A SPECTROFLUOROMETRIC STUDY OF MORIN AND 5,7-DICHLORO-8-QUINOLINOL COMPLEXES OF LANTHANUM (III)

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ABSTRACT

A SPECTROFLUOROMETRIC STUDY OF MORIN AND 5, 7-DICHLORO-8-QUINOLINOL COMPLEXES OF LANTHANUM (III)

by Lawrence LaRoy Fleck

Lanthanum (III) forms fluorescent complexes with morin or 5, 7-dichloro-8-quinolinol. The various factors which affect the fluorescence of these complexes in 50 percent dioxane-50 percent water and the nature of these complexes were investigated.

At pH 5.5 lanthanum (III) forms a 1:2 complex with morin (lanthanum (III):reagent). The value of the equilibrium "constant" for this complex is approximately 0.6.

Solutions of lanthanum (III)-morin at the optimum pH of 5.5 are excited to nearly equal fluorescence intensities by the 365, 405 and 436 mμ mercury radiations yielding a fluorescence band whose peak is between 505 and 510 mμ. Under these conditions morin is relatively non-fluorescing. Fluorescence intensity of the complex increased with increasing dioxane content. The fluorescence intensity varies linearly when the concentration of lanthanum (III) is varied between 0-80 γ with the morin concentration fixed at 400 γ per 25 ml. Morin concentrations greater than 400 γ decreased the fluorescence intensity of the complex by concentration quenching. The addition of up to 130 γ of samarium (III), which forms a non-fluorescing complex with morin, to 25 ml. solutions containing 40 γ lanthanum (III) and 400 γ morin does not significantly change the fluorescence intensities originating from these solutions when excited by 365 mμ radiation. The rate of change in the fluorescence intensity of the complex corresponds to 1.8 fluorescence intensity units decrease per degree centigrade increase over the temperature interval 9-50°.

The absorption band peaks of the complex and morin are at 410 and 356 mμ, respectively, and the absorptivities of the complex and morin at 410 mμ are 4.00×10^4 and 2.28×10^3 liters per mole-cm., respectively. The pKa of morin is 6.7.

The species, which is responsible for the fluorescence yield of 50 percent dioxane-50 percent water solutions containing lanthanum (III) and 5,7-dichloro-8-quinolinol at pH 9, is probably the 1:3 complex (lanthanum (III):reagent). The complex is excited by the 365 mμ mercury radiation to yield a fluorescence band peak between 526 and 530 mμ, while the absorption maximum is at 388 mμ. For solutions containing 1000 γ of reagent, the lanthanum (III) concentration range in which the fluorescence intensity changes linearly with concentration is approximately 0-70 γ of lanthanum (III) per 25 ml. Maximum fluorescence intensity readings for solutions containing 80 γ of lanthanum (III) are obtained when the reagent concentration is varied between 800-1500 γ per 25 ml. Above 1500 γ of reagent, the fluorescence intensity decreases by concentration quenching. When 0-35 γ of samarium (III) are added to solutions containing 40 γ lanthanum (III) and 1500 γ of reagent in 25 ml., no appreciable change in intensity is noted. Maximum fluorescence readings are obtained in solutions containing between 50 and 55 percent dioxane. Addition of sodium carbonate to solutions of the complex reduces the fluorescence intensity, therefore, carbonate free base must be used in the development of the complex at pH 9. An increase in temperature decreases the fluorescence intensity 3.0 fluorescence intensity units per degree centigrade over the temperature range 14-45°.

The logarithms of the consecutive formal stability constants, K_1 and K_2, for the lanthanum (III)-5,7-dichloro-8-quinolinol complexes are 7.3 and 6.5, respectively. The value of K_3 can not be determined by a titrimetric method since lanthanum (III) tends to hydrolyze at higher pH

values. The pK_{NH} and pK_{OH} of 5, 7-dichloro-8-quinolinol at 25^{o} are estimated to be 1.6 and 9.11, respectively.

Both morin and 5, 7-dichloro-8-quinolinol have potential analytical applications as fluorometric reagents for the determination of microgram quantities of lanthanum (III) in pure solutions or in solutions containing trace amounts of samarium (III).

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES - Continued

FIGURE Page

INTRODUCTION

When many organic and some inorganic substances are excited by a relatively high-energy source, usually an ultraviolet light source, fluorescence, a particular type of photoluminescence, is observed. In this phenomenon there is only a relatively short, finite time delay between the absorption and emission of radiant energy.

Fluorescence is produced in molecules by the absorption of radiant energy of a frequency within the normal absorption band of the molecule, usually in the ultraviolet region of the spectrum. The absorbed radiation excites the molecule to one of many vibrational energy levels of an upper excited electronic singlet state. (A singlet state in an unsaturated molecule is considered to be characterized by a pair of unsaturation (π) electrons having paired, antiparallel, spins (23).) Within a period of less than 10^{-12} second (26), the energy above the lowest vibrational level of the first excited electronic singlet state may be dissipated by a non-radiative transition. The molecule, within a period of about 10^{-9} second, then returns to an excited vibrational energy level of the ground electronic singlet state. This latter transition, which is a singlet $\longrightarrow$ singlet transition, gives rise to a fluorescence band (23, 26). Since the fluorescence transition usually involves less energy than the absorption transition, the fluorescence band appears at longer wavelengths than the absorption band.

The intensity or power of the fluorescence radiation I_f, emitted by a solution of the fluorescing solute in a non-fluorescing solvent, is related to the concentration by the relationship $I_f = QI_o (1-10^{-abc})$, where I_o is the intensity of the incident radiation, Q is the quantum efficiency of the absorbed radiation in producing fluorescence, $\underline{a}$ is the absorptivity, and $\underline{b}$ is the cell width. For low concentrations it has been shown, that by means of an exponential series transformation, the above equation simplifies to $I_f = QI_o abc$ (10). Consequently, at low concentrations the fluorescence intensity I_f is proportional to $\underline{c}$, the concentration.

A relatively limited number of inorganic substances fluoresce in both the crystalline state and in solution with their characteristic line or band spectra. Among these substances are the tripositive lanthanide ions of samarium, europium, terbium, and dysprosium (5, 40), and gadolinium (40). However, the trivalent salts of praseodymium, neodymium, holmium, erbium, and thulium are reported to be luminescent only in liquid or solid solution and as activators in crystal phosphors (40). Furthermore the tripositive ions of lanthanum, cerium, ytterbium, and lutetium do not yield line fluorescence spectra as pure salts or in crystal phosphors (40).

Although a number of the tripositive lanthanide ions fluoresce, extensive quantitative analytical applications of this phenomenon have not been exploited. The main reason for the apparent lack of interest in determination of the lanthanides by measurement of their characteristic line fluorescence is that exciting radiation wavelengths shorter than 250 mμ are necessary to obtain adequate energies for excitation. The lanthanide ion absorptions are weak and narrow for wavelengths longer than 250 mμ and as a result excitation of moderate intensities require great intensities of exciting light. For wavelengths shorter than 250 mμ, the lanthanide ions are relatively strongly absorbing and have continuous absorption bands (9, 18). Unfortunately, the uncontrolled nature of the exciting sources presently available, such as arc or spark discharges for wavelengths shorter than 250 mμ, exclude accurate measurements of the fluorescence intensities (11).

A few organo-lanthanide complexes can be excited in the near ultraviolet and visible region of the spectrum to emit intense fluorescence which is characteristic of the complex. Lanthanum (III), gadolinium (III), and lutetium (III) are the only tripositive lanthanide ions which contain sufficiently stable cation electronic configurations to yield an observable fluorescence which is characteristic of their complexes. The remaining tripositive lanthanide ions, having other than zero, seven or fourteen 4f

electrons, are considered to diminish the fluorescence yields of the complex due to the interaction between the electronic field of the metal ion and the optical electrons of the organic entity responsible for the fluorescence in the complex. This strong "internal quenching"* effect of the tripositive lanthanide ions other than lanthanum (III), gadolinium (III), and lutetium (III) is apparently caused by the deactivation of the fluorescence of the complex by an "intramolecular energy transfer" (27, 47, 58) process which is brought about by spin-orbital interactions (8). In qualitative detection tests, lanthanum (III), gadolinium (III), and lutetium (III) were found to be the only tripositive lanthanide ions which formed fluorescent complexes with morin (2′, 4′, 3, 5, 7-pentahydroxyflavone (37) and 8-quinolinol (37, 43).

To the present time, most of the work done on the fluorescence of lanthanide complexes has been of a qualitative nature in which the various factors which affect fluorescence have been disregarded. It was the purpose of this study to initiate a systematic study in which, (1) organic reagents are tested as to whether or not they form fluorescent complexes with lanthanide ions, (2) the characteristics of the complexes are established, and (3) the various factors, such as the affect of solvent, pH of solution, reagent concentration, temperature, and excitation wavelength, which affect the fluorescence are tested. Lanthanum (III) was selected as the representative member of lanthanide ions which form fluorescent complexes with some organic compounds. The organic compounds selected were morin, 8-quinolinol, and 5, 7-dichloro-8-quinolinol.

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*Quenching is the phenomenon by which substances showing fluorescence have their emission reduced or extinguished by substances added to the solutions, changes in temperature, solvent, or physical state, or increase in concentration of absorbing species. When it is due to none of these, however, but represents a real reduction of "quantum yield" due to conversion of light energy of the excited species, it may be called "true quenching" (6).

HISTORICAL

Several books concerning the theoretical and practical aspects of fluorescence have been written. Pringsheim in a book entitled "Fluorescence and Phosphorescence" (40) discussed the theoretical aspects of fluorescence and phosphorescence and reviewed existing information on many substances which are known to fluoresce. In this book, one complete chapter is devoted to the fluorescence of the lanthanide ions in crystals, solutions, and in phosphors. In a book "intended for the use of students and practical workers," entitled "Fluorescence of Solutions" by Bowen and Wokes (6), the most significant facts pertaining to fluorescence theory and the practical aspects of fluorescence measurement have been considered. In "The Chemical Aspects of Light," Bowen (5) has presented, insofar as possible, a non-mathematical treatment of the modern concepts of the interaction of light with matter. The author (5) also discussed the transformation of absorbed radiation and its relation to fluorescence. Although there are several other books or chapters within books which are devoted to fluorescence, the above noted works contain a reasonably complete treatment of the subject.

In a series of review articles by White (48, 49, 50, 51, 52, 53, 54, 55), covering the period of 1939 to 1960, organic and inorganic applications of fluorometric analysis and the fundamental developments in instrumentation are discussed. Approximately 1000 references are cited in these review articles.

Morin has been used extensively as a reagent for the fluorometric analysis or fluorometric detection of a relatively large number of metals (2, 8, 19, 42, 46).

Sill and Willis (41) have reported a fluorometric method for the determination of beryllium (II) by complexation with morin in a basic solution which contained EDTA to complex traces of impurities contained in the solvent. Bismuth, silver, mercury, gold and platinum metals

interfered in this method. Yttrium (III), scandium (III), lanthanum (III), and lithium (I) were reported to yield a green fluorescence which was similar to beryllium(II), while thorium (IV) and zirconium (IV) produced a yellowish fluorescence when complexed with morin. The authors reported that ions such as lanthanum (III) and thorium (IV) formed fluorescent complexes with morin even in the presence of EDTA. Cerium (IV), praseodymium (III), neodymium (III), samarium (III) and uranium (VI) reduced the fluorescence intensities of the beryllium (II) morin solutions.

Fletcher and Milkey (29) conducted a very thorough fluorometric study of the thorium (IV)-morin complex formed in slightly acidic ethanol-water solutions. The effect on the fluorescence of the complex by such variables as concentration of acid, ethanol content, thorium (IV), morin and complex concentrations, time, temperature and wavelengths of exciting radiation were studied to determine experimental conditions which yield maximum fluorescence. Equations which account for the contribution to the total fluorescence by three components in this system when the fluorescence is measured in a transmission-type fluorometer were derived.

Analytical fluorescence tests employing morin as a reagent for the detection of small amounts of aluminum (III), berylium (II), gallium (III), germanium (IV), tin (IV), antimony (V), thorium (IV) and zirconium (IV) have been worked out by Patrovsky (35).

Geiger and Sandell (16) have developed a fluorometric method for the determination of submicrogram quantities of zirconium (IV) with morin in 2 M hydrochloric acid, ethanol-water solutions. The various experimental factors which affect the fluorescence of the complex were tested. More than 30 elements including lanthanum (III) were individually tested to determine their effect on the fluorescence of the complex. Solutions containing one or two mg. of lanthanum (III) in the presence of 300 γ of morin did not yield any measurable fluorescence.

The influence of various anions on the morin fluorescence test for aluminum (III), gallium (III), beryllium (II) and zinc (II) were evaluated by Bishop (3). Citrate, fluoride, oxalate, phosphate, tartrate or vanadate ions diminish the fluorescence intensity of complexes formed between any of the above listed cations and morin. The presence of any individual anion which forms a complex or a precipitate with any of the above listed cations can be detected by its quenching of the metal ion-morin complex fluorescence.

Pollard, et al. (38) employed paper chromatograms for separation and organic complexing agents for identification in a study to develop tests for distinguishing between lanthanon groups and for identifying certain individual lanthanides. The morin and 8-quinolinol complexes of lanthanum (III), gadolinium (III), and lutetium (III) were the only ones which could be detected visually by their fluorescences.

Lederer (24, 25) studied the separation of lanthanides by paper chromatography. The location of the individual lanthanides on the chromatograms was accomplished by illuminating the chromatogram, which had been treated with a solution of 8-quinolinol, with ultraviolet light and noting where fluorescence appeared. Lanthanum (III), erbium (III), and lutetium (III) complexes emitted a green fluorescence, while all other lanthanide complexes appeared only as brown or black spots.

White, et al. (56) have determined the optimum pH of solutions, absorption band peaks, maximum excitation wavelengths, and the fluorexcence band peaks for a number of metal chelates including aluminum (III)-morin, beryllium (II)-morin and lithium (I)-8-quinolinol.

Several investigators have studied the fluorescence of metal ion-8-quinolinol complexes (12, 31, 32, 57). Ohnesorge and Rogers obtained spectrofluorometric data on a number of group III A metal-8-quinolinol complexes. The effects of acidity (33) and different solvents (34) on the fluorescence of these complexes were studied.

Popovyelt and Rogers (39) obtained the fluorescence spectra, intensities, and "efficiencies" of zinc (II) chelates of 8-quinolinol, 8-quinolinol-5-sulfonic acid, 2-methyl-8-quinolinol, and 5, 7-dichloro-8-quinolinol as a function of solvent and substituents. The fluorescence spectra of 2-methyl-8-quinolinol chelates with gallium (III), indium (III), magnesium (II) and cadmium (II) were also obtained. The solvents tested were chloroform, ethanol, carbon tetrachloride, dimethylformamide, ether, water, and tetrahydrofuran. The fluorescence intensities were found to be highest in "inert" solvents.

Nishikawa (30, 31, 32) in a series of papers on fluorometric analysis discussed the fluorescence behavior of chloroform solutions or suspensions of metal chelates of the metals in groups I, II, III, and IV in the periodic table. The organic reagents which were employed in these studies included 8-quinolinol, 5, 7-dichloro-8-quinolinol, 5, 7-dibromo-8-quinolinol, and 5, 7-diiodo-8-quinolinol.

Nishikawa (31) also discussed the relationship between fluorescence of metal ion-8-quinolinol complexes and the position of the metal in the periodic table. The author stated that the following relationships exist between the position of metallic elements in the periodic table and the ability of their ions to form fluorescent complexes:

1). Group I, II, III and IV metal ions form fluorescent complexes.

2). Group IIa, IIIa and IIb metal ions form the most intensely fluorescing complexes.

3). Within a group, the lower the atomic number of the metal, the stronger will its complex fluoresce.

4). Metal ions which have a high magnetic susceptibility do not yield fluorescent complexes.

Fassel and Heidel (11) have developed a method for the determination of 0.005 to 100 per cent terbium in complex lanthanide mixtures by measurement of fluorescence intensity at 545 mμ, the maximum peak of the strongest fluorescence band emitted when aqueous solutions containing terbium (III) are irradiated with ultraviolet light.

EXPERIMENTAL

INSTRUMENTATION

The spectrofluorometer used in this work is a modification of one constructed in this laboratory by Thommes (44). The only change in the instrument was the replacement of the Farrand grating monochromator and associated light source with a high resolution Bausch and Lomb Model 33-86-40 grating monochromator, and a Hanovia S-H high pressure mercury arc and the required Hanovia 110/120 volt constant voltage transformer. Figure 1 shows a block diagram of the modified instrument and the excitation and fluorescent emission radiation light paths.

The Bausch and Lomb monochromator was connected by a light tight seal to the cell compartment by means of a rubber 0-ring gasket. The monochromator and a quartz cylindrical converging lens which was mounted in the cell compartment to replace the factory equipped collective lens, were positioned so that the narrow, nearly parallel beam of light from the source was focused on the center of the cell. This arrangement was found to give satisfactory operation.

The S-H mercury arc served as the source for excitation in all the experimental work. This lamp emits seven mercury lines which are sufficiently intense for fluorescence work. These are the 436, 405, 365, 313, 303, 265, and 254 mμ lines.

Clear window silica cells, 10 x 20 x 50 mm., purchased from the Farrand Optical Company, were employed for all fluorescence measurements made in this study. The cells could be reproducibly positioned in the instrument cell compartment by means of a specially constructed cell holder. These cells are quite transparent to all the principle mercury lines listed above.

The procedure employed in obtaining fluorescence intensity measurements was as follows:

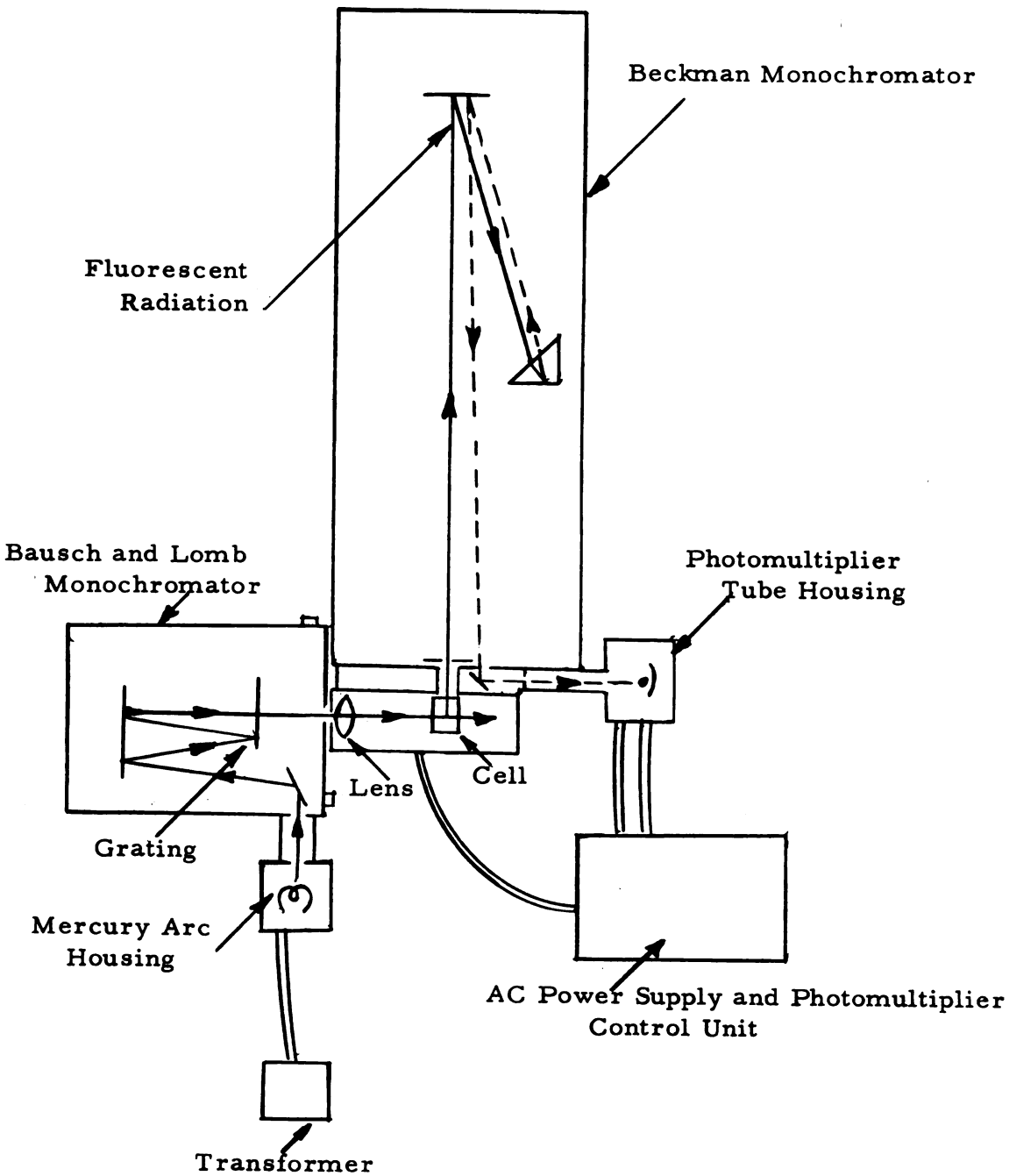

Figure 1. Block Diagram and Radiation Paths of Spectrofluorometer

1) The mercury arc lamp, AC Power Supply, and Beckman DU instrument were allowed to warm up for at least thirty minutes prior to use in order to insure maximum instrument stability.

2) The photomultiplier sensitivity control knob was set to the "full" position and the Beckman DU sensitivity control knob set three turns from its clockwise limit.

3) The Bausch and Lomb monochromator entrance and exit slits were set to 1.0 and 0.5 mm., respectively, and the Beckman DU slit adjusted to approximately 1.0 mm.

4) The Beckman DU instrument was zeroed with the "dark current" control knob before each series of measurements. The instrument was calibrated with a 0.4 γ per ml. dichlorofluorescein standard solution by the following procedure. The cell containing the standard solution was placed in the cell holder, the phototube shutter was opened and with the selector switch in the 0.1 position, slight adjustments were made in the Beckman DU slit width so that a reading of 50 on the o/oT scale was obtained. For this calibration procedure the Beckman DU and Bausch and Lomb monochromators were set at 530 and 365 mμ, respectively.

5) After the instrumental operational variables were established through the calibration procedure, the fluorescence intensities of the solutions investigated were obtained at the desired excitation and emission wavelengths. No corrections for the variations of photomultiplier tube sensitivity for various wavelengths were applied to fluorescence spectral data.

A Beckman DU or a DK-2 spectrophotometer was used for all absorption measurements. The Beckman DU, however, was employed exclusively for the single wavelength measurements required for the stability and complex composition studies. Matched one cm silica cells were employed in all these measurements.

A Sargent constant temperature bath, equipped with a number 7530 thermo-regulator and associated Sargent number S-2770 heater control

unit, was maintained at 25° ± 0.2 for the preparation and storage in flasks of all solutions employed in this study. After thermal equilibrium of the flasks and contents was attained, aliquots were withdrawn from the flasks and the single wavelength fluorescence measurements were made within one minute after sample withdrawal.

A Beckman Model G pH meter with a glass-saturated calomel electrode pair was used for all measurements except those employed in the chelate stability studies. The pH meter which was standardized at pH 4 or 7 with Beckman standard buffer solutions, gave satisfactory performance for all solutions containing less than 70 per cent dioxane. Erratic electrode behavior was noted for solutions of very high dioxane content.

The apparatus employed in the alkalimetric titrations, which were performed for the determination of the chelate stabilities of the lanthanum (III)-5, 7-dichloro-8-quinolinol system, consisted of a Beckman Model G pH meter equipped with a macro saturated calomel and glass electrode pair and a titration vessel which was maintained at 25° in the constant temperature bath. The electrical lead of the glass electrode was shielded with grounded aluminum foil to eliminate any stray electrical pickup. The electrode pair was mounted in a polyethylene disk which was one-quarter inch thick and four inches in diameter and was equipped with ports for a nitrogen inlet tube, two semi-micro burets tips, and an electric motor driven glass stirring paddle. The electrode assembly was positioned on top of either a 250 or 400 ml. beaker and the cell unit rigidly mounted in the 25° water bath. The Beckman pH meter was calibrated as previously indicated at pH 4 and 7 and was found to give satisfactory operation for this system.

REAGENTS

The purification of a number of chemicals employed in this investigation was not deemed necessary due to their initial high degree of purity. The chemicals, labeled purity and source are:

Dichlorofluorescein	Eastman Kodak, white label
Lanthanum sesquioxide	Optical Grade, Heavy Minerals Co., Chattanooga, Tennessee
Morin dihydrate	Doctor Theodor Schuchardt, München, Germany
Perchloric acid	70-72 percent Bakers Analyzed Reagent
Potassium acid phthalate	Primary Standard, Bakers Analyzed Reagent, oven-dried for 2 hours at 105°
Samarium sesquioxide	Labeled purity 99.9 per cent, Michigan Chemical Corporation, Saint Louis, Michigan
Sodium acetate trihydrate	A.C.S., Fisher Certified Reagent
Sodium carbonate	A.C.S., Fisher Certified Reagent

Matheson Coleman and Bell 8-quinolinol was purified by sublimation under the reduced pressure of a water aspirator in a large sublimation apparatus maintained in a water bath at approximately 70-72°. The melting point of the pure white needles of the sublimed 8-quinolinol was found to be 74-74.5° (u.c.); American Chemical Society specifications, 72.5-73.5°.

The 5,7-dichloro-8-quinolinol was prepared by chlorination of 8-quinolinol dissolved in ethanol (17). The reagent was recrystallized variously from glacial acetic acid, acetone, and absolute alcohol and dried in an oven at 105° for approximately two hours.

Commercial grade dioxane was purified by a modification of the procedure of Hess and Frahm (21). A mixture of 54 ml. of concentrated hydrochloric acid, 400 ml. of distilled water, and 4 l. of dioxane was refluxed for 12 hours, during which time a slow stream of nitrogen was

bubbled through the solution to sweep out aldehydes. The solution was cooled and potassium hydroxide pellets added slowly with mixing until the solution became saturated and a second layer formed. The dioxane was decanted, treated with fresh potassium hydroxide pellets to remove adhering aqueous liquor and transferred to a large screw capped bottle containing anhydrous calcium chloride. The following day the dioxane was filtered into a clean flask, refluxed for 12 hours over calcium hydride, and distilled. A 100 to 100.5° fraction was collected and the dioxane stored in an amber colored screw capped bottle.

The distilled water employed throughout this investigation was allowed to pass through a "Crystalab Deeminizer" ion exchange column in order to remove possible metal ion impurities contained in the water.

Preparation of Reagent Solutions

A 0.1 M stock solution of ammonium hydroxide was prepared from distilled reagent grade ammonia and stored in a polyethylene bottle equipped with an ascarite absorption bulb and glass siphon assembly.

Approximately 0.1 M carbonate free sodium hydroxide was prepared from a saturated solution of the reagent grade pellets and standardized with potassium acid phthalate by titration to the phenolphthalein endpoint. The sodium hydroxide solution was also stored in a polyethylene bottle equipped with an ascarite absorption bulb and a glass siphon assembly.

A 0.4 γ per ml. solution of dichlorofluorescein in four percent ethanol, which was employed as the fluorescent standard, was prepared by diluting an aliquot of a stock solution made by dissolving a weighed quantity of the reagent in 95 percent ethanol.

Individual stock solutions of lanthanum (III) or samarium (III) perchlorate in dilute perchloric acid were prepared by dissolving the required amounts of freshly ignited oxides in 1 M perchloric acid.

Aliquots of these stock solutions were taken for the preparation of the working solutions. The lanthanum stock solution was analyzed by precipitation of the lanthanum (III) with 8-quinolinol according to the procedure of Pirtea (36). The analyzed solution contained 0.0938 moles of lanthanum (III) perchlorate per liter. Since the samarium was used only in interference tests, it was not analyzed quantitatively.

Solutions of morin, 8-quinolinol, and 5,7-dichloro-8-quinolinol of the desired concentrations were prepared by dissolving a weighed quantity of the solid material in dioxane. The reagents adopted for use in the analytical studies contained respectively, 0.2, 1.0, 1.0 mg. of morin, 8-quinolinol, and 5,7-dichloro-8-quinolinol per ml. of dioxane. Even though there was no evidence that these reagent solutions were unstable, fresh reagent solutions were prepared every two or three weeks.

EXPERIMENTAL PROCEDURES

Introduction

The spectrofluorometric study of the complexes which form between lanthanum (III) and the organic reagents, morin, 8-quinolinol, and 5,7-dichloro-8-quinolinol, was made with dioxane-water solutions. In establishing the optimum conditions for the reactions between lanthanum (III) and the organic reagents, the individual effects of the more important experimental variables were established. In studying these effects, the reagents were added in the order described in the solution preparation procedures.

Preliminary investigations of the effect of pH and dioxane content on the fluorescence of a lanthanum (III)-organo complex were carried out on the lanthanum (III)-8-quinolinol system. In the pH region of maximum fluorescence, this complex is not sufficiently soluble in solutions containing less than 60-70 percent dioxane for satisfactory study.

When sufficient lanthanum (III) and 8-quinolinol was introduced into a 50-50 DW* medium and the pH adjusted to attain maximum fluorescence, a suspension rather than a solution was obtained. Since the fluorescence intensities of the complex are highly pH dependent and, as previously indicated, the pH meter readings are erratic and non-reproducible for solutions containing greater than 60 percent dioxane, no further studies on the lanthanum (III)-8-quinolinol system were made.

The Effect of Temperature

The effect of temperature on the fluorescence of morin and 5, 7-dichloro-8-quinolinol complexes of lanthanum (III) was estimated by heating the solutions contained in 25 ml. glass stoppered bottles in a water bath or cooling in an ice bath to approximate desired temperature for recording the fluorescence intensities. The temperature was measured by a mercury thermometer which was immersed in the fluorescence cell immediately before and immediately after obtaining the fluorescence readings. A plot was then constructed of the average of the two temperature readings against the fluorescence intensities. For both complexes, a nearly linear decrease in fluorescence intensities with increasing temperature was observed. From the respective plots, a relative temperature coefficient expressed as fluorescence intensity units decrease per degree centigrade increase was evaluated for each of the complexes over the temperature range in which the fluorescence decreased linearly.

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*Hereafter a 50 percent dioxane-50 percent water solvent will be designated as 50-50 DW solvent. During this investigation no attempt was made to correct for the volume contraction on mixing dioxane and water. When equal volumes of dioxane and water were mixed, the error was found to be 1.5 percent at 25°. Freiser stated that the error was less than 2 percent (7).

Lanthanum (III)-morin System

In preliminary investigations, dilute ethanol-water and dioxane-water solutions of lanthanum (III)-morin or morin were tested to determine which solvent system would be most satisfactory for the spectrofluorometric and spectrophotometric study of the complex. The results of these studies indicate that higher fluorescence intensities are obtained when some definite amounts of lanthanum (III) and morin are dissolved in a dioxane-water medium than when the same quantities of lanthanum (III) and morin are dissolved in an equivalent volume of an ethanol-water solvent. Furthermore, morin dissolved in ethanol-water fluoresced noticeably throughout the pH range in which the complex fluoresced, whereas the morin dissolved in dioxane-water did not. For these reasons a dioxane-water solvent system was employed throughout this entire investigation.

Solution Preparation Procedure

The 50-50 DW solutions of lanthanum (III)-morin or morin were prepared in either 25 or 50 ml. volumetric flasks. Unless otherwise noted the concentrations were expressed in units of micrograms, γ, per 25 ml. of solution.

A measured volume of the 0.2 mg. per ml. of dioxane stock solution of morin was introduced into a 25 ml. volumetric flask and sufficient dioxane added so that the total volume in the flask was 12.5 ml. The required volume of a lanthanum (III) stock solution was added and the total perchloric acid content adjusted to 0.1 meq. by adding the necessary amount of 0.1 M perchloric acid. Then 0.65 ml. of 0.2 M sodium acetate and sufficient water were added to bring the liquid level nearly to the encircling mark on the neck of the flask, the flask was tightly stoppered, inverted several times, and placed in the 25° water bath for at least one-half hour. Finally, water was added to

the flask to bring the liquid level to the mark, the flask stoppered and the contents thoroughly mixed. The flask with its contents was again placed in the bath until needed for the measurements.

The above procedure was employed in the preparation of all solutions with the exception of those prepared for the effect of pH and the nature of the complex studies. For these solutions the sodium acetate and the perchloric acid were not added and the pH was adjusted with dilute perchloric acid or ammonia only.

Lanthanum (III)-5, 7-dichloro-8-quinolinol System

Preliminary investigations had shown that the lanthanum (III)-5, 7-dichloro-8-quinolinol complexes are sufficiently soluble in dioxane-water solutions for a spectrofluorometric study in this medium. No other solvents were tested for studies on the complex.

Solution Preparation Procedure

The 50-50 DW solutions of lanthanum (III)-5, 7-dichloro-8-quinolinol were prepared in a similar manner employed in the morin study. A measured volume of the 1.0 mg. per ml. stock solution of 5, 7-dichloro-8-quinolinol in dioxane was added to each 25 ml. volumetric flask. The total dioxane content in the flask was adjusted to 12.5 ml. and a measured volume of a lanthanum (III) perchlorate stock solution was added. Then, 0.5 ml. of 0.1 M perchloric acid was added and the solution made basic by the dropwise addition of 1.25 ml. of 0.1 M ammonia solution while carefully swirling the flask to prevent the formation of a high local concentration of the hydroxide. Water was then added slowly while swirling the flask until the liquid level was nearly to the encircling mark on the neck of the flask. The flask was tightly stoppered, inverted several times, and placed in the 25° water bath for nearly one-half hour. Finally, water was added to the

flask to bring the liquid level to the mark, the flask stoppered and the contents thoroughly mixed. The flask and its contents was again placed in the bath until needed for the measurments. The single wavelength fluorescence measurements were obtained one-half hour after addition of the ammonia.

The above procedure was employed for the preparation of all solutions except those for the effect of pH and the evaluation of the stability constants studies. The effect of pH study was carried out in the same manner as that employed for the lanthanum (III)-morin system.

Evaluation of the Complex Stability Constants

The technique of Freiser and co-workers (15), which is an adaption of the method of Calvin and Wilson (7) and Bjerrum (4) for the determination of stabilities for chelating agents possessing more than one acidic or basic group, was employed for the evaluation of the stabilities of the lanthanum (III)-5, 7-dichloro-8-quinolinol complexes. Information required to evaluate the consecutive stability constants and to establish the composition of the complexes, was obtained from alkalimetric titrations of solutions containing the metal ion and the chelating agent.

Titration Procedure: A weighed quantity of 5, 7-dichloro-8-quinolinol was transferred to the titration vessel and 105 ml. of dioxane added. To this was added a measured volume of the 0.00938 M lanthanum (III) perchlorate in dilute perchloric acid stock solution and water so that the total volume of water was 105 ml. The titration vessel and contents were purged with nitrogen for about ten minutes and the titration begun. A nitrogen atmosphere was maintained above the solution during titration. Sodium hydroxide was added in small increments by means of a semi-micro buret which was equipped with

a small ascarite absorption bulb. The volume of the titrant was decreased to 0.05 ml. increments in regions of rapidly changing pH values. With each addition of base an equal volume of dioxane was added to maintain the desired dioxane-water ratio. The solution was stirred continuously except when pH readings were taken.

DISCUSSION OF RESULTS

LANTHANUM (III)-MORIN SYSTEM

In Figure 2, curves A and B show the absorption spectra of morin and the lanthanum (III)-morin complex, respectively, while curve C shows the fluorescence spectrum of the complex when exposed to 365 mμ radiation. In order to force the formation of the complex a large excess of lanthanum (III) (16 mg.) was employed.

The complex which forms at a pH of 5.5 in 50-50 DW can be excited to nearly equal fluorescence intensities by the 365, 405 or 436 mμ mercury radiations. No appreciable fluorescence radiation can be detected when the complex is exposed to ultraviolet radiation shorter in wavelength than 365 mμ.

The absorption peaks of the complex and of the reagent are at 410 and 356 mμ, respectively, while the fluorescence maximum occurs between 505 and 510 mμ. Single wavelength absorbance and fluorescence measurements of the complex were taken at their respective peaks.

Effect of Experimental Variables on the Absorbance and Fluorescence of the Complex

Although the addition of sodium acetate and perchloric acid to solutions containing lanthanum III and morin resulted in approximately a 30 percent decrease in the fluorescence readings, the acetate buffer was used in order to facilitate the preparation of the solutions at pH 5.5. The addition of sodium acetate did not shift the absorption peaks of the reagent or of the complex.

The effect of the dioxane content on the fluorescence and absorbance of the complex was studied and the results shown in Figure 3. The fluorescence of the complex in solution at I_{365}^{505*} increases nearly linearly with

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*For notations of the type I_{365}^{505}, the subscript indicates the wavelength of excitation and the superscript denotes the wavelength at which the fluorescence radiation is recorded.

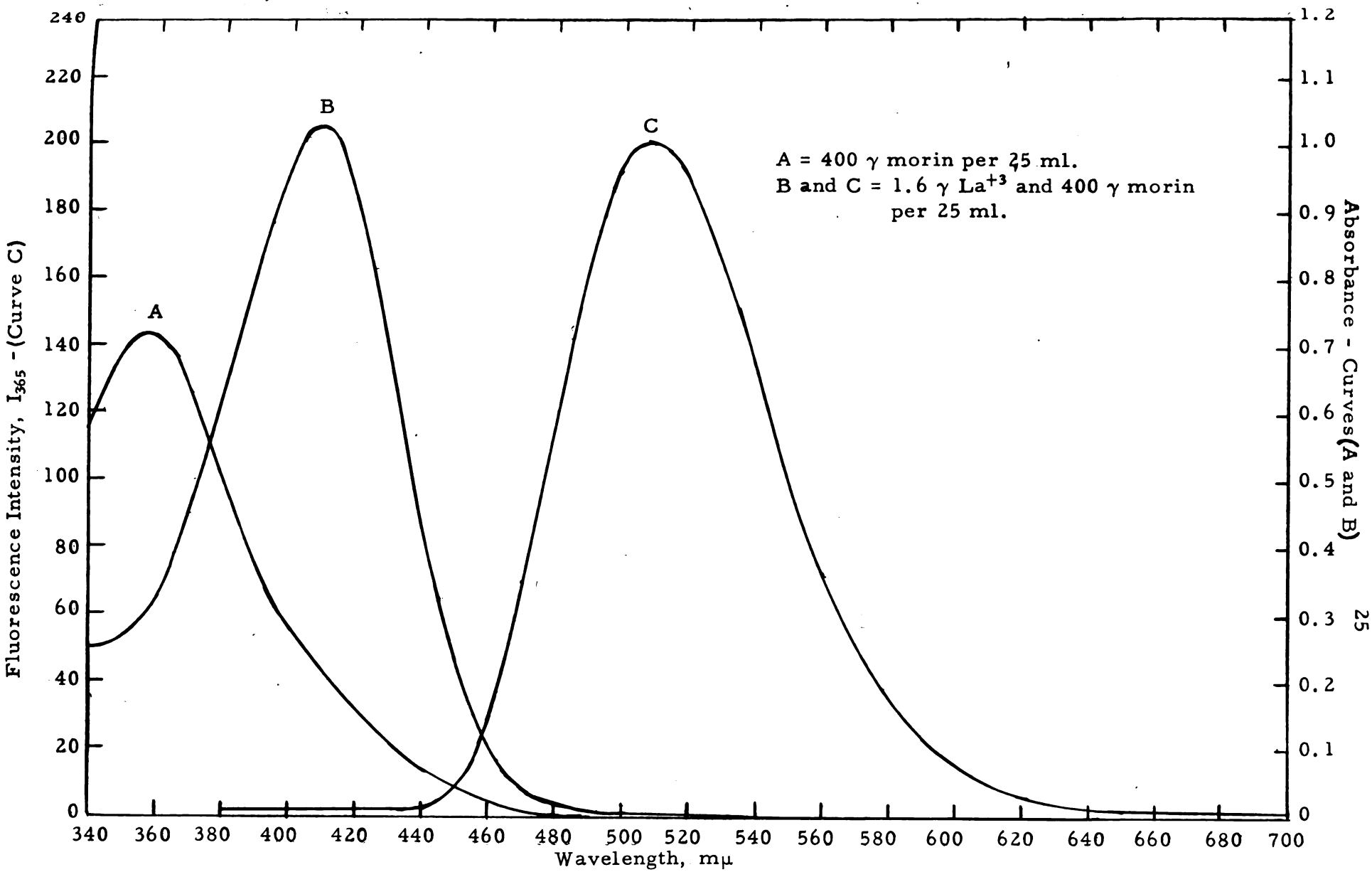

Figure 2. Absorption and Fluorescence Spectra of Lanthanum (III)-Morin and the Absorption Spectrum of Morin in 50-50 DW at pH 5.5.


100
1.0
A and B = 3.2 mg La+3 and 200 γ

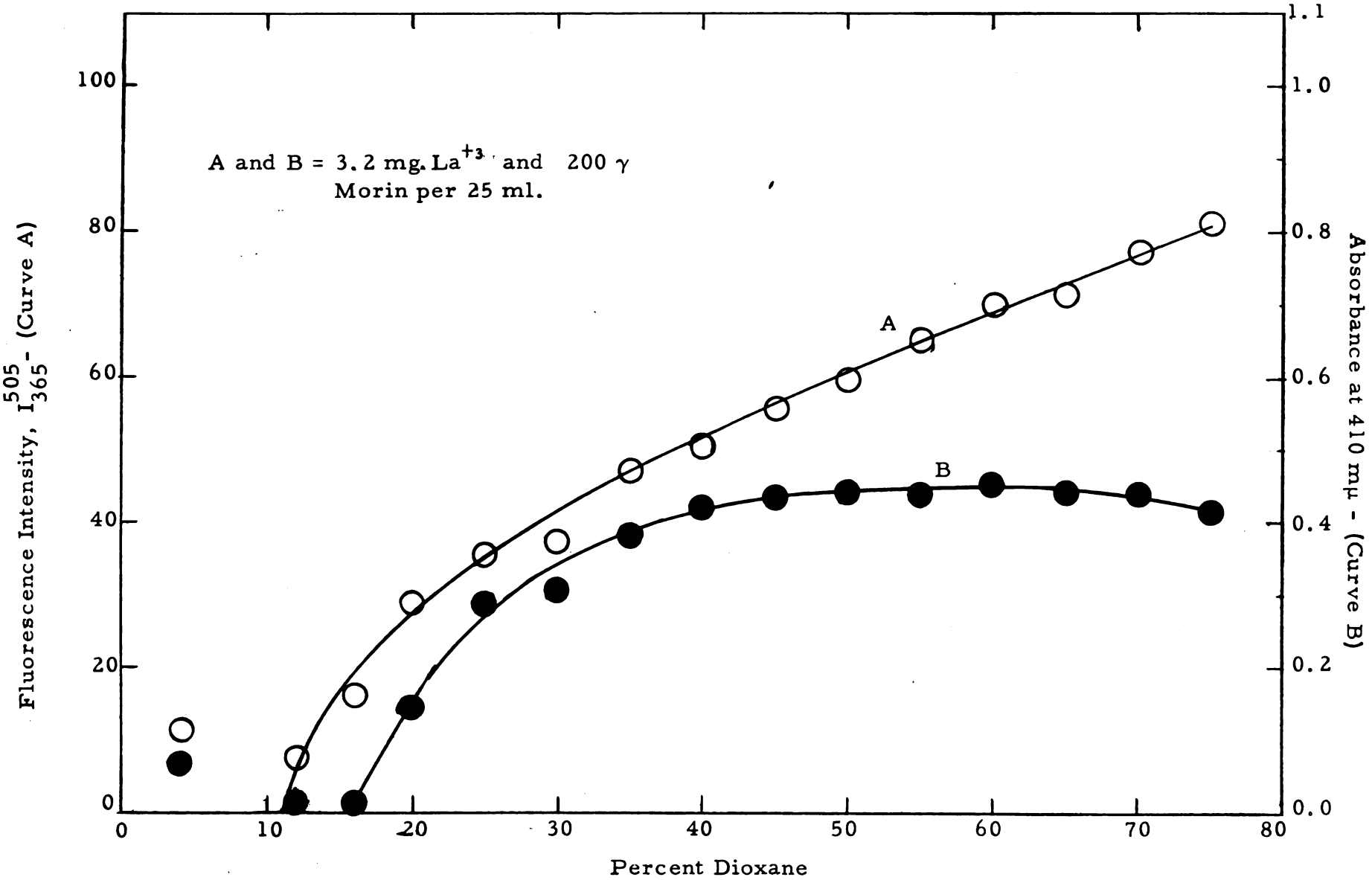

Figure 3. Effect of Dioxane Content on the Fluorescence and Absorbance of Lanthanum (III)-Morin Complex at pH 5.5

increasing dioxane content, however, the absorbance at 410 mμ reaches a maximum at about 40 percent and remains essentially constant up to at least 75 percent dioxane. A 50 percent dioxane-50 percent water solution was selected as the solvent medium for the remaining studies for the following reasons:

1) Maximum absorbances are attained in 50-50 DW.
2) At high dioxane contents, about 60 percent and above, erratic and non-reproducible pH readings are obtained.
3) Several investigators have stated that nearly absolute pH values are obtained in 50-50 DW (13, 14, 15). Freiser has stated that corrections must be applied to pH readings for solutions containing greater than 50 percent dioxane (14).

In Figure 4, curve A shows the effect of pH on the absorbance of lanthanum (III)-morin solutions at 410 mμ and curve B shows the effect on solutions containing pure morin. The absorbances of the lanthanum (III)-morin solutions reach a maximum at approximately pH 6 and then decrease. Comparison of the absorption spectra for these solutions over the pH range covered shows that the absorption peak shifts to longer wavelengths at higher pH values.

An estimation of the ionization constant for the dissociation of morin can be obtained from curve B, Figure 4. At the pH value where the absorbance is half its maximum value, morin exists half in the dissociated and half in the associated form. Therefore, the pH at the half absorbance value should correspond to the pKa of morin. From the plot, the pH at the half absorbance value yields a pKa of 6.7. Unfortunately no pKa value for morin in 50-50 DW could be found in the literature with which to compare the above results.

The effect of pH on the fluorescence radiation of lanthanum (III)-morin and pure morin solutions at 505 mμ, when the solutions are exposed to 365 and 436 mμ radiation, is shown in Figure 5. The curve for I_{405}^{505} is quite similar to I_{436}^{505} but of slightly lower intensity, hence it was not

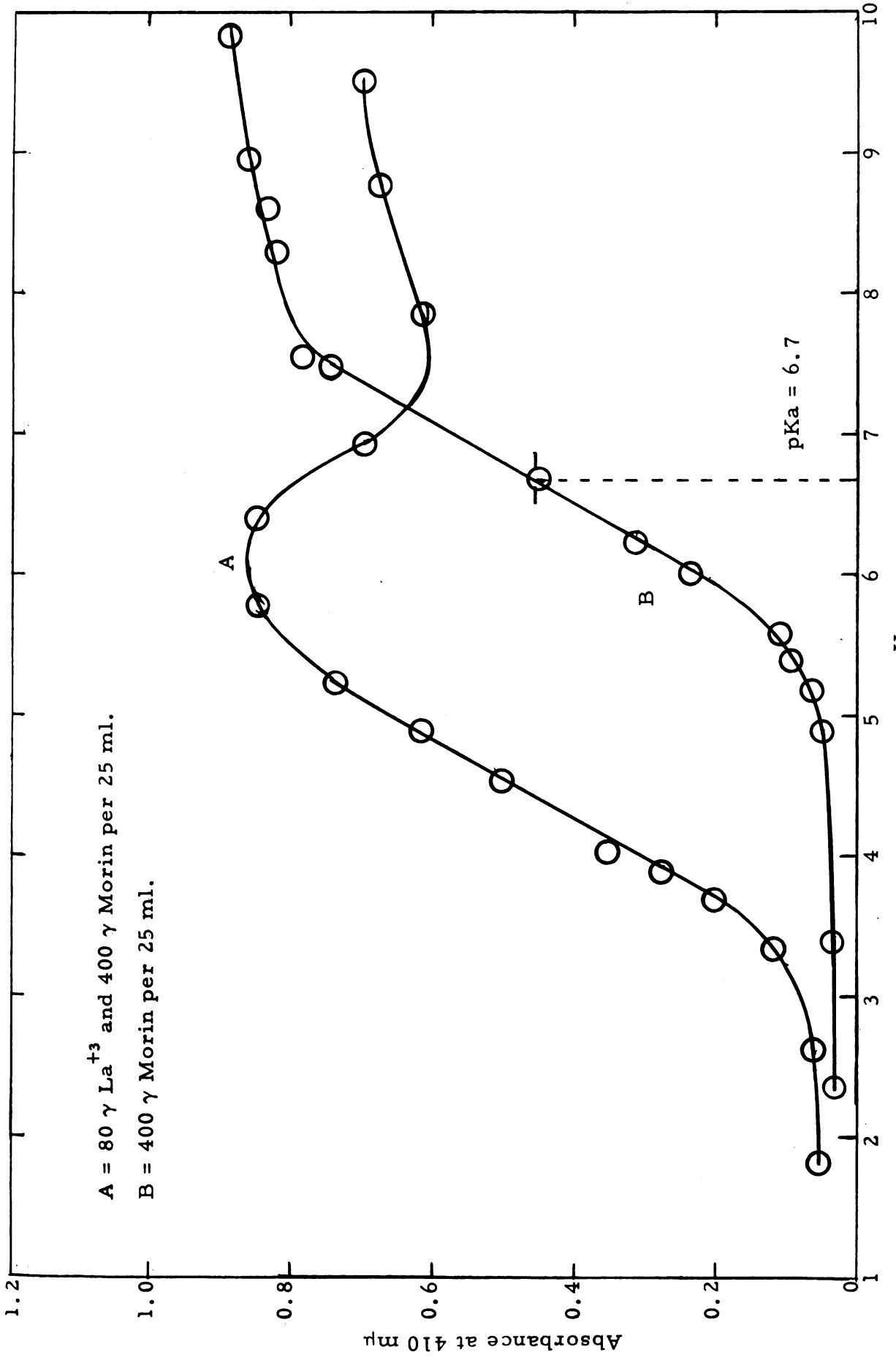

Figure 4. Effect of pH on the Absorbance of Lanthanum (III)-Morin and Morin in 50-50 DW at 410 mμ.

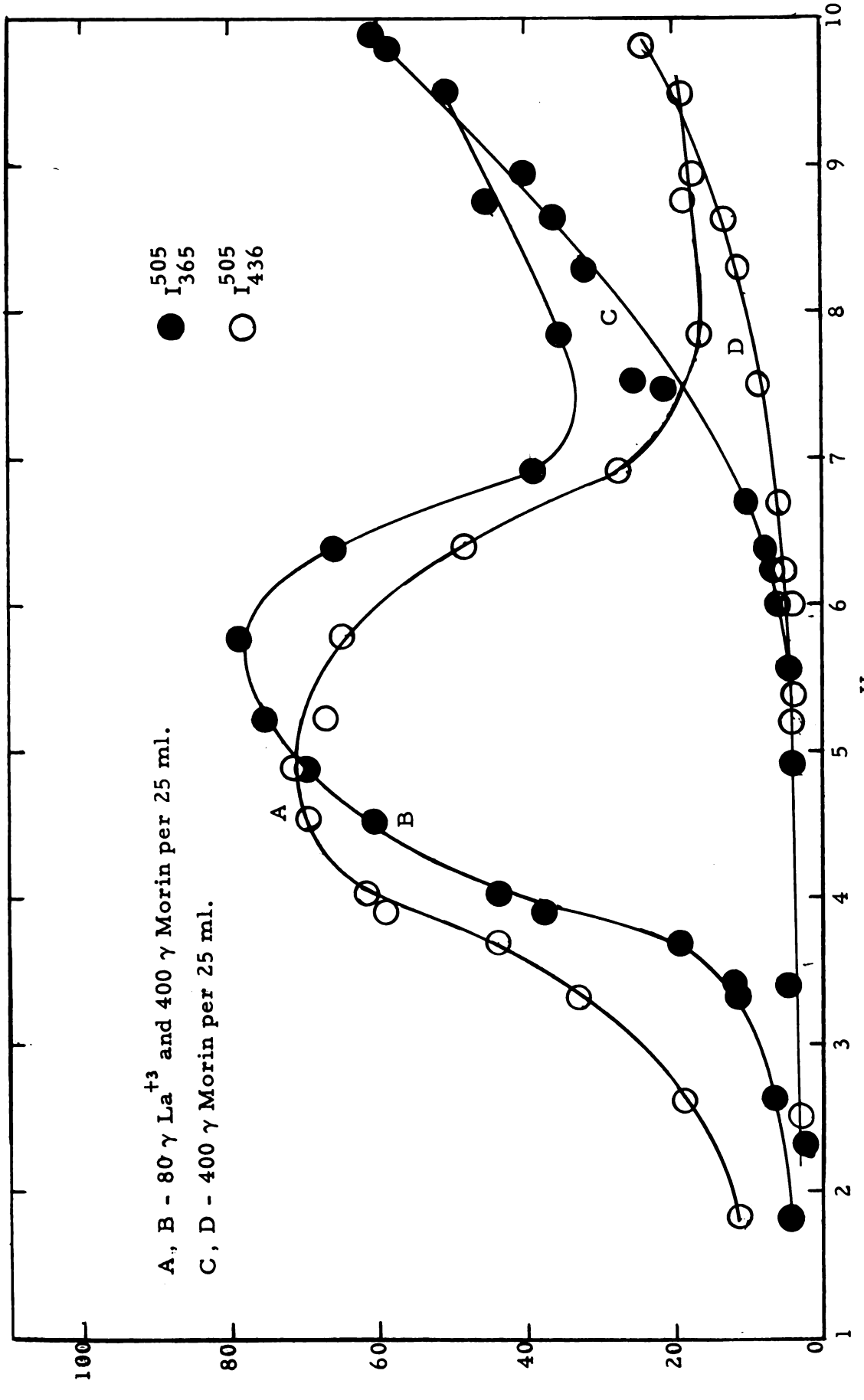

Figure 5. Effect of pH on the Fluorescence of Lanthanum (III)-Morin and Morin in 50-50 DW.

included. The pure morin solutions, curves C and D, do not fluoresce at a pH of 5.5, consequently the excess reagent does not interfere in fluorescence measurements on the complex.

The bathochromic shift in the absorbance spectra and the decrease in the fluorescence intensity of the complex as the pH is increased above 6, suggests that another species, which is non-fluorescing, is being formed. The maximum fluorescence intensities at I_{365}^{505} and I_{436}^{505} and the optimum absorbance at 410 mμ of the complex is attained at a pH of about 5.5; hence the pH was adjusted to this value in the solution preparation procedure.

In Figure 6, curve A shows the effect of the change of lanthanum (III) concentration on the absorbance of 400 γ of morin, while curves B and C show the same effect on the fluorescence. A nearly linear increase in the absorbance and fluorescence of lanthanum (III) morin solutions is attained in the approximate region 0 to 80 γ of lanthanum (III). The gradual change in slope of the absorption curve with increasing lanthanum (III) concentration above approximately 80 γ suggests that the complex formed is relatively weak.

Figure 7 shows the effect of the change in lanthanum (III) concentration on the fluorescence of 1000 γ of morin and clearly indicates the effect of concentration quenching. As lanthanum (III) is added to the solution containing the morin, the morin concentration is decreased and the concentration of the complex is increased. Thus quenching by morin absorption of the 365 mμ radiation is decreased and more excitation radiation is made available to the complex. Consequently, since morin absorbs more strongly at 365 mμ than an equivalent amount of complex and does not absorb appreciably at 436 mμ, I_{365}^{505} increases at a greater rate with increasing lanthanum (III) concentration, above 50 γ lanthanum (III), than I_{436}^{505} due to concentration quenching.

Figure 8 shows the effect of the variation in morin content on the fluorescence of 80 γ of lanthanum (III) per 25 ml. Maximum fluorescence

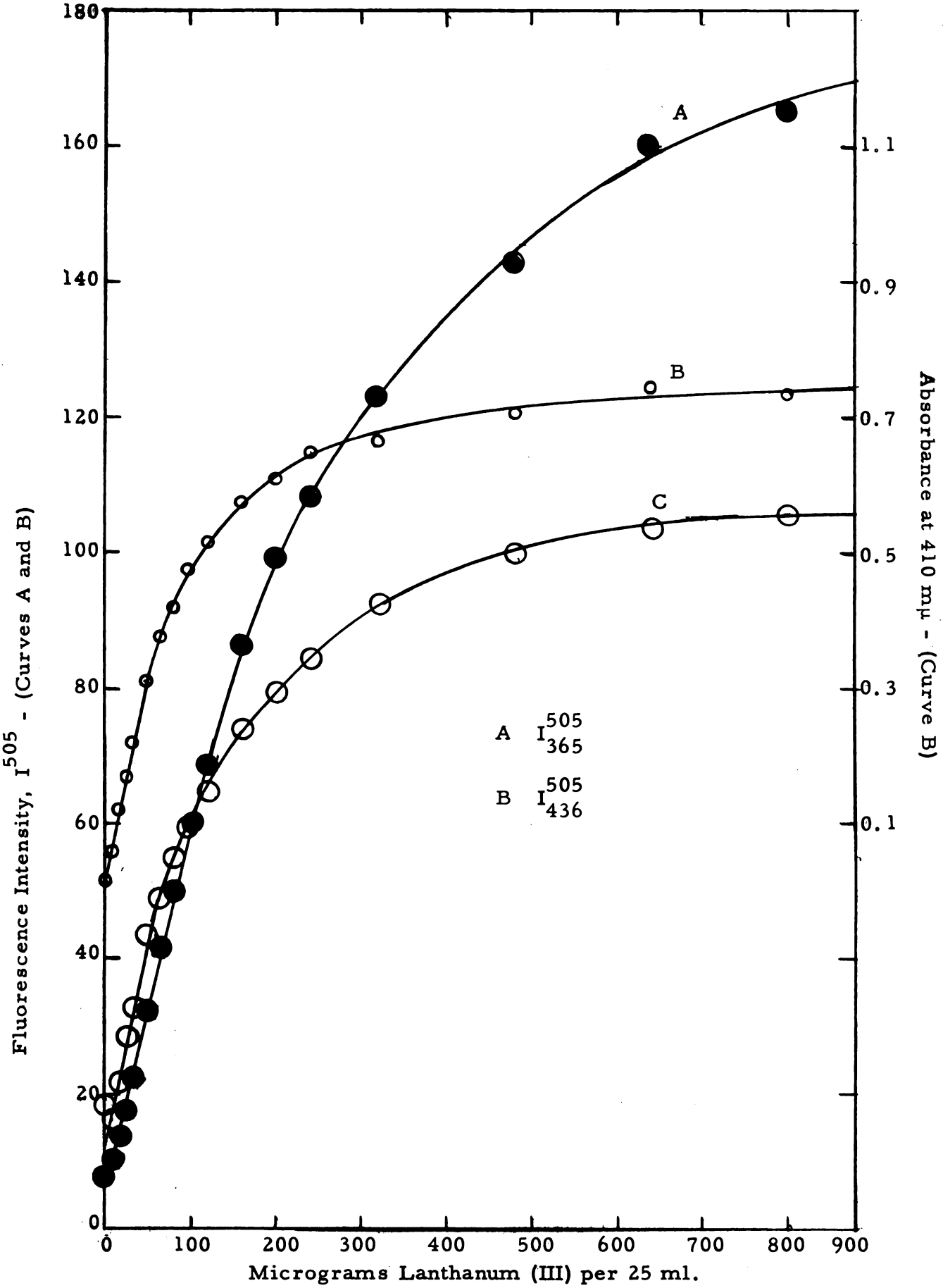

Figure 6. Effect of Lanthanum (III) on the Fluorescence and Absorbance of 400 γ Morin per 25 ml. in 50-50 DW at pH 5.5

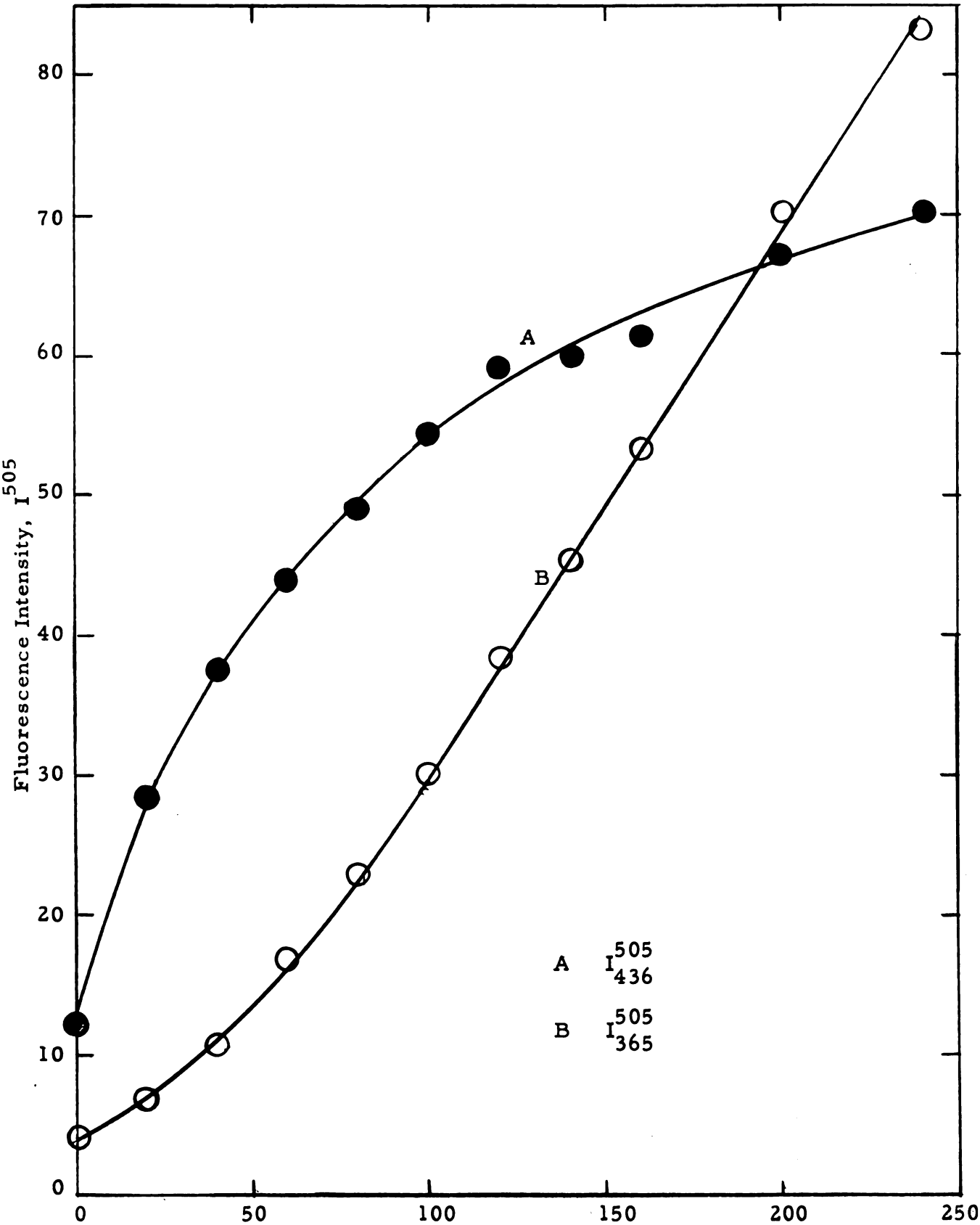

Figure 7. Effect of Lanthanum (III) on the Fluorescence of 1000 γ Morin per 25 ml. in 50-50 DW at pH 5.5

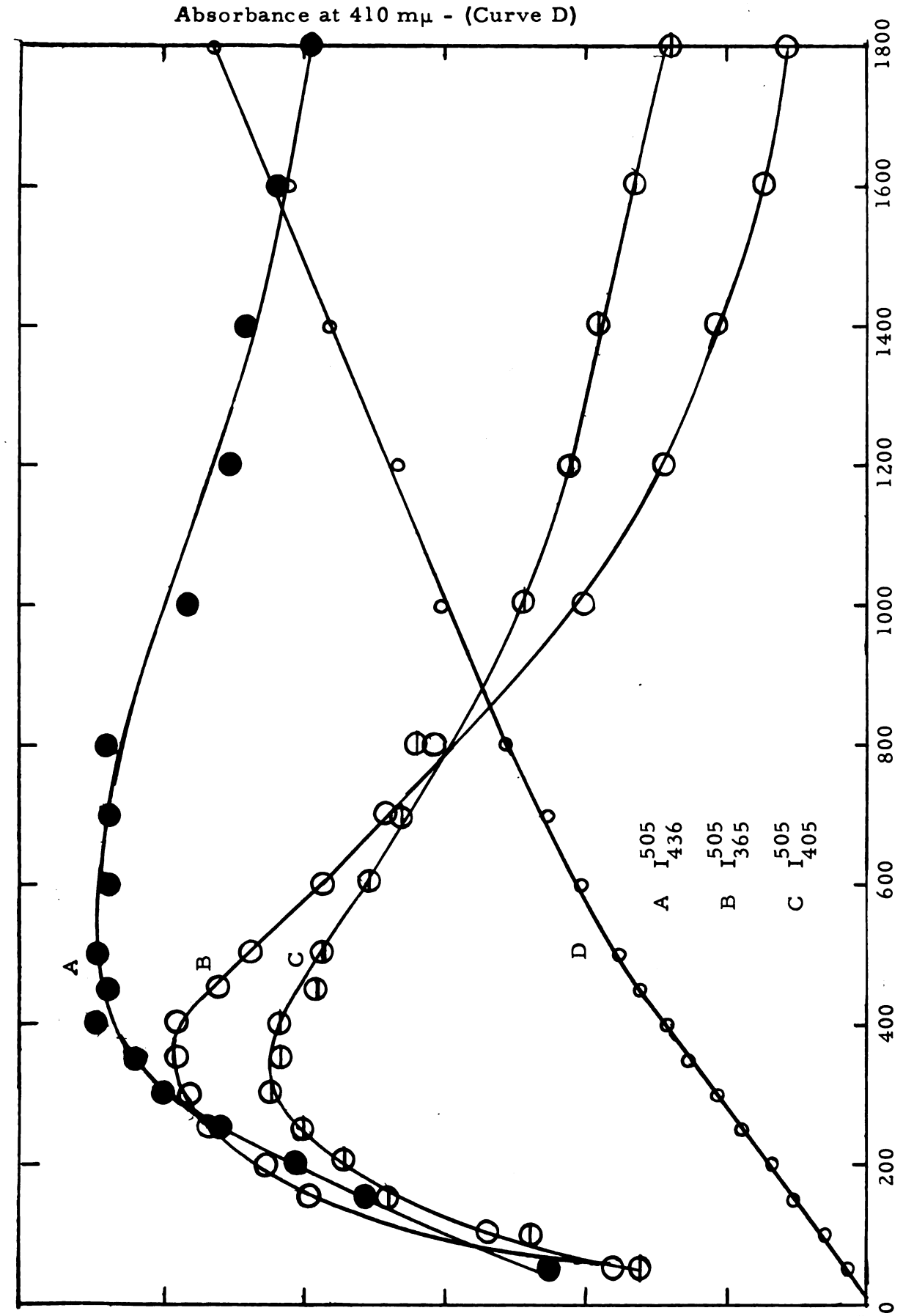

Figure 8. Effect of Morin on the Fluorescence and Absorbance of 80 γ Lanthanum (III) per 25 ml. in 50-50 DW at pH 5.5.

intensities, I_{436}^{505}, I_{405}^{505} and I_{365}^{505}, were obtained for the three excitation wavelengths at approximately 400 γ of morin, while above 400 γ the fluorescence intensities decrease due to concentration quenching. For purposes of comparison, the effect of the morin concentration on the absorbance of the complex is also shown in this figure.

The effect of change in temperature on the fluorescence intensity at I_{365}^{505} of the complex (1.6 mg lanthanum (III) and 400 γ morin per 25 ml.) at pH 5.5 was obtained over the temperature interval 9 to 50°. the fluorescence intensities of 9° and 50° were 228 and 152, respectively and decreased linearly with increasing temperature. From 12 measurements made in the above temperature range, the rate of change corresponds to about a 1.8 fluorescence intensity units decrease per degree centigrade increase.

Samarium (III) was employed in order to study the effect that a lanthanide ion which forms a non-fluorescing complex with morin* would have on the fluorescence of the lanthanum (III)-morin complex. Figure 9 shows the results of this study on solutions containing 40 γ of lanthanum (III) and 400 γ of morin and Figure 10 illustrates the same effect on 80 γ of lanthanum (III) and 1000 γ of morin.

As Figure 9 shows, the fluorescence intensity of the lanthanum (III) complex remains essentially constant at I_{365}^{505} and decreases at I_{436}^{505} as the samarium (III) content is varied from 0 to 137 γ per 25 ml. At the lanthanum (III) and morin concentration levels employed, these results indicate that at least a two to threefold excess of samarium (III) would not cause an appreciable error in the determination of lanthanum when the complex is exposed to 365 mμ radiation.

Figure 10 clearly illustrates the fluorescence quenching effect due to the formation of a samarium (III) complex in lanthanum (III)-morin

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*A 50-50 DW solution of samarium (III)-morin, which contained 86 γ samarium (III) and 400 γ of morin gave approximately the same fluorescence intensity at I_{436}^{505} as a blank solution containing no samarium (III).

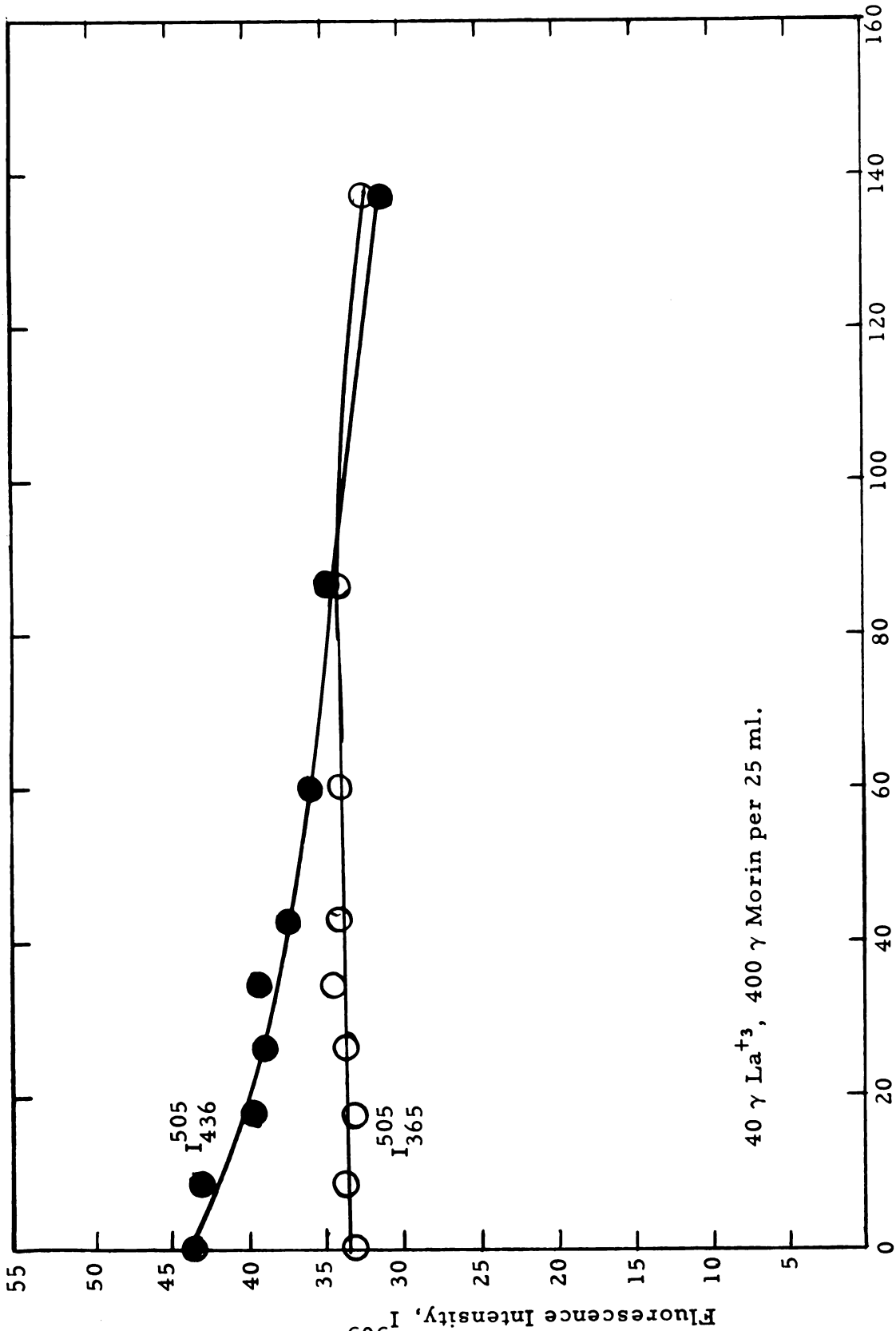


Figure 9. Effect of Samarium (III) on the Fluorescence of Lanthanum (III)-Morin in 50-50 DW at pH 5.5.

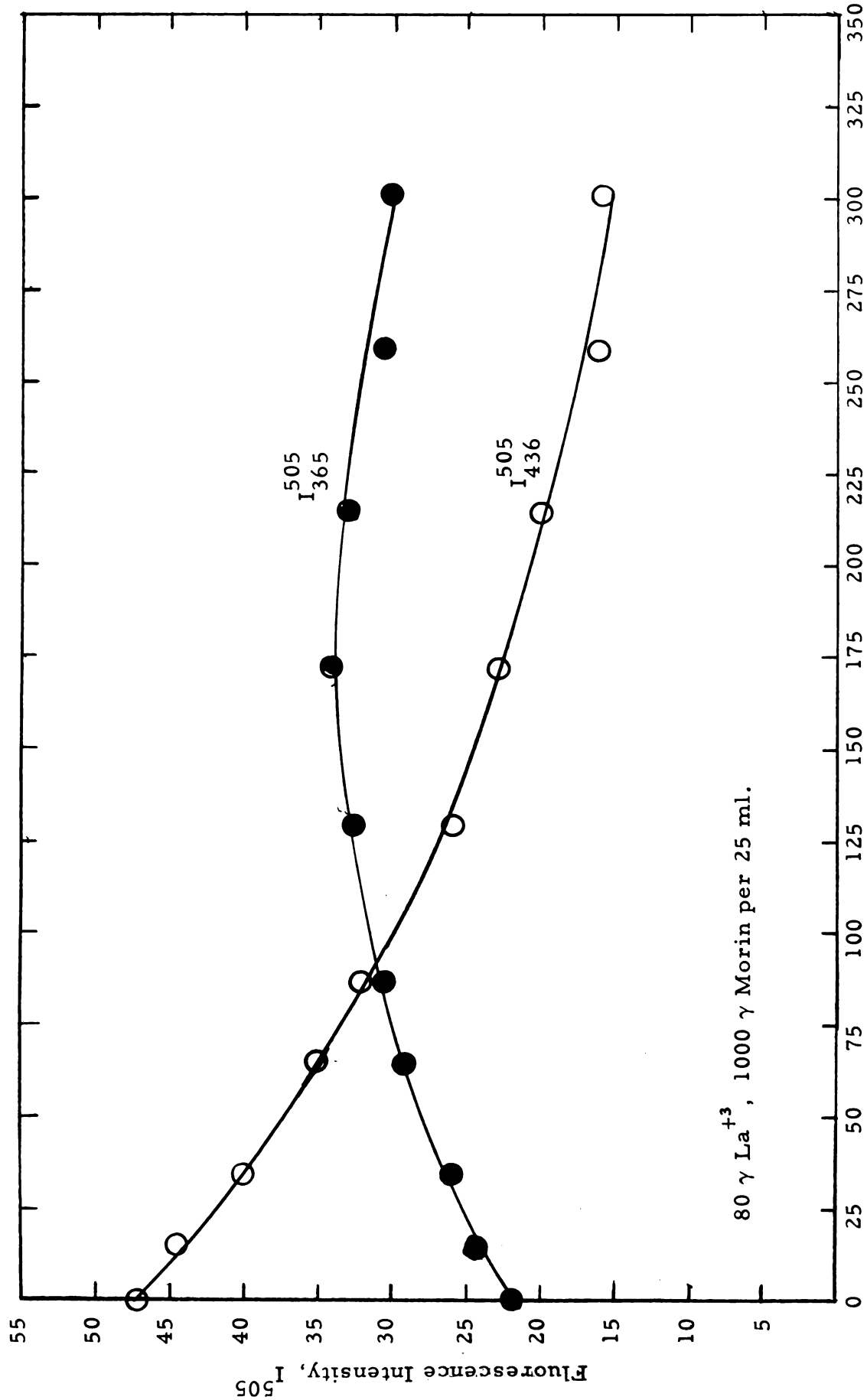


Figure 10. Effect of Samarium (III) on the Fluorescence of Lanthanum (III)-Morin in 50-50 DW at pH 5.6.

solutions. The addition of the samarium (III) to these solutions reduces the uncombined morin concentration and results in a decrease in absorbance at 365 mμ and an increase at 436 mμ. Since, as indicated in Figure 8, there is appreciable quenching of the lanthanum (III)-morin fluorescence at 80 γ of lanthanum (III) and 1000 γ of morin per 25 ml, the fluorescence intensity of the lanthanum (III)-morin complex would be expected to increase at I_{365}^{505} and decrease at I_{436}^{505} when samarium is added as shown in Figure 10. The slight decrease in fluorescence readings at I_{365}^{505}, when more than 200 γ of samarium (III) is added, is probably due to the competition between samarium (III) and lanthanum (III) for morin.

A fluorescence intensity-time study was made on a solution of the complex, which was prepared by the solution preparation procedure and contained 80γ lanthanum (III) and 400 γ morin per 25 ml., to determine the stability of the complex. The fluorescence of the solution was monitored for one week. No significant change in fluorescence readings with time was noted at either I_{365}^{505} or I_{436}^{505}. The fluorescence readings at five minutes after solution preparation at I_{436}^{505} was 64.0 and that after one week, 63.5. Therefore, it would appear that the complex is formed immediately after preparation and is stable for periods up to at least one week.

Nature of the Reaction

In order to ascertain the number of complexes which might be formed and to determine whether the reaction is stoichiometric, nineteen solutions containing 400 γ of morin and amounts of lanthanum (III) varying between 0 and 1.6 mg. per 25 ml. as indicated were prepared by the solution preparation procedure. The absorbance spectra from 340 to 425 mμ for these solutions are shown in Figure 11.

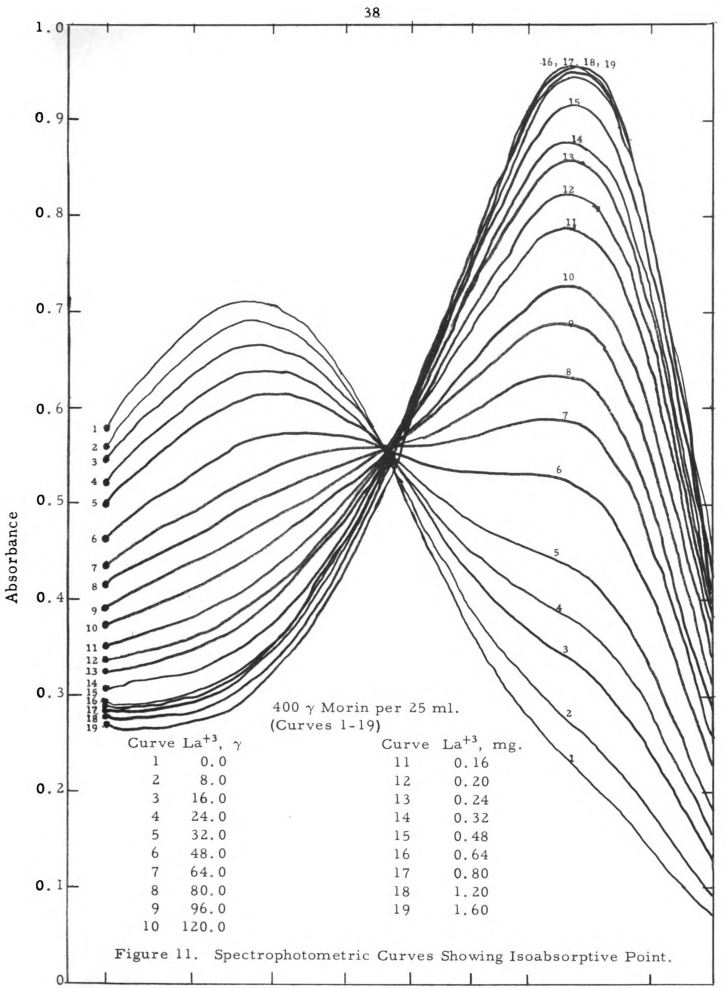


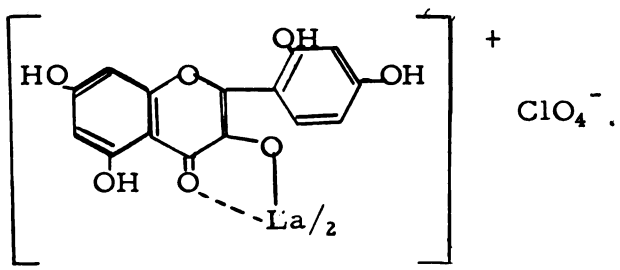
Figure 11. Spectrophotometric Curves Showing Isoabsorptive Point.

The family of curves in Figure 11 yield an isoabsorptive point at 377 mμ which suggest the formation of a single complex and indicates that an equilibrium exists between two colored species (28). The decrease in the absorbance st 356 mu, the morin peak, and the increase at 410 mμ, the complex peak, as the concentration of lanthanum (III) is increased, suggests an equilibrium of the type $La^{+3} + xMH \rightleftharpoons La(M)_x^{+3-x} + xH^+$ is involved--where MH = morin.

Composition of the Complex and Estimation of the Equilibrium Ratio, K'eq

Job's method of continuous variation (22) was employed to determine the empirical formula of the complex which forms at a pH of 5.5. The total molar concentration of the lanthanum (III) and morin was maintained constant at 6.0×10^{-5} moles per liter for a series of eighteen solutions, while the individual concentrations of lanthanum (III) and morin were varied. The molar concentrations of lanthanum (III) and of morin per 50 ml. were plotted against Y, the corrected absorbance which is due only to the complex.

Job (22) and Vosburgh and Cooper (45) have shown that the value of Y at each wavelength is a maximum when the greatest amount of complex is formed. Figure 12 shows the results obtained when Y for the complex is plotted at three different wavelengths, 410, 390 and 356 mμ. The maximum or minimum value of Y appears at a ratio of one mole lanthanum (III) to two moles morin and indicates that the general formula of the complex is LaM_2^+. The structure of the complex is probably of the same type as that suggested by Fletcher and Milkey for the thorium(IV)–morin complex (28); that is



$+ \quad ClO_4^-$.

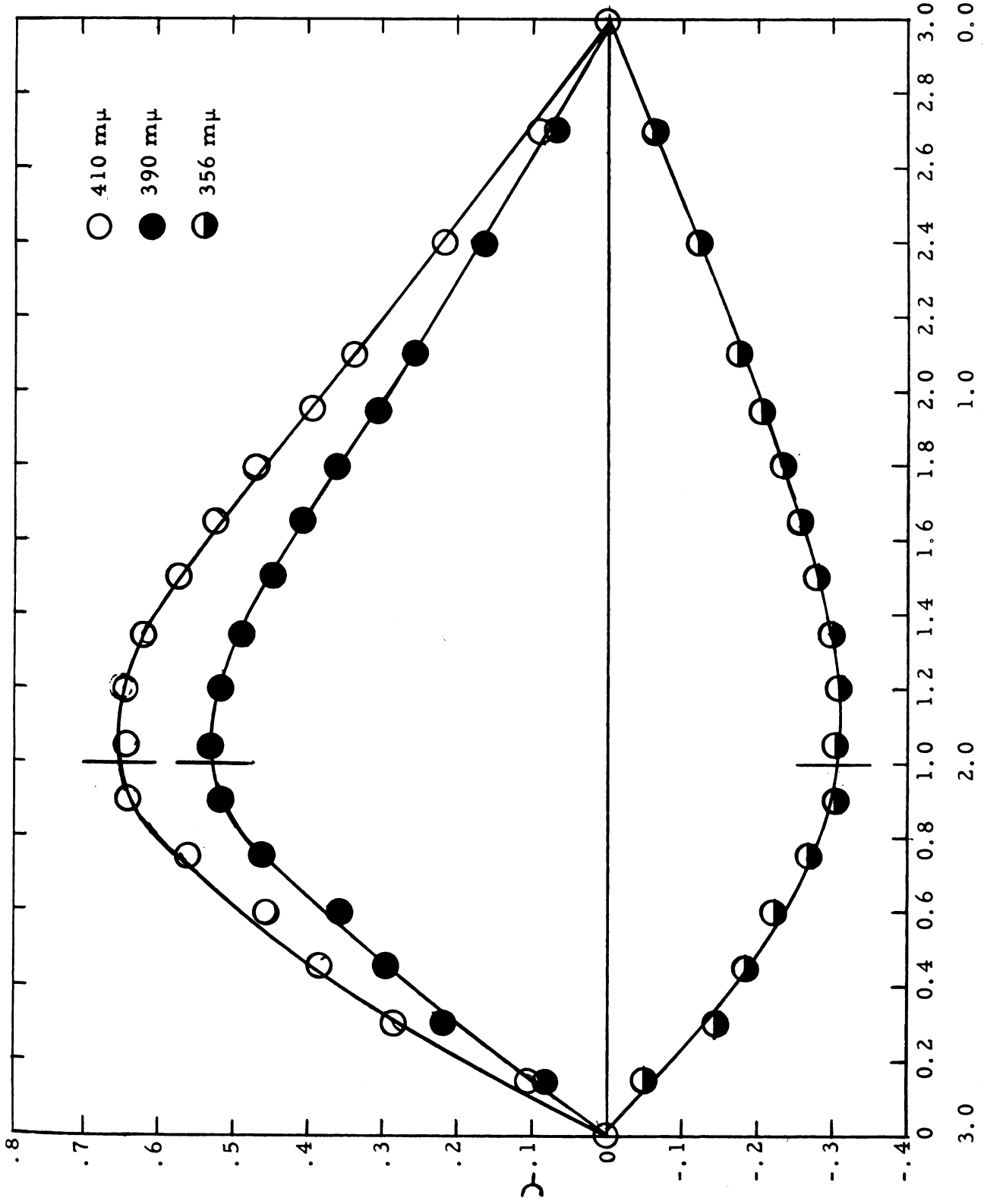


Figure 12. Determination of Composition of the Lanthanum (III)-Morin Complex in 50-50 DW at pH 5.5 by Method of Continuous Variations.

In order to substantiate the above results, the composition of the complex was also determined at pH-5.5 by the slope ratio method of Harvey and Manning (20). Two series of lanthanum (III)-morin solutions were prepared and the absorbance at 410 mμ recorded. The first series contained a large excess of lanthanum (III) (30.6 mg.), which should ensure essentially complete reaction with the morin to form the complex, and amounts of morin varying between 0 and 1014 γ per 50 ml. The second series contained a relatively large excess of morin (2.0 mg.), which should be enough to react with essentially all of the lanthanum (III), and lanthanum (III) varying between 0 and 41.5 γ per 50 ml.

Figure 13 shows the absorbance of 410 mμ, the absorption maximum for the complex, plotted against the moles of morin per liter, curve 1, and the moles of lanthanum (III) per liter, curve 2, for the slope ratio method. The slope of curve 1, which represents the rate in change in absorbance per mole of morin, is 1.98×10^4, while the slope of curve 2, which represents the rate in change in absorbance per mole of lanthanum (III), is 3.96×10^4. On the basis of the slopes of the two curves it can be concluded that two moles of morin combine with one mole of lanthanum (III), since

$$\frac{\text{Slope curve 1}}{\text{Slope curve 2}} = \frac{1.98 \times 10^4}{3.96 \times 10^4} = \frac{\Delta \text{ A/mole morin}}{\Delta \text{ A/mole lanthanum (III)}} = \frac{\text{1 mole lanthanum (III)}}{\text{2 moles morin}}$$

The molar absorptivities for pure morin and the complex at pH 5.5 were determined from plots of absorbance against concentration. Assuming that when morin is added to a large excess of lanthanum (III) in solution, the morin is completely consumed in complex formation. For each two moles of morin added, one mole of complex is formed. On the basis of this assumption, curve 1 of Figure 13 can be used to evaluate the molar absorptivity of the complex. Thus the molar

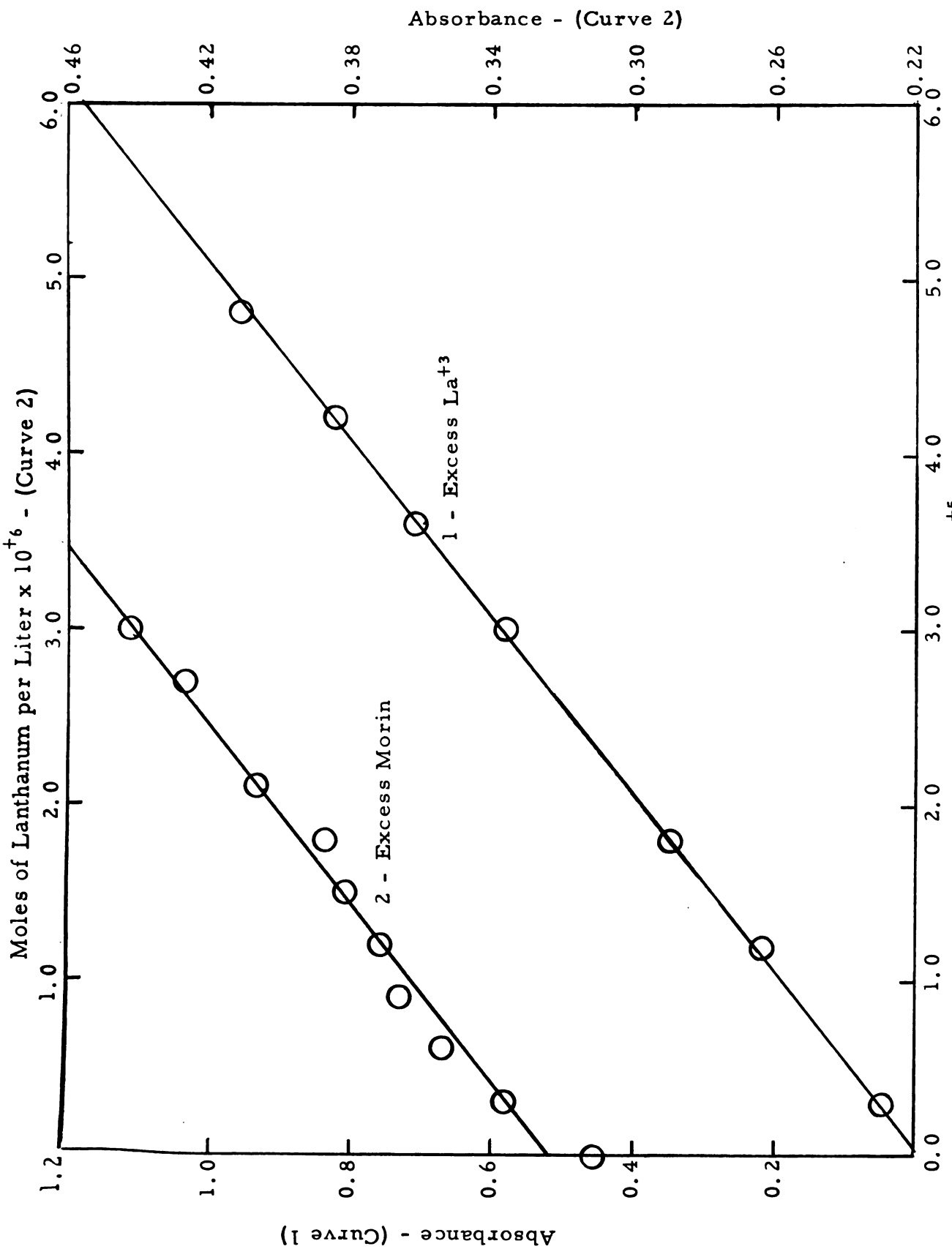


Figure 13. Determination of Composition of the Lanthanum (III)-Morin Complex in 50-50 DW at pH 5.5 by Slope Ratio Method.

absorptivity of the complex will be equal to an absorbance value selected from the curve divided by one-half of the corresponding morin concentration. The data for the molar absorptivity of morin was obtained from the absorbance values of a series of solutions which varied in morin content from 1.2×10^{-5} to 7.0×10^{-5} moles per liter. Both the complex and morin were found to obey Beer's Law. The absorptivities at 410 mμ are:

$a_{La(M)_2^+}$ for complex = 4.00×10^4 liters/mole cm.

$a_{M\ H}$ for morin = 2.28×10^3 liters/mole cm.

The formal equilibrium constant expression for the lanthanum (III)-morin complex for the reaction, $La^{+3} + 2\ M.H \rightleftharpoons La(M)_2^+ + 2H^+$ is

$$Keq = \frac{[La(M)_2^+]\,[H^+]^2}{[La^{+3}]\,[M\ H]^2} .$$

An estimation of the value of this constant can be made employing the equations of Fletcher and Milkey (28).

In the lanthanum (III)-morin system only two components absorb light: morin, M H and the complex, $La(M)_2^+$.

If X = moles of complex per liter

$(M\ H)$ = total moles of morin added per liter

(La^{+3}) = total moles of lanthanum (III) added per liter

Y = moles of uncombined morin per liter

Z = moles of uncombined lanthanum (III) per liter

A_{410} = absorbance at 410 mμ of solutions containing the complex

b = internal cell length in cm. (1.00 cm.)

$a_{M\ H}$ = molar absorptivity for morin at 410 mμ and pH 5.5

$a_{LaM_2^+}$ = molar absorptivity for the complex at 410 mμ and pH 5.5

then

$$A_{410} = a_{M\ H}[(M\ H) - (2X)] + a_{LaM_2}[X]$$

and

$$X = \frac{A_{410} - a_{M\,H}\,(M\,H)}{a_{LaM_2} - 2a_{M\,H}}$$

$$Y = (M\,H) - 2[X]$$

$$Z = (La^{+3}) - [X]$$

Employing the above equations, the equilibrium ratio, K'eq was calculated from data obtained from the study involving the 18 solutions which were employed in the determination of the composition of the complex. The values needed to evaluate the equilibrium ratio and the values of the equilibrium ratio are tabulated in Table I.

The equilibrium ratio values, K'eq, vary between 0.02 and 5.4 with the majority of the values ranging between 0.25 and 1.6. Excluding the extreme values designated in Table I, the average K'eq is 0.6 and indicates that the complex is weak in comparison to the average value of 1×10^6 found by Fletcher and Milkey (28), for the thorium (III)-morin complex in a dilute ethanol-water system.

LANTHANUM (III)-5, 7-DICHLORO-8-QUINOLINOL SYSTEM

In Figure 14, curves B, D and F show the absorption spectra from 260 to 620 mμ for 1000 γ of 5, 7-dichloro-8-quinolinol per 25 ml. in 50-50 DW at several pH values and curves C and E show the effect of the addition of 80 γ of lanthanum (III) at pH 8.8 and 6.8, respectively, on the same amount of reagent in 50-50 DW. Under various conditions of acidity, three different species of 5, 7-dichloro-8-quinolinol have been reported (1, 12).

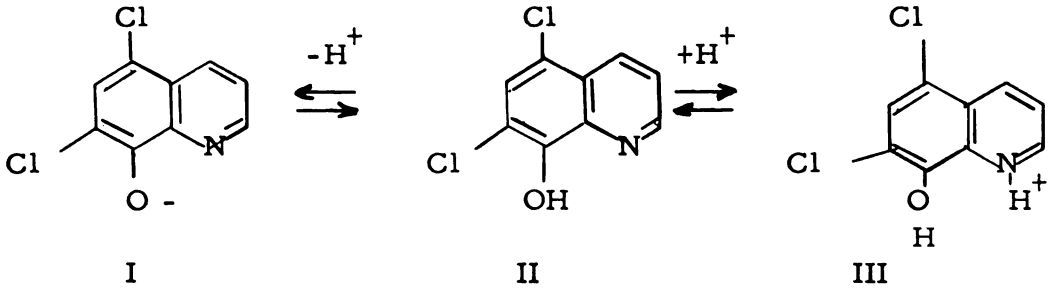


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Table I. Estimated Equilibrium Ratio, K'_{eq}, Value of the Lanthanum (III)-Morin Complex in 50 Percent Dioxane-Water at 25°.

Trial	Total $[La^{+3}]$ Added M/lx10^6	Total [MH] Added M/lx10^6	$[H^+]$ M/lx10^6	Z M/lx10^6	M/lx10^6	$[LaM_2^+]$ M/lx10^6	$A_{410\ m\mu}$[1]	K'eq
1	3.00	57.0	3.16	0.43	51.9	2.57	0.221	0.02[2]
2	12.0	48.0	3.16	0.70	25.4	11.3	0.508	0.25
3	15.0	45.0	3.02	1.2	17.4	13.8	0.509	0.35
4	18.0	42.0	2.82	2.4	10.8	15.6	0.648	0.44
5	21.0	39.0	3.31	5.5	8.0	15.5	0.636	0.48
6	24.0	36.0	3.02	8.1	4.2	15.9	0.644	1.00
7	27.0	33.0	2.69	11.7	2.4	15.3	0.618	1.60
8	30.0	30.0	2.95	15.8	1.6	14.2	0.570	3.10[2]
9	33.0	27.0	2.88	20.0	1.0	13.0	0.522	5.40[2]
10	36.0	24.0	2.63	24.4	0.8	11.6	0.468	5.10[2]
11	39.0	21.0	3.02	29.3	1.7	9.66	0.390	1.00
12	42.0	18.0	2.63	33.8	1.6	8.22	0.332	0.66
13	48.0	12.0	2.88	42.7	1.4	5.32	0.215	0.53
14	54.0	6.0	3.47	51.9	1.9	2.06	0.087	0.13
15	3750	36.0	3.09	3732	0.4	17.8	0.710	0.28
16	3750	42.0	3.09	3730	0.8	20.6	0.822	0.08
17	3750	48.0	3.16	3706	0.2	23.9	0.958	1.60
18	3750	30.0	3.02	3736	1.0	14.5	0.580	0.04[2]

[1] Absorbance values and data for trials 1-14 were obtained from solutions used in continuous variation study, while for trials 15-18 from solutions used in slope ratio study.

[2] Not included in calculated average of 0.6.

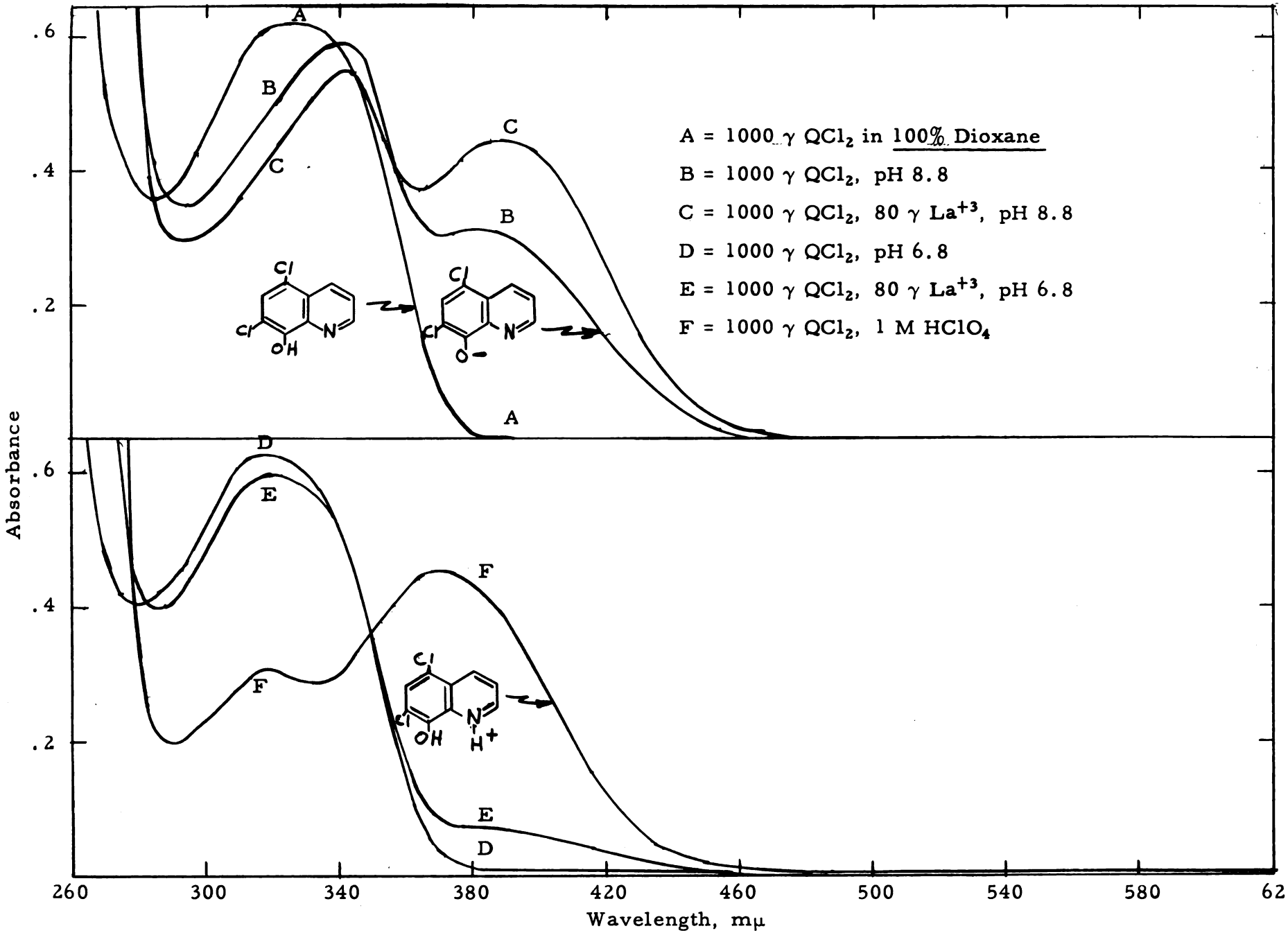

Figure 14. Absorption Spectra of 5, 7-Dichloro-8-quinolinol (QCl_2) and Lanthanum (III)-QCl_2 in 25 ml. of 50-50 DW.

The absorption band centering around 390 mμ for the reagent at pH 8.8 (curve B) is due to species I, while the peak at 370 mμ, the reagent in 1 M perchloric acid (curve F), is due to species III. Species II, the neutral molecule, is represented by curve A, the reagent dissolved in pure dioxane which has an absorption peak at 326 mμ and does not absorb in the visible region above 380 mμ. The addition of 80 γ of lanthanum (III) to 1000 γ of 5, 7-dichloro-8-quinolinol (curves C and E) results in an increase in the absorption in the region of 388 mμ. This increase suggests that lanthanum (III) forms a complex or complexes with the reagent at both pH 6.8 and 8.8 and that the complex absorption peak is at approximately 388 mμ.

The fluorescence spectra of 80 γ lanthanum (III) and 1000 γ of 5, 7-dichloro-8-quinolinol per 25 ml. at pH 9.0, (curve A), and at 6.1, (curve B), when exposed to 365 mμ radiation are shown in Figure 15. The fluorescence peak for curve A appears between 526 and 530 mμ, while the peak for curve B is shifted to a slightly longer wavelength centering about 535 mμ. The peak fluorescence intensity at the higher pH value is more than two and one-half times that obtained at the lower one.

The optimum excitation wavelength for lanthanum (III)-5, 7-dichloro--8-quinolinol is at 365 mμ. The fluorescence intensity at I_{365}^{530} is nearly three to four times that at I_{313}^{505}, I_{405}^{505}, and I_{436}^{505}, while no appreciable fluorescence can be detected when the solutions are exposed to radiation below 365 mμ. On the basis of the above results, single wavelength fluorescence measurements were taken at I_{365}^{530}.

Effect of Experimental Variables on the Fluorescence of the Complexes

Figure 16 shows the effect of the variation of dioxane content on the fluorescence of lanthanum (III)-5, 7-dichloro-8-quinolinol solutions buffered at pH 9 with ammonium hydroxide-ammoniumperchlorate.

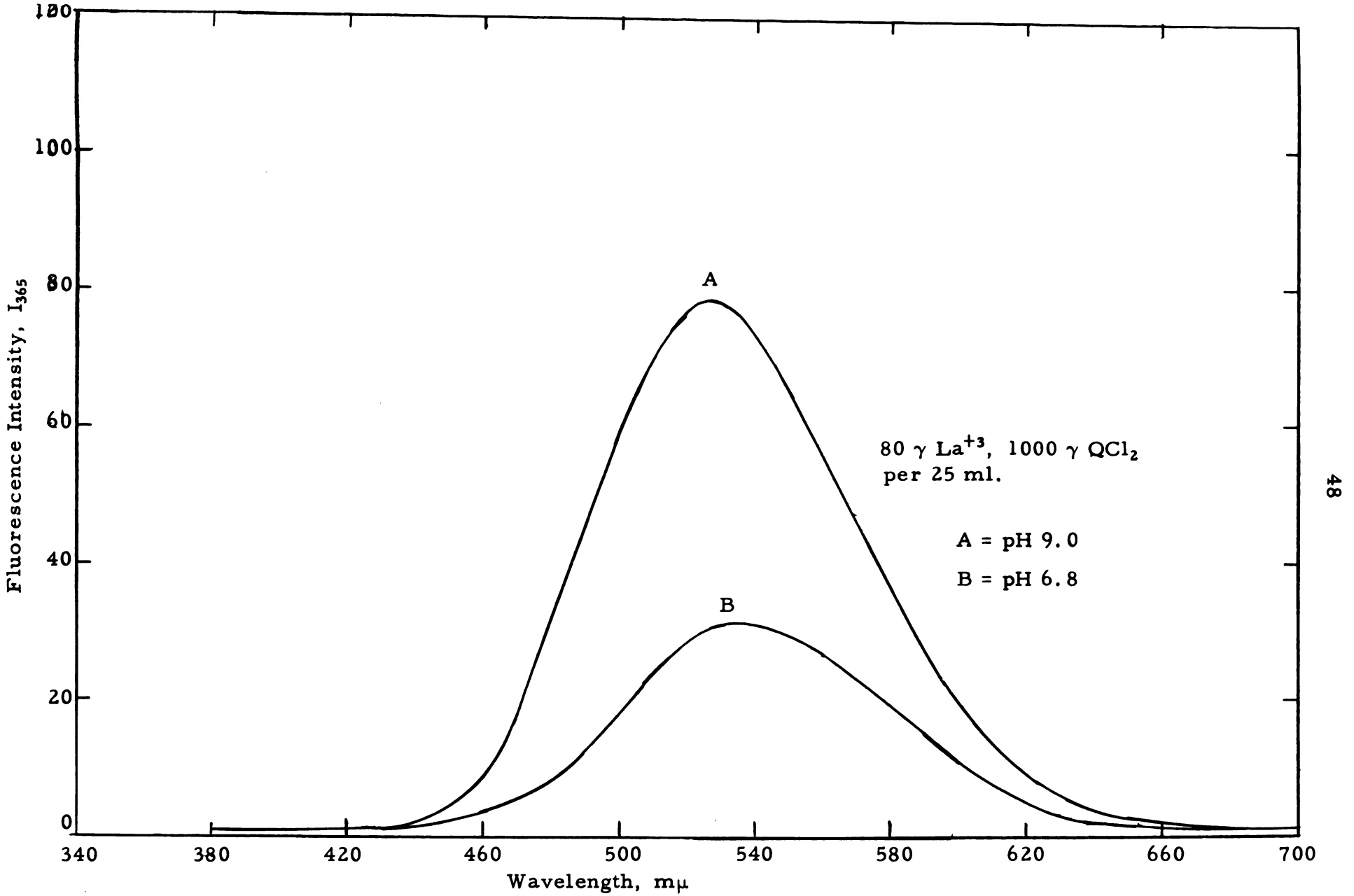

Figure 15. Fluorescence Spectra of Lanthanum (III)-5, 7-Dichloro-8-quinolinol in 50-50 DW.

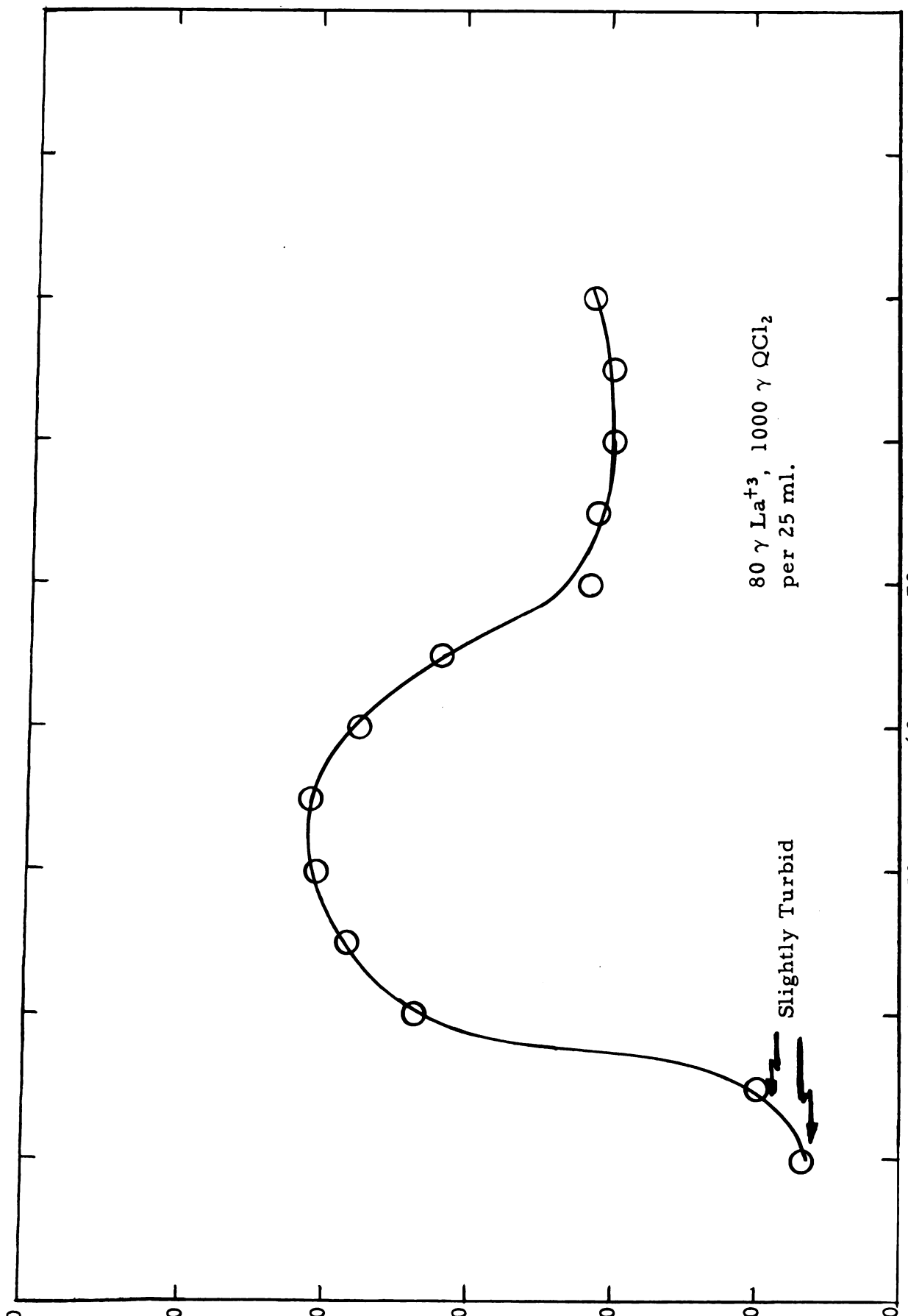

Figure 16. Effect of Dioxane Content on the Fluorescence of Lanthanum (III)-5, 7-Dichloro-8-quinolinol at pH 9.0 in 50-50 DW.

A slight turbidity, which undoubtably accounts for the low fluorescence readings, is formed in solutions containing less than 40 percent dioxane. The absorbance readings at 388 mμ, the complex maximum, against dioxane content for the same solutions employed in obtaining the data for Figure 16 showed a decrease in absorbance values with an increase in dioxane content. Since maximum fluorescence intensities are attained in solutions containing between 50 and 55 percent dioxane, 50-50 DW was selected as the solvent for this study.

The effect of pH on the fluorescence of pure 5, 7-dichloro-8-quinolinol and lanthanum (III)-5, 7-dichloro-8-quinolinol at I_{365}^{520} is shown in Figure 17. Lanthanum (III)-5, 7-dichloro-8-quinolinol, curve A, fluoresces in the approximate pH region 4.5 to 10 with a maximum and nearly constant fluorescence intensity between pH 8.7 to 9.5, while the pure reagent, curve B, does not yield appreciable fluorescence over the entire pH range covered. Since solutions of the reagent or of lanthanum (III) do not fluoresce, lanthanum (III)-5, 7-dichloro-8-quinolinol complexes are responsible for the fluorescence yields shown in curve A.

The effect of the change in lanthanum (III) concentration on the fluorescence of 1000 γ of 5, 7-dichloro-8-quinolinol in 50-50 DW at pH 8.8 is shown in Figure 18. A nearly linear increase in the fluorescence of the lanthanum (III)-5, 7-dichloro-8-quinolinol solutions is attained in the approximate region 0 to 70 γ of lanthanum (III). Above approximately 100 γ of lanthanum (III), the fluorescence intensity tends to reach a limiting value.

Figure 19 shows the effect of the variation of 5, 7-dichloro-8-quinolinol content on the fluorescence of the complex at pH 8.8. A maximum and optimum fluorescence reading is attained at about 800 to 1500 γ of 5, 7-dichloro-8-quinolinol for 80 γ lanthanum (III) per 25 ml. Above approximately 1500 γ the intensity of fluorescence gradually decreases due to concentration quenching.

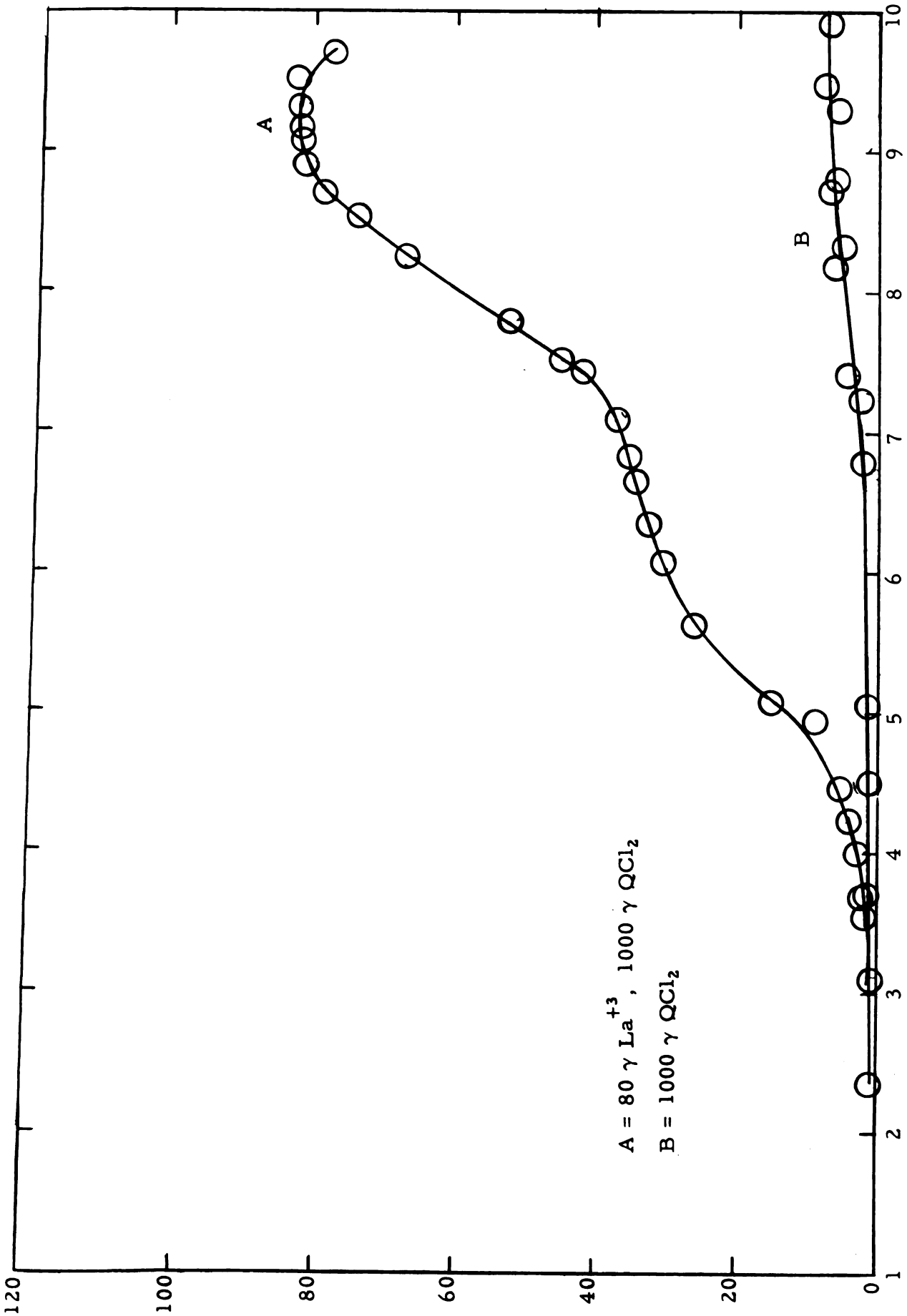

Figure 17. Effect of pH on the Fluorescence of 5,7-Dichloro-8-quinolinol and Lanthanum (III)-5,7-Dichloro-8-quinolinol in 25 ml. of 50-50 DW.

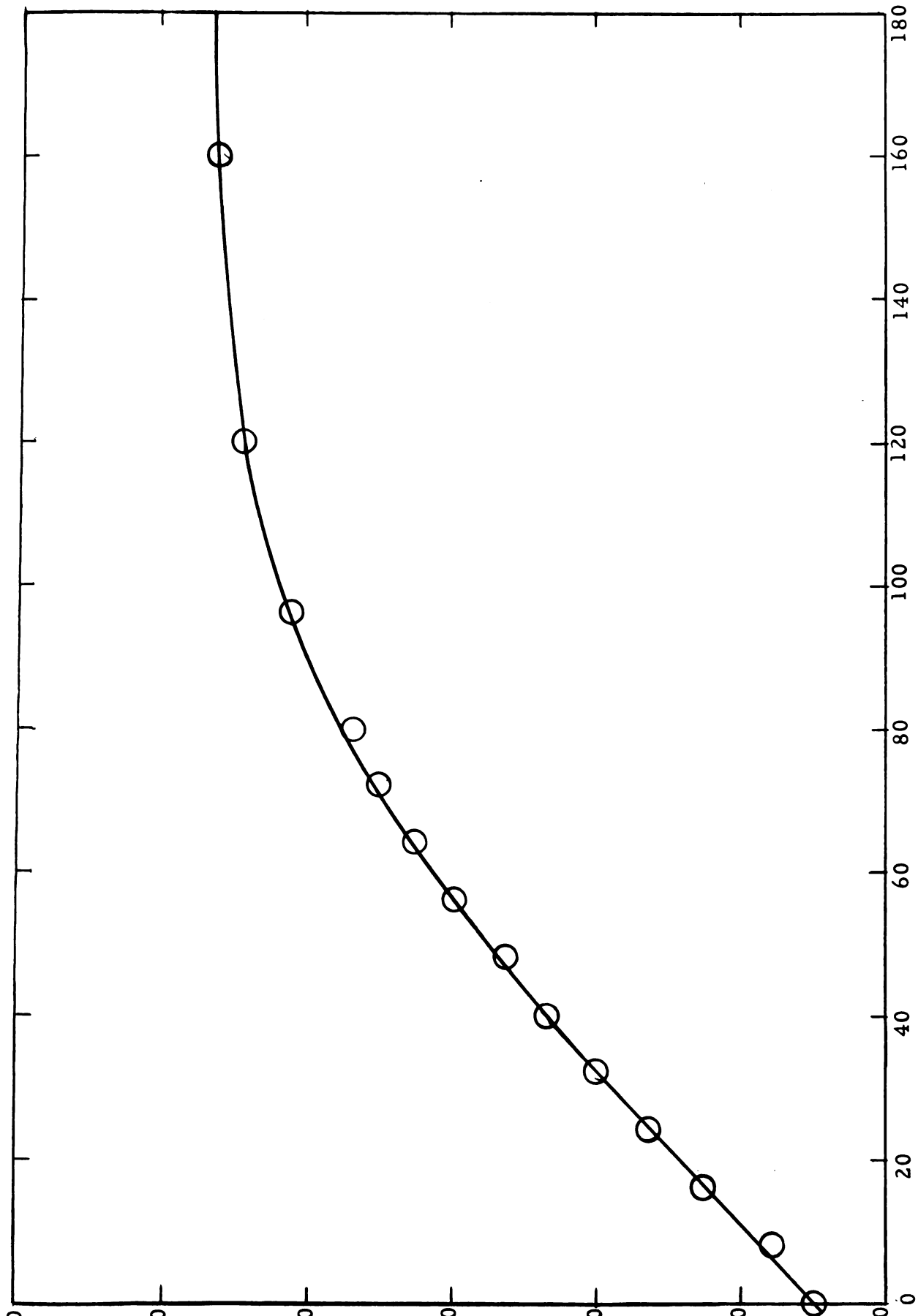

Figure 18. Effect of Lanthanum (III) on the Fluorescence of 1000 γ 5,7-Dichloro-8-quinolinol in 50-50 DW at pH 8.8.

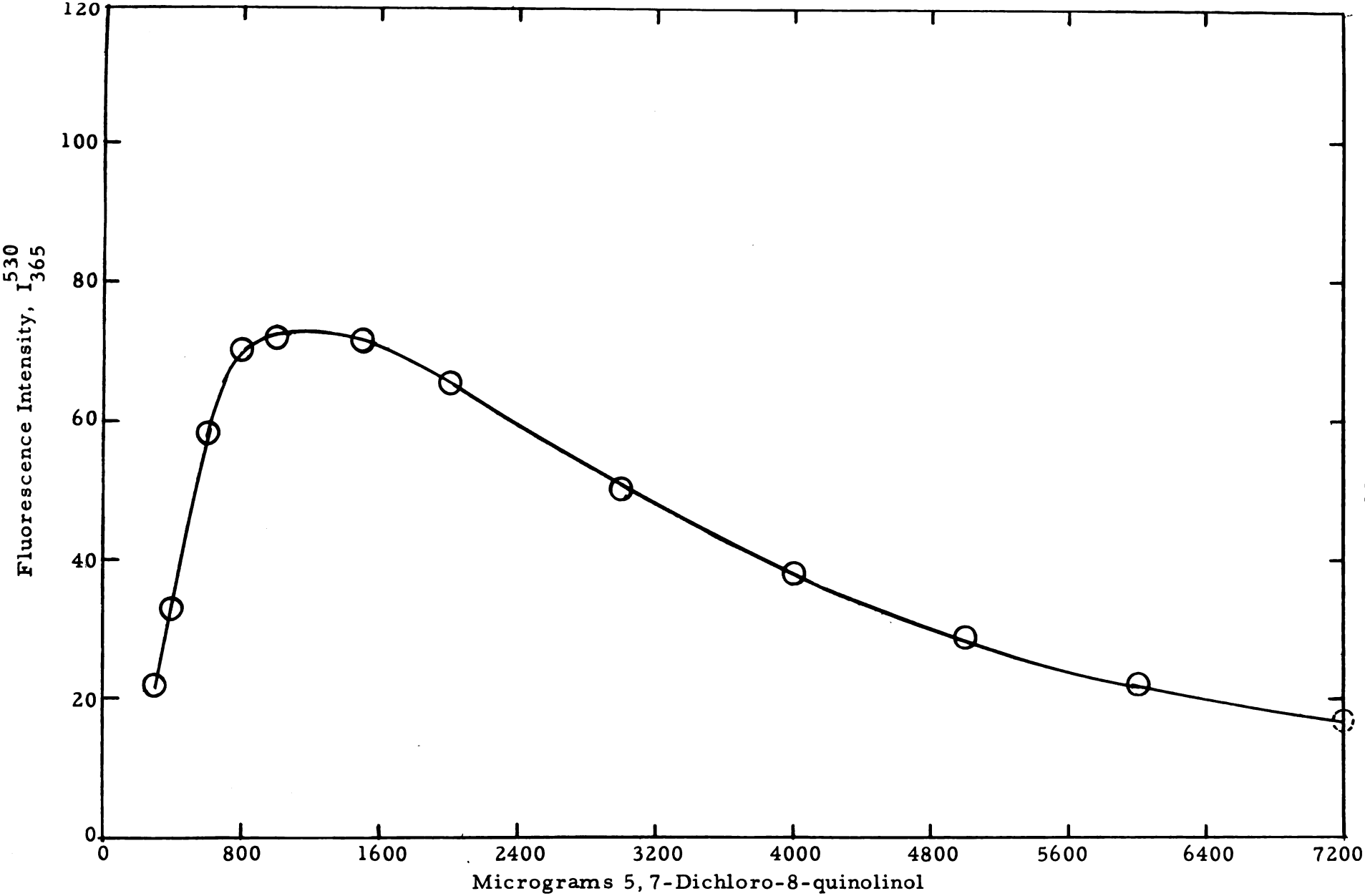

Figure 19. Effect of 5, 7-Dichloro-8-quinolinol on the Fluorescence of 80 γ Lanthanum (III) per 25 ml in 50-50 DW at pH 8.8.

Solutions which were prepared by the solution preparation procedure and contained 80 γ of lanthanum (III) and 1000 γ of 5,7-dichloro-8-quinolinol at a pH of 8.8 were employed to study the effect of the variation in temperature on the fluorescence. A plot of temperature from 14 to 45^{o} against fluorescence intensity readings (not shown here) indicated a nearly linear decrease in fluorescence intensity with increasing temperature increase. This decrease corresponded to approximately 3.0 fluorescence intensity units per degree centigrade increase over nearly the entire temperature range covered. It would appear on the basis of the above results that constant temperature conditions should be maintained for any quantitative fluorescence study on the complex.

In preliminary investigations the observation was made that solutions of the complex at pH 9, which were prepared from ammonia repeatedly exposed to the atmosphere, gave significantly lower fluorescence readings than those prepared from ammonia which was protected from the atmosphere. This suggested that carbon dioxide absorbed in ammonia, quenches the fluorescence of the complex and therefore the effect of carbonate was studied.

Since sodium ions would not be expected to produce fluorescence quenching, high purity sodium carbonate was selected to test the effect of carbonate on the fluorescence of the complex. The only modification introduced into the solution preparation procedure was the addition of varying amounts of sodium carbonate (0-5 mg.) and 0.1 M ammonia to attain a pH of 9.

Table II shows the results of the effect of carbonate on the fluorescence of the complex and specifies the amounts of reagents required per 25 ml. The results of this study clearly indicate that carbonate has a pronounced quenching effect on the fluorescence since an increase in carbonate content causes a decrease in the fluorescence intensity.

Table II. Effect of Sodium Carbonate on the Fluorescence Intensity of Lanthanum (III)-5, 7-Dichloro-8-quinolinol in 50 percent Dioxane-Water at pH 9.0.
80 γ Lanthanum (III), 1000 γ 5, 7-Dichloro-8-quinolinol per 25 ml.

Trial	Mg. Na_2CO_2	Ml. 0.1 M NH_4OH	pH	I_{365}^{530}
1*	0.0	1.25	8.95	78.2
2	0.0	1.25	8.95	78.2
3	1.0	1.25	9.10	70.0
4	2.0	0.87	8.98	44.7
5	3.0	0.68	8.96	35.1
6	4.0	0.49	9.02	28.1
7	5.0	0.31	9.03	22.3

*Water boiled to remove dissolved carbon dioxide for this trial.

The effect of samarium (III) on the fluorescence of solutions containing 40 γ of lanthanum (III) and 1500 γ of 5, 7-dichloro-8-quinolinol per 25 ml. was studied. The readings at I_{365}^{530} for 0.0, 17, 34, 52 and 71 γ of samarium (III) per 25 ml. were 46.2, 45.4, 45.9, 43.8 and 41.5, respectively, and indicate that samarium (III) in concentrations approximately equal to lanthanum (III) can be tolerated without causing a significant error. However, for concentrations of samarium (III) greater than this amount a slight decrease in the fluorescence intensities is noted.

A fluorescence intensity-time study was made on the lanthanum (III)-5, 7-dichloro-8-quinolinol solutions in order to ascertain its stability. The results of this study are shown in Table III for solutions prepared by the solution preparation procedure, trials I and II, and for one non-buffered solution, trial III, prepared at pH 9 by omitting perchloric acid and adding only dilute ammonia. The results indicate that the fluorescent complex develops within at least ten minutes after addition of ammonium hydroxide and yields essentially constant fluorescence readings for approximately one to two hours. The fluorescence intensity gradually diminishes after one or two hours of standing and shows a 30 percent decrease after standing about 24 hours. On the basis of the above results all fluorescence measurements were made between one-half to one hour after development of the complex.

Evaluation of the Lanthanum (III)-5, 7-dichloro-8-quinolinol Stability Constants

To gain information regarding the possible species responsible for the fluorescence of the lanthanum (III)-5, 7-dichloro-8-quinolinol system, the formal stepwise stability constants, K_1 and K_2, were evaluated by the method of Freiser and co-workers (15). Since lanthanum (III) tends to hydrolyze above pH 6, the constant K_3 could not be accurately determined by this method.

Table III. Fluorescence Stability Data on 50 percent Dioxane-Water Solutions of Lanthanum (III)-5, 7-Dichloro-8-quinolinol at pH 9.0.

80 γ Lanthanum (III), 1000 γ 5, 7-Dichloro-8-quinolinol per 25 ml.

Time After Development of Complex	Trial 1 I_{365}^{530}	Trial 2 I_{365}^{530}	Trial 3 I_{365}^{530}
10 minutes	82.6	78.5	82.1
20 minutes	81.2	80.2	82.2
30 minutes	80.8	80.2	81.0
1 hour	80.2	79.8	81.8
2 hours	77.2	77.2	----
22 hours	----	----	59.2
24 hours	63.8	65.5	----

Figure 20, curve C, shows the pH titration curve for 4.99 ml. of a 0.00938M lanthanum (III) perchlorate stock solution. For purposes of comparison the titration curves of 5, 7-dichloro-8-quinolinol and lanthanum (III)-5, 7-dichloro-8-quinolinol are also shown in this figure. The first titration break in curve C corresponds to the titration of perchloric acid and the second one to the precipitation of lanthanum (III) hydroxide. The acid concentration of the stock solution was determined from this curve and from samples which were titrated to the methyl red endpoint with standard sodium hydroxide. The concentration of the acid was found to be 0.0751 moles per liter. The hydrolysis curve of lanthanum (III) is almost identical to that obtained by Freiser et al. (15).

The acid dissociation constants of the protonated nitrogen K_{NH} and of the phenol, K_{OH}, which are required for the calculation of the formal stability constants of the 5, 7-dichloro-8-quinolinolate complexes, were evaluated in 50-50 DW at 25°. The pK_{OH} was determined graphically from curves of pH against ml. of sodium hydroxide which are required to titrate acidified samples of the reagent. The pH at the half neutralization point corresponds to the pK_{OH} and yields values of 9.10 and 9.12 for two trials as shown in Figure 20, curves B and D. The average K_{OH} was found to be 7.8×10^{-10}.

The pK_{NH} was estimated from a plot of pH against absorbance at 369 mμ for a series of solutions containing 1000 γ of 5, 7-dichloro-8-quinolinol per 25 ml. The acidity of the solutions were adjusted with dilute perchloric acid. The pH at half absorbance value, which corresponds to the pK_{NH}, was found to be 1.6 as shown in Figure 21.

The consecutive stability constants, K_1 and K_2, for the reaction of lanthanum (III) with 5, 7-dichloro-8-quinolinol are

$$K_1 = \frac{[La(QCl_2)^{+2}]}{[La^{+3}]\,[QCl_2^-]} \quad \text{and} \quad K_2 = \frac{[La(QCl_2)_2^+]}{[(La(QCl_2)^{+2})][(QCl_2^-)]}$$

and can be calculated employing the equations derived by Freiser, et al. (15).

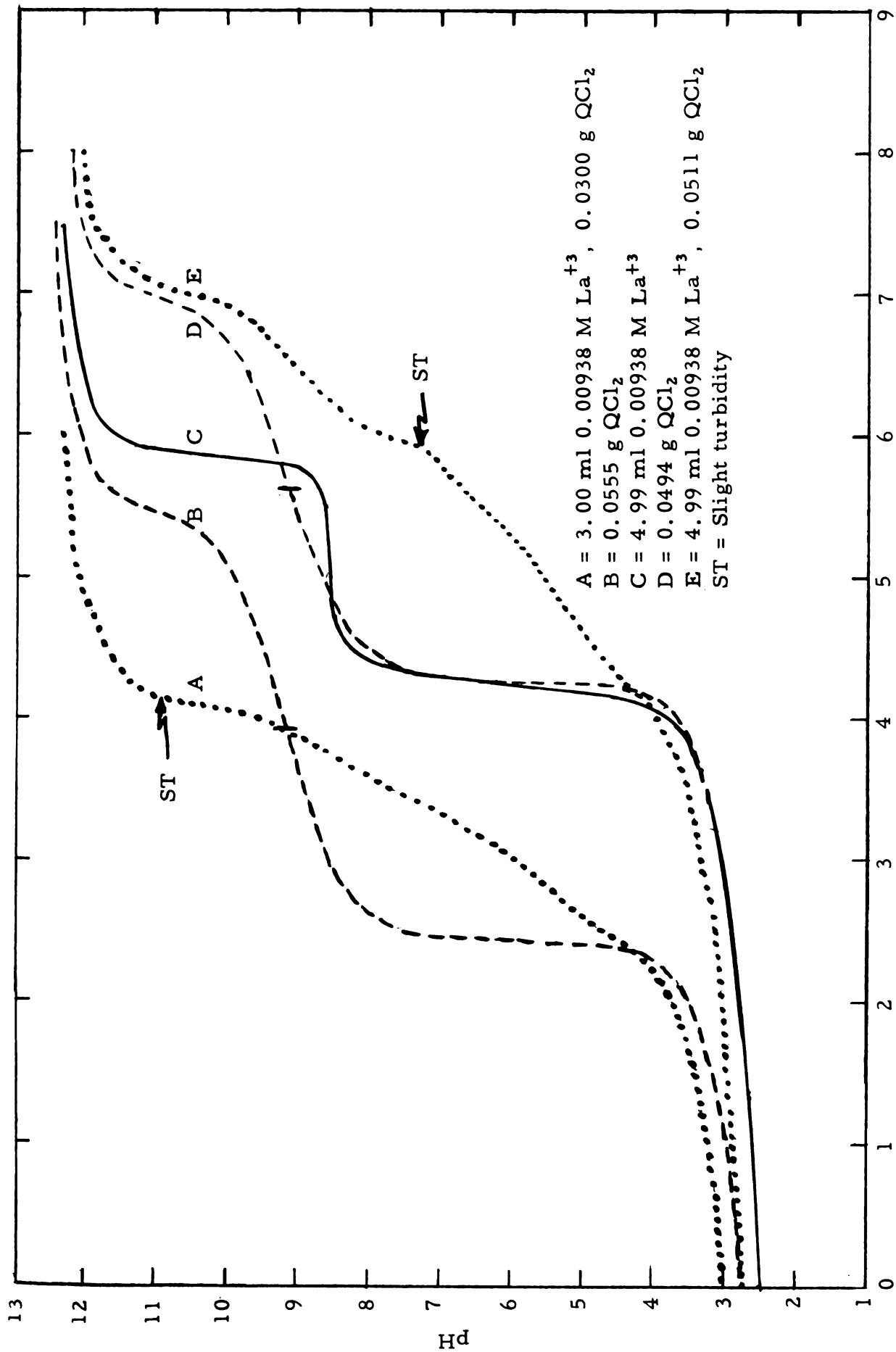

Figure 20. pH Titrations for Lanthanum (III), Lanthanum (III)-5,7-Dichloro-8-quinolinol and 5,7-Dichloro-8-quinolinol in Dilute Perchloric Acid Solutions of 50-50 DW.

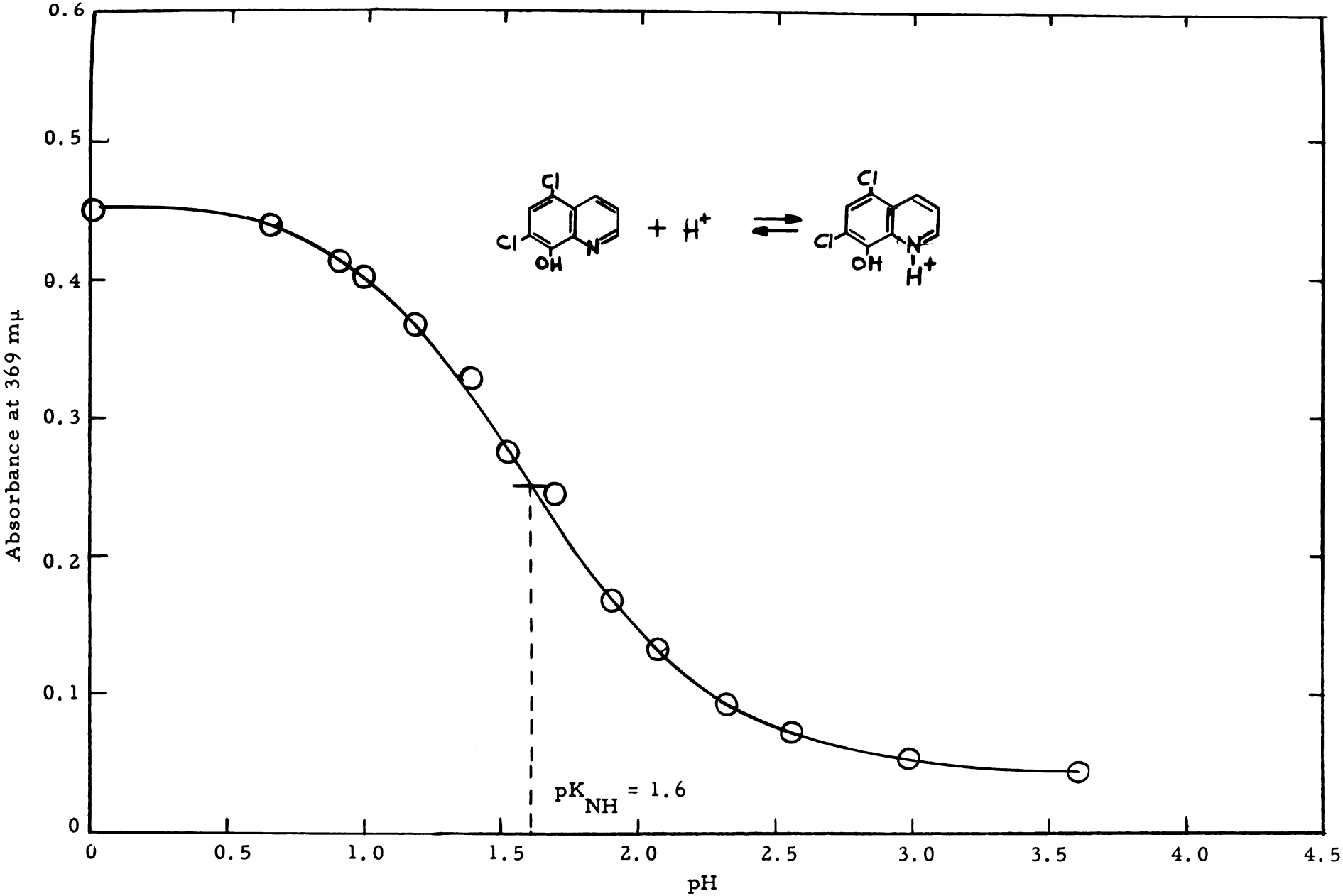

Figure 21. Effect of pH on the Absorbance at 369 mμ of 1000 γ 5, 7-Dichloro-8-quinolinol in 25 ml. of 50-50 DW.

The equations, which were developed for reagents such as 8-quinolinol from equations expressing material balances of metal and reagent, charge balance, and the acid dissociation constants K_{NH} and K_{OH}, are:

$$\bar{n} = \frac{1}{T_{M^{+3}}} \left[T_{HR} - S \left(\frac{K_{NH} + H^+}{K_{NH} + 2H^+} \right)\right.$$

and

$$R^- = \frac{K_{NH} \times S \times K_{OH}}{H^+ (K_{NH} + 2H^+)}$$

where

$\bar{n}$ = average number of reagent species, R^-, bound to M^{+3}

A = total moles per liter of the acid added

$T_{M^{+3}}$ = total moles per liter of the metal ion

T_{HR} = total moles per liter of reagent

$S = T_{HR} + A - Na^+ + OH^- + H^+$

$[Na^+]$ = calculated from amount of base added

$[OH^-] = \frac{K_\omega}{(H^+)}$

(H^+) = from measured pH.

The logarithms of the stepwise formal stability constants K_1 and K_2 are obtained from a plot of $\bar{n}$ against pR^- at values of $\bar{n} = 1/2$ and $3/2$, respectively.

In Figure 20 curves A and E show the pH titration curve of lanthanum (III) perchlorate and approximately a fivefold excess of 5,7-dichloro-8-quinolinol with 0.0862 N sodium hydroxide. The data from these titrations were employed to calculate $\bar{n}$ and pR^- for the complex. Table IV tabulates some calculated values of $\bar{n}$, which are between 0.07 and 2.88, and pR^- and gives the concentrations of the reagents which were employed.

Figure 22 shows the results obtained when $\bar{n}$ is plotted against $pQCl_2^-$ for the data given in Table IV. The log K_1 values obtained

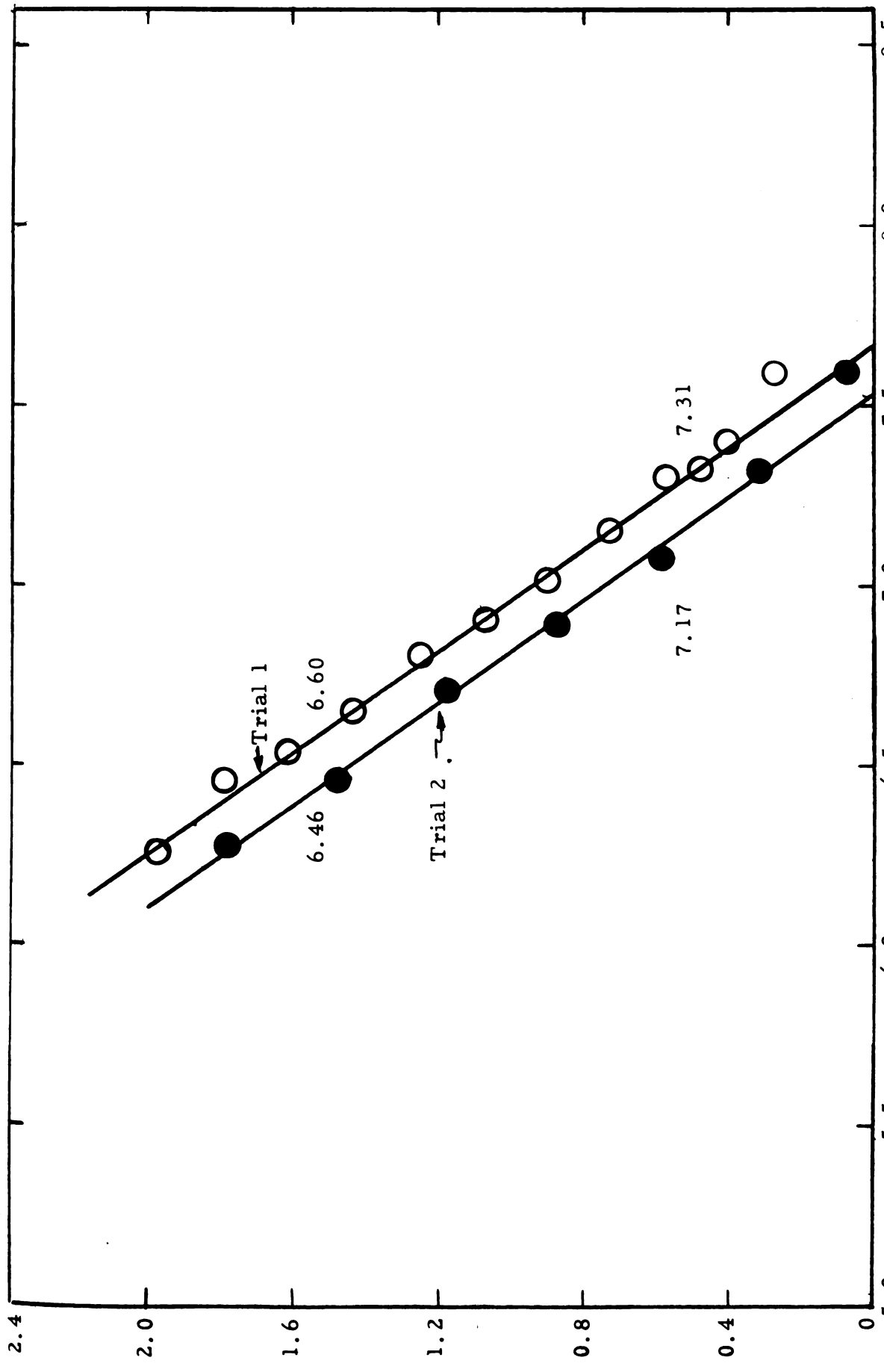

Figure 22. Plot for the Evaluation of log K_1 and log K_2 for the Lanthanum (III)-5, 7-dichloro-8-quinolinol Complexes in 50-50 DW.

Table IV. pR^- and $\bar{n}$ Values for Titration of Lanthanum (III) Perchlorate-5, 7-Dichloro-8-quinolinol in 50 Percent Dioxane-Water with 0.0862 N Sodium Hydroxide at 25°.

Ml. NaOH	Volume of Soln.	pH	$[H^+]$ M/1x10^6	$[OH^-]$ M/1x10^9	$[Na^+]$ M/1x10^3	(Acid) M/1x10^3	$T_{(La^{+3})}$ M/1x10^4	$T_{(HR)}$ M/1x10^3	pR^-	$\bar{n}$
Trial 1 - 0.0511 g. 5, 7-Dichloro-8-quinolinol; 4.99 ml. 0.00938 M $La(ClO_4)_3$ in Dilute $HClO_4$; 105.0 ml. Dioxane; 99.9 ml. H_2O.										
4.30	218.5	4.50	31.6	0.316	1.696	1.669	2.142	1.093	7.59	0.27
4.40	218.7	4.69	20.4	0.489	1.734	1.667	2.140	1.092	7.42	0.41
4.45	218.8	4.78	16.6	0.602	1.753	1.666	2.139	1.091	7.32	0.48
4.50	218.9	4.83	14.8	0.675	1.772	1.665	2.138	1.091	7.30	0.57
4.60	219.1	4.99	10.2	0.976	1.810	1.664	2.136	1.089	7.15	0.73
4.70	219.3	5.13	7.41	1.35	1.848	1.663	2.134	1.083	7.01	0.90
4.80	219.5	5.25	5.25	1.91	1.885	1.661	2.132	1.088	6.90	1.07
4.90	219.7	5.40	3.98	2.51	1.923	1.659	2.130	1.087	6.80	1.25
5.00	219.9	5.57	2.69	3.28	1.960	1.658	2.128	1.086	6.65	1.43
5.10	220.1	5.70	2.00	5.00	1.998	1.651	2.126	1.085	6.54	1.61
5.20	220.3	5.86	1.56	6.42	2.035	1.655	2.124	1.084	6.46	1.79
5.30	220.5	6.03	0.933	10.7	2.072	1.654	2.122	1.088	6.26	1.97
5.80	221.6	7.02	0.0955	105.0	2.260	1.646	2.112	1.077	5.42	2.88
Trial 2 - 0.0300 g. 5, 7-Dichloro-8-quinolinol; 3.00 ml. 0.00938 M $La(ClO_4)_3$ in Dilute $HClO_4$; 105.0 ml. Dioxane; 101.9 ml. H_2O.										
2.50	214.9	4.71	19.5	0.513	1.003	1.012	1.309	0.652	7.59	0.07
2.60	215.1	5.40	10.0	1.00	1.042	1.011	1.318	0.652	9.32	0.31
2.70	215.3	5.25	5.62	1.78	1.081	1.010	1.317	0.651	7.07	0.58
2.80	215.5	5.49	3.24	3.08	1.120	1.009	1.306	0.650	6.89	0.87
2.90	215.7	5.71	1.95	5.13	1.159	1.008	1.305	0.650	6.71	1.17
3.00	215.9	5.99	1.02	9.80	1.198	1.007	1.303	0.649	6.46	1.47
3.10	216.1	6.23	0.589	17.0	1.237	1.007	1.302	0.649	6.28	1.78
3.20	216.3	6.60	0.251	39.8	1.275	1.006	1.301	0.648	5.93	2.07
3.30	216.5	6.93	0.118	84.8	1.314	1.005	1.300	0.647	5.64	2.31
3.40	216.7	7.30	0.0501	200.0	1.350	1.004	1.300	0.647	5.33	2.62

from this plot are 7.17 and 7.31 and the log K_2 values are 6.46 and 6.60. The average log K_1 and log K_2 values are 7.24 and 6.53, respectively. Freiser, et al. (15) estimate that the stability constants determined by this procedure are accurate to within 0.2 log K units.

SUMMARY AND CONCLUSIONS

INTRODUCTION

A spectrofluorometric investigation of the complexes which form when lanthanum (III) is added to 50-50 DW solutions of morin or 5, 7-dichloro-8-quinolinol was undertaken. The various factors which affect the fluorescence of the active species were investigated and the composition and stability of the fluorescent complexes were established. All of these studies were carried out at 25°. A 50-50 DW solvent ratio was selected as the solvent medium for these studies.

LANTHANUM (III)-MORIN SYSTEM

The absorption band peaks of morin and of the lanthanum (III)-morin complex in 50-50 DW at pH 5.5 are at 410 and 356 mμ, respectively, while the fluorescence maximum of the complex occurs between 505 and 510 mμ. The complex can be excited to nearly equal fluorescence intensities by the 365, 405, and 436 mμ mercury radiations. Single wavelength fluorescence measurements were made at I_{365}^{505} and I_{436}^{505}.

The effect of the dioxane content on the fluorescence of solutions of lanthanum (III)-morin at pH 5.5 was established. The fluorescence intensity of the complex at I_{365}^{505} increased nearly linearly with increasing dioxane content, while the absorbance at 410 mμ reached a maximum value of about 40 percent and remained essentially constant up to at least 75 percent dioxane.

The optimum pH for the spectrofluorometric study of the lanthanum (III)-morin solutions was established. The maximum fluorescence intensities at I_{365}^{505} and I_{436}^{505} of the complex and minimum fluorescence intensities of morin were obtained for solutions maintained at pH 5.5. This pH also corresponded to the optimum value for absorption measurements on the complex, therefore, the pH of the solutions were adjusted to 5.5 for all studies on the complex or morin.

The pK_a of morin in 50-50 DW was estimated from a plot of absorbance against pH for a series of solutions containing 400 γ per 25 ml. The estimated pK_a value is 6.7.

The effect of lanthanum (III) concentration on the fluorescence intensities at I_{365}^{505} and I_{436}^{505}, and the absorbance at 410 mμ on 400 γ or 1000 γ of morin per 25 ml. was established. A nearly linear increase in the fluorescence intensities and the absorption measurements for solutions containing 400 γ of morin is attained in the approximate region 0 to 80 γ of lanthanum (III) per 25 ml. For solutions containing 1000 γ of morin, considerable concentration quenching of the fluorescence was noted.

The effect of the morin content on the fluorescence of 80 γ of lanthanum (III) per 25 ml. was determined. Maximum fluorescence intensities were attained at approximately 400 γ of morin. Above 400 γ of morin the fluorescence intensities decreased due to concentration quenching.

The fluorescence intensity of the lanthanum (III)-morin complex at I_{365}^{505} decreased nearly linearly with increasing temperature. The rate of change in the fluorescence intensity corresponds to about 1.8 fluorescence intensity units decrease per degree centigrade increase over the temperature interval studied (9 to 50°).

The effect of samarium (III), which forms a non-fluorescing complex with morin, on the fluorescence of solutions containing 40 γ of lanthanum (III) and 400 γ of morin or 80 γ of lanthanum (III) and 1000 γ of morin per 25 ml. was determined. For solutions containing 40 γ of lanthanum (III) and 400 γ morin, a two to threefold excess of samarium (III), did not significantly change the fluorescence intensity readings of the lanthanum (III)-morin complex at I_{365}^{505}. However, at I_{436}^{505}, the fluorescence intensity decreased with increasing samarium (III) concentration. The decrease in the fluorescence intensity of the lanthanum (III)-morin complex when excited with 436 mμ radiation is probably due

to quenching of the 436 mμ excitation radiation by the samarium (III)-morin complex which absorbs strongly at this wavelength. At 80 γ lanthanum (III) and 1000 γ morin, small changes in samarium (III) concentration caused a relatively large change in the fluorescence intensity of the lanthanum (III)-morin complex. On the basis of the above results and those obtained in the effect of lanthanum (III) concentration on the fluorescence of morin studies, microgram quantities of lanthanum (III) in pure solutions or in solutions containing low concentrations of samarium (III) or possibly other lanthanides which also form non-fluorescing complexes with morin can be determined fluorometrically.

When lanthanum (III) is added to 50-50 DW solutions of morin and the pH adjusted to 5.5, a stable complex is formed. The complex formed immediately after addition of the reagents and was stable for at least one week.

When amounts of lanthanum (III) varying between 0 and 1.6 mg per 25 ml. were added to solutions containing 400 γ morin and the pH adjusted to 5.5, the absorption spectra gave a series of curves yielding a single isoabsorptive point at 377 mμ. This family of curves with a single isoabsorptive point suggests that an equilibrium exists between lanthanum (III) and morin and that a single complex is formed.

Job's method of continuous variations (22) and the slope ratio of Harvey and Manning (20) was employed to determine the empirical formula of the fluorescent complex. These studies indicate that lanthanum (III) forms a 1:2 complex (Lanthanum (III):morin) with morin at pH 5.5 and that the complex, $La(M)_2^+$, is the species responsible for fluorescence in the lanthanum (III)-morin solutions.

The equilibrium ratio, K'eq, for the lanthanum (III)-morin complex was estimated from the absorbance, pH, and reagent concentrations data compiled in the composition of the complex studies. The average calculated K'eq ratio was found to be 0.6.

LANTHANUM (III)-5, 7-DICHLORO-8-QUINOLINOL SYSTEM

The absorption spectra from 260 to 620 mμ of 50-50 DW solutions which were adjusted to various pH values and contained 1000 γ 5, 7-dichloro-8-quinolinol or 80 γ lanthanum (III) and 1000 γ 5, 7-dichloro-8-quinolinol per 25 ml. were obtained. The absorption band peaks for solutions of the reagent or the complex at pH 8.8 centered around 342 and 388 mμ. The addition of lanthanum (III) to solutions of 5, 7-dichloro-8-quinolinol at pH 8.8 resulted in an enhancement of the absorption peak of the reagent at 388 mμ and suggested the formation of a complex or complexes.

The fluorescence spectra of 80 γ lanthanum (III) and 1000 γ 5, 7-dichloro-8-quinolinol per 25 ml. at pH 9.0 and 6.1 were obtained when the solutions were exposed to 365 mμ radiation. At pH 9.0 the fluorescence peak appeared between 526 and 530 mμ, while that at pH 6.1 was shifted to a slightly longer wavelength centering about 535 mμ. The peak fluorescence intensity at the higher pH value was more than two and one-half times that obtained at the lower one. The optimum excitation-wavelength for the complex is at 365 mμ, therefore single wavelength fluorescence measurements were measured at I_{365}^{530}.

The effect of dioxane content on the fluorescence of lanthanum (III)-5, 7-dichloro-8-quinolinol solutions buffered at pH 9 with ammonium hydroxide-ammonium perchlorate was evaluated. Maximum fluorescence intensities were obtained in solutions containing between 50 and 55 percent dioxane.

Lanthanum (III)-5, 7-dichloro-8-quinolinol fluoresces in the pH region 4.0 to 10 with maximum and nearly constant fluorescence intensities at pH 6 to 7.2 and 8.7 to 9.5, while the pure reagent shows no significant fluorescence over the entire pH range investigated (pH 2.3 to 9.7). Since the reagent is non-fluorescent, the existence of two plateau regions suggested that more than one lanthanum (III)-5, 7-dichloro-8-quinolinol fluorescent complex is formed.

The effect of the change in lanthanum (III) concentration on the fluorescence of 1000 γ of reagent per 25 ml. at pH 8.8 was evaluated. A nearly linear increase in the fluorescence intensity of the complex was attained in the approximate region 0 to 70 γ of lanthanum (III).

In a series of solutions adjusted to pH 8.8 and containing 80 γ of lanthanum (III) per 25 ml., maximum fluorescence intensity readings were obtained for 800 to 1500 γ of 5, 7-dichloro-8-quinolinol. For solutions containing greater than 1500 γ of reagent, the fluorescence intensity of the complex decreased due to concentration quenching.

The fluorescence intensity of the lanthanum (III)-5, 7-dichloro-8-quinolinol complex decreased with increasing temperature. This nearly linear decrease corresponded to about 3.0 fluorescence intensity units per degree centigrade increase over the temperature range 14-45°. On the basis of this result, constant temperature conditions should be maintained for any fluorescence study on the complex.

The effect of carbonate on the fluorescence of the lanthanum (III)-5, 7-dichloro-8-quinolinol complex in 50-50 DW at pH 9.0 was ascertained. The addition of carbonate to these solutions (0 to 5 mg. sodium carbonate) resulted in decrease in the fluorescence intensity of the complex. Approximately a 10 percent reduction in fluorescence intensity was noted when 1.0 mg. of sodium carbonate was added to solutions containing 80 γ lanthanum (III) and 1000 γ 5, 7-dichloro-8-quinolinol per 25 ml. On the basis of these results, carbonate-free base should be employed in the preparation of the complex.

The effect of samarium (III) on the fluorescence of solutions containing 40 γ lanthanum (III) and 1500 γ of 5, 7-dichloro-8-quinolinol per 25 ml. was investigated. Samarium (III) in concentrations approximately equal to lanthanum (III) can be tolerated without causing a significant error in the fluorescence intensity reading of the complex.

The lanthanum (III)-5, 7-dichloro-8-quinolinol complex develops within at least ten minutes after preparation of the complex and yields

essentially constant fluorescence readings for about one to two hours. Therefore, fluorescence measurements on the complex should be obtained within at least one to two hours after preparation of the complex.

The acid dissociation constants of the protonated nitrogen K_{NH} and of the phenol in 50-50 DW solutions, K_{OH}, which were required for the calculation of the stability constants of the 5, 7-dichloro-8-quinolinol complexes, were evaluated. The average K_{OH} and K_{NH} was found to be 7.8×10^{-10} and 2.5×10^{-2} respectively.

The consecutive formal stability constants K_1 and K_2 for the lanthanum (III)-5, 7-dichloro-8-quinolinol system were evaluated by the method of Freiser, et al. (15). The stability constant, K_3, could not be accurately determined by this method since lanthanum (III) tends to hydrolyze at higher pH levels. The average log K_1 and log K_2 values are 7.24 and 6.53, respectively.

On the basis of normal valency considerations in which the maximum coordination number of six is recognized for lanthanum (III), and on the basis of $\bar{n}$ values greater than two attained at pH values greater than about seven, it is concluded that the species responsible for the fluorescence of the lanthanum (III)-5, 7-dichloro-8-quinolinol system at pH 9 is probably a 1:3 complex (lanthanum (III):reagent).

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