Coumarins as Fluorescent Indicators of Metal Ions

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Abstract

Methyleneiminodiacetic acid derivatives of 7-hydroxy-5-methyl-3-carbethoxycoumarin and 7-hydroxy-5-methyl-3-phenyl coumarin were synthesized and isolated in pure form. Variations in fluorescence and absorbance as a function of pH and the effects of selected metal ions on the fluorescence of the compounds were studied. These highly fluorescent materials find use as fluorescent indicators in EDTA titrations of calcium in the presence of magnesium.

Introduction

Many methods employed to locate the end-point in titrimetric determinations of water hardness when EDTA is used as the titrant are not entirely satisfactory. Included are the appearance of permanent suds caused by the presence of soap and the disappearance of calcium oxalate turbidity. When Eriochrome Black T was introduced by Biedermann and Schwarzenbach (3) it was adopted immediately for determining the endpoint in EDTA titrations of calcium plus magnesium. At pH 10 the magnesium-indicator compound is red. It is converted to blue, the color of the free indicator, at the end-point.

Determinations of calcium alone in mixtures of calcium and magnesium must be performed at pH 13 or higher where magnesium exists as non-dissociated magnesium hydroxide. Indicators used to mark this end-point were introduced by Schwarzenbach (1) and consist of an acid-base colorimetric indicator to which half of an EDTA molecule, a methyleneiminodiacetic acid group, has been added. Upon combination with metal ions the indicators undergo a color change. Fluorescent indicators of metal ions are obtained if half of an EDTA molecule is added to a fluorescent acid-base indicator. Metallofluorescent indicators are extremely sensitive to the presence of very small quantities of metal ions. Examples of this type of indicator include Calcein, introduced by Diehl and Ellinboe (4), and Calcein Blue, introduced by Wilkins (7). Unfortunately, these indicators experience ring opening in alkaline solution and loss of indicator function results.

This research was undertaken with the aim of developing indicators more highly fluorescent and more resistant to ring opening than those presently used.

Experimental Work

Apparatus and Reagents

Measurements of pH were made with a Corning Model 10 pH meter standardized against standard buffers. Fluorescence spectra were obtained using an Aminco-Keirs Spectro-phosphorimeter that had been converted to a spectrofluorometer. Absorption spectra were obtained on a Cary Model 15 recording spectrophotometer. Additions of small volumes of reagents were made with a Micro Metric Model SB 2

microburet and a Model S5Y syringe. 7-Hydroxy-5-methyl-3-phenylcoumarin was prepared according to the method of Balaiah *et al.* (2). Melting point: 235.5-237.5°C, reported 233°C. 7-Hydroxy-5-methyl-3-carbethoxycoumarin was prepared according to the method of Rao and Seshardi (5). Melting point: 199-202°C, reported 193-194°C.

Synthesis of Methyleneiminodiacetic Acid Derivatives of 7-Hydroxy-5-methyl-3-Carbethoxycoumarin and of 7-Hydroxy-5-Methyl-3-Phenylcoumarin

To 370 ml of glacial acetic acid was added 0.03 mole of the coumarin of choice, 0.045 mole of disodium iminodiacetate monohydrate and 0.045 mole of 37% formaldehyde solution. The reaction was allowed to proceed at 70°C for 18 hours with constant stirring. The reaction mixture was then allowed to cool and the yellow crystalline material was filtered and washed with deionized water. The material was recrystallized by dissolving it in the minimum amount of KOH and filtered to remove insoluble impurities. The pH of the filtrate was adjusted to 4 by dropwise addition of dilute HCl. The precipitate was filtered, washed with deionized water and acetone and recrystallized two more times in the same manner. The derivative of the carbethoxy compound decomposes at 248°C. Analysis (Spang Microanalytical Laboratory): found C 53.73, H 4.77, N 3.29; $C_{18}H_{19}NO_9 \cdot 1/2H_2O$ requires C 53.73, H 5.01, N 3.48. The derivative of the phenyl compound decomposes at 243°C. Analysis (Spang Microanalytical Laboratory): C 62.09, H 4.73, N 3.34; $C_{21}H_{19}NO_7 \cdot 1/2H_2O$ requires C 62.12, H 4.96, N 3.45.

Potentiometric Titration

Neutralization equivalents were obtained by adding known amounts of the desired indicator to 75 ml of 0.1 M KNO_3 and titrating with NaOH.

Fluorescence Study

Excitation and emission spectra of the parent and indicator molecules were obtained at one half pH unit intervals ranging from pH 1.5 to 13.0. Solutions on which the spectra were obtained were prepared by mixing 0.25 ml of 0.01 M EDTA to sequester any metal ions present, 5 ml of buffer solution, the appropriate volume of 1.55×10^{-3} M stock solution of fluorescent material, and diluting to 25 ml with 0.1 M KNO_3.

Effect of Magnesium, Calcium, Strontium and Barium of Fluorescence

The effect of magnesium, calcium, strontium, and barium on the fluorescence of the indicator molecules in 0.8 M KOH was studied by measuring relative fluorescence of solutions prepared by mixing amounts of indicator that were used in the preceeding work, adding 15 ul of 1.47×10^{-2} M EDTA, 125 ul of the appropriate 3.11×10^{-3} M metal nitrate stock solution, and diluting to 25 ml with 0.8 M KOH.

Absorbance Study

The absorbance spectra of the compounds were obtained at one half pH unit intervals ranging from 1.5 to 13. Solutions on which spectra were run were prepared by mixing appropriate volumes of

3.11 x 10^{-3} M indicator stock solution, 5 ml of buffer solution, and diluting to 25 ml with 0.1 M KNO_3. The pH of each of the solutions was checked after the spectra were run.

Results and Discussion

In the potentiometric titration of the products obtained from the Mannich condensation of iminodiacetic acid and formaldehyde with 7-hydroxy-5-methyl-3-carbethoxycoumarin and 7-hydroxy-5-methyl-3-phenylcoumarin one end-point was observed. This break corresponds to the neutralization of the second replaceable proton, an hydroxyl proton. The molecular weights of the compounds calculated from the volume of alkali required to reach the end-point are 403.2 and 405.0, respectively. These experimental values agree well with theoretical values for one coumarin molecule containing one methyleneiminodiacetic acid group and one half molecule of water: 402.3 and 406.4, respectively.

The fluorescence excitation spectra of the parent coumarin molecules and of the methyleneiminodiacetic acid derivatives show one band, the wavelength of maximum excitation shifting from shorter wavelength in acid solution to longer wavelength in alkaline solution. The emission spectra of all of the compounds exhibit one band. In the case of the compounds containing the carbethoxy group this band does not shift with changes in pH. A shift in the wavelength of fluorescence emission is noted in the case of the phenyl derivatives. The wavelengths of maximum fluorescence excitation in acid and alkaline solution and the wavelengths of maximum fluorescence emission in acid and alkaline solution are listed in Table 1.

TABLE 1. *Wavelengths of maximum absorbance and of maximum fluorescence excitation and emission in acid and alkaline solution for 7-hydroxy-5-methyl-3-carbethoxycoumarin·H_2O, 7-hydroxy-5-methyl-3-phenylcoumarin·H_2O, 7-hydroxy-5-methyl-3-carbethoxycoumarinmethyleneiminodiacetic acid ·1/2H_2O, and 7-hydroxy-5-methyl-3-phenylcoumarinmethyleneiminodiacetic acid·1/2H_2O.*

	Maxima			
	Fluorescense Excitation (Absorbance)		Fluorescence Emission	
Compound	Acid Solution nm	Alkaline Solution nm	Acid Solution nm	Alkaline Solution nm
7-Hydroxy-5-methyl-3-carbethoxycoumarin·H_2O	360 (356)	402 (407)	449	449
7-Hydroxy-5-methyl-3-phenylcoumarin·H_2O	349	392 (265,387)	469	479
7-Hydroxy-5-methyl-3-carbethoxycoumarinmethyleneiminodiacetic acid·1/2H_2O	360 (355)	409 (404)	447	447
7-Hydroxy-5-methyl-3-phenylcoumarinmethyleneiminodiactic acid·1/2H_2O	348 (255,344)	392 (267,387)	472	475

The intensity of the emitted light varies with pH, the intensity of fluorescence at the emission maxima and at the excitation maxima in acid and alkaline solutions was measured (Figs. 1 thru 4). A plateau is observed for all of the compounds at acidic pH values extending over a range of approximately three pH units. The parent molecules of the compounds under study exhibit another plateau in alkaline solutions. In the case of the methyleneiminodiacetic acid derivatives a maximum is observed. The shift in the fluorescence excitation wavelength for all of the compounds is attributed to the ionization of the phenolic proton. The maxima are attributed to the neutralization of the ammonium ion which is accompanied by a decrease in fluorescence.

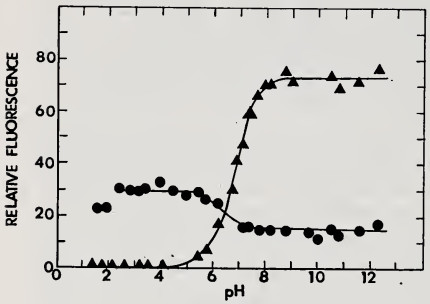

FIGURE 1

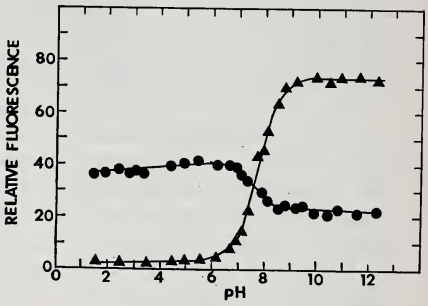

FIGURE 2

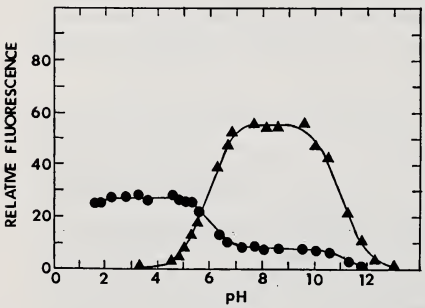

FIGURE 3

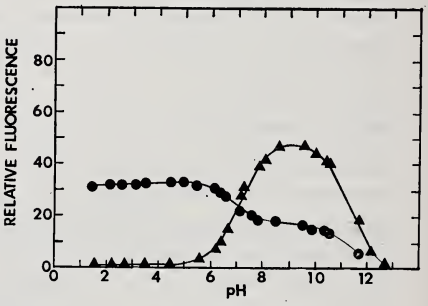

FIGURE 4

FIGURE 1. *Variation in intensity of fluorescence of 7-hydroxy-5-methyl-3-carbethoxycoumarin $\cdot H_2O$ with pH.*
—●—●— *Excitation monochromator set at 360 nm*
—▲—▲— *Excitation monochromator set at 402 nm*

FIGURE 2. *Variation in intensity of fluorescence of 7-hydroxy-5-methyl-3-phenylcoumarin $\cdot H_2O$ with pH.*
—●—●— *Excitation monochromator set at 349 nm*
—▲—▲— *Excitation monochromator set at 392 nm*

FIGURE 3. *Variation in intensity of fluorescence of 7-hydroxy-5-methyl-3-carbethoxycoumarinmethyleneiminodiacetic acid $\cdot 1/2H_2O$ with pH.*
—●—●— *Excitation monochromator set at 360 nm*
—▲—▲— *Excitation monochromator set at 409 nm*

FIGURE 4. *Variation in intensity of fluorescence of 7-hydroxy-5-methyl-3-phenylcourmarin-methyleneiminodiacetic acid $\cdot 1/2H_2O$ with pH.*
—●—●— *Excitation monochromator set at 348 nm*
—▲—▲— *Excitation monochromator set at 392 nm*

In 0.8 M KOH the indicators are non-fluorescent. The addition of calcium restores fluorescence. Addition of strontium restores less than half of the fluorescence restored by calcium. Addition of barium restores less than one sixth the fluorescence restored by calcium. Addition of magnesium does not restore fluorescence because at pH 13 magnesium is present as non-dissociated magnesium hydroxide.

The fluorescence of the calcium-indicator compounds decreases on standing in 0.8 M KOH. This is attributed to opening of the lactone ring. The presence of phenyl and carbethoxy substituents at position 3 slows down the rate of ring opening which is very rapid for 7-hydroxycoumarin; nevertheless, the steady drop in fluorescence renders these compounds unsuitable for use in spectrofluorometric analyses. The compounds serve as excellent indicators in titrimetric determinations of calcium, when barium and strontium are absent, because only dramatic quenching of fluorescence at the end-point is required.

Both of the synthesized indicators are more fluorescent than Calcein Blue. The phenyl coumarin is approximately twice as fluorescent as Calcein Blue. The carbethoxy coumarin is approximately four times as fluorescent as Calcein Blue. These values correlate well with those established for the parent fluorescent molecules (6).

The absorption spectra of the carbethoxy compounds show one strong band, the wavelength of maximum absorbance shifting from shorter wavelength in acid solution to longer wavelength in alkaline solution. Below pH 7.5 and at the concentrations used in the absorbance study 7-hydroxy-5-methyl-3-phenylcoumarin precipitates. The absorption spectrum of this compound in alkaline solution exhibits two bands. As expected the absorbance of its methyleneiminodiacetic acid derivative also exhibits two bands. Inspection of the spectra of the acid and base forms of this compound shows a shift in the wavelength of the two bands to longer wave lengths in going from acid to alkaline solution. These shifts correspond to similar ones in the fluorescence excitation spectra of the compounds. The wavelengths of absorbance maxima in acid and maxima in acid and alkaline solution for each of the compounds are given in Table 1.

Absorbance changes with pH, the absorbance at the wavelength of maximum absorbance in acid solution decreases with increasing pH and the absorbance at the wavelength of maximum absorbance in alkaline solution increases with increasing pH. This change in the absorption spectra corresponds to the change observed in the fluorescence spectra and is attributed to neutralization of the phenolic proton. Only one inflection point is observed in each curve.

In conclusion, two compounds that can serve as indicators to signal the end-point in titrimetric determinations of calcium when EDTA is used as the titrant have been isolated in pure form and studied. Both compounds are more fluorescent than Calcein Blue and both are able to function as indicators for longer periods of time in alkaline solution than Calcein Blue. At pH 13 the calcium-indicator compounds are brilliantly fluorescent. Upon removal of calcium fluorescence disappears.

Acknowledgements

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