A SPECTROFLUOROMETRIC METHOD FOR THE DETERMINATION OF ALUMINUM (III) USING FLAVONOL AS THE REAGENT

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ABSTRACT

A SPECTROFLUOROMETRIC METHOD FOR THE DETERMINATION OF ALUMINUM (III) USING FLAVONOL AS THE REAGENT

by Doris Ann Wambach

The feasibility of fluorometrically determining aluminum using flavonol as a reagent in a 3:2 water to ethanol mixed solvent was tested.

Without controlling the pH, spectrophotometric and spectrofluorometric data indicate the formation of species of the 1:1 and 1:3, aluminum to flavonol, stoichiometries in the solvent. The 1:1 aluminum to flavonol chelate has no visible color, but exhibits a blue fluorescence. The 1:3 aluminum to flavonol chelate has a yellow color, but appears to be non-fluorescent. In a study of the effect of various factors on the stabilities of these chelates, it was found that they are much more stable if they are protected from the light.

The spectrofluorometric method was based on pH adjustment of solutions containing an excess amount of flavonol compared to the aluminum concentration. At high acidity, the species of these solutions changes from the yellow, non-fluorescent, 1:3, aluminum to flavonol, chelate, to a colorless, fluorescent species.

A reproducible calibration curve in the aluminum concentration range of 0.5 to 7.0 x 10^{-5} M was obtained from solutions prepared under the following conditions: with a 20 x 10^{-5} M flavonol concentration, the water-ethanol ratio maintained at 3:2, the pH adjusted to 3.50 ± 0.05, and allowed to stand protected from the light for at least five hours before the fluorescence intensities were measured. Many substances were found to interfere under these conditions. This method determines aluminum in the concentration range of 0.5 to 7.0 x 10^{-5} M with a relative mean deviation of 3.8 parts per hundred.

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By

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A THESIS

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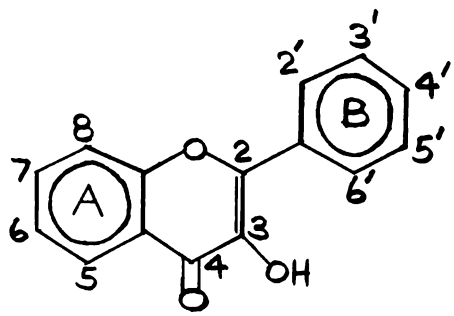
INTRODUCTION

Flavonol (3-hydroxyflavone) is not a naturally occurring substance as are many polyhydroxy or polymethoxy substituted flavones. The natural occurrence, syntheses and reactions of flavonoid compounds has been reviewed (5,11,12,17,21). Flavonol has been synthesized and has the following structure:



Since a hydroxy group is present at the 3 position, as well as a carbonyl oxygen in the 4 position, flavonol could form chelates with various metal ions. The formation of such chelates with several metal ions has been studied. A recent study in this laboratory involved the chelation reactions of flavonol and aluminum ions in absolute ethanol using spectrophotometric and spectrofluorometric techniques (15). In this medium fluorescent and absorbing species were formed.

The present study is concerned with the feasibility of developing a spectrofluorometric method for the determination of aluminum in a water-ethanol solvent.

A water-ethanol mixture was chosen as the solvent since the aluminum content of a sample would probably be determined in an aqueous solution, rather than an ethanolic solution. Water alone was not used as the solvent, since the solid flavonol is not soluble in water. Flavonol remains dissolved when an ethanolic solution is added to water.

Flavonol was selected as the chelating agent to determine if absorbing and fluorescent species corresponding to those obtained in absolute ethanol, are formed with aluminum in the water-ethanol solvent. Since it was found that such species do form, the possibility of developing a spectrofluorometric method for the determination of aluminum was investigated.

HISTORICAL

Goppelsroeder (7) discovered that in the reaction between aluminum and morin (2',3,4'5,7,pentahydroxy-flavone) a species formed, which exhibited an intense greenish fluorescence. White and Lowe (18) based the first quantitative fluorometric method for the determination of aluminum on the use of morin. A water-ethanol solvent was used in this method and the pH was adjusted to 3.3 with acetic acid. The amount of morin and ethanol, temperature and pH had to be carefully controlled. A concentration range of 0.1 to 1.2 mg of aluminum per liter can be determined. Many common ions interfered in this method.

Since then other reagents have been proposed for use in fluorometric methods for aluminum. Pontachrome Blue Black R was used as a reagent to produce fluorescence on reaction with aluminum by Weissler and White (16). The concentration range that can be determined by this method is 0.0002 to 0.025 mg aluminum per 50 ml of solution buffered at a pH of 4.8. About an hour was required before full fluorometric intensity was attained. This method was found to be extremely sensitive and subject to fewer interferences than the morin determination.

Goon et al. (6) used 8-quinolinol as the reagent

for a fluorometric determination of aluminum. In this method the concentration range of aluminum that can be determined was given as 0 to 16 μ g per 100 ml and the pH was controlled at 8.0 ± 1.5. The authors found that the fluorescence did not vary critically with time, nor with the amount of reagent used, as is the case with other fluorometric methods for aluminum. They also determined that the sensitivity of this method was equal to, but no better than in the Pontachrome Blue Black R method.

Other reported methods for the fluorometric determination of aluminum have been based on using the above three reagents. Simons, Monaghan and Taggart (14) used Pontachrome Blue Black R for the determination of aluminum in surface sea water. Collat and Rogers (3) tested the feasibility of determining aluminum and gallium in a mixture of their oxinates. Morin was used by Will (20) to determine aluminum in high-purity boiler water condensate. Noll and Stefanelli (9) used 8-quinolinol for a fluorometric and spectrophotometric determination of aluminum in industrial water.

The reaction between flavonol and various metal ions to produce a fluorescent species has also been a basis for reported methods. Coyle and White (4)

reported a fluorometric determination of tin with flavonol. Bottei and Trusk (2) suggested flavonol as a fluorometric reagent for tungsten. The emission, excitation, and absorption spectra of several flavone-metal chelates were examined by White, Hoffman, and Magee (19).

EXPERIMENTAL

Instrumentation

The following instruments were employed to make the appropriate measurements:

Beckman Zeromatic pH meter with glass and saturated calomel electrodes to measure the apparent pH of the water-ethanol solutions.

Beckman Model DB Spectrophotometer equipped with a Sargent SR recorder to obtain visible and ultraviolet absorption spectra.

Cary Model 14 Recording Spectrophotometer to obtain visible and ultraviolet absorption spectra.

Beckman 1R5A Infrared Spectrophotometer to obtain infrared absorption spectra.

Spectrofluorometer (13), employing a Hanovia mercury arc source, a Bausch and Lomb excitation monochromator, a Beckman DU with an AC power supply as an emission monochromator and a IPUA5 photomultiplier tube as a detector.

Chemicals

The following chemicals were used without further purification in the syntheses and preparations of reagent solutions described in this study:

Acetic acid, glacial	Baker's Analyzed Reagent J. T. Baker Chemical Co.
Aluminum nitrate monohydrate	Analytical Reagent Grade Allied Chemical
Ammonium chloride	Baker's Analyzed Reagent J. T. Baker Chemical Co.
Ammonium fluoride	Baker's Analyzed Reagent J. T. Baker Chemical Co.

Ammonium hydroxide	Reagent Grade Fisher Scientific Company
Benzaldehyde	The Matheson Co., Inc.
Beryllium nitrate, dihydrate	c.p. Fisher Scientific Company
Calcium chloride	Reagent Grade Allied Chemical
Chromium chloride, hexahydrate	Fisher Scientific Company
Cobalt nitrate, hexahydrate	Baker's Analyzed Reagent J. T. Baker Chemical Co.
Cupric nitrate, trihydrate	Mallinckrodt Chemical Works
Ethanol, absolute	Commercial Solvents Corp.
Ethanol, 95%	Commercial Solvents Corp.
Ethylenediaminetetracetic acid, disodium salt, monohydrate	Analytical Reagent Grade J. T. Baker Chemical Co.
Hydrochloric acid	Baker's Analyzed Reagent J. T. Baker Chemical Co.
Hydrogen peroxide, 30%	Baker's Analyzed Reagent J. T. Baker Chemical Co.
o-Hydroxyacetophenone	K and K Chemical Company
Iron wire	Analytical Reagent Mallinckrodt Chemical Works
Lead nitrate	Fisher Certified Reagent Fisher Scientific Company
Methanol	Baker's Analyzed Reagent J. T. Baker Chemical Co.
Nickelous nitrate, hexahydrate	Baker's Analyzed Reagent J. T. Baker Chemical Co.

Potassium iodide	Baker's Analyzed Reagent J. T. Baker Chemical Co.
Potassium permanganate	Analytical Reagent Grade Mallinckrodt Chemical Works
Quinine sulfate	Mallinckrodt Chemical Works
Silver nitrate	A. C. S., D. F. Goldsmith Chemical & Metal Corp.
Sodium bromate	Matheson Company, Inc.
Sodium bromide	Fisher Scientific Company
Sodium carbonate, monohydrate	Baker's Analyzed Reagent J. T. Baker Chemical Co.
Sodium chloride	Baker's Analyzed Reagent J. T. Baker Chemical Co.
Sodium citrate, dihydrate	Baker's Analyzed Reagent J. T. Baker Chemical Co.
Sodium hydroxide	Baker's Analyzed Reagent J. T. Baker Chemical Co.
Sodium molybdate, dihydrate	Analytical Reagent Mallinckrodt Chemical Works
Sodium phosphate, dibasic	N.F. Allied Chemical Company
Sodium sulfate, decahydrate	Mallinckrodt Chemical Works
Sodium tartrate, dihydrate	Baker's Analyzed Reagent J. T. Baker Chemical Co.
Thorium nitrate	Baker's Analyzed Reagent J. T. Baker Chemical Co.
Zirconium nitrate	Central Scientific Company
Zinc nitrate	Baker's Analyzed Reagent J. T. Baker Chemical Co.

Zinc sulfate	Reagent Grade Allied Chemical Company

Soluble chlorides of dysprosium, lanthanum, samarium and ytterbium, previously prepared in the laboratory, were dissolved in absolute ethanol and their concentrations were determined by titrating with EDTA solution.

Preparation of Flavonol

Previously synthesized flavonol was used in a portion of this study. The remainder of the flavonol used was prepared according to the methods of Oyamada (10) and Kostanecki (8) as given in the previous study (15), except that the flavonol was purified by sublimation instead of recrystallization from aqueous ethanol.

The flavonol preparation was analyzed by the Spang Microanalytical Laboratory. Found: 75.52% C, 4.32% H; calculated: 75.61%, 4.23% H.

Stock solutions of flavonol in 95% ethanol were prepared by direct weighing of the flavonol.

Preparation of Stock Solutions

Standard ethylenediamineteracetic acid (EDTA) solution was prepared by direct weighing of the disodium salt hydrate.

A zinc sulfate solution was standardized by

titration with the EDTA solution using Eriochrome Black T indicator.

Solutions of the aluminum salt were prepared by dissolving the appropriate amount of hydrated aluminum nitrate in distilled water to give approximately 0.01 M solutions. A measured excess of the standard EDTA solution was added to aliquots of the aluminum solution. The pH of the solutions was then adjusted to between 7 and 8, a few drops of Eriochrome Black T indicator were added, and the excess EDTA was titrated immediately with the standard zinc sulfate solution. The concentrations of the aluminum solutions were calculated and the stock solutions of the desired aluminum ion concentrations were prepared by dilution.

Stock solutions of the 3:2 volume ratio water to ethanol solvent were prepared by mixing 1200 ml of distilled water and 800 ml of 95% ethanol.

Stock solutions of the 1:1 acetic acid were prepared by mixing equal portions of glacial acetic acid and solvent.

Experimental Procedures

Calibration of the Spectrofluorometer

The spectrofluorometer was calibrated with a solu-

tion containing 24.1×10^{-3} mg/ml of quinine sulfate in 0.1 N sulfuric acid. The following instrument settings were employed in the calibration procedure: entrance slit, excitation monochromator, 1.0 mm; exit slit, excitation monochromator, 0.5 mm; excitation wavelength, 365 mμ; emission wavelength, 540 mμ; slit, emission monochromator (DU), 0.3 mm; selector switch, DU, 0.1; percent transmittance, 60%; AC power supply, power switch, on, sensitivity switch, full. With the phototube shutter closed, the dark current was adjusted to zero. The calibration solution was now placed in the sample compartment and with the shutter open, the sensitivity control on the DU was adjusted to give a zero reading.

Spectrophotometric Titrations

An aliquot of the reagent to be titrated was placed in each of a series of volumetric flasks. To these aliquots were added successively larger increments of the titrant. As required to maintain the 3:2 solvent ratio, appropriate amounts of water or ethanol were added. The contents of the flasks were then diluted to volume with solvent and the appropriate measurements were made on these solutions. No correction for dilu-

tion was necessary with this procedure.

Apparent pH Measurements

In the apparent pH measurements, the meter was calibrated with an aqueous pH 7 buffer solution. The electrodes were then allowed to equilibrate for a minimum of fifteen minutes in the solvent before any readings were taken.

Bates, Paabo and Robinson (1) report a procedure which correlates the apparent pH of alcohol-water solvents, including ethanol-water solvents, to the actual pH. According to their procedure, the actual pH of this ethanol-water solvent is approximately 0.10 pH unit greater than the apparent pH. In this study apparent pH is meant by all references to pH values, and the actual pH can be calculated by applying the correction factor.

Temperature Control

Unless otherwise stated, no temperature control was employed during any of the measurements.

RESULTS AND DISCUSSION

Determination of the Water to Ethanol Ratio for the Solvent

The volume per cent of water to ethanol was varied in a series of solutions containing constant and approximately equal concentrations of aluminum and flavonol. Figure 1 shows the dependence of the fluorescence intensity on the per cent of water at I_{365}^{455}.* With increasing water content, the fluorescence intensity steadily increases, reaching a maximum at 65 per cent water and then rapidly decreases. Approximately 60 per cent water was chosen as a suitable solvent. In the region of 60 per cent water, small variations of the water content give smaller variations in the fluorescence intensity, while still giving a relatively high fluorescence intensity.

In preparing the individual chelate solutions, after the addition of aliquots of the aqueous aluminum and ethanolic flavonol stock solutions to volumetric flasks, water or ethanol was added as required to maintain the 3:2 volume ratio. Then solvent, previously prepared by mixing water and ethanol in a volume ratio

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*For notations of the type I_{365}^{455}, the subscript indicates the wavelength of excitation and the superscript denotes the wavelength at which the fluorescence radiation is recorded.

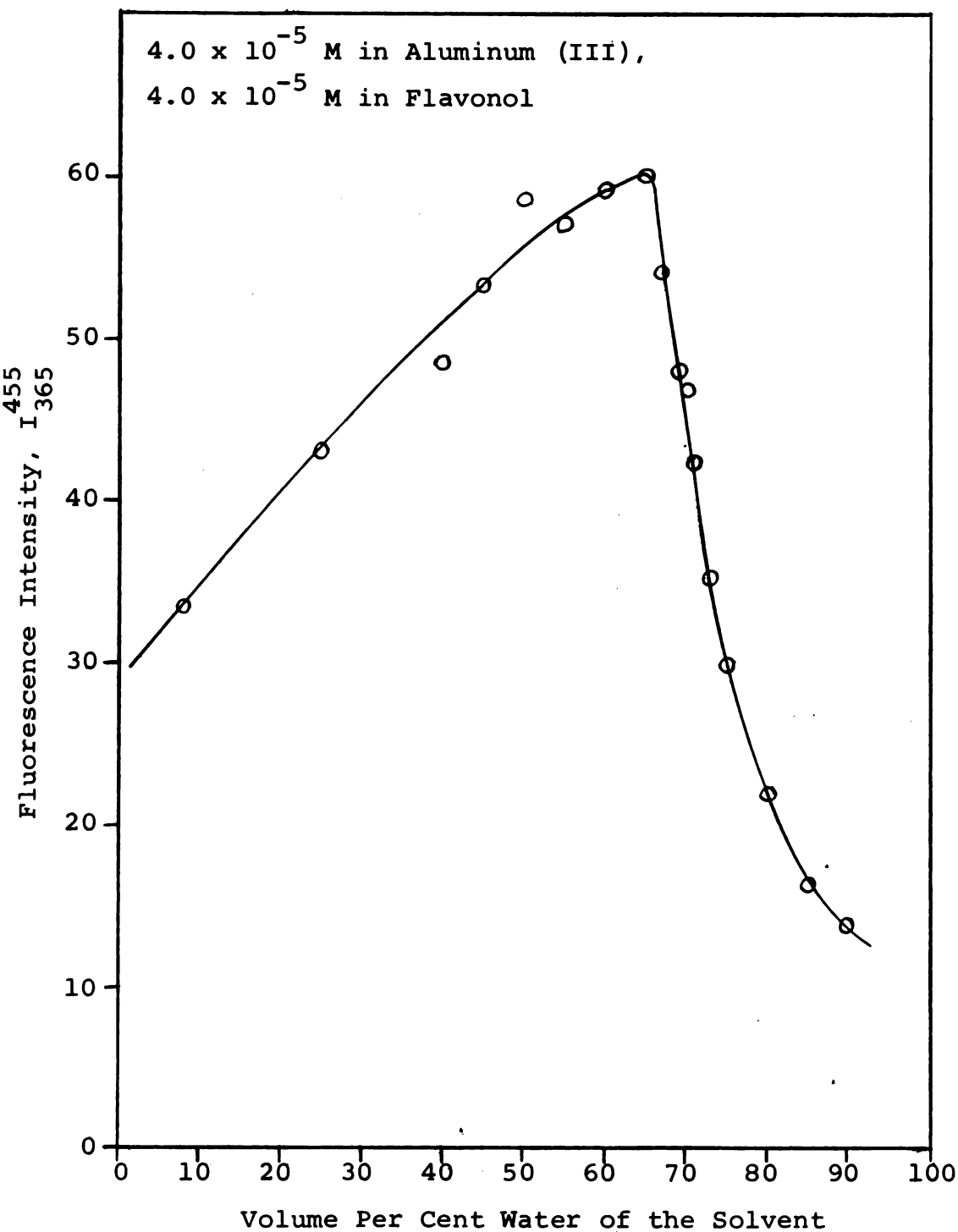

FIGURE 1. Dependence of the Fluorescence of the 1:1 Aluminum to Flavonol Chelate on the Water Volume Per Cent of the Water-Ethanol Solvent.

of 3:2, was added to dilute the solution to volume.

The Absorption and Fluorescence Spectra of Flavonol in the Water-Ethanol Solvent

The absorption (Figure 2) and fluorescence (Figure 3) spectra of flavonol were obtained in the water-ethanol solvent. The electronic absorption bands are located at 201, 238-243, 310 and 344 mμ. The absorption bands of flavonol in absolute ethanol have been reported to be at 201, 238-242, 306 and 344 mμ (15). The slight bathochromic shift of the 306 mμ band to 310 mμ in the water-ethanol solvent can probably be attributed to the differences in solvents.

The fluorescence spectrum of flavonol has two weak bands, one at 410 mμ and the other at 525 mμ in the water-ethanol solvent compared to one at 410 mμ and another at 535 mμ reported in absolute ethanol. Again the differences in solvents are probably responsible for the slight shift of the 535 mμ band to 525 mμ in the water-ethanol solvent.

Determination of the Stoichiometries of the Aluminum-Flavonol Chelates

Without the addition of acid or base, the stoichiometries of the chelates were investigated employing spectrophotometric and spectrofluorometric titrations

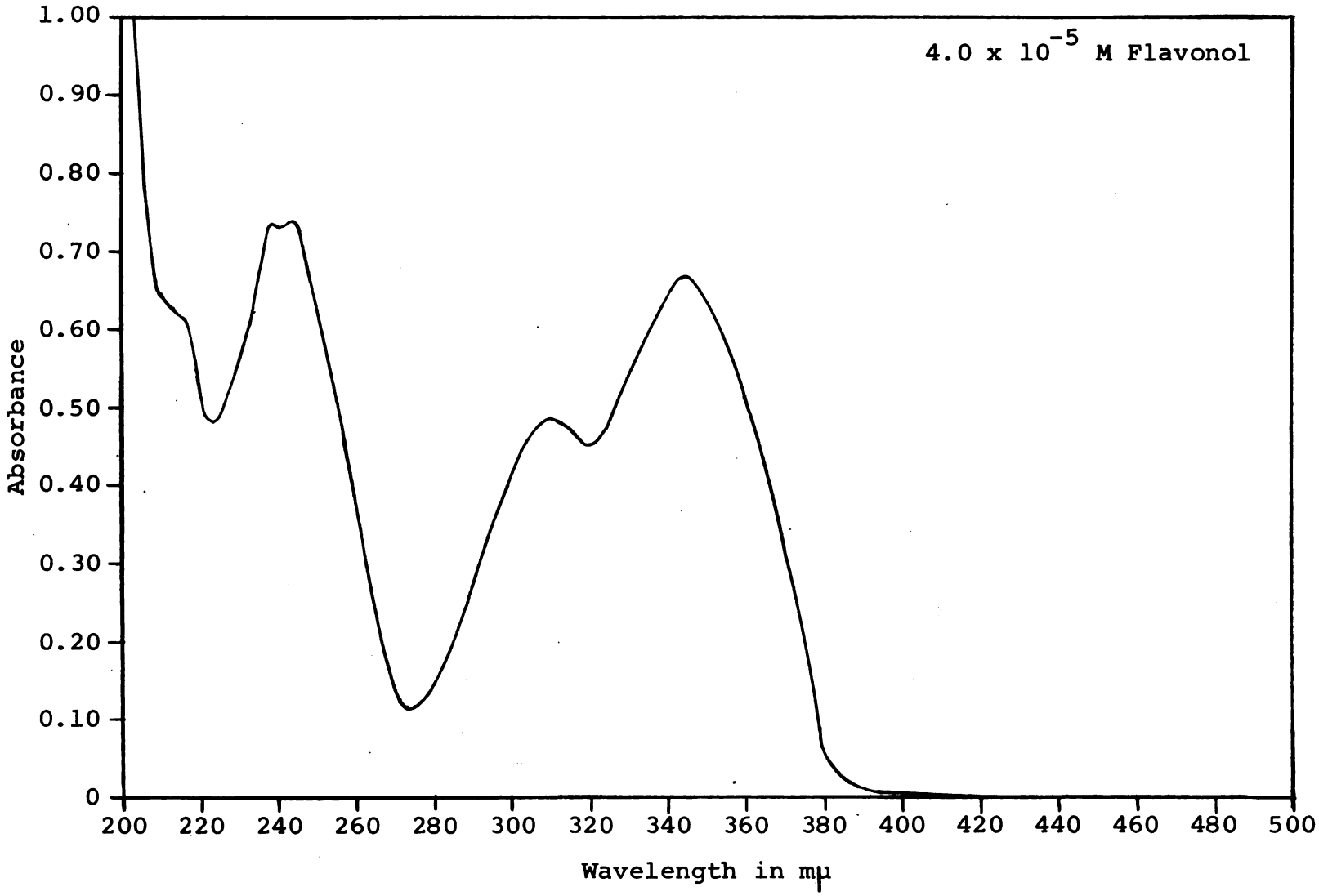

FIGURE 2. Absorption Spectrum of Flavonol in the 3:2 Water-Ethanol Solvent.

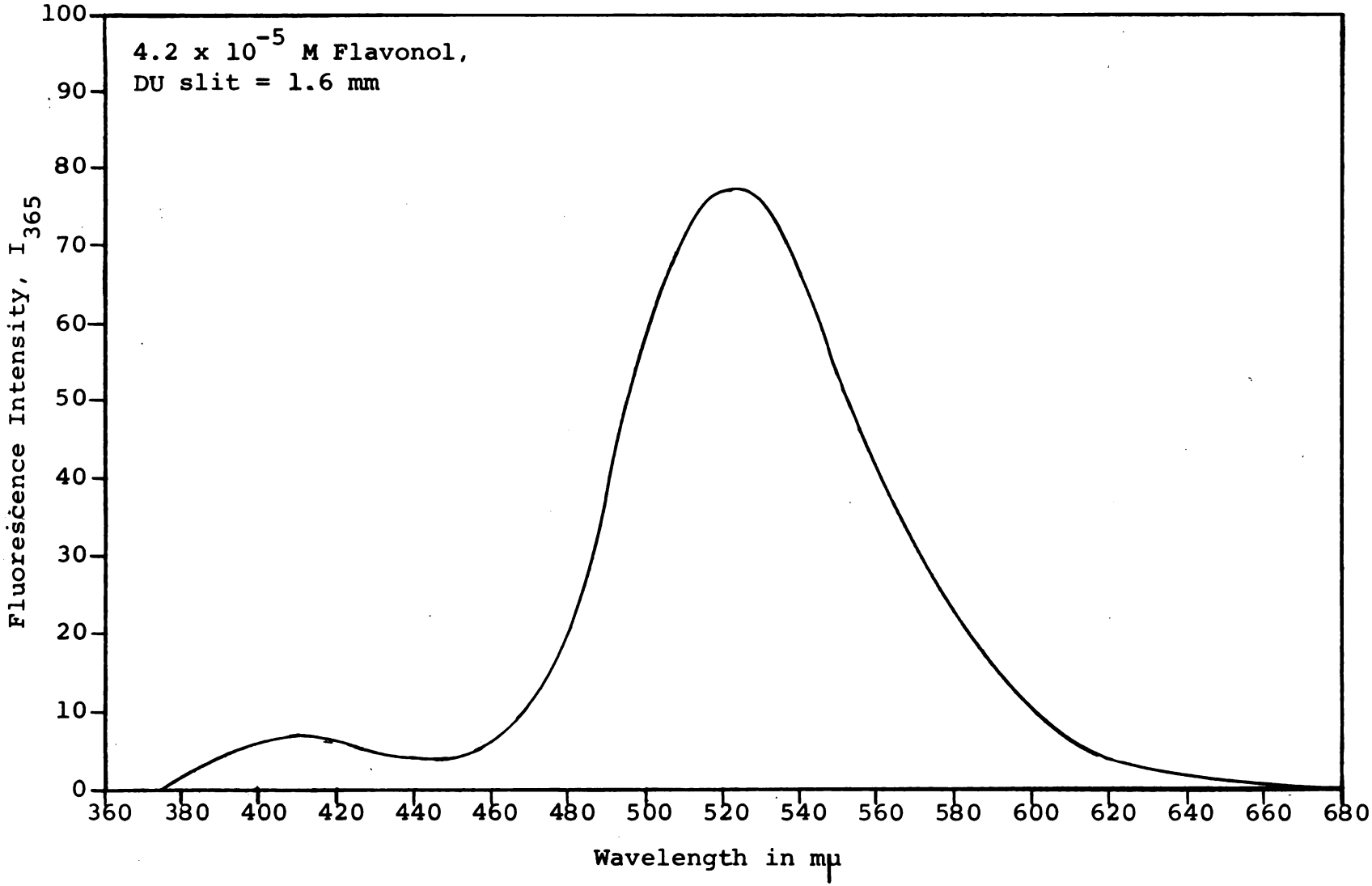

FIGURE 3. Fluorescence Spectrum of Flavonol in the 3:2 Water-Ethanol Solvent.

of the same solutions. The spectrophotometric titrations followed the increase in absorbance values of the chelate peak, which appeared in the region of 395 to 405 mμ. The spectrofluorometric titrations followed the change in fluorescence intensities of the chelate emission peak at 455 mμ as excited by 365 mμ radiation. Figure 4 shows the chelate emission spectrum which can be excited by 365 and 405 mμ radiation. Excitation of the chelate by 365 mμ radiation produces a greater relative emission intensity than that for 405 mμ radiation.

The titration of aluminum ions with flavonol in the mole ratio range of 0 to 2.5, flavonol to aluminum, is given in Figure 5. A straight line is obtained from the spectrophotometric data, indicating that a chelate is not formed in this mole ratio range. But the spectrofluorometric titration indicates the formation of a chelate with the stoichiometry of 1:1, aluminum to flavonol.

In the aluminum to flavonol mole ratio range of 0 to 2.5, Figure 6 shows the titration of flavonol with aluminum ions. The spectrophotometric titration indicates the 1:1, aluminum to flavonol, stoichiometry. Due to the order of this titration, aluminum ions titrating flavonol, the spectrofluorometric data does not indicate

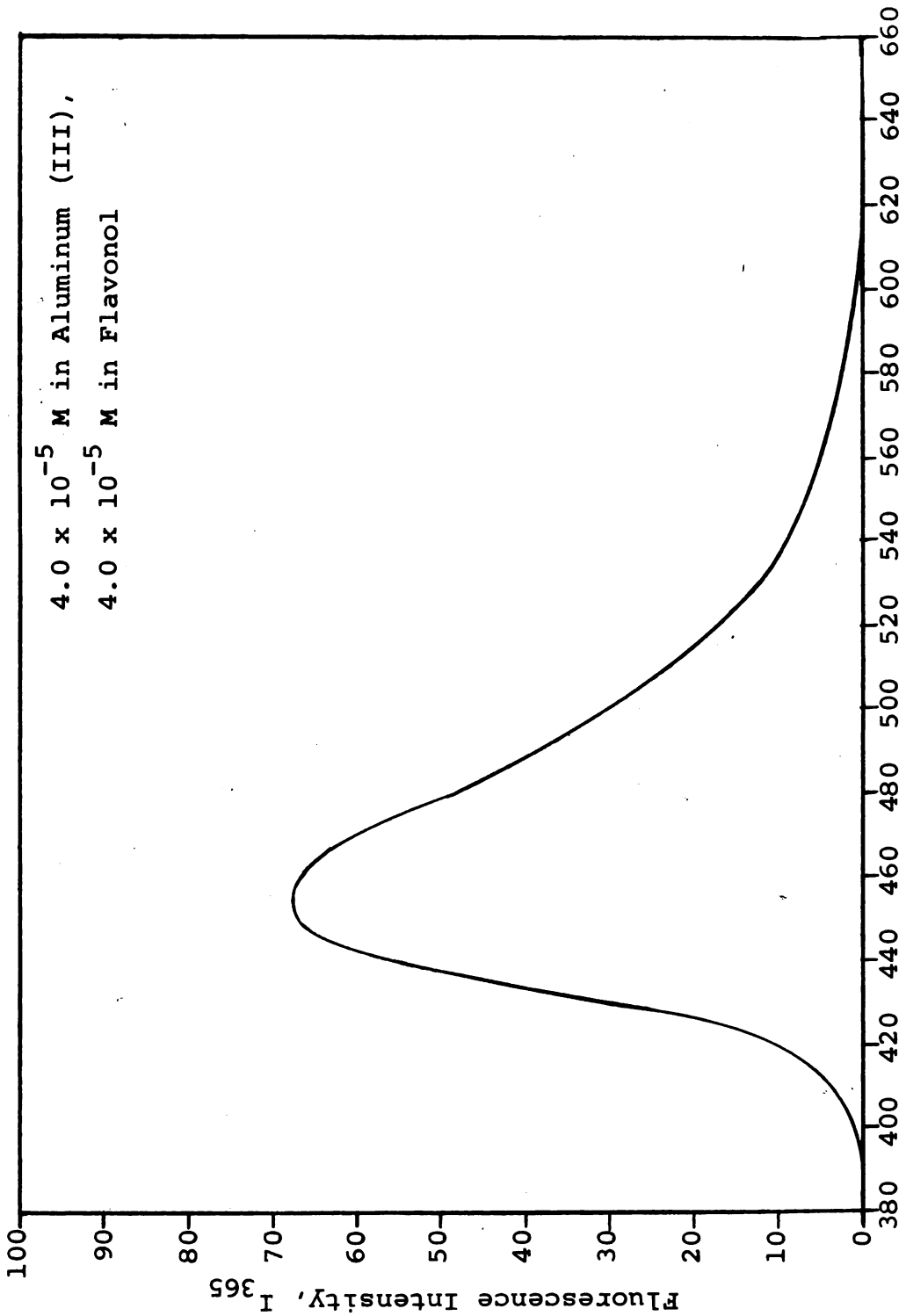

FIGURE 4. Fluorescence Spectrum of the 1:1 Aluminum-Flavonol Chelate in the 3:2 Water-Ethanol Solvent.

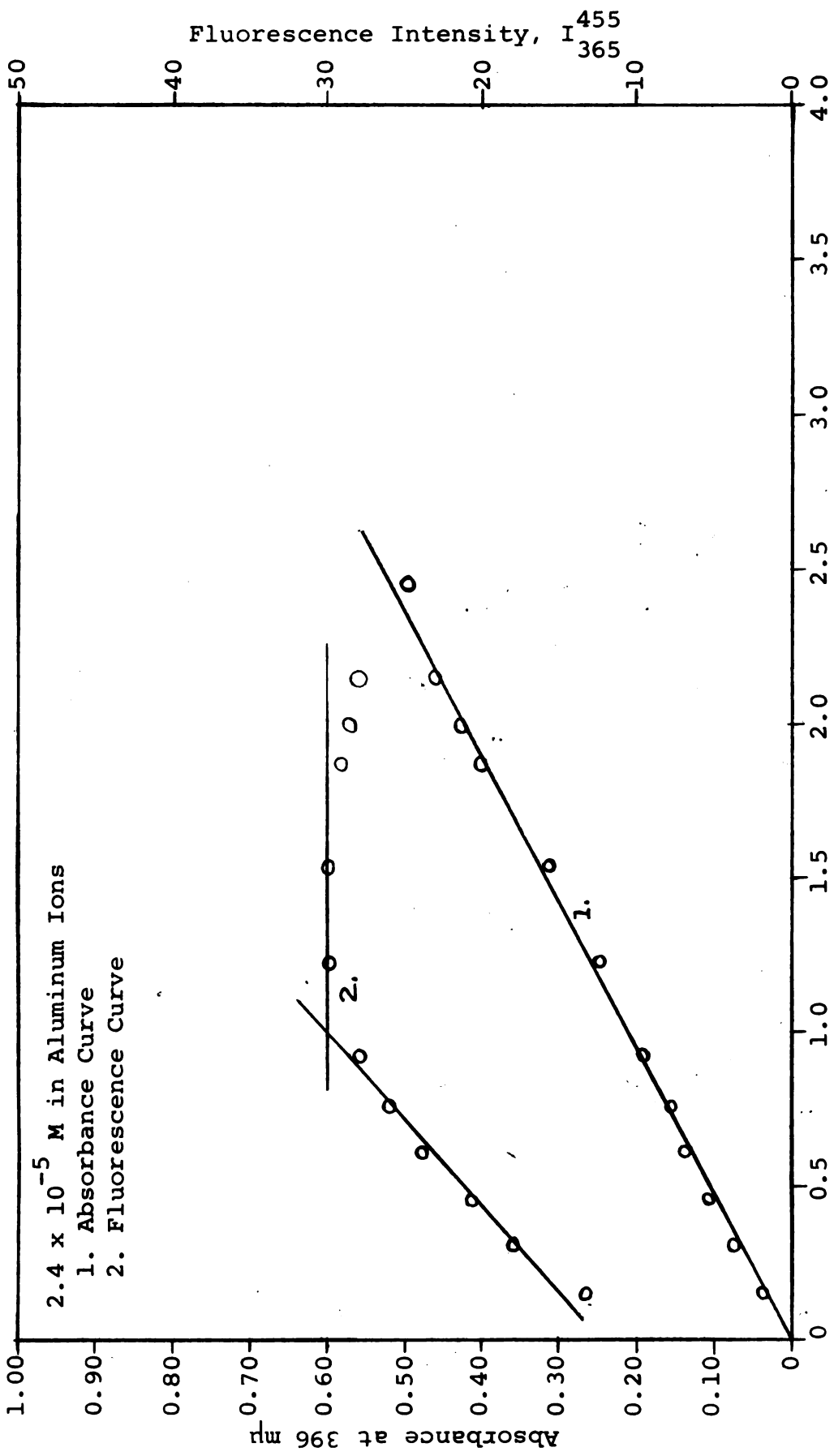

FIGURE 5. Spectrophotometric and Spectrofluorometric Titration Curves for Aluminum (III) Titrated with Flavonol Indicating the 1:1 Aluminum to Flavonol Species.

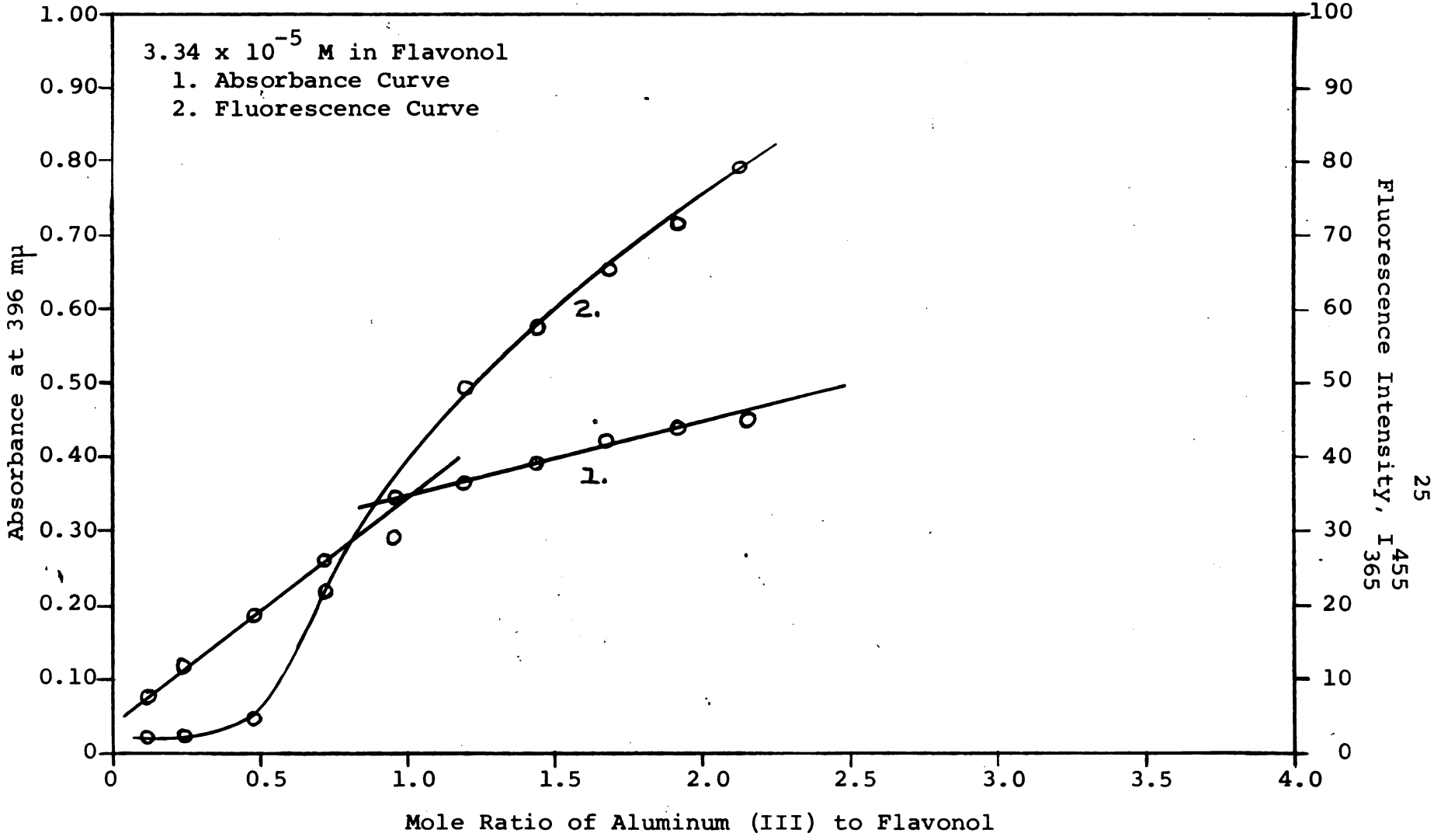

FIGURE 6. Spectrophotometric and Spectrofluorometric Titration Curves for Flavonol Titrated with Aluminum Ions Indicating the 1:1 Aluminum to Flavonol Species.

the stoichiometry of the chelate formed. In the region of low aluminum to flavonol mole ratios, the fluorescence obtained is probably due to the combined effect of the increasing aluminum concentration and the decrease in quenching by the excess flavonol as more of the flavonol is chelated. Consequently a definite break is not observed in the titration curve at the 1:1 aluminum to flavonol ratio. The curve merely shows how the fluorescence increases as a result of these effects.

The above titrations indicate the formation of a chelate with a 1:1 aluminum to flavonol stoichiometry. It appears to be a weak complex since only the spectrophotometric titration of flavonol with aluminum ions gives a break at this ratio. The 1:1 chelate is only a stable species when it is forced, as is the case when the aluminum ions are present in excess. In the titration of aluminum with flavonol, additional flavonol readily chelates with the aluminum ions to form higher chelates. Thus the titration curve shows no break at the 1:1 flavonol to aluminum mole ratio.

From the spectrofluorometric data, the 1:1 aluminum to flavonol chelate appears to be fluorescent species. A solution of the chelate has no visible color, but it exhibits a blue fluorescence. The relative intensity

of the fluorescence of the chelate is much stronger than that of flavonol.

Figure 7 shows the titration of aluminum with flavonol in the flavonol to aluminum mole ratio of 1.5 to 5.0. The spectrophotometric titration indicates a stoichiometry of 1:3, aluminum to flavonol. The spectrofluorometric curve does not indicate the stoichiometry of the chelate formed. It shows a fairly constant fluorescence in the region of lower flavonol to aluminum mole ratios and then a decrease in fluorescence with increasing flavonol concentrations. The decrease in fluorescence may be due to the formation of a non-fluorescent species, quenching of the fluorescence by the presence of excess flavonol, or the combined effects of these factors.

The titration of flavonol with aluminum ions in the aluminum to flavonol mole ratio range of 0 to 1.1 is shown in Figure 8. A stoichiometry of 1:3, aluminum to flavonol is indicated by the spectrophotometric titration. The 1:3 stoichiometry is also suggested by the spectrofluorometric titration, but the break is not as sharp as the spectrophotometric one. This observation can probably be attributed to some quenching of the fluorescence by the flavonol.

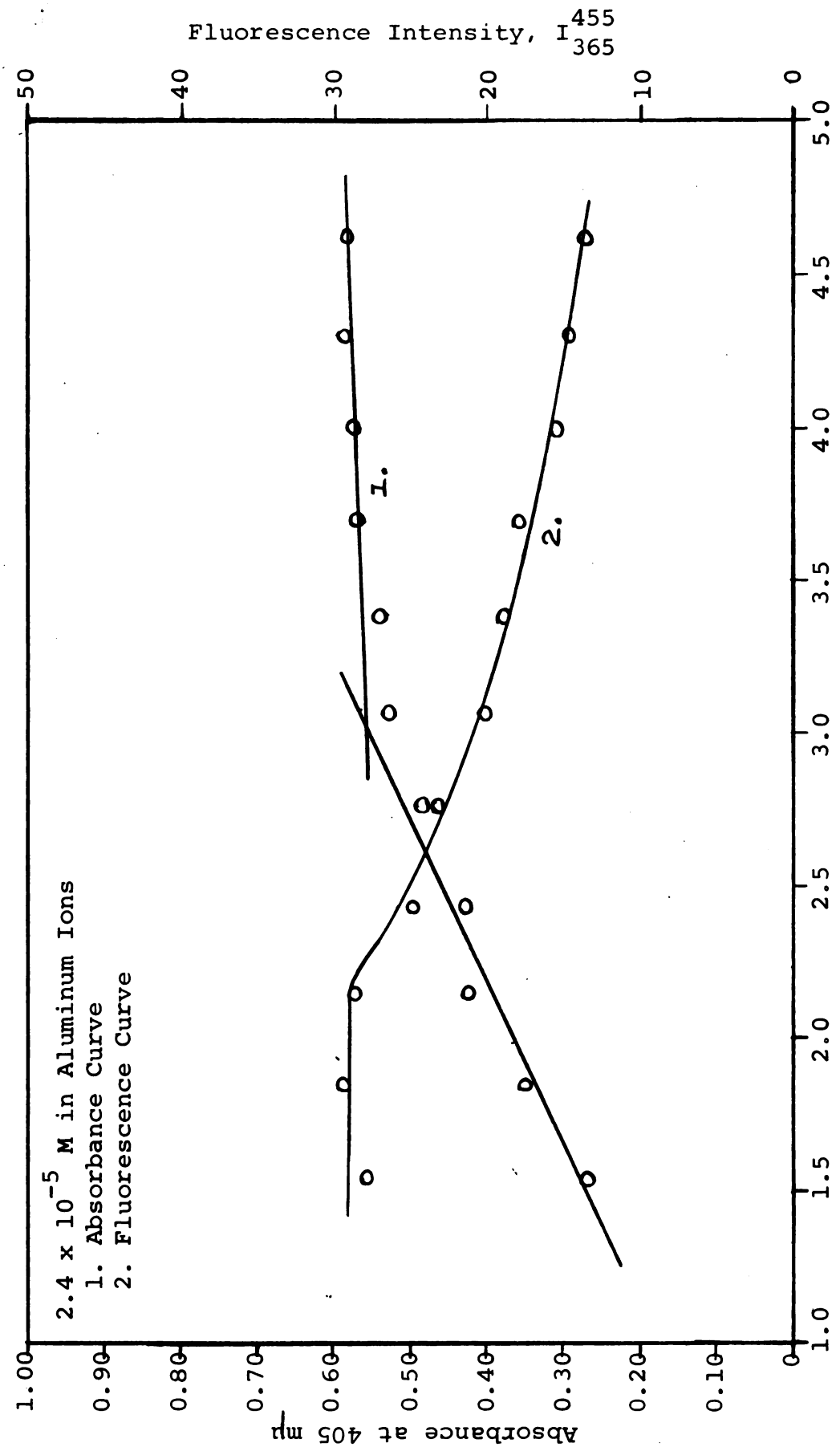

FIGURE 7. Spectrophotometric and Spectrofluorometric Titration Curves for Aluminum (III) Titrated with Flavonol Indicating the 1:3 Aluminum to Flavonol Species.

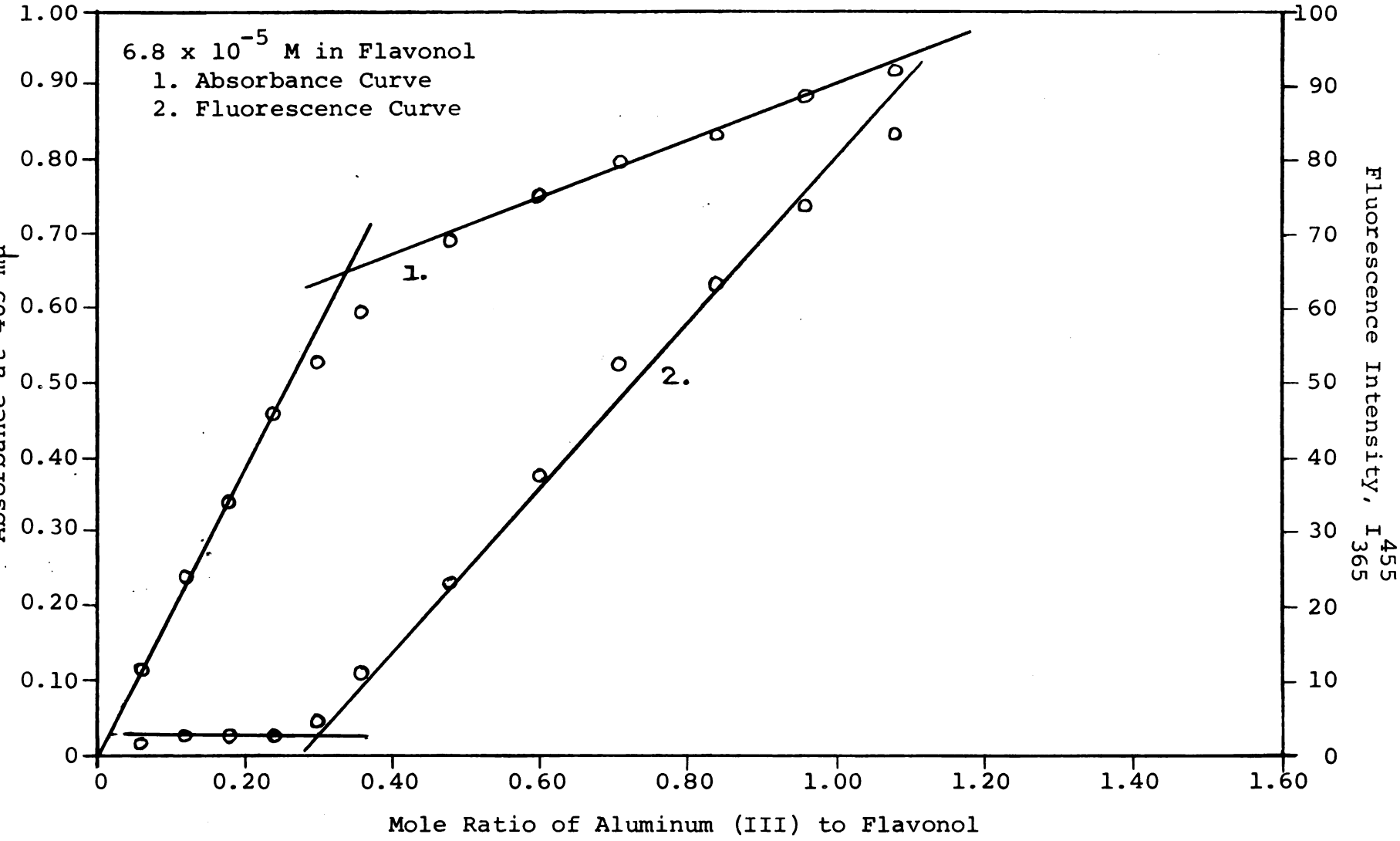

FIGURE 8. Spectrophotometric and Spectrofluorometric Titration Curves for Flavonol Titrated with Aluminum Ions Indicating 1:3 Aluminum to Flavonol Species.

From the above titrations, evidence for the formation of a strong chelate with a 1:3, aluminum to flavonol, stoichiometry is obtained. In this chelate the maximum number of available sites of the aluminum ion are coordinated. The 1:3 chelate appears to be a strong chelate since it is indicated in the spectrophotometric curves of both titrations, that of aluminum titrated with flavonol and flavonol with aluminum ions. Only the spectrofluorometric curve for the titration of flavonol with aluminum ions indicated the 1:3 chelate due to the factors explained above.

The spectrofluorometric data suggests that the 1:3 chelate is a non-fluorescent species. In the titration of aluminum with flavonol, as the mole ratio of flavonol to aluminum approaches 3:1, the fluorescence intensity decreases and continues to decrease as more of the chelate is formed. In the titration of flavonol with aluminum ions, as the mole ratio of aluminum to flavonol becomes less than 0.3, where the 1:3 chelate is formed, the fluorescence intensity rapidly decreases and becomes negligible. Some of this decrease in fluorescence may be attributed to a quenching effect by the presence of excess flavonol. But is doubtful if in this case, especially in the region of the 1:3 chelate,

whether flavonol is present in a large enough excess or if the quenching effect alone could cause such a rapid decrease in fluorescence. Also, a solution of this chelate has a yellow visible color and appears to exhibit no blue fluorescence. For the 1:3 aluminum to flavonol chelate, attempts to use 365, 405 and 436 mμ as the excitation radiation for producing fluorescence proved unsuccessful.

Absorption Spectra of the Aluminum-Flavonol Chelates

The formation of the 1:1 aluminum to flavonol chelate results in the appearance of a band at 396 mμ in the absorption spectrum (Figure 9). When the 1:3 aluminum to flavonol chelate is formed, the new absorption band appears at 405 mμ (Figure 10). In the previous spectrophotometric titrations, as the aluminum to flavonol ratios changed, the shift from one chelate absorption band to the other band was observed.

Determination of the Molar Absorptivities of the Aluminum-Flavonol Chelates

In the determination of the molar absorptivities of the aluminum-flavonol chelates, it was necessary to provide for the complete formation of the chelate. For the 1:1 chelate, solutions were prepared containing a

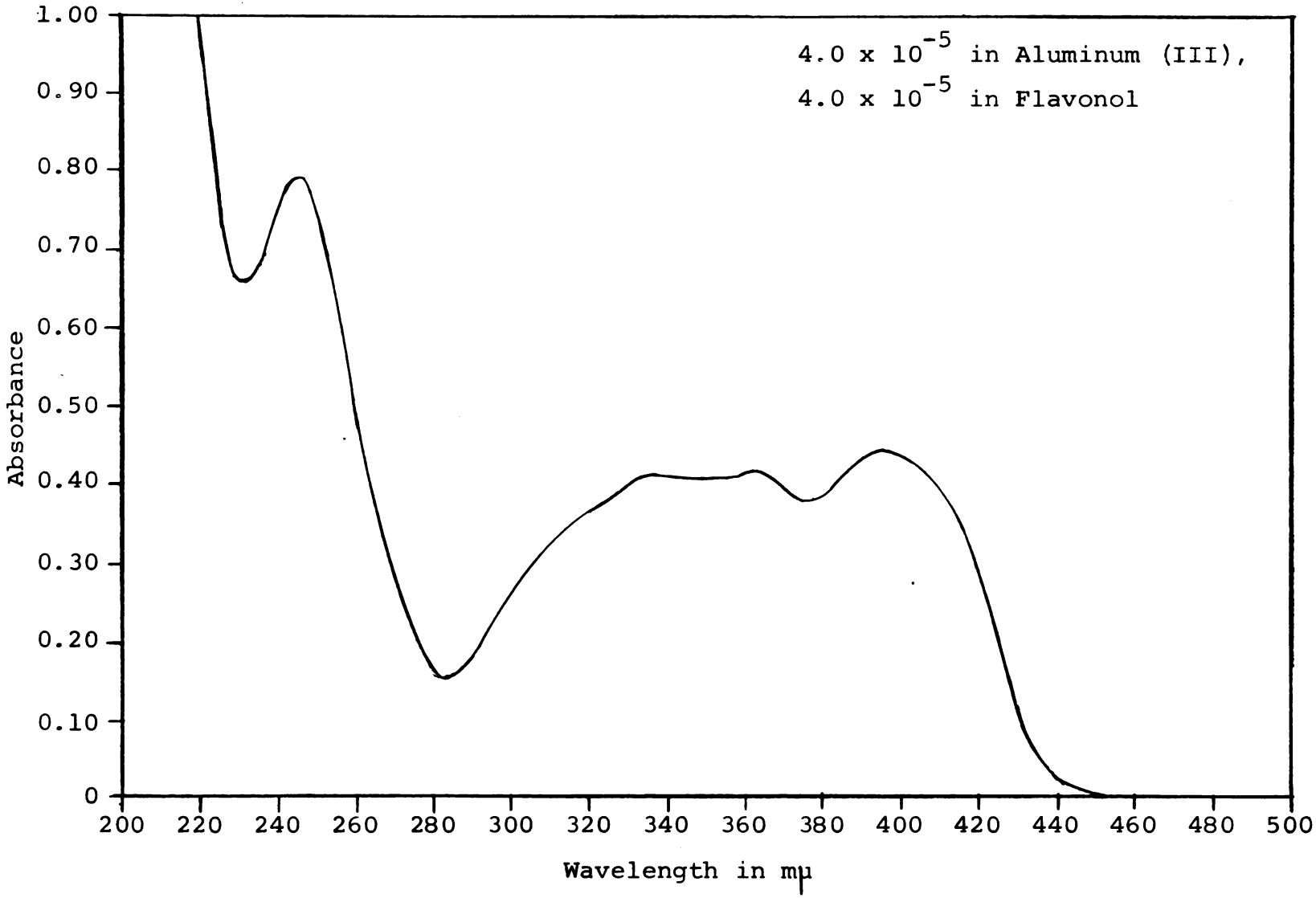

FIGURE 9. Absorption Spectrum of the 1:1 Aluminum to Flavonol Chelate in the 3:2 Water-Ethanol Solvent.

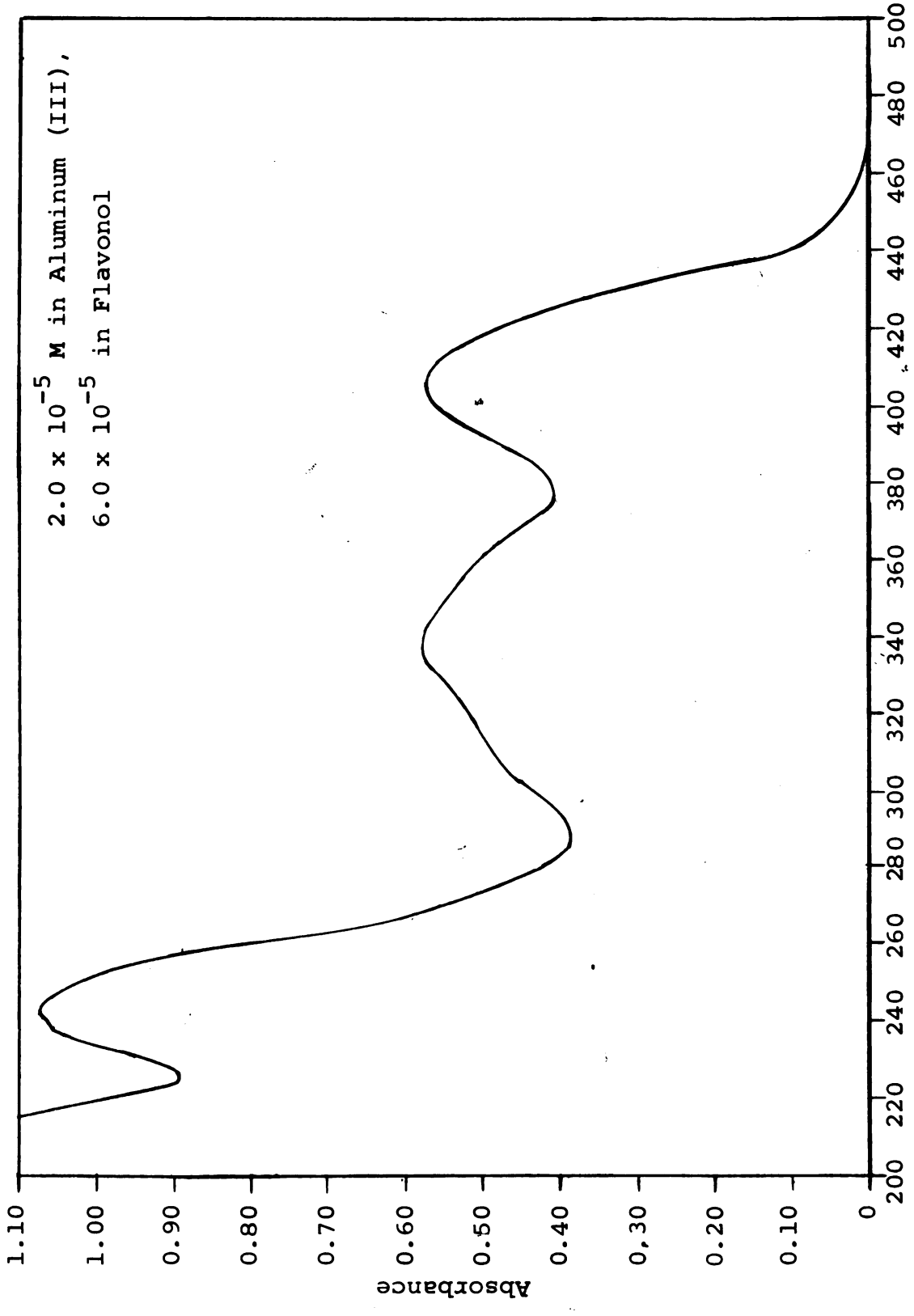

FIGURE 10. Absorption Spectrum of the 1:3 Aluminum to Flavonol Chelate in the 3:2 Water-Ethanol Solvent.

constant flavonol concentration. The aluminum ion concentration was increased so that a limiting absorbance value of the 396 mμ chelate peak was obtained at high aluminum to flavonol ratios.

A similar procedure was employed for determining the limiting absorbance value of the 1:3 aluminum to flavonol chelate. The aluminum concentration remained constant as the flavonol concentration was increased. At high flavonol to aluminum ratios the limiting absorbance value of the 405 mμ chelate was obtained. It was observed that the concentration solubility limit of flavonol in the water-ethanol solvent is approximately 24×10^{-5} M.

The molar absorptivities of the chelates were calculated from the limiting absorbance values and are tabulated below:

	Molar Absorptivity	log
1:1 Chelate	$396 = 1.82 \times 10^4$	4.260
1:3 Chelate	$405 = 4.00 \times 10^4$	4.602

Determination of the Stability of the Aluminum-Flavonol Chelates

The effect of various factors on the stability of solutions containing the aluminum to flavonol concentration ratios of 5:1, 1:1, 1:2, 1:3 and 1:5 were observed.

Changes in the chelate absorption peak were followed with time to determine the effect of light on the solutions. Two sets of the above solutions were prepared, one was protected from the light, and the other set was exposed to ordinary light, including sunlight. It was noted that an induction time of approximately 30 minutes was required for most solutions to reach maximum chelation.

For the solutions not protected from the light, the solution of the aluminum to flavonol concentration ratio of 5:1 was fairly stable for about four hours. But the other solutions, containing more flavonol, were not even stable for an hour. The decrease in the absorbance value of the chelate peak was accompanied by a decrease in the flavonol absorbance peak. This observation indicates that the flavonol in the solution was being decomposed by the light. A possible explanation is that the light exerts or causes the aluminum to exert a catalytic effect on the oxidation of flavonol.

As the flavonol decomposed, the yellow color of the 1:3 aluminum to flavonol chelate, in the solutions containing aluminum to flavonol concentration ratios of 3:1 and 5:1, rapidly faded. The solutions became clear and exhibited a blue fluorescence. Also the chelate

absorbance peak of these solutions shifted upon standing from 405 to 396 mμ, indicating that the stoichiometry of the chelate changes from 1:3 to 1:1 aluminum to flavonol.

For the solutions protected from the light, the solutions having the aluminum to flavonol concentration ratios of 5:1, 1:1, 1:2 and 1:3, chelated to a greater extent for about six hours. Then the amount of chelation decreased somewhat and leveled off, remaining fairly constant for at least two days. In all cases, a decrease in the absorbance value of the chelate peak was accompanied by an increase in the absorbance value of the flavonol peak. This fact indicates that the total amount of flavonol remains constant.

The solution of the concentration ratio 1:5, aluminum to flavonol, protected from the light, required about 20 hours to achieve maximum chelation. Then the amount of chelation leveled off and remained constant for at least three days. On standing, the chelate absorption peak of the solutions having the concentration ratios 1:3 and 1:5, aluminum to flavonol, remained at 405 mμ, indication that the 1:3 aluminum to flavonol stoichiometry of the chelate did not change. These solutions also maintained their yellow color.

The change in fluorescence intensities of two sets of solutions, prepared as described above, was followed with time. This experiment was performed to determine if the fluorescence stability followed the same trends as those indicated by the absorbance experiment. Again, one set of solutions was protected from the light, while the other was not. The same stability trends were observed, as the fluorescence intensities of the solutions protected from the light remained fairly constant on standing. But on exposure to light, the fluorescence intensities of the solutions rapidly changed.

The absorbance experiment previously indicated that on exposure to light, solutions containing the aluminum to flavonol concentration ratios of 1:3 and 1:5, show a shift in the stoichiometry of the chelate from 1:3 to 1:1, aluminum to flavonol. This shift occurs as the flavonol is decomposed by the light and consequently only sufficient flavonol becomes available for the formation of the chelate with the 1:1 stoichiometry. The fluorescence experiment further substantiates this shift in the chelate stoichiometry. The fluorescence intensities of the above solutions greatly increased on exposure to light, whereas those solutions

protected from the light were non-fluorescent. The increase in fluorescence is probably due to the shift from the non-fluorescent 1:3 chelate to the 1:1 fluorescent chelate.

To determine the effect of heating on the chelate, one set of the series of five solutions were prepared as explained above. The solutions were protected from light, heated to 42°C, and maintained at this temperature. The solutions were cooled to room temperature before the absorption values were recorded. Since the same stability was indicated by the fluorescence data and the absorption data, the effect of heating the solutions was only determined by changes in the absorbance values. It is assumed that the fluorescence stability would follow the same trends.

For the solutions of the aluminum to flavonol concentration ratios of 5:1 and 1:1, the absorbance values of the 1:1 chelate peak decreased rather rapidly with time. The decrease in the chelate absorption peak was accompanied by an increase in the flavonol absorption peak. This observation indicates that while heating the solutions decreases the stability of the chelate, it does not affect the flavonol.

For the other solutions, the absorbance values of

the 1:3 chelate peak also decreased with time, and these solutions became cloudy after approximately three hours. The appearance of cloudiness in the solutions was accompanied by an increasing formation of an absorption peak at 438 mμ, which was not observed in similar solutions at room temperature. Along with the formation of the 438 mμ peak, the 1:3 chelate absorption peak of 405 mμ shifted to 410 mμ. As this shift occurred, the absorbance value decreased to a minimum on being heated for about 23 hours. After the shift was complete, the absorbance value increased. Figure 11 shows the absorption spectrum of a 1:3 aluminum to flavonol chelate after heating, where this shift has occurred and the peak at 438 mμ has formed. Again a decrease in the absorbance value of the chelate peak was accompanied by an increase in the absorbance value of the flavonol peak.

At room temperature the appearance of an absorption peak at 438 mμ, along with the shift of the chelate peak was observed for some solutions in other experiments. These solutions also became cloudy after standing for several hours. But these solutions contained a high flavonol to aluminum concentration ratio, much higher than those used in the stability experiments.

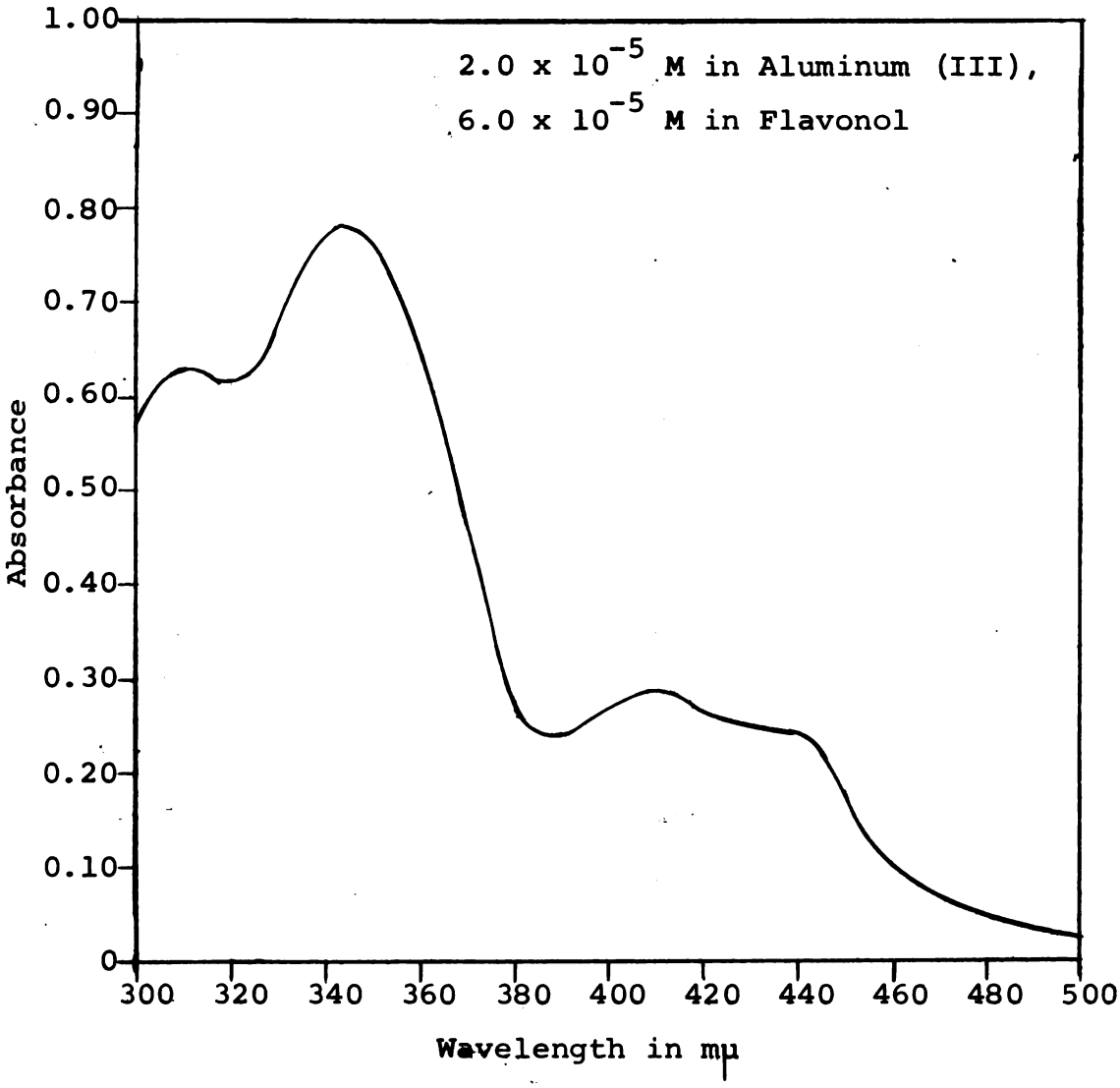

FIGURE 11. Absorption Spectrum of the 1:3 Aluminum to Flavonol Chelate on Heating.

Solutions of the aluminum to flavonol concentration ratios of 1:1 and 1:3 were exposed to 365 mμ radiation to determine if the fluorescence intensity changes during the time required to obtain a reading. It was found that the fluorescence intensity remains constant for at least an hour.

Determination of the Conditions for the Spectrofluorometric Method

In the development of a suitable spectrofluorometric method for the determination of aluminum using flavonol, several conditions should be fulfilled. These conditions are that an excess amount of the reagent should be present, the reagent should form a fluorescent species with the aluminum and the fluorescence of the species should be proportional to the concentration of aluminum present. If possible, the method should not be highly dependent on any variable, and the number of variables should be at a minimum.

From the study of the nature of the aluminum-flavonol chelates in the water-ethanol solvent, it is apparent that all these conditions cannot be met. When flavonol is present in an amount in excess to the aluminum concentration, the 1:3 aluminum to flavonol chelate forms. This chelate has been shown to be a

non-fluorescent species and therefore it is not suitable for use in a spectrofluorometric method.

But a spectrofluorometric method was developed based on the introduction of another variable, that of pH control. Figure 12 shows the dependency of the absorbance and fluorescence values on pH in solutions containing the aluminum to flavonol concentration ratio of 1:1. Before pH adjustment, the pH of the solutions was about 4.6. The fluorescence curve shows that the fluorescence intensity of this solution is extremely dependent on the pH values. The fluorescence intensity rapidly decreases in either direction from the narrow maximum range at pH 4.3 to 4.4.

As shown by the absorption curve, the absorbance values of the chelate peak are also very dependent on the pH and reach a maximum in the narrow pH range of 4.6 to 4.7. The difference in the pH range where maximum fluorescence and absorbance values are obtained for the chelate probably occurs because fluorescence can be affected by factors which do not affect absorption. After 4.7, as the pH increases, the absorption curve slowly decreases and reaches a minimum about pH 8. In the pH range of 8.5 to 9.0, the absorption peak shifted from the 1:1 chelate peak at 396 mμ to a 408 mμ peak.

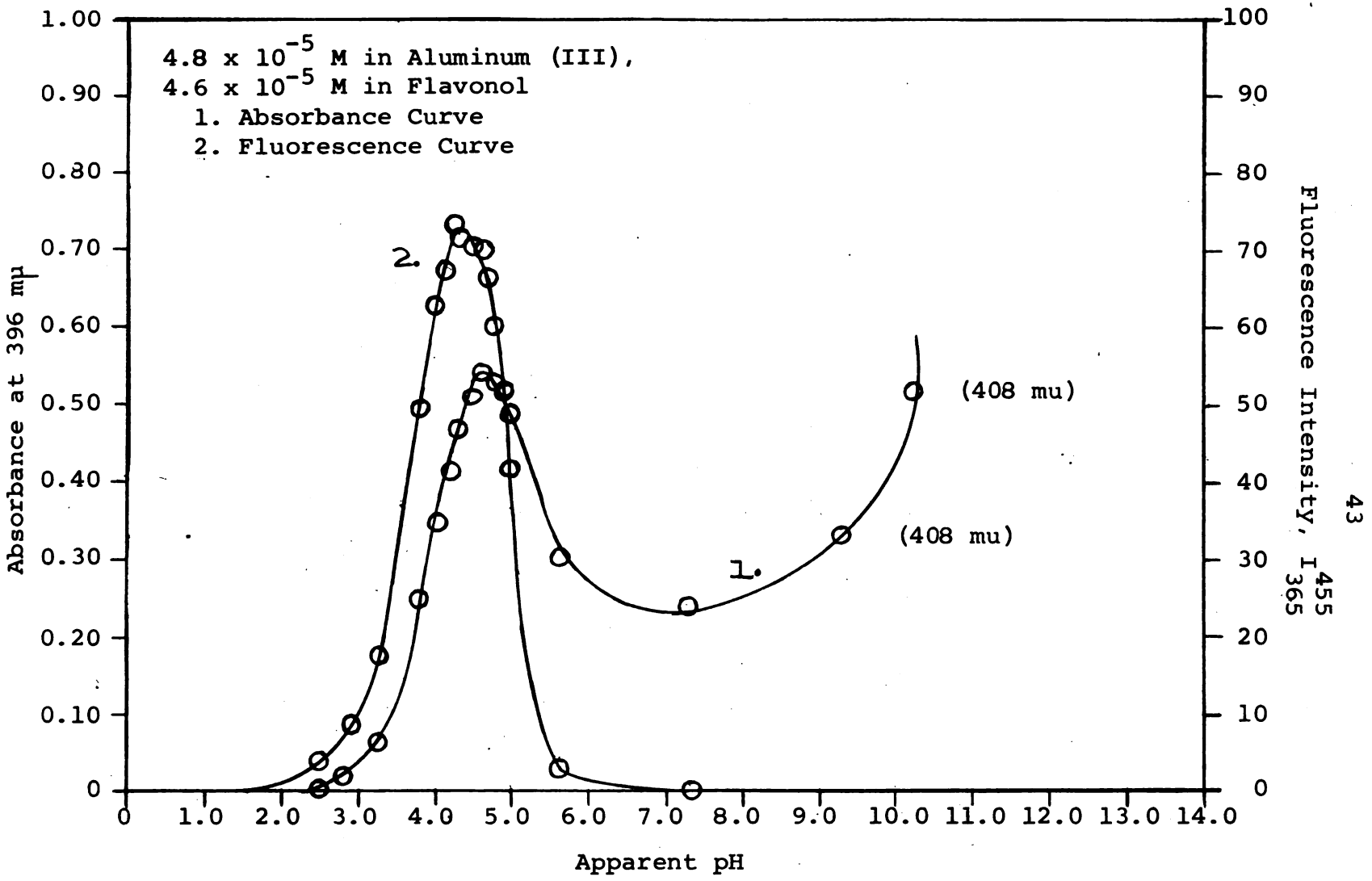

FIGURE 12. Effect of Apparent pH on the Absorbance and Fluorescence Values of the 1:1 Aluminum to Flavonol Chelate in the 3:2 Water-Ethanol Solvent.

The flavonol absorption peak at 344 mμ disappeared. Also in this pH range, the appearance of the solutions changed from having no visible color to a deep yellow color. At pH's higher than 9.0, the absorption peak remained at 408 mμ and the solutions remained yellow.

The appearance of the absorption peak at 408 mμ may be attributed to the formation of the flavonol anion. Under basic conditions, the visible absorption spectrum of flavonol shows a shift from the 344 mμ band to 408 mμ. Also the solutions had a deep yellow color, compared to having no visible color under neutral and acidic conditions.

The dependency of the chelate absorbance and fluorescence values on pH, in solution containing the aluminum to flavonol concentration ratio of 1:5, is shown in Figure 13. Again before pH adjustment, the pH of the solutions was about 4.6. From the fluorescence curve, it appears that a weakly fluorescent species forms in the pH range of 2.0 to 4.5. The fluorescence intensity reaches a maximum in the pH range 3.4 to 3.6 and rapidly decreases as the pH increases or decreases.

The absorption curve shows two breaks, a sharp one in the pH region of 3.5 to 4.5 and a gentle one at 8.5 to 10.0. Before the first break, the pH range of

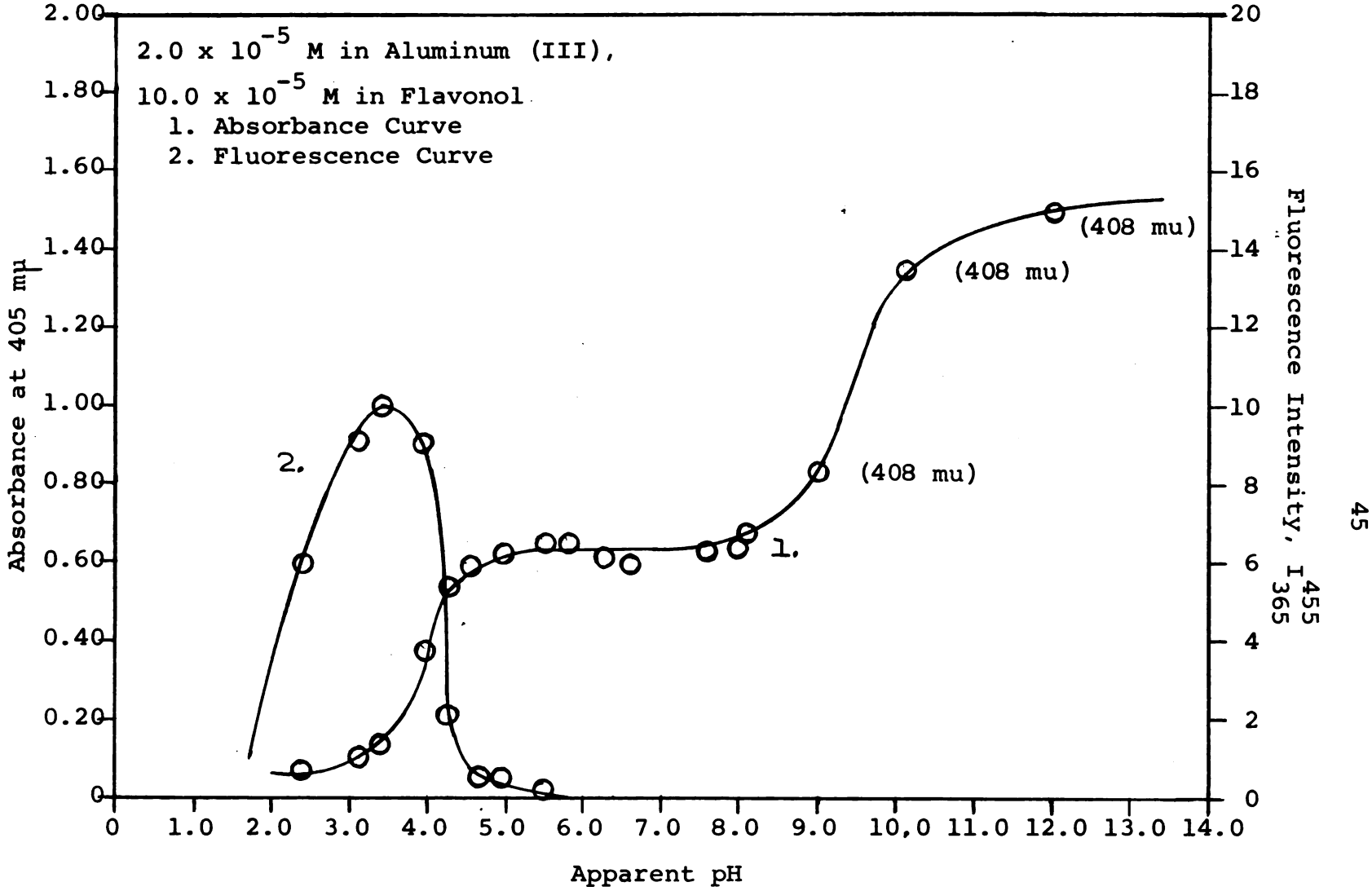

FIGURE 13. Effect of Apparent pH on the Absorbance and Fluorescence Values of the 1:3 Aluminum to Flavonol Chelate in the 3:2 Water-Ethanol Solvent.

2.0 to 4.5 coincides with the region where the fluorescence is observed. In this region, the chelate absorption peak appears as a shoulder at about 400 mμ to the flavonol peak (Figure 14). The absorbance values of the chelate peak are fairly low. The solutions of this chelate had no visible color, but exhibited a blue fluorescence.

In the pH region between the two breaks, 4.5 to 8.5, the solutions had a yellow color and were non-fluorescent. The chelate absorption peak was at 405 mμ. These observations indicate that the chelate in this pH range has the 1:3, aluminum to flavonol, stoichiometry, especially since the pH of the solutions used to determine the 1:3 stoichiometry was about 4.6.

Again the break in the pH range of 8.5 to 10.0 is attributed to the formation of the flavonol anion. The yellow color of the solutions deepened. The absorption peak shifted from the 405 mμ chelate peak to 408 mμ as the 344 mμ flavonol absorption peak disappeared.

From the above observations, a spectrofluorometric method was based on the fluorescent species formed by adjusting a solution, containing an excess flavonol concentration, to a low pH. It was noted that this fluorescent species exhibited a stronger fluorescence intensity

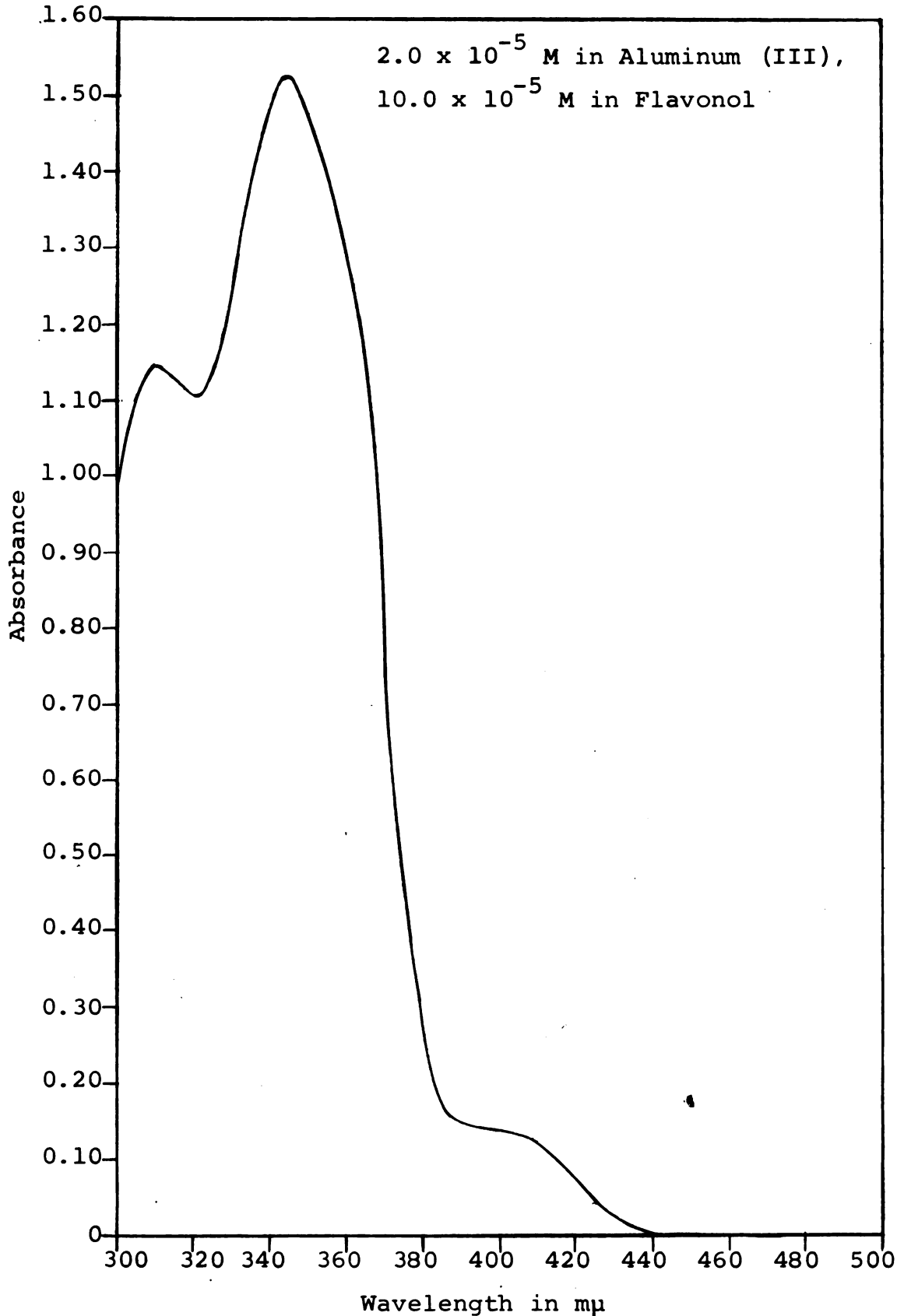

FIGURE 14. Absorption Spectrum of the Aluminum-Flavonol Chelate at the Apparent pH of 3.4.

when excited by 405 mμ radiation than that by 365 mμ radiation. This is in contrast to the chelate of the aluminum to flavonol stoichiometry of 1:1, where 365 mμ excitation radiation produced the stronger fluorescence intensity. The emission spectra of both of the fluorescent species was the same, the emission peak being at 455 mμ. Consequently in the method, 405 mμ was used as the excitation radiation. Flavonol does not fluoresce when excited by 405 mμ radiation, thus a possible interference by the presence of excess flavonol is eliminated.

In obtaining the fluorescence intensity readings, the spectrofluorometer was calibrated with the quinine sulfate standard as previously described. Then the exciting wavelength was changed to 405 mμ, the slit opening was set at 0.40, and the fluorescence intensities were measured.

The effect on the chelate absorbance and fluorescence values of varying the excess flavonol concentration, while maintaining a constant aluminum concentration and adding the same amount of 1:1 acetic acid to a series of solutions, is shown in Figure 15. The amount of flavonol added to these solutions was limited since previous experiments showed that the solubility limit in the water-ethanol solvent is approximately 24×10^{-5} M.

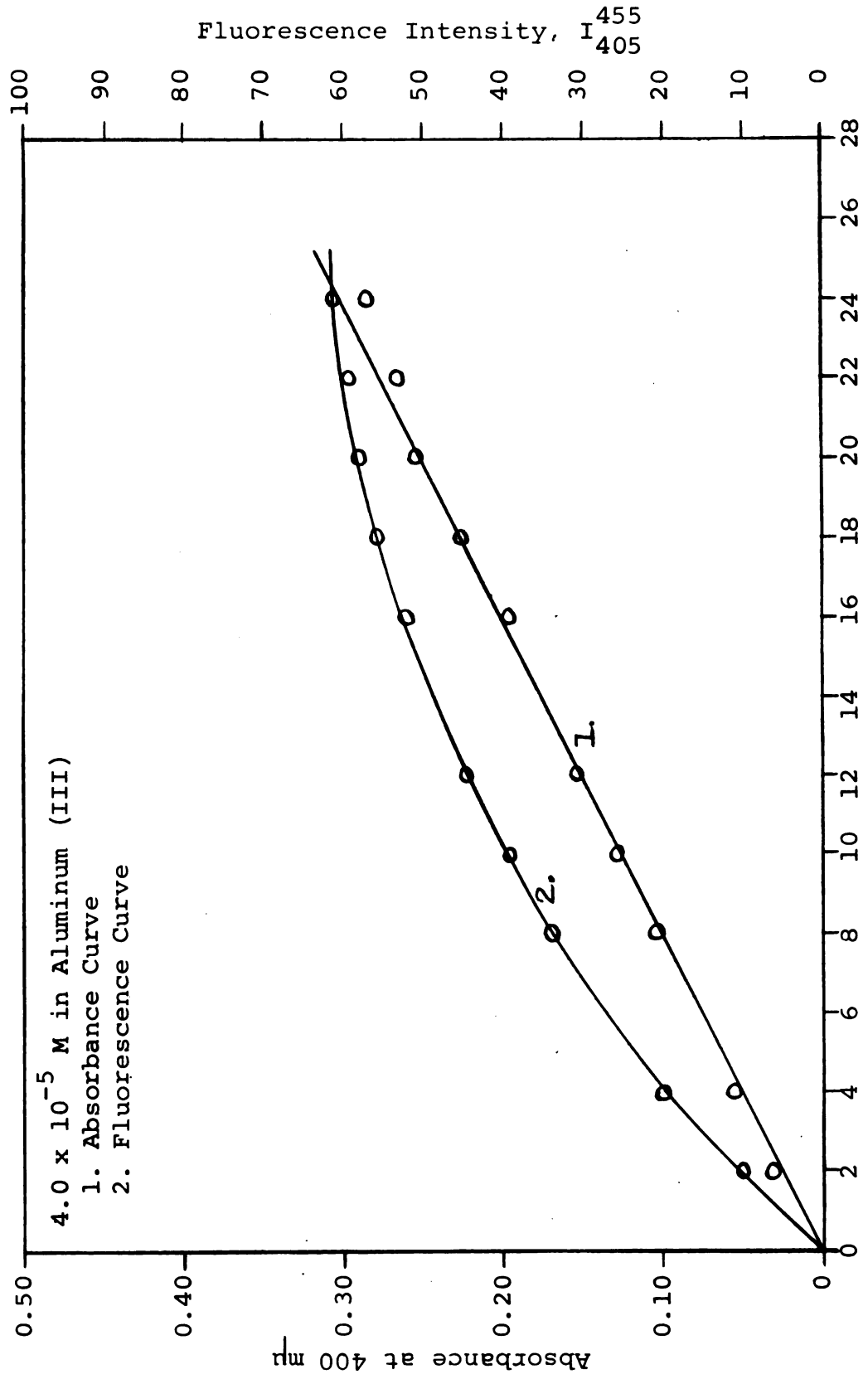

FIGURE 15. Dependence of the Absorbance and Fluorescence Values of the Aluminum-Flavonol Chelate at Apparent pH 3.45 - 3.55 on the Flavonol Concentration.

A straight line is obtained from the absorbance values, indicating that the amount of chelate formed is a linear function of the flavonol concentration. From the fluorescence data, a smooth curve showing negative deviation from linearity is obtained. The curvature of this line increases as the flavonol concentration increases due to an increase in the quenching effect by the flavonol.

From this experiment, 20×10^{-5} M flavonol was chosen as the excess concentration to be used in the method. At this flavonol concentration the desired large excess of flavonol is present, forcing a greater extent of the available aluminum ions into the chelate form, as shown by the absorbance data. Accordingly, a relatively higher fluorescence intensity is obtained due to the formation of more chelate than at lower flavonol concentrations. But in the region of 20×10^{-5} M flavonol, the fluorescence intensity does not increase as rapidly with increasing flavonol concentration as occurs in the lower flavonol concentration region. This smaller dependence of the chelate fluorescence on the flavonol concentration is desirable, since small variations in the flavonol concentration would not appreciably affect the fluorescence values.

Figure 16 shows the effect of small variations in pH on the chelate absorbance and fluorescence values for a series of solutions in which a constant aluminum concentration was maintained. The excess flavonol concentration in these solutions was 20×10^{-5} M. The absorbance values show that the amount of the fluorescent species formed is very dependent on the pH. In the pH range of 3.0 to 3.7, a straight line is obtained, but at higher pH's the line curves abruptly as the yellow, 1:3, aluminum to flavonol, chelate forms.

The fluorescence intensity of the fluorescent species also shows a high degree of dependence on the pH. The fluorescence curve reaches a maximum value at a pH of approximately 3.5. The fluorescent intensity is fairly constant only for the small pH range of about 3.45 to 3.55. After the pH of 3.6 the fluorescence intensity begins to decrease, and rapidly decreases in the region of 3.7 to 4.0. This is the pH region where the non-fluorescent, yellow, 1:3 chelate forms.

Therefore the pH of the solutions must be carefully controlled to obtain reproducible absorbance and fluorescence values. For the method, the pH should be adjusted to 3.50 ± 0.05, since this is the range in which fairly constant and maximum fluorescence values

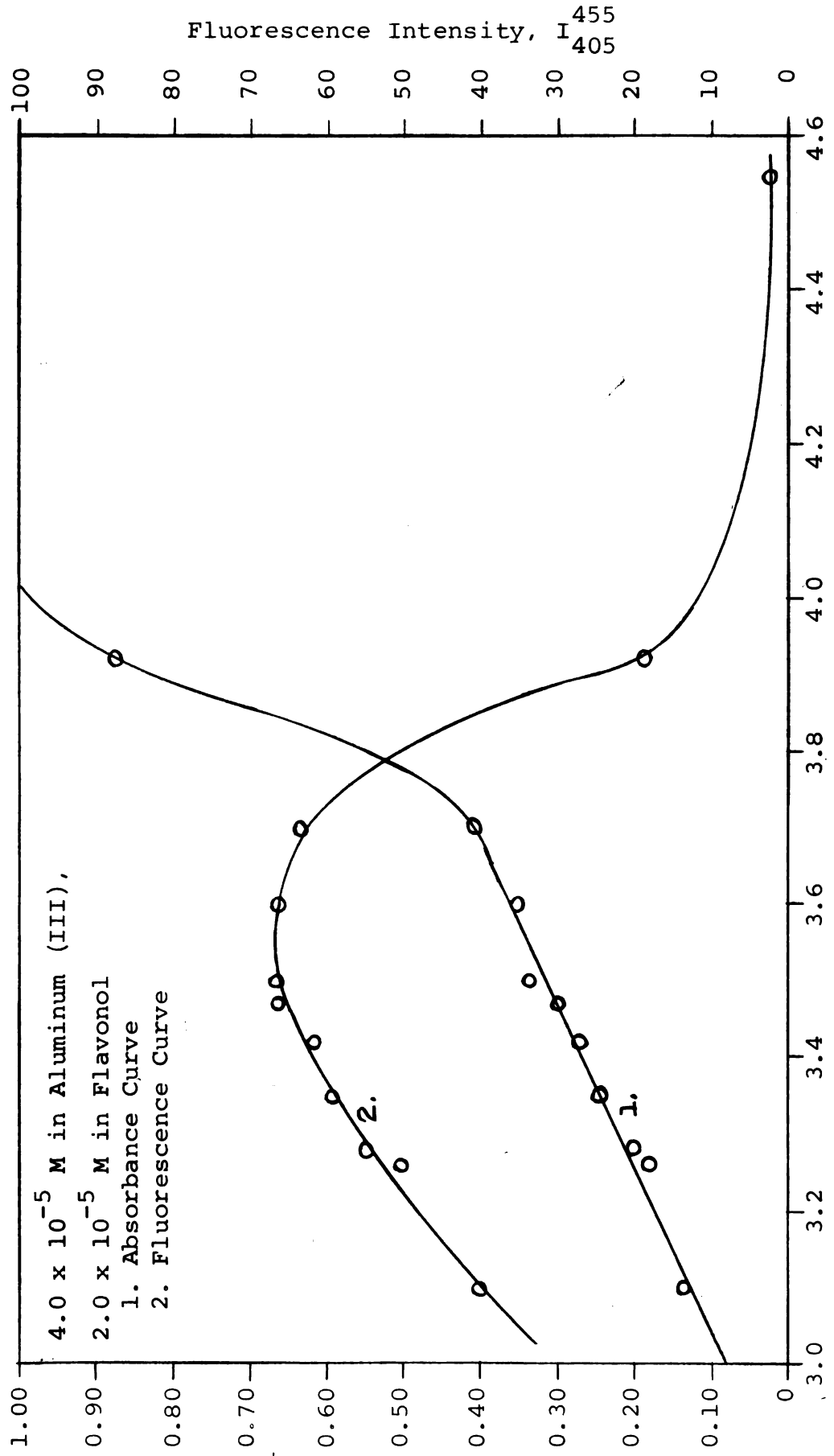

FIGURE 16. Dependence of the Absorbance and Fluorescence Values of the Aluminum-Flavonol Chelate on Small Variations in Apparent pH.

are obtained. The amount of 1:1 acetic acid required to adjust a solution of the aluminum-flavonol species to the pH of 3.50 was determined. Then a series of solutions in the aluminum concentration range of 1.0 to 10 x 10^{-5} M, approximately the aluminum range to be determined by this method, and containing the flavonol concentration of 20 x 10^{-5} M, were prepared. The previously determined amount of 1:1 acetic acid was added to each solution. Approximately the same pH reading was obtained for each of the solutions.

Consequently the following procedure was adopted for adjusting the pH of the solutions. The solutions were prepared as previously described, but before they were diluted to volume, the pre-determined amount of 1:1 acetic acid was added. A procedure of adjusting each solution to the pH of 3.50 only introduces the possibility of additional errors and is too time consuming.

The stability of the fluorescent species, obtained by pH adjustment of solutions containing 2.0 and 6.0 x 10^{-5} M aluminum and 20 x 10^{-5} M flavonol, was observed by following the changes in absorbance and fluorescence values with time. The changes in the chelate absorbance values were followed for two sets of solutions. One set of solutions was protected from the light and the

other set was not. The solutions, which were exposed to the light, were very unstable due to the decomposition of the flavonol, as previously observed in the solutions of the 1:1 and 1:3, aluminum to flavonol, chelates.

In the solutions protected from the light, the chelate absorption values steadily increased for about five hours. The amount of chelation slowly continued to increase, even after two days. But after the first five hours, the rate of chelation was so slow that the absorbance value of the chelate peak does not significantly increase within 24 hours. The changes in the fluorescence intensities of the solutions protected from the light followed the same pattern as that for the chelate absorption values. Therefore it is suggested that all the respective measurements be made at approximately the same time after preparing the solutions, allowing at least five hours for a greater extent of chelation.

Determination of the Calibration Curve

The above determined conditions were included in the procedure for preparing the solutions from which the calibration curve was obtained. According to the

order of addition to a volumetric flask, the procedure for preparing the solutions is as follows: a suitable aliquot of the aluminum stock solution was added; if necessary, a volume of water or ethanol was added to maintain the 3:2 water to ethanol solvent ratio; an aliquot of the flavonol stock solution was added such that the resulting solution had a flavonol concentration of 20×10^{-5} M; the pre-determined amount of 1:1 acetic acid was added; and the solution was diluted to volume with the solvent. The apparent pH was measured as a check on the pH control. The solutions were allowed to stand, protected from the light, for about 14 hours before the absorbance and fluorescence measurements were made.

The concentration range of aluminum to be determined by this spectrofluorometric method was chosen as 0.5 to 7.0×10^{-5} M. The lower aluminum concentration limit was set at 0.5×10^{-5} M from the chelate absorbance and fluorescence values obtained on varying the aluminum ion concentration from 0.1 to 4.0×10^{-5} M, as shown in Figure 17. Both the absorbance and fluorescence curves show that the respective values obtained for the chelate in solutions of aluminum concentration lower than 0.5×10^{-5} M, are very small. Therefore these concentrations

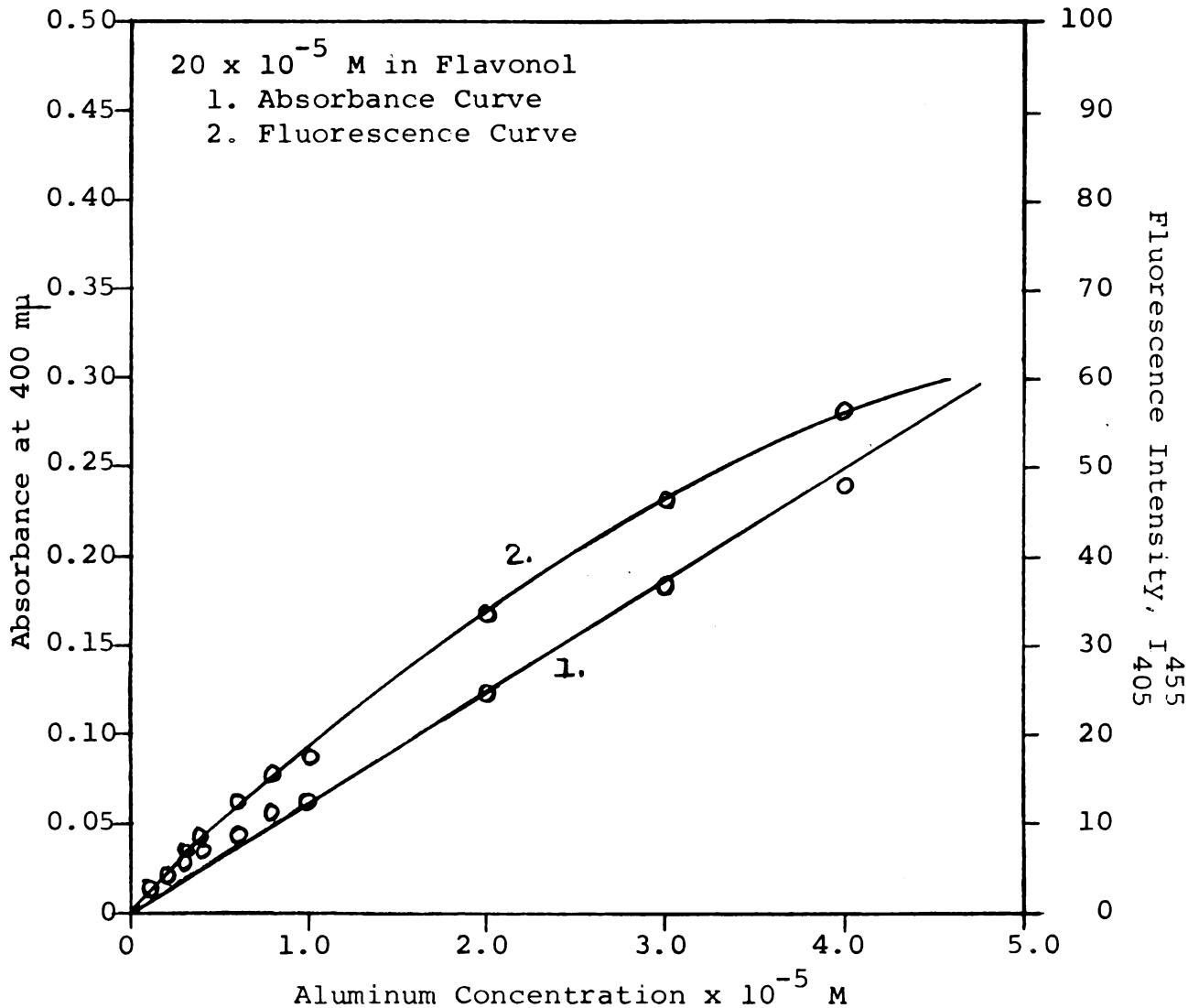

FIGURE 17. Dependence of the Absorbance and Fluorescence Values of the Aluminum-Flavonol Chelate at Apparent pH 3.45 - 3.55 on the Aluminum (III) Concentration.

could not be determined with sufficient accuracy to be included in the concentration range of this method.

The higher aluminum concentration limit was set at 7.0×10^{-5} M from the absorbance and fluorescence curves (Figure 18) of varying the aluminum concentration from 0.8 to 10.4×10^{-5} M. A straight line is obtained for the chelate absorbance values. For the fluorescence data, with increasing aluminum concentration, a slightly curved line of negative deviation from linearity is obtained to about 7.0×10^{-5} M, after which the curvature of this line increases. While the slightly curved line to 7.0×10^{-5} M in aluminum ions would be suitable for use as a fluorescence calibration curve, the greater curvature of the line at higher concentrations would not be suitable.

Figure 19 shows the spectrophotometric and spectrofluorometric curves obtained for a series of solutions prepared according to the procedure given above in the aluminum concentration range of 0.5 to 7.0×10^{-5} M. With increasing aluminum concentration, a straight line is obtained from the chelate absorbance values. The fluorometric values result in a line which is linear to about 3.0×10^{-5} M, after which the line curves slightly with a negative deviation. The calibration curves were

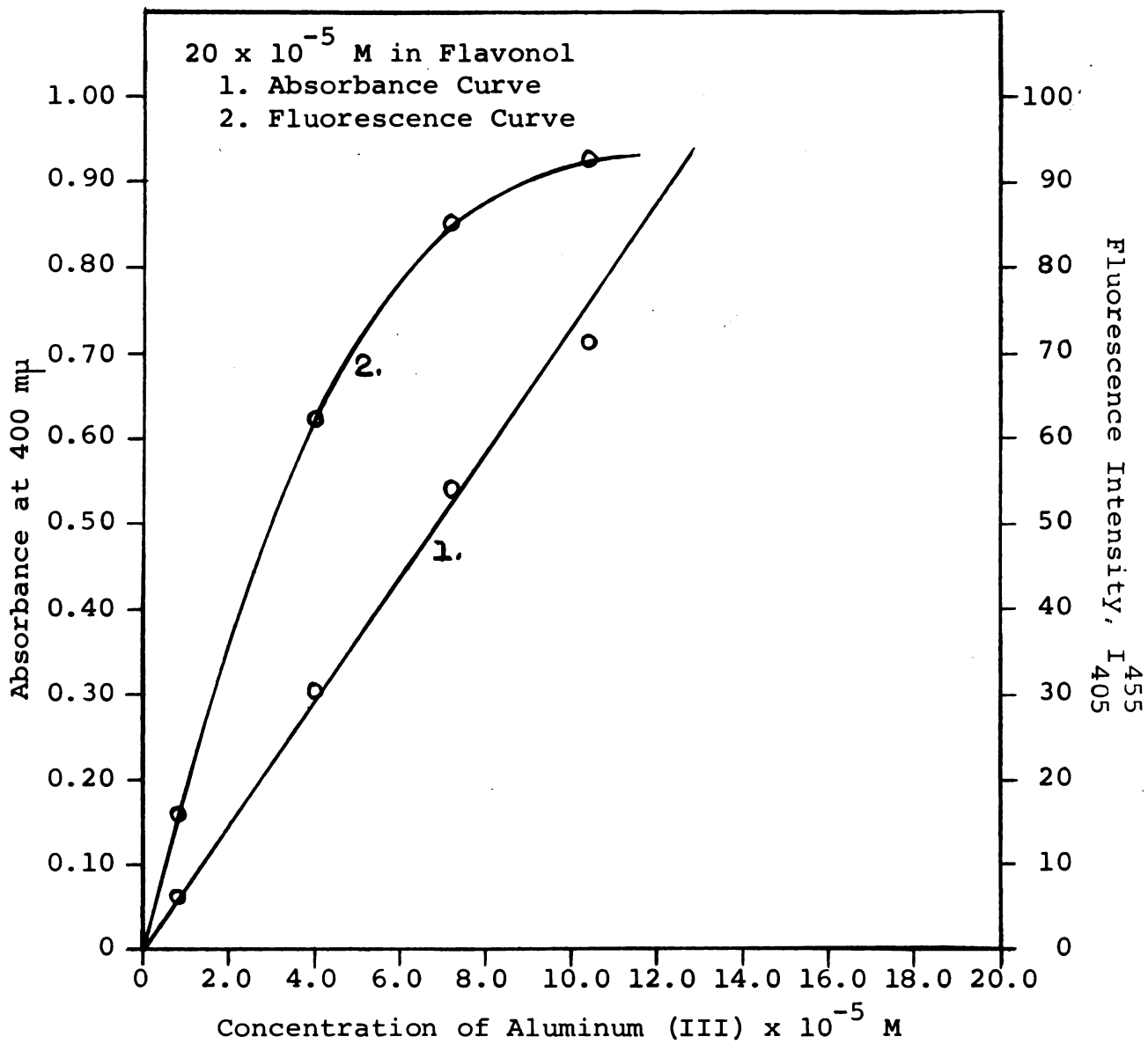

FIGURE 18. Dependence of the Absorbance and Fluorescence Values of the Aluminum-Flavonol Chelate at Apparent pH 3.45 - 3.55 on the Aluminum (III) Concentration.

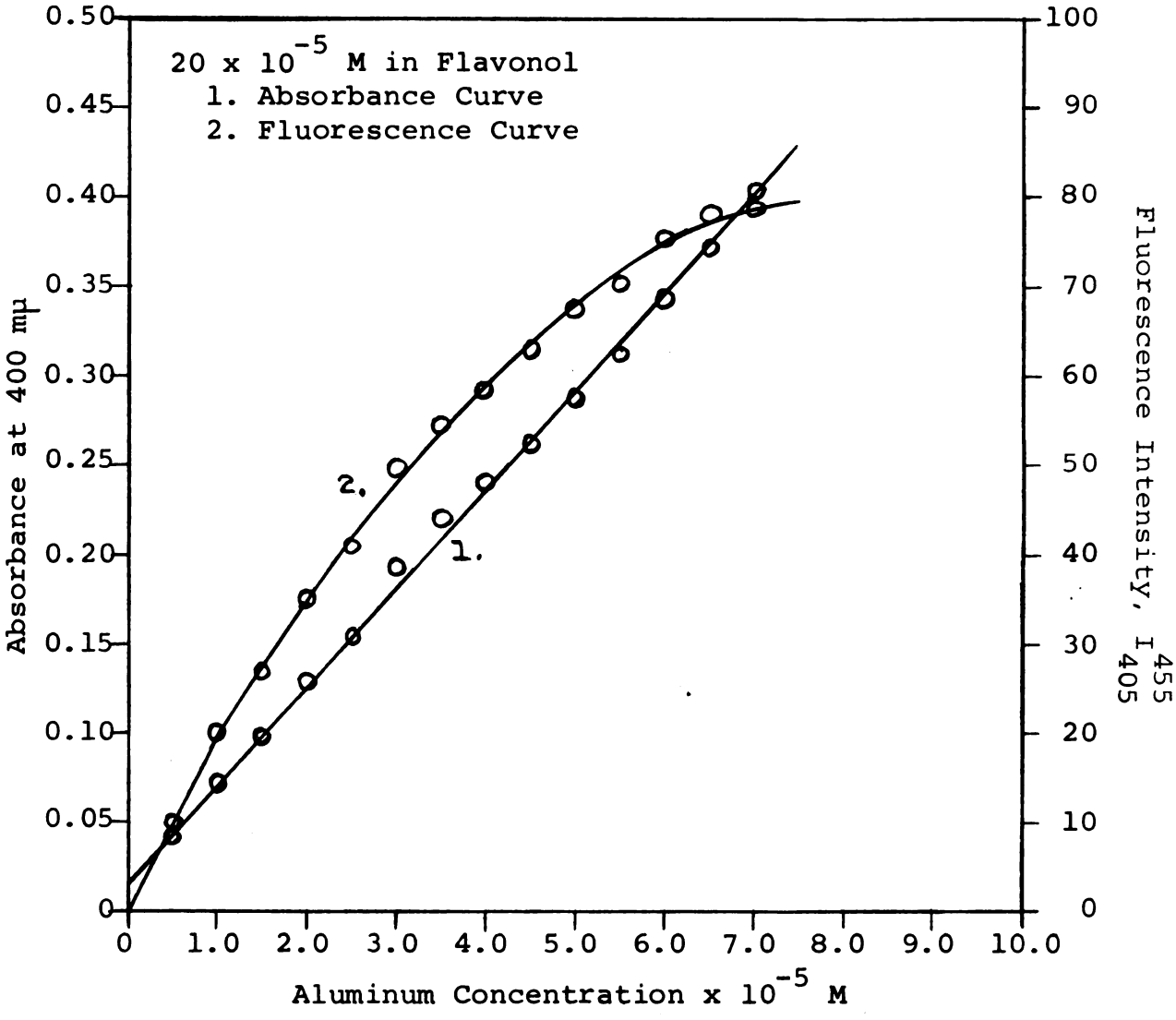

FIGURE 19. Spectrophotometric and Spectrofluorometric Calibration Curves for the Aluminum-Flavonol Chelate at Apparent pH 3.45 to 3.55.

found to be reproducible.

Although a straight line was obtained from the absorbance values, indicating that the amount of the chelate formed is a linear function of the aluminum concentration, the fluorescence values resulted in a curved line. This discrepancy in the lines is probably due to the differences in the factors which affect absorbance and fluorescence. The measured chelate absorbance values are directly proportional to the amount of chelate present. The emission of fluorescence depends not only on the amount of chelate present, but it is also affected by self-absorption of the emission radiation and by quenching effects of the solvent and other species in the solution.

Determination of Interferences

In the procedure for determining whether a particular substance is an interference, an amount of the substance equivalent to approximately 4×10^{-4} M, was added to the volumetric flask. Then the solution was prepared to contain the aluminum concentration of 4.0×10^{-5} M as previously described. The absorbance and fluorescence values were measured to determine if the substance is an interference under the conditions of the

spectrofluorometric method.

Of the substances examined, it was determined that the following ions are interferences: beryllium, copper, chromium, dysprosium, iron, lanthanum, lead, nickel, samarium, thorium, ytterbium, zinc, zirconium, bromate, carbonate, citrate, fluoride, molybdate, permanganate, phosphate, and tartarate.

The following ions are not interferences: ammonium, calcium, cobalt, silver, bromide, chloride, and iodide.

The ions which were determined to be interferences for this method closely parallel those for the fluorometric method for the determination of aluminum using morin. Therefore it is anticipated that other substances which were not examined here, but shown to be interferences in the morin method (18), would also interfere in this method.

Determination of the Relative Mean Deviation

To determine the standard deviation of this spectrofluorometric method, twenty times each, solutions of four aluminum concentrations, 0.48, 2.00, 5.00 and 7.00 x 10^{-5} M were prepared and the fluorescence intensities were measured.

The results are summarized below:

Aluminum (III) Ion Concentration, Molarity	Number of Trials	Mean Deviation x 10^5	% Relative Mean Deviation
0.48×10^{-5}	20	0.02	4.2
2.00×10^{-5}	20	0.05	2.5
5.00×10^{-5}	20	0.20	4.0
7.00×10^{-5}	20	0.25	3.6
			Mean = 3.8

From the table it is observed that this spectrofluorometric method determines the aluminum concentration of 0.5 to 7.0×10^{-5} M with a relative mean deviation of 3.8 parts per hundred.

SUMMARY AND CONCLUSION

A 3:2 water to ethanol volume ratio was chosen for the solvent from a spectrofluorometric study in which the water to ethanol ratio was varied in a series of solutions containing approximately equal concentrations of aluminum and flavonol.

In the water-ethanol solvent, the electronic absorption bands of flavonol are located at 201, 238-243, 310 and 344 mμ. The fluorescence spectrum consists of two weak bands, one at 410 mμ and the other at 525 mμ. These spectra are similar to those obtained for flavonol in absolute ethanol, with the slight shifts observed in the spectra being attributed to the differences in the solvents.

From spectrofluorometric and spectrophotometric titrations, in which the pH was not controlled, evidence was obtained for chelates of the aluminum to flavonol stoichiometry of 1:1 and 1:3. The 1:1 aluminum to flavonol chelate appears to be a weak chelate which is stable only under conditions forcing its formation. A solution of the 1:1 chelate has no visible color, but exhibits a blue fluorescence with the emission peak at 455 mμ. The absorption peak at 396 mμ, which appears on chelation, was assigned to the 1:1 chelate, and has a molar absorptivity of 1.82×10^4.

The 1:3 aluminum to flavonol chelate appears to be a strong chelate in which the maximum number of available sites of the aluminum ion are coordinated. A solution of the 1:3 chelate has a yellow color, but is non-fluorescent. The 405 mμ absorption peak, which appears on chelation, was assigned to the 1:3 chelate and has a molar absorptivity of 4.00×10^4.

The effect of various factors on the stability of the aluminum-flavonol chelates was studied. It was found that the chelates are unstable on exposure to light due to the decomposition of flavonol. The light may have a catalytic effect or may cause the aluminum to have a catalytic effect on the oxidation of flavonol.

For solutions of the chelates which were protected from the light, the chelates appear to form in a reversible reaction. About six hours were required to obtain a constant amount of chelation, which remains constant for at least two days. But for solutions which contain a large excess of flavonol, much more time, about 20 hours, is required to obtain maximum chelation. Once a constant amount of chelation is obtained, it remains constant for at least three days.

Chelates in solutions protected from light are not stable on heating, but the total amount of flavonol

appears to be unaffected. The solutions of the 1:3 aluminum to flavonol chelate become cloudy on standing for about three hours. The visible absorption spectrum exhibits the following simultaneous changes on heating, a shift of the 405 mμ chelate peak to 410 mμ and a peak appears at 438 mμ which forms a double peak with the one at 410 mμ.

It was determined that on exposure for one hour to 365 mμ radiation, the fluorescence intensities of the chelates remained unchanged. Therefore in the time required to measure the fluorescence intensity of a solution, the 365 mμ excitation radiation will cause no change in the relative intensity value.

The aluminum-flavonol chelates which form in the water-ethanol solvent, without pH control, are not suitable for use in a spectrofluorometric method for the determination of aluminum. But a method was developed based on pH adjustment of solutions containing an excess of flavonol. It was shown that a solution of excess flavonol, on increasing acidity, changes from the yellow, non-fluorescent, 1:3, aluminum to flavonol, chelate to a species which has no visible color, but exhibits a blue fluorescence. Maximum fluorescence intensity of this species is obtained in the pH range

of 3.45 to 3.55. This species exhibits a stronger relative intensity when excited by radiation of 405 mμ than by 365 mμ, whereas the relative intensity of the 1:1 aluminum to flavonol chelate is stronger when excited by radiation of 365 mμ than by 405 mμ. In the absorption spectrum, the chelate peak for the pH adjusted species appears as a shoulder at about 400 mμ of the flavonol peak.

The excess concentration of flavonol to be used in this method was chosen as 20×10^{-5} M. A factor involved in choosing this concentration was that the concentration solubility limit of flavonol in the water-ethanol solvent is about 24×10^{-5} M. As opposed to a lower concentration of flavonol, this concentration forces a greater extent of the available aluminum ions into the species form and therefore a comparatively higher fluorescence intensity is obtained. In the concentration range of 20×10^{-5} M, the dependence of the fluorescence intensity on the flavonol concentration is smaller than at lower concentrations.

In the stability study of the pH adjusted species, solutions exposed to light were unstable, again due to the decomposition of flavonol by the light. For solutions protected from light, the amount of chelation

increased fairly rapidly for about five hours, after which the rate of chelation was very slow. But the amount of chelation slowly increased, even after standing for two days, although the change within 24 hours is not very significant. Therefore for reproducible results, all solutions should stand for approximately the same length of time before measuring the fluorescence intensities.

The concentration range of aluminum to be determined by this spectrofluorometric method was chosen as 0.5 to 7.0 x 10^{-5} M. The lower concentration limit was selected as 0.5 x 10^{-5} M since the spectrofluorometric and spectrophotometric data indicated that at lower concentrations only a small amount of the species was formed and could not be accurately determined. The higher concentration limit was chosen as 7.0 x 10^{-5} M when the fluorescence curve on increasing aluminum concentration exhibited a slight negative deviation from linearity to about 7.0 x 10^{-5} M, but after this concentration the amount of deviation increased.

A calibration curve was obtained from solutions prepared according to the following conditions: in the aluminum concentration range of 0.5 to 7.0 x 10^{-5} M, with the water-ethanol volume ratio maintained at 3:2,

with the flavonol concentration of 20×10^{-5} M, and the pH adjusted to 3.45 to 3.55. The solutions were protected from the light and were allowed to stand at least five hours. The fluorescence intensity as excited by 405 mμ radiation was measured within 24 hours. The spectrofluorometric calibration curve was shown to be reproducible. Upon calculation of the relative mean deviation, this method determines aluminum in the concentration range of 0.5 to 7.0×10^{-5} M with a relative mean deviation of 3.8 parts per hundred.

This study shows that a method using flavonol as the reagent in a spectrofluorometric determination of aluminum is feasible. The flavonol method appears to have no advantage over the morin method and suffers from all of the disadvantages of the morin method. The fluorescence of the species used in this method, in addition to being subject to many interferences, is highly dependent on several variables. These variables are the water to ethanol ratio of the solvent, the concentration of flavonol in the solution, the apparent pH of the solution, and the inconvenience of allowing the solution to stand at least five hours before the fluorescence intensities are measured.

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