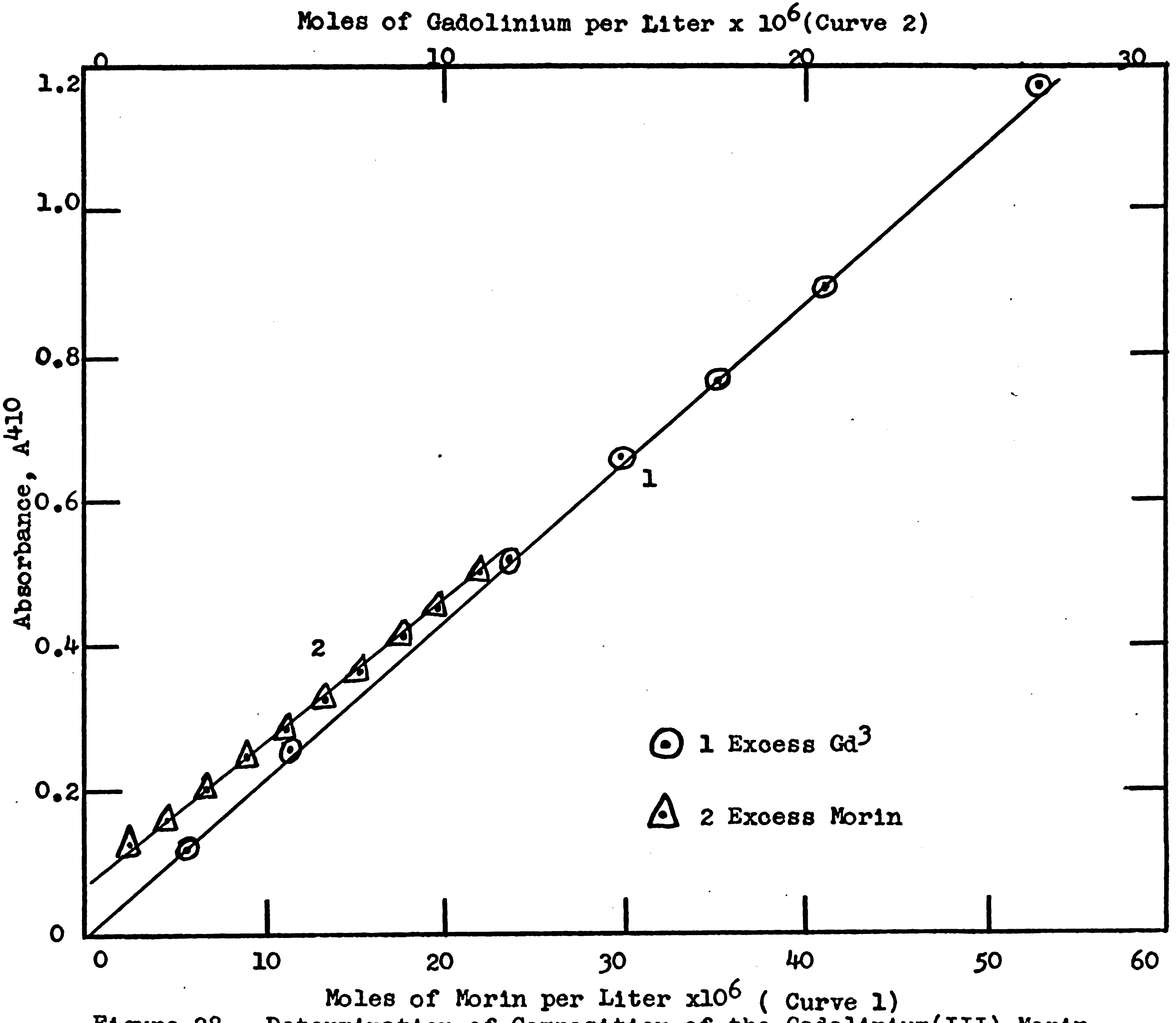


Figure 28. Determination of Composition of the Gadolinium(III) Morin Complex in 50-50 DW at pH 4.25 by Slope Ratio Method.

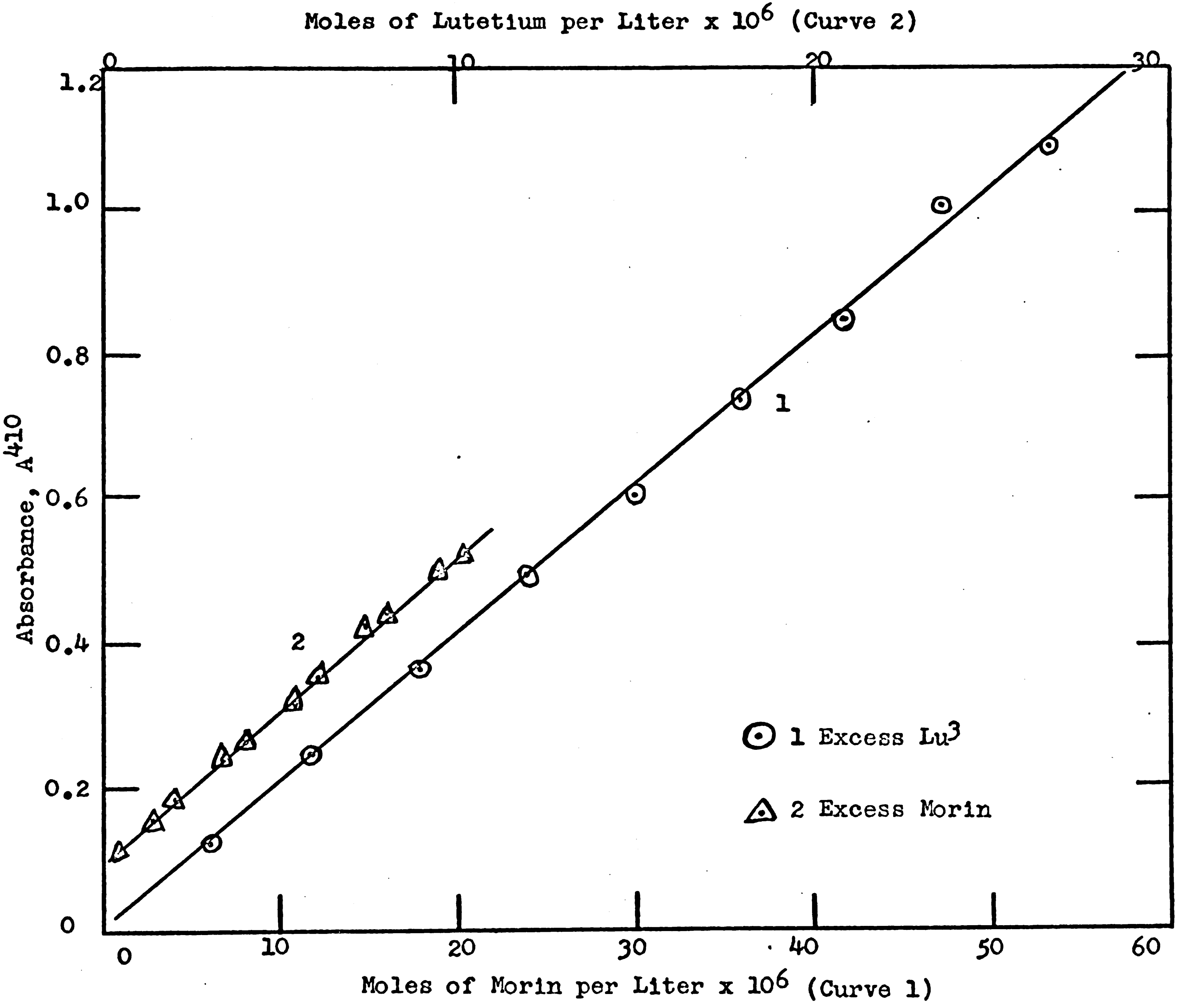


Figure 29. Determination of Composition of the Lutetium(III) Morin Complex in 50-50 DW at pH 4.25 by Slope Ratio Method

Condition	Slope of the Plotted Line	
	Gadolinium	Lutetium
Excess lanthanide ion	2.16×10^4	2.06×10^4
Excess morin	4.00×10^4	4.30×10^4
Slope ratio	1/1.85	1/2.06

The data confirm the one to two lanthanide ion to morin ion ratio for the complexes formed which produce the fluorescence.

Equilibrium Constant of the Complex

The absorptivity of morin (8.25×10^2 L/mole cm) and of the complexes (GdM_2^+ -- 4.45×10^4 L/mole cm and LuM_2^+ -- 4.17×10^4 L/mole cm) calculated using the formula $a = A/bc$, as indicated in the experimental section, were used in the calculations suggested by Milkey and Fletcher (17) to determine the concentration of the components at pH 4.25 ± 0.05. These data are shown in the Appendix. This information was used to calculate the equilibrium constants for the complexes which are included in the Appendix. The average values are 32.9 for the lutetium complex and 14.8 for the gadolinium complex. These are of the same order of magnitude but slightly higher than the equilibrium constant for the lanthanum complex reported by Fleck (7).

The calculation was also carried out using the data obtained in the Slope Ratio Method. These data gave very erratic results because the reaction was forced to completion with a large excess of one component. The resulting small concentration of uncombined second component was used in the calculation of the equilibrium constant. Errors in this small concentration lead to large variations in the calculated equilibrium constant values. The magnitude of the variation was of the order of fifty fold.

SUMMARY

Since the complexes formed by gadolinium and lutetium with morin were both investigated, all general statements made will refer to both of the complexes. Specific references will be made where differences were noted.

Absorption peaks for morin and the complexes were recorded at 355 and 410 mμ, respectively. The fluorescence spectra of the complexes maximized in the 505-510 mμ region. Morin exhibits little if any fluorescence except at high pH values.

The pH of the solution has drastic effects on both the fluorescence and absorbance of the complexes. Maximum fluorescence intensity of the gadolinium complex I_{365}^{510} occurs at pH 4.5 while that of the lutetium complex occurs at pH 4.0. It is of interest to note that in both cases the I_{436}^{510} maximum fluorescence intensity occurs approximately a half of a pH unit lower. The absorption maximum occurs at pH 4.8 and 4.2 for the gadolinium and lutetium complexes, respectively. This maximum is partially due to a shift in absorption to longer wavelengths with increasing pH.

The absorbance changes nearly linearly with increasing morin concentration over a range of 0 to 500 γ in the presence of approximately 85 γ of the lanthanide ion per 25 ml of solution. Maximum fluorescence intensity was noted at approximately 300 γ morin for gadolinium and 200 γ morin for the lutetium. Above these values the fluorescence intensity falls off due to concentration quenching by the morin. I_{365}^{510} falls very rapidly due to the strong absorption of the exciting radiation by free morin in this region.

In the presence of 400 γ morin both the fluorescence and absorbance intensity of the complex increases linearly over a range of 0 to 100 γ of the lanthanide ion per 25 ml solution.

The effect of anions on the fluorescence and absorbance of the complexes falls into two categories: 1) those having little or no effect (chloride, bromide, iodide, carbonate, acetate and tetraborate), and

2) those which inhibit the complex formation completely (sulfate, phosphate, oxalate, and tartrate).

The fluorescence intensity increases with increasing dioxane concentration up to the point where the adjustment of the pH of the solution is no longer meaningful. The fact that the pH values of the high dioxane concentration solutions is in error is borne out by observed shifts in the absorption peak which indicates a higher pH than that recorded. Similar effects were noted in the absorption measurements.

The three lanthanide ions, holmium (III), ytterbium (III), and europium (III) form nonfluorescing complexes with morin. The increase in absorption at 410 mμ with increasing concentration of these ions in the presence of gadolinium or lutetium proves this point. The fluorescence intensity I_{436}^{510} remains essentially unchanged in the presence of increasing amounts of these three lanthanide ions. However when the total lanthanide ion concentration moves above the 100 γ per 25 ml range, the complexes present in the solution absorb a considerable amount of 436 mμ radiation and reduce the fluorescence intensity. The fluorescence intensity I_{365}^{510} initially increases with increasing concentration of these three lanthanide ions. This is due to the removal of free morin from solution through the formation of the additional complex. Free morin absorbs strongly in the 365 mμ range and reduces the excitation radiation.

Both complexes were stable for from two to five days as indicated by fluorescence measurements.

Increasing the sensitivity of the instrument lowers the detection limit by increasing the instrument response for a given amount of fluorescence. A very suitable working curve was produced over the range of 0-10 γ of lanthanide ion per 25 ml. One complex is formed between the lanthanide ions and morin as indicated by the formation of one isoabsorptive point (17) in the absorption spectra of a series of solutions of constant morin concentration and varying lanthanide ion concentration.

Job's (13) method of continuous variations and Harvey and Manning's (11) slope ratio method indicate this to be a 1:2 lanthanide ion : morin complex. This relationship holds true at pH 4.25 where fluorescence occurs, as well as at pH 5.2 and 7.0 where the complex no longer exhibits the strong fluorescence. This would indicate a change in the structure of the complex rather than the formation of a different complex is responsible for the change in fluorescence character of the system.

The equilibrium constants, K_{eq}, 32.9 for the lutetium complex and 14.8 for the gadolinium complex are slightly higher but of the same order as the value reported for the lanthanum complex (7).

CONCLUSIONS

The fluorescent complexes formed by gadolinium and lutetium with morin at pH 4.25 in a 50-50 mixture of dioxane and water are suitable for use in the determination of these cations singly over a concentration range of 1-100 γ per 25 ml of solution.

The three fluorescing complexes formed with morin by lanthanum, gadolinium, and lutetium are in general affected in the same way by all the variables investigated. However, they might be separated to some extent through careful pH control, multiple measurements at different pH values and excitation wavelengths and the use of simultaneous equations. The presence of the non-fluorescing lanthanide complexes would not interfere unless present in relatively large amounts.

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APPENDIX

Estimated Equilibrium Ratio, K'eq, Values of the Gadolinium(III) Morin Complex in 50-50 DW at 25°.

Trial	Total $[Gd^{3}]$ Added M/1x10^{6}	Total $[MH]$[1] Added M/1x10^{6}	$[H^{+}]$ M/1x10^{5}	Z[2] M/1x10^{6}	Y[3] M/1x10^{6}	$[GdM_2^{+}]$ M/1x10^{6}	A[4] 410 mμ	K'eq
1	4.3	38.9	5.5	2.3	34.9	2.0	0.088	2.2[5]
2	8.7	34.6	6.3	4.2	25.7	4.5	0.192	6.4[5]
3	10.8	32.4	5.5	5.4	21.5	5.5	0.235	6.6[5]
4	13.0	30.3	6.3	6.7	17.8	6.3	0.273	9.4
5	14.0	29.2	5.4	7.4	15.9	6.7	0.287	10.3
6	15.1	28.1	6.0	8.3	14.5	6.9	0.292	14.0
7	17.3	25.9	6.3	10.6	12.5	6.7	0.290	16.0
8	21.6	21.6	5.6	15.3	9.0	6.3	0.272	16.1
9	25.9	17.3	5.3	20.4	6.3	5.5	0.237	19.1
10	30.3	13.0	5.5	25.8	4.0	4.5	0.193	33.5[5]
11	34.6	8.7	6.0	31.9	3.2	2.7	0.114	30.6[5]
12	38.9	4.3	6.2	37.7	1.9	1.2	0.051	35.9[5]

[1] MH = Morin

[2] Z = Moles of uncombined Gadolinium(III) per liter

[3] Y = Moles of uncombined Morin per liter

[4] Absorbance values and data were obtained from solutions used in the continuous variation study.

[5] Not included in the calculation of the average value.

Estimated Equilibrium Ratio, K'eq, Values of the Lutetium(III)·Morin Complex in 50-50 DW at 25°.

Trial	Total $[Lu^3]$ Added $M/1x10^6$	Total $[MH^1]$ Added $M/1x10^6$	$[H^+]$ $M/1x10^5$	Z^2 $M/1x10^6$	Y^3 $M/1x10^6$	$[LuM_2^+]$ $M/1x10^6$	A^4_{410} mμ	K'_{eq}
1	4.3	38.9	5.8	1.2	32.7	3.1	0.132	8.1[5]
2	8.7	34.6	5.6	2.2	22.5	6.4	0.266	18.2[5]
3	10.8	32.4	5.3	3.6	17.9	7.2	0.297	17.3[5]
4	13.9	30.3	6.3	5.1	14.5	7.9	0.323	29.0
5	14.0	29.2	6.3	6.0	13.2	8.0	0.328	30.8
6	15.1	28.1	6.3	7.0	11.0	8.0	0.329	37.3
7	17.3	25.9	5.6	9.0	9.4	8.3	0.340	32.5
8	21.6	21.6	6.0	14.4	7.2	7.2	0.298	34.2
9	25.9	17.3	5.6	19.5	4.4	6.4	0.268	34.6
10	30.3	13.0	5.3	25.6	3.7	4.7	0.199	37.4
11	34.6	8.7	6.0	31.9	3.3	2.7	0.122	28.1
12	38.9	4.3	6.3	38.0	2.5	0.9	0.055	25.2[5]

[1] MH = Morin

[2] Z = Moles of uncombined Lutetium(III) per liter

[3] Y = Moles of uncombined Morin per liter

[4] Absorbance values and data were obtained from solutions used in the continuous variation study.

[5] Not included in the calculation of the average value.

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