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## Fluorescence Studies of Cobalt (III) Complexes

Arnold T. Weibel

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FLUORESCENCE STUDIES OF  
COBALT(III) COMPLEXES

by  
Arnold T. Weibel

A Thesis  
Submitted to the  
Faculty of the School of Graduate  
Studies in partial fulfillment  
of the  
Degree of Master of Arts

Western Michigan University  
Kalamazoo, Michigan  
March, 1968

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Arnold T. Weibel

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## INTRODUCTION

### Theory of Fluorescence and Phosphorescence

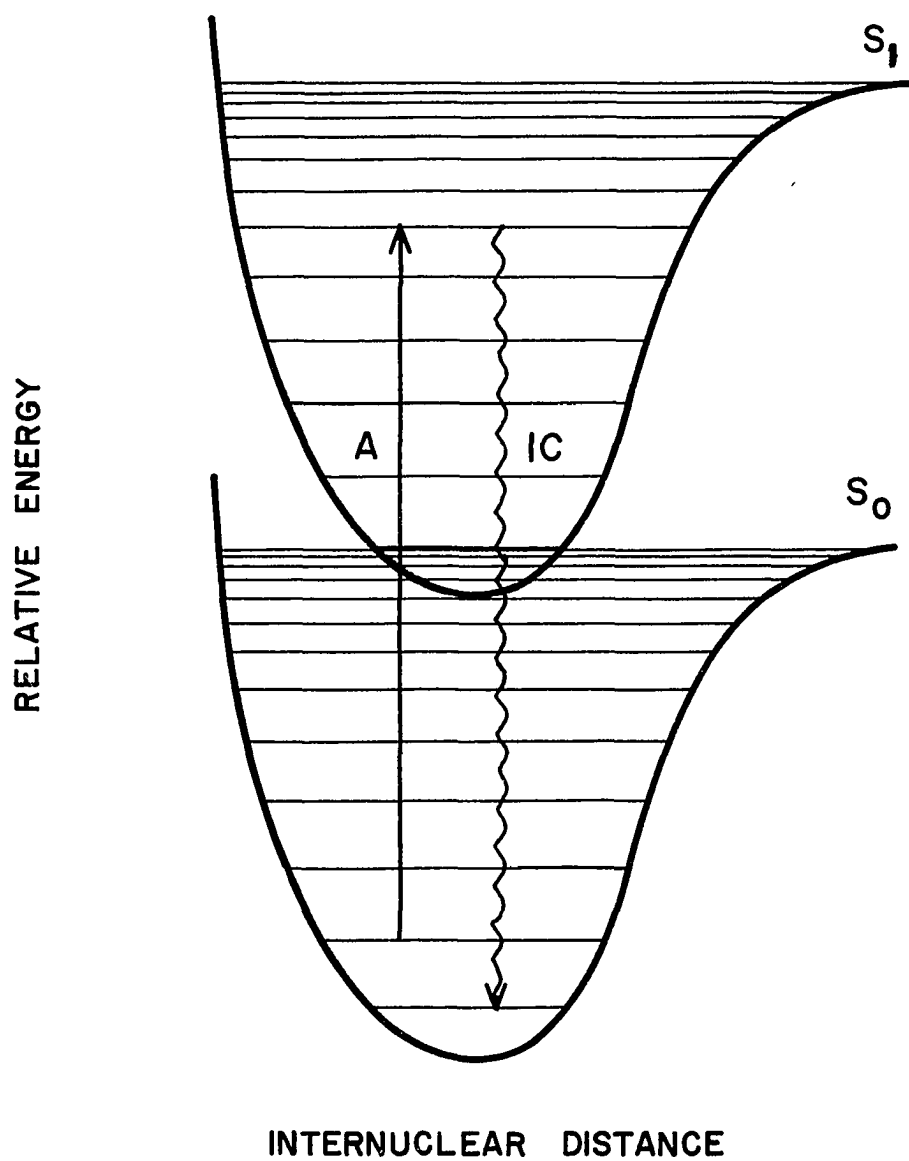
The excitation of a molecular species by absorption of electromagnetic radiation is followed by a deexcitation process which may be either radiative or nonradiative in character (1). The absorption process requires approximately  $10^{-15}$  seconds, and is followed by nonradiative vibrational-rotational relaxation (VRR) to the lowest vibrational-rotational level of the excited state. Vibrational-rotational relaxation requires about  $10^{-13}$  to  $10^{-11}$  seconds. However, when two electronic states of the same multiplicity have overlapping vibrational levels, a process of non-radiative deexcitation known as internal conversion (IC) may occur as shown in Figures 1 and 2. In those cases where there is no overlap of vibrational levels of two electronic states of the same multiplicity, further deexcitation occurs radiatively by means of fluorescence emission (F) as shown in Figure 2. The process of fluorescence emission occurs in  $10^{-9}$  to  $10^{-7}$  seconds. Certain molecules have overlapping vibrational levels of two electronic states with different multiplicities. In these cases a form of nonradiative decay known as intersystem crossing (ISC) may occur, which lowers the energy of the excited molecule as seen in Figure 3.

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(1) Hercules, D. M., "Fluorescence and Phosphorescence Analysis", Interscience Publishers Inc., New York (1966) Ch. 1 and 4.

Radiative decay between electronic states of different multiplicities is said to be forbidden or a process of low probability. Transitions of this type, known as phosphorescence (P), require approximately  $10^{-4}$  to 10 seconds. Other processes of deexcitation which are often found to compete with fluorescence and phosphorescence will be discussed later.

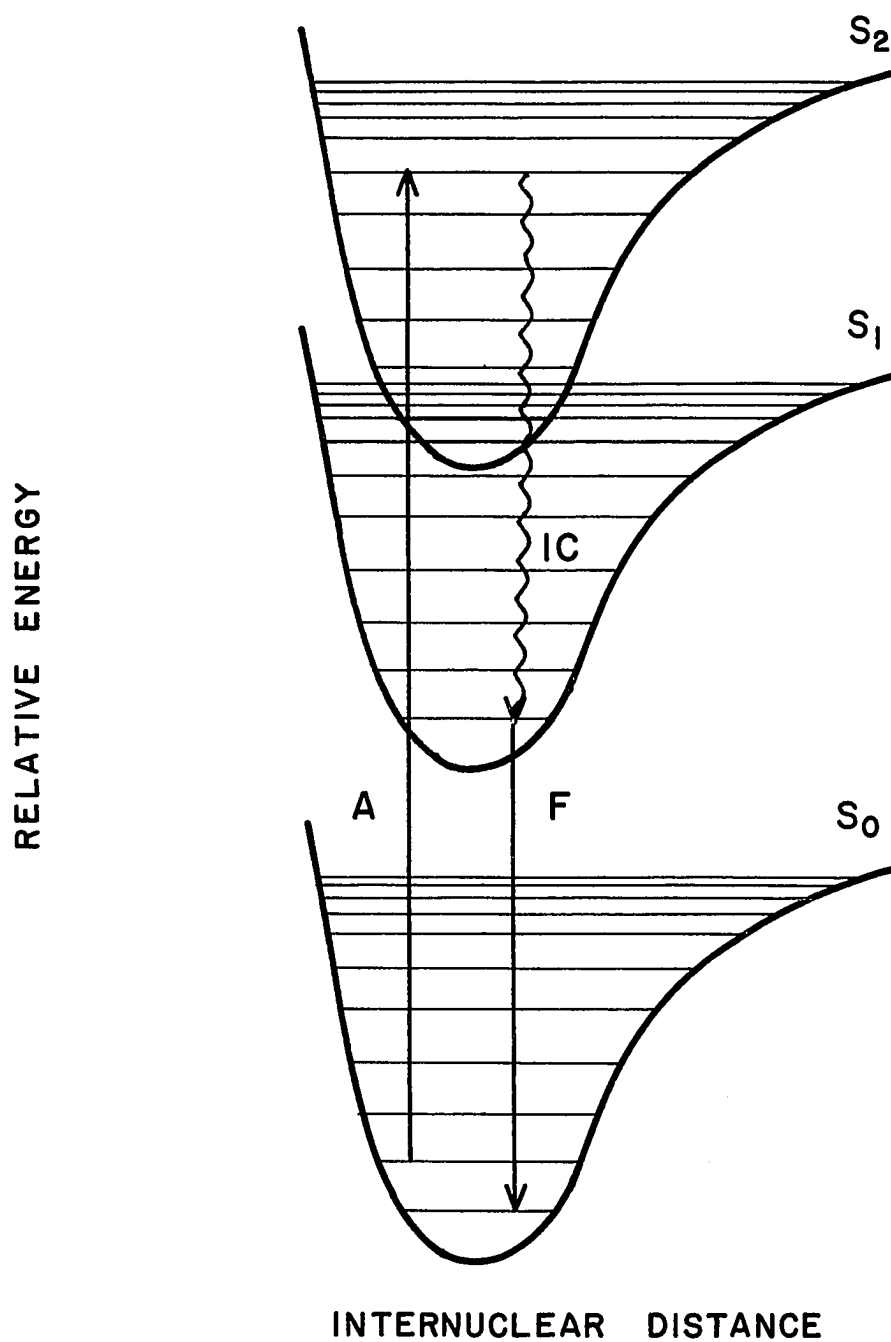
FIGURE I



S - singlet state  
A - absorption  
IC - internal conversion

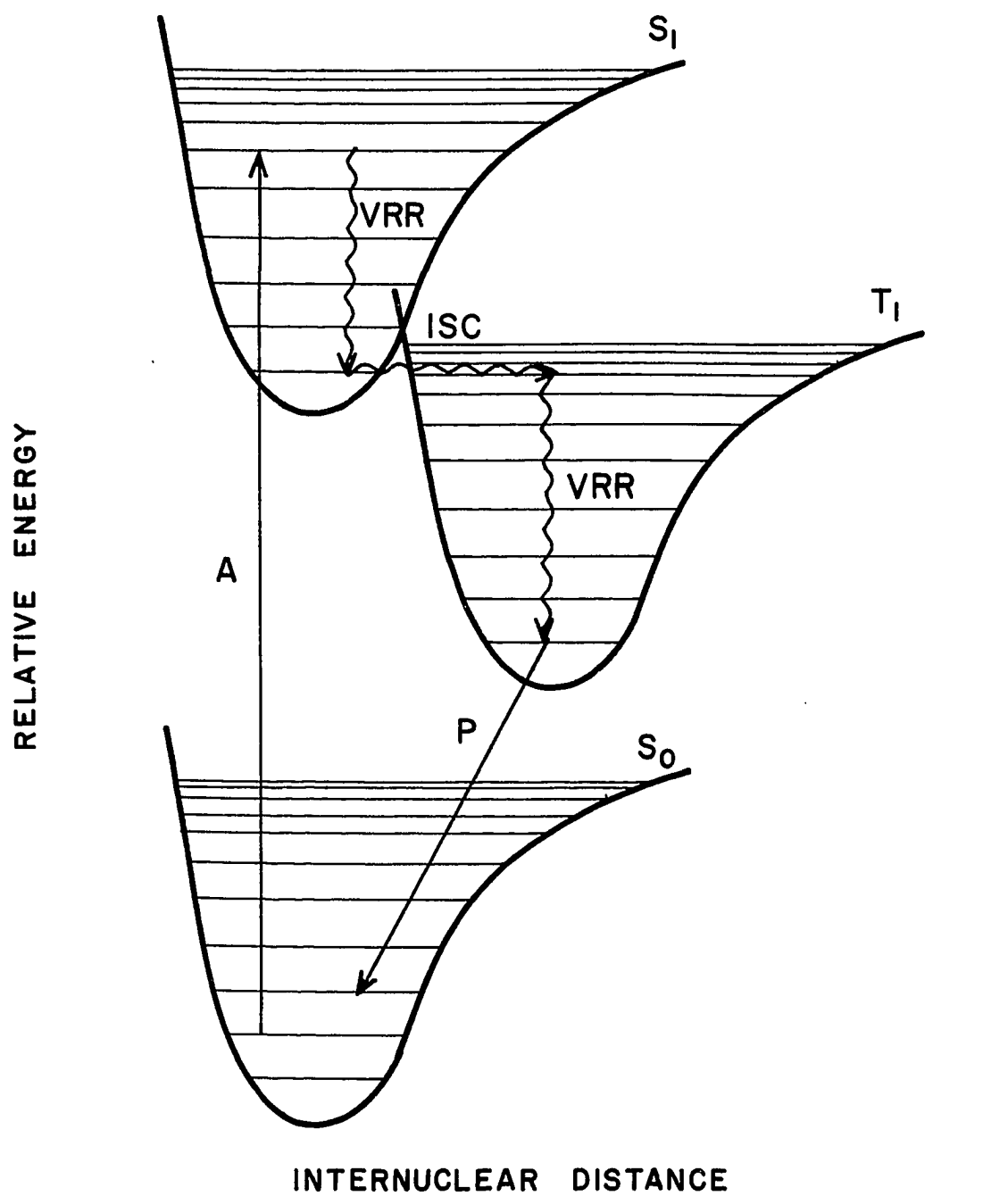


FIGURE 2



- S - singlet state
- A - absorbance
- IC - internal conversion
- F - fluorescence

FIGURE 3



- S - singlet
- T - triplet
- A - absorption
- VRR - vibrational-rotational relaxation
- ISC - intersystem crossing
- P - phosphorescence

## Historical

### Micro determination of cobalt

The most frequently used methods for the determination of small amounts of cobalt are methods utilizing ultraviolet and visible absorption spectrophotometry. Most of the methods which have been developed are useful in the range of one to ten parts per million. These spectrophotometric methods, while often being simpler in procedure than many of the competitive methods mentioned later, tend to be less sensitive and frequently exhibit more interferences. A few of the common interfering ions encountered in these methods are iron(III), copper(II), nickel(II), lead(II), and chromium(III). Some of the more extensively used chromogenic reagents have been thiocyanate (2,3), 1-nitroso-2-naphthol (3,4), nitroso-R salt (5), and diacetylmonoxime or  $\alpha$ -benzil monoxime (6). Also, a differential spectrophotometric method has been reported which utilizes the ammonium hexathiocyanato-cobaltate(III) complex (7).

- 
- (2) Young, R. S., and Hall, A. J., Ind. Eng. Chem., Anal. Ed., 18, 264 (1946).
  - (3) Lundquist, R., Markle, G. E., and Boltz, D. F., Anal. Chem., 27, 1731 (1955).
  - (4) Claassen, A. and Daamer, A., Anal. Chim. Acta., 12, 547 (1955).
  - (5) Shipman, W. H., and Lai, J. R., Anal. Chem., 28, 1151 (1956).
  - (6) Calzolari, C. and Donda, A., Ann. Chim. (Rome), 44, 280 (1954).
  - (7) Brueck, A., Lauer, K. F., and LeDuigou, Y., U.S. At. Energy Comm. EUR-581.f (1964).

Several polarographic methods for cobalt in this same concentration range have been reported. Mikula and Codell(8) have described a method for the simultaneous determination of cobalt(II), nickel(II), and copper(II). A method has been reported by Meites (9) for the determination of cobalt in the presence of large amounts of nickel.

Atomic absorption spectrophotometry has been utilized in recent years for cobalt determinations. A study of some of the experimental parameters affecting the use of various cobalt emission lines have been reported (10).

Emission spectroscopy is one of the more sensitive methods for the determination of cobalt. Thiers and Williams (11) reported a method for the determination of 1 to 500 nanograms of cobalt in biological samples after a preliminary separation from interfering metals by ion exchange techniques.

Neutron activation analysis is perhaps the most sensitive method for many of the elements. As little as three nanograms of cobalt have been determined by this technique (12).

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(8) Mikula, J. J. and Codell, M., Anal. Chem., 27, 729 (1955).

(9) Meites, L., Anal. Chem., 28, 404 (1956).

(10) Harrison, W. W., Anal. Chem., 37, 1168 (1965).

(11) Thiers, R. E. and Williams, J. F., Anal. Chem., 27, 1725 (1955).

(12) Yull, H. P., Anal. Chem., 37, 129 (1965).

### Micro determination of cobalt by fluorescence

Earlier workers have described various methods which utilize fluorescence for the determination of cobalt. Zamochnick and Rechnitz (13) reported an indirect method in which the fluorescence of an aluminum complex with Super-Chrome Blue-Black Extra was diminished by cobalt(II). Wallach and Steck (14) have developed an indirect method for cobalt(II) involving the diminution of the fluorescence of a substituted fluorescein derivative. Babko and Lukovskya (15) have found that the total emission from a chemiluminescent reaction between hydrogen peroxide and luminol is dependent on the cobalt(II) concentration. No reports of a direct determination of cobalt utilizing a fluorescent cobalt compound have been found.

- 
- (13) Zamochnick, S. B. and Rechnitz, G. A., Z. anal. Chem., 199, 424 (1964).  
(14) Wallach, D. F. and Steck, T. L., Anal. Chem., 35, 1035 (1963).  
(15) Babko, A. K. and Lukovskya, N. M., Industrial Laboratory, 29, 409 (1963).

## SPECTROPHOTOMETRIC STUDIES

### Introduction

A partial Orgel diagram for cobalt(III) complexes, which have a  $d^6$  configuration in a strong crystalline field (16), is shown in Figure 4. This diagram shows the relative energies between the ground state and four of the excited states. The ground state is a singlet, the two upper excited states are singlets, and the two lower excited states are triplets. After excitation to one of the singlet states occurs, deexcitation to the ground state could occur through fluorescence emission. Since the triplet states lie relatively close to the singlet states it is possible that intersystem crossing could occur followed by deexcitation through phosphorescence emission.

### Experimental

#### Absorption spectra

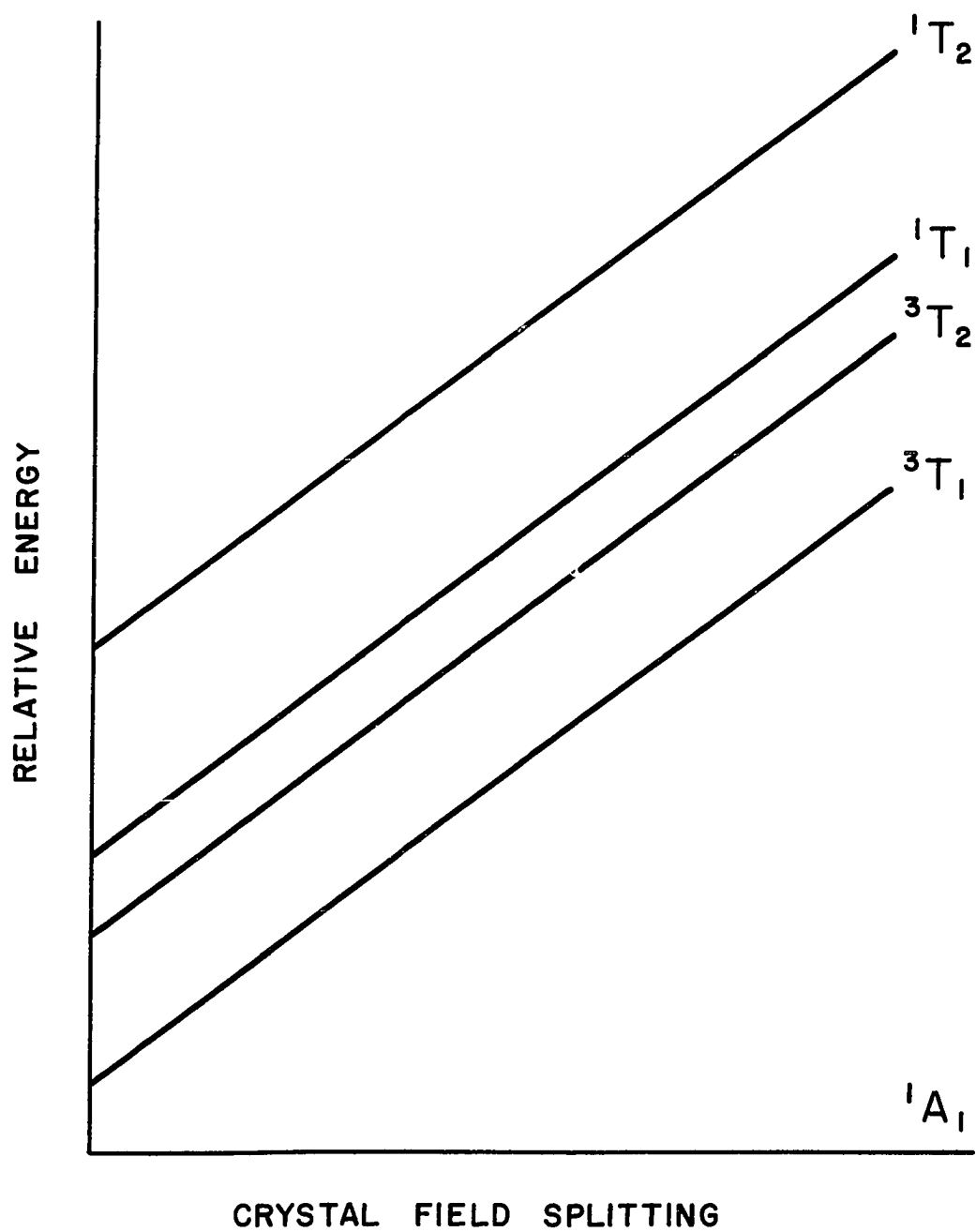
Absorption spectra of numerous cobalt(III) complexes were measured to determine at which wavelengths they exhibited absorption maxima and precisely what their respective molar absorptivities were. This information is particularly useful in determining the excitation wavelengths for fluorescence and phosphorescence studies.

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(16) Dunn, T. M., "Modern Coordination Chemistry", Lewis, J. and Wilkins, R. G., Eds., Interscience Publishers Inc., New York (1960) p250.

FIGURE 4

## PARTIAL ORGEL DIAGRAM



These spectra were also ultimately used for the purpose of selecting a cobalt(III) complex to be used in the thin-layer chromatographic studies.

Potassium hexacyanocobaltate(III) was prepared according to Grauer (17), while the potassium chloropentacyano-cobaltate(III) complex was prepared by the procedure of Adamson (18). Tris (2,4-pentanedione) cobalt(III) was prepared according to Bryant and Fernelius (19) and recrystallized twice by their procedure. The cobalt peroxy-carbonate complex was prepared in solution as needed using a procedure described by Telep and Boltz (20). Other complexes studied were provided by Dr. D. W. Cooke of the Department of Chemistry at Western Michigan University.

The absorption spectra used in this study were measured between 2000 and 8000 angstroms on a Cary Model 14 recording spectrophotometer using one centimeter quartz cells.

Solutions of tris (2,4-pentanedione) cobalt(III) were prepared using 95 percent ethanol which had been redistilled to remove fluorescent impurities. Solutions of all the other complexes were prepared with doubly distilled water.

- 
- (17) Brauer, G., Ed., "Handbook of Preparative Inorganic Chemistry", Vol 2, Academic Press (1965) p1541.
- (18) Adamson, A. W., J. Am. Chem. Soc., 78, 4260 (1956).
- (19) Bryant, B. E. and Fernelius, W. C., Inorg. Syn., 5, 188 (1957).
- (20) Telep, G. and Boltz, D. F., Anal. Chem., 24, 945 (1952).



The absorption maxima and the corresponding molar absorptivities of the compounds studied are shown in Table 1. Absorptivities for the potassium chloropentacyanocobaltate(III) maxima are given as approximate values because a small unknown amount of potassium hexacyanocobaltate(III) was present.

Those absorption maxima in the range of 3000 to 6000 angstroms, with molar absorptivities between 50 and 300 arise from ( $^1A_1 \rightarrow ^1T_1$  and  $^1A_1 \rightarrow ^1T_2$ ) singlet-singlet transitions (21), while those maxima between 2000 and 3000 angstroms with molar absorptivities of approximately  $10^4$  are apparently the result of charge transfer transitions (22). A few of the compounds exhibited weak absorption in the vicinity of 8000 angstroms which may be attributed to forbidden ( $^1A_1 \rightarrow ^3T_1$  and  $^1A_1 \rightarrow ^3T_2$ ) singlet-triplet transitions (21).

### Fluorescence

All fluorescence spectra were obtained with an Aminco-Bowman Spectrophotofluorometer. The slits were set for maximum light intensity and the photomultiplier microphotometer was set for maximum sensitivity in an effort to detect even the slightest luminescence. A fluorescent impurity was detected in the distilled water which necessitated redistillation from acidic permanganate

- 
- (21) Ballhausen, C. J., "Introduction to Ligand Field Theory", McGraw-Hill Book Co., Inc., New York (1962) p259.
  - (22) Orgel, L. E., "An Introduction to Transition-Metal Chemistry", John Wiley and Sons Inc., New York (1960) p99-102.

Table 1  
Absorption Maxima of Cobalt(III) Complexes

	$\lambda$ (Å)	$\nu$ (cm <sup>-1</sup> )	$\epsilon$
[Co(NH <sub>3</sub> ) <sub>6</sub> ]Cl <sub>3</sub>	4770	21000	56.3
	3400	29400	45.0
	1950	51300	2.90x10 <sup>4</sup>
[Co en <sub>3</sub> ]Cl <sub>3</sub>	4670	21400	59.4
	3420	29300	53.3
	2120	47200	1.52x10 <sup>4</sup>
[Co en <sub>2</sub> (NO <sub>2</sub> ) <sub>2</sub> ]NO <sub>2</sub>	4340	23000	164
	3390	29500	2.88x10 <sup>3</sup>
	2500	40000	1.71x10 <sup>4</sup>
	2040	49000	2.88x10 <sup>4</sup>
[Co en <sub>2</sub> CO <sub>3</sub> ]ClO <sub>4</sub>	5100	19600	136
	3600	27800	124
	2250	44400	1.42x10 <sup>4</sup>
[Co en <sub>2</sub> (OAc) <sub>2</sub> ]ClO <sub>4</sub> (trans)	5400	18500	60.5
	4500	22200	29.5
	3570	28000	74.0
	2350	42600	1.81x10 <sup>4</sup>
[Co trien CO <sub>3</sub> ]ClO <sub>4</sub>	5080	19700	91.3
	3600	27800	80.0
	2380	42000	7.39x10 <sup>3</sup>

Table 1 (continued)

	$\lambda$ (Å)	$\nu$ (cm <sup>-1</sup> )	$\epsilon$
K[Co (EDTA)]	5350	18700	278
	3810	26300	187
	2270	44100	1.88x10 <sup>4</sup>
K <sub>3</sub> [Co(CN) <sub>6</sub> ]	3150	31800	203
	2600	38500	143
	2000	50000	2.25x10 <sup>4</sup>
K <sub>3</sub> [Co(CN) <sub>5</sub> Cl]	3800	26300	100 *
	3100	32300	300
	2050	48800	1x10 <sup>4</sup>
Co (acac) <sub>3</sub>	5930	16900	133
	2580	38800	3.58x10 <sup>4</sup>
	2300	43500	4.25x10 <sup>4</sup>
Co( peroxy-carbonate)	6400	15600	172
	4420	22600	172
	2600	37500	1.53x10 <sup>4</sup>

\* Approximate values due to presence of an impurity

in order to remove any detectable fluorescence. A similar impurity was found in the 95 percent ethanol thus necessitating its redistillation also. Redistillation of the ethanol was accomplished using a 20 centimeter Vigreux column. Methanol was distilled in the same manner.

The excitation wavelengths for this impurity were at 2350 and 2900 angstroms and the emission maximum was at 3400 angstroms. The fluorescence intensity prior to purification was approximately one thousand times greater in ethanol than in the distilled water. No attempts were made to identify this impurity.

Solubilities were measured in several solvents for each of the complexes studied. Most of these complexes were found to be very soluble in water, but insoluble or only slightly soluble in methanol, ethanol, glycerol, acetone, dimethyl sulfoxide, dioxane, and dimethylformamide. The only exception was tris (2,4-pentanedione) cobalt(III) which was found to be very soluble in benzene, slightly soluble in ethanol and methanol, and insoluble in the other solvents previously mentioned.

In view of the preceeding solubility studies the solvents used in the fluorescence studies were restricted to water, ethanol, and methanol. When water was used as the solvent the solutions were checked for fluorescence at pH values of 0, 3, 6, 9 and 11. The pH of the solutions was adjusted using dilute solutions of hydrochloric acid or potassium hydroxide.

The concentrations of the cobalt complexes were made such that the absorbance at the absorption maximum would be approximately

0.5. This was done to minimize problems arising from self-quenching. This required two concentrations for each complex, one for the singlet-singlet transition and one for the charge transfer transition. All fluorescence measurements were made between 2000 and 8000 angstroms with each solution initially being measured by setting the excitation wavelength at the absorption maxima and scanning the longer wavelengths for emission spectra. The solutions were then measured with the excitation wavelength set at 2000 angstroms and increased by 200 angstroms after each successive scan of the emission wavelength.

Adamson and Sporer (23) have reported that potassium chloropentacyanocobaltate(III) solutions fluoresced. Solutions of this compound were prepared and studied; however, no fluorescence was observed. None of the compounds which were investigated exhibited any detectable fluorescence.

### Phosphorescence

All the phosphorescence studies were made using an Aminco-Bowman Spectrophotofluorometer with a phosphoroscope attachment. This attachment permits working at low temperatures as well as preventing any fluorescent or scattered light from reaching the detector.

A temperature of  $-95^{\circ}\text{C}$  was obtained by suspending the sample cell in an acetone-liquid nitrogen bath. Ethanol, methanol,

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(23) Adamson, A.W. and Sporer, A.H., J. Am. Chem. Soc., 80, 3865 (1958).

sulfuric acid and phosphoric acid were used as solvents in these studies because they harden to give a clear glass which does not crack at  $-95^{\circ}\text{C}$ . Studies at liquid nitrogen temperature were not possible since glasses of these solvents crack at these temperatures. Phosphorescence measurements were made at all wavelengths between 2000 and 8000 angstroms in the same manner as described for the fluorescence measurements. No significant phosphorescence was detected in any of the complexes studied.

### Conclusions

In many compounds the absence of luminescence is due to internal conversion to the ground state from either a singlet or a triplet state (24). Intersystem crossing can also compete with fluorescence from the zeroth vibrational level of an excited singlet state and is more probable if the singlet-triplet energy difference is small. The probability of intersystem crossing to a triplet state is increased by increasing the singlet state lifetime, having a paramagnetic atom present, or an atom with a high atomic weight (24). All of these factors decrease fluorescence intensity and may increase phosphorescence intensity. Nonradiative de-excitation of a triplet state by internal conversion is more probable than for a singlet state because the energy difference to the ground state is frequently less, thus increasing vibrational

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(24) Allison, J. B. and Becker, R. S., J. Chem. Phys., 32, 1410 (1960).

coupling between the excited state and the ground state. More important, the lifetime of the triplet state is greater, which increased the probability of collisional deactivation (25).

Increasing solute-solvent interaction by increasing ligand polarity or solvent polarity will decrease fluorescence intensity because collisional deactivation becomes more probable (26). An increase in the molecular weight of the solvent or a decrease in the temperature will often decrease the frequency of collision, thus decreasing collisional deactivation.

Nonradiative transfer also competes with fluorescence and can occur with or without collision. In the case of metal chelates this is usually a transfer of energy from the ligand to the metal ion and is an important path of energy loss for a number of metals (27).

Photochemical reactions may also occur since the excited states are more reactive (28,29). These reactions are more probable from the triplet state because the lifetime is longer.

All these processes appear to be possible for cobalt(III) complexes. Collisional deactivation is probably one of the more important processes in these studies since they were performed in

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(25) Hercules, D. M., op. cit., Ch 1.

(26) Halverson, F., Brinen, J. S. and Leto, J. R., J. Chem. Phys., 41, 157 (1964).

(27) Paris, J. P. and Brandt, W. W., J. Am. Chem. Soc., 81, 5001 (1959).

(28) Schlafer, H. L., J. Phys. Chem., 69, 2201 (1965).

(29) Adamson, A. W., J. Phys. Chem., 71, 798 (1967).

water, methanol, and ethanol. Photodecomposition would also seem to be an important process since many cobalt(III) chelates are known to undergo photochemical reactions (30,31,32). These reactions include substitution of ligands with solvent molecules, racemization of cis-trans isomers, and reduction of cobalt(III) to cobalt(II) accompanied by oxidation of the ligand (33,34). The specific type of reaction which occurs is dependent upon the frequency of the radiant energy being absorbed.

- 
- (30) Adamson, A. W., Discuss. Faraday Soc., 29, 163 (1960).  
(31) Spees, S. T., and Adamson, A. W., Inorg. Chem., 1, 531 (1962).  
(32) Endicott, J. F. and Hoffman, M. Z., J. Am. Chem. Soc., 87, 3348 (1965).  
(33) Klein, D. and Moeller, C. W., Inorg. Chem., 4, 394 (1965).  
(34) Taylor, W. C. and Moeller, C. W., Inorg. Chem., 4, 398 (1965).



## THIN-LAYER CHROMATOGRAPHY OF COBALT(III)

### Introduction

In view of the difficulties associated with obtaining a fluorescent cobalt complex, previous workers in the area have approached the analysis indirectly. Since cobalt(III) complexes exhibit strong charge transfer bands in the ultraviolet region, a system involving quenching by a cobalt(III) complex would seem reasonable. The use of a fluorescent thin-layer chromatography plate accomplishes this with the added advantage of simultaneous separation and analysis.

### Experimental

#### Reagents

All chemicals used in these studies were reagent grade unless otherwise specified. A commercial bleach solution which was used for sodium hypochlorite was found to contain no detectable amounts of cobalt or other metals which would interfere. Thin-layer plates used in this study are commercially available from Distillation Products Industries (Eastman Kodak Co.). They consist of a 100 micron layer of silica gel with a polyvinyl alcohol binder on a 200 micron sheet of polyethyleneterphthalate. The silica gel coating contains a fluorescent indicator of lead-manganese activated calcium silicate.

### Apparatus

A fluorescence spectrum of the thin-layer plates was obtained using an Aminco-Bowman spectrophotofluorometer. A Turner Model 111 filter fluorometer with a thin-layer scanning attachment was used for all quantitative fluorescence measurements of the thin-layer plates. The normal slit, which is 10 by 15 millimeters, was modified to 2 by 8 millimeters in order to give better resolution of the spots on the thin-layer plates. The primary filter isolated the 2537 angstrom line of the mercury excitation source. The secondary filter permitted only wavelengths greater than 5350 angstroms to reach the detector.

### Preliminary work

Two important requirements were considered in the selection of the cobalt(III) complex used in this study. The first requirement was that the complex should absorb radiation strongly at the excitation and emission wavelengths of the fluorescent indicator. Strong absorption is necessary to produce a great amount of quenching. The excitation wavelength is more important because the absorptivities of the cobalt(III) complexes are much greater in this region. It is also necessary that the reagents used to form the complex should be optically transparent in these regions. The second requirement was that the cobalt(III) complexes should be easily formed in dilute solution. It is also desirable that

other metal ions, if present, should not interfere with the formation of the cobalt(III) complex.

Comparison of the absorption spectra revealed that several of the cobalt(III) complexes absorbed at or near the excitation wavelength of the fluorescent indicator, but only two of these are easily formed in dilute solutions of cobalt. These are the ethylenediaminetetracetate (EDTA) complex and the peroxy-carbonate complex. Figure 5 shows the relationship between the excitation and emission spectra for the fluorescent indicator and the absorption spectrum for cobalt(III) EDTA.

The stability of the peroxy-carbonate complex decreases when the bicarbonate concentration is much below 20 percent. Iron(III), chromium(III), copper(II), titanium(IV), vanadium(V), and uranium(VI) ions also formed peroxy complexes which absorb in this region. For these reasons the cobalt(III) EDTA complex was selected for this study.

The developing solvent was selected to give maximum separation between cobalt and interfering ions. Water and ethanol were found to give little or no separation between cobalt and copper. The retardation factors ( $R_f$ ) of a series of dioxane-water mixtures saturated with EDTA are given in Table 2. The best separation was obtained with a mixture of dioxane and water in a volume ratio of 5:2. This ratio of dioxane and water is somewhat less than 0.01 molar in EDTA at saturation. An impurity observed at the solvent front was found not to originate from the

FIGURE 5

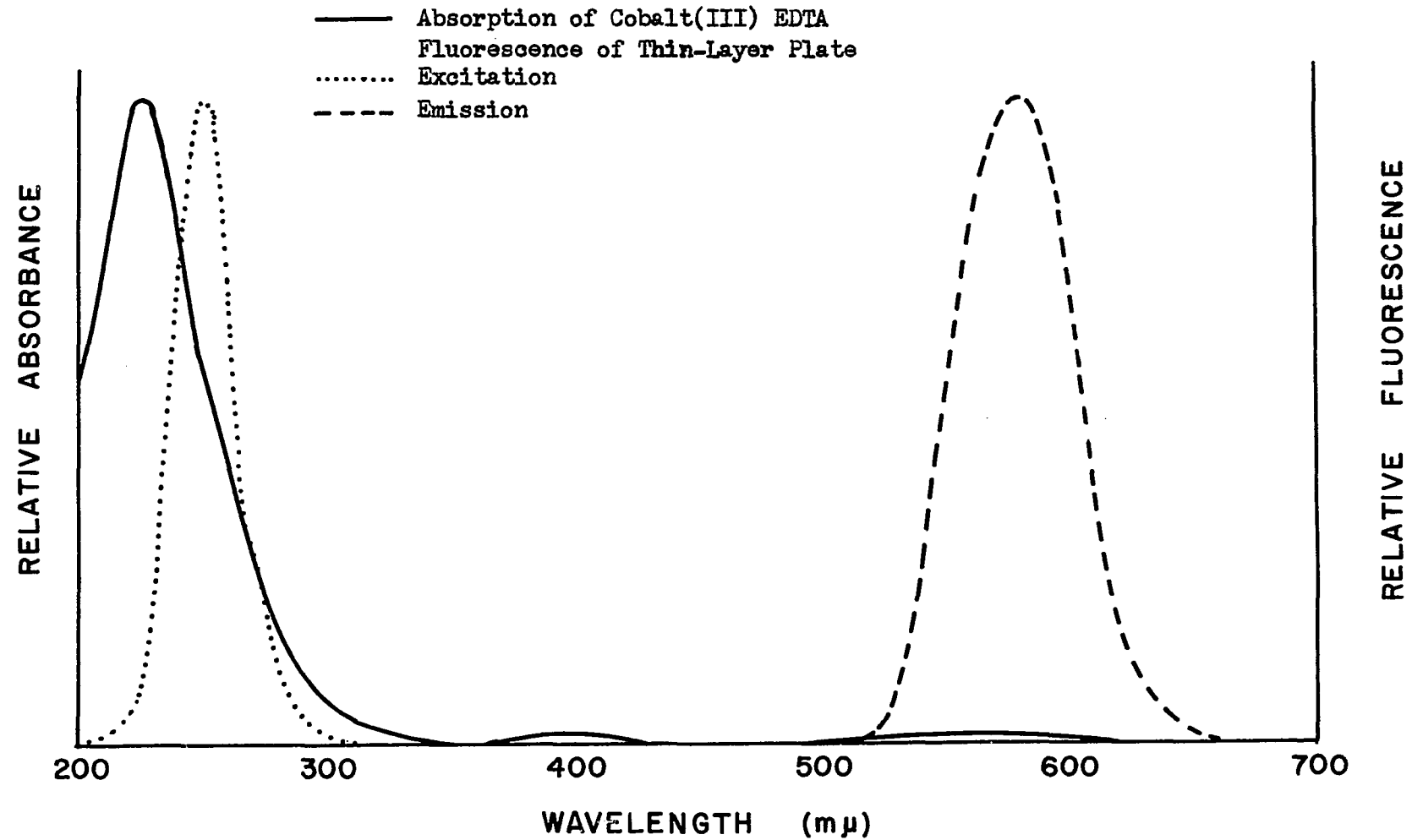


Table 2

## Retention Data

<u>Volume Ratio</u> <u>Dioxane : Water</u>	<u>Metal</u>	<u>R<sub>f</sub></u>
1 : 0	Co(III)	0.00
	Cu(II)	0.00
3 : 1	Co(III)	0.42
	Cu(II)	0.00
5 : 2	Co(III)	0.57
	Cr(III)	0.00
	Fe(III)	0.32
	Cu(II)	0.31
	Pb(II)	0.29
2 : 1	Co(III)	0.67
	Cu(II)	0.47
	Fe(III)	0.36
1 : 1	Co(III)	0.82
	Cu(II)	0.76
	Fe(III)	0.84
0 : 1	Co(III)	0.85
	Cu(II)	0.92

thin-layer coating nor was it removed by distillation of the dioxane or the water. The EDTA would appear to be the probable source of this impurity.

### Procedure

The following is a recommended general procedure and is the same as was used in this work. The sample solution containing cobalt and other metal ions should be adjusted to between pH 4 and 7 with dilute solutions of hydrochloric acid or potassium hydroxide so that cobalt(II) hydroxide will not precipitate. A measured amount of this solution containing 0.25 to 2.5 milligrams of cobalt is transferred to a 50 milliliter volumetric flask and sufficient EDTA is added to complex all the metal ions present and provide an excess of 25 to 50 percent. The solution was made 0.02M in potassium dihydrogen phosphate to buffer it to pH 5. The sodium hypochlorite concentration is made 0.07M to oxidize the cobalt(II) to cobalt(III), and the solution is diluted to volume with distilled water. Addition of the hypochlorite solution after the EDTA is added is done to prevent formation of cobalt(III) hydroxide. This pH problem can be avoided with hydrogen peroxide but the oxidation requires more than two days. Thin-layer separation can be carried out on these solutions immediately after dilution although they appear to be stable for several weeks.

The thin-layer plates are used as received without oven activation or other pretreatment. Samples of one microliter each

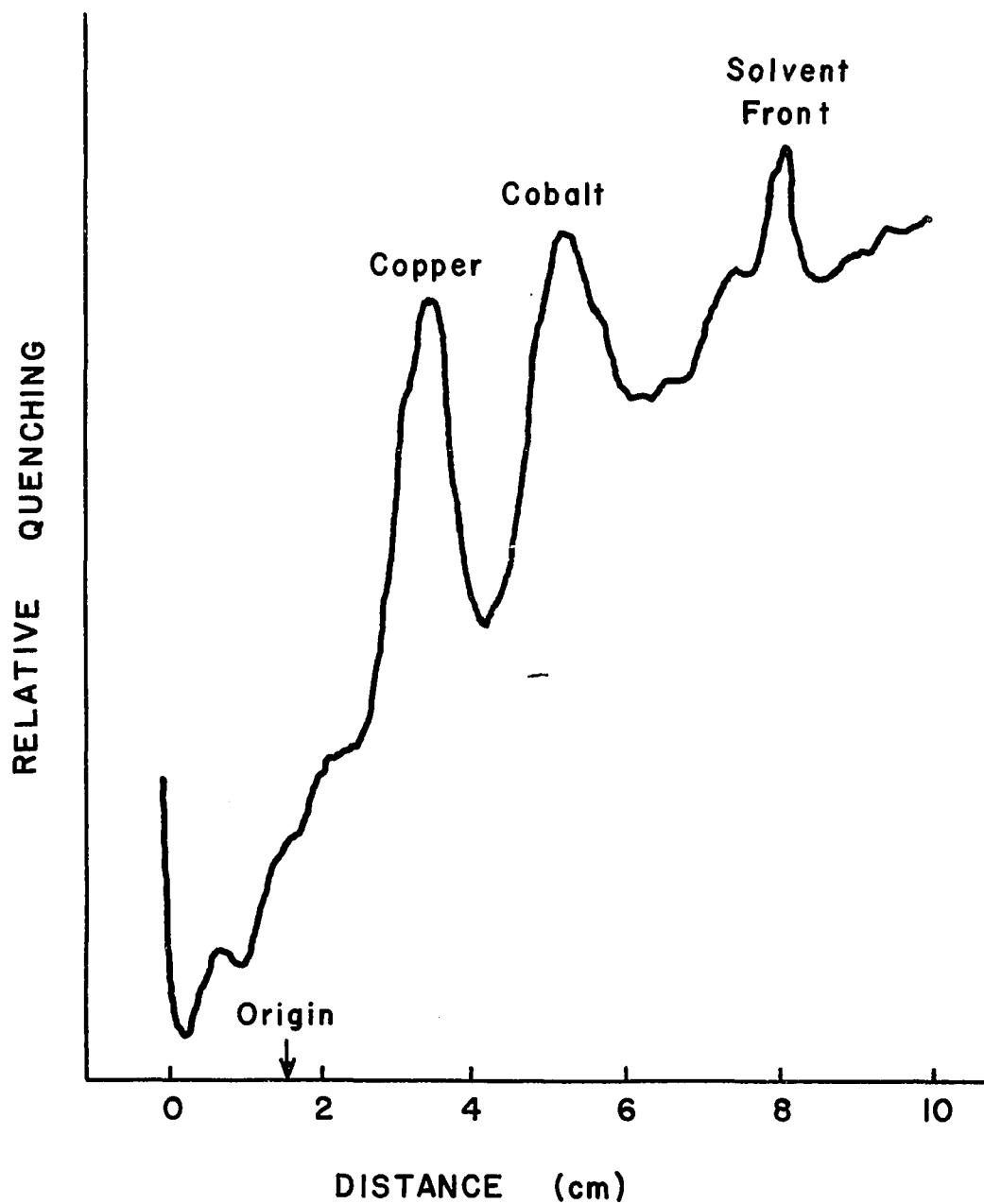
are spotted on the plates using a ten microliter syringe. The plates are then developed using the apparatus from an Eastman Chromagram Kit. The developing solvent used in this work is a mixture of dioxane and water in a volume ratio of 5 : 2 saturated with EDTA. The plates are developed for 45 minutes during which time the solvent moves about eight centimeters past the origin. The cobalt(III) EDTA spots are nearly circular with a diameter of 6 to 7 millimeters. After air drying for about one half hour, the plates are scanned to measure the decrease in fluorescence caused by the presence of the cobalt(III) EDTA complex. This decrease is recorded as a peak on a strip chart recorder and the area under the peak measured with a planimeter.

## Results

### Statistical Data

The solutions used in the statistical study of fluorescence quenching due to cobalt(III) EDTA were prepared using the following concentrations : 0.02M EDTA, 0.02M potassium dihydrogen phosphate, 0.07M sodium hypochlorite, 47 to 933 parts per million cobalt(II) perchlorate. Ten samples of one microliter each were spotted on a plate. A separate plate was used for each of the nine concentrations. After development and scanning, the area under each peak was measured. Figure 6 shows the typical results of scanning a plate. These areas were normalized due to variations of the baseline on different parts of the plate. Normalization is

FIGURE 6  
TYPICAL SCAN OF A THIN-LAYER PLATE





achieved by dividing the measured area under the peak by the chart reading at its baseline then multiplying by the full chart value. Three methods for determining the amount of cobalt present were tested. These were the measurement of peak height, calculation of the area under the peak from the peak height times the width at half height, and measurement of the peak area using a planimeter. The planimeter measurement appeared to give the best precision since the shape of the peaks varied greatly due to variations in the slope of the baseline.

The statistical data obtained are given in Table 3 and are plotted in Figure 7. At lower concentrations the quenching is directly proportional to the weight of cobalt present; however, at higher concentrations the quenching approaches a maximum. The point at 600 nanograms of cobalt is lower in relation to the others, possibly because it was run on a plate from an earlier batch which had less fluorescent indicator.

#### Interference studies

The solutions used in the interference studies were prepared using the following concentrations: 0.03M EDTA, 0.02M potassium dihydrogen phosphate, 0.07M sodium hypochlorite, 186 parts per million cobalt(II) perchlorate, 1000 parts per million of each interfering metal ion. One spotting was made with cobalt containing the interfering metal ion and four spottings were made with cobalt alone. The plates were developed, scanned, and the areas under the cobalt peaks were determined.

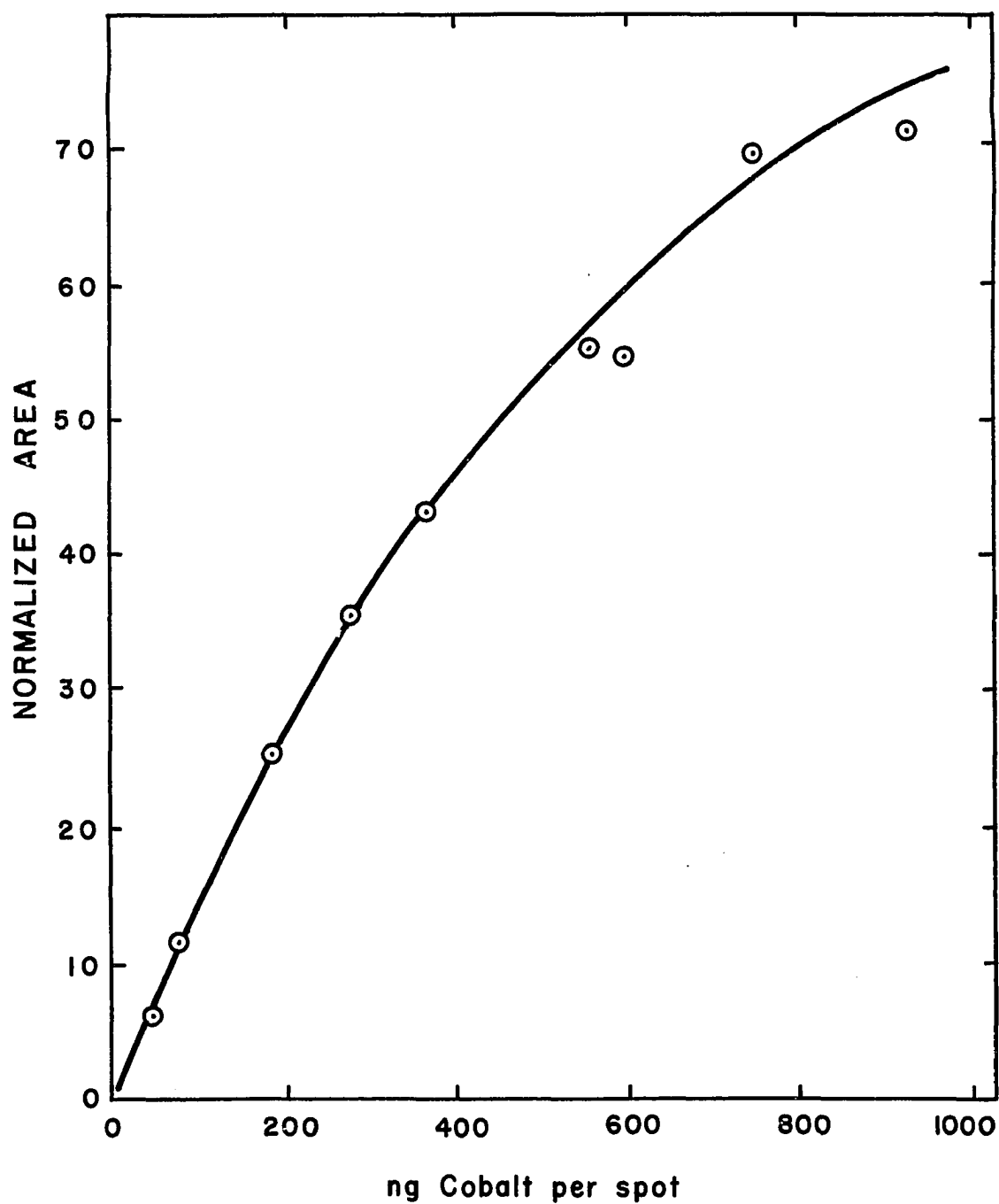
Table 3

## Statistical Data

<u>Sample Number</u>	<u>Number of Determinations</u>	<u>Weight of Cobalt (ng)</u>		<u>Standard Deviation</u>		<u>Average Area</u>	<u>Standard Deviation</u>	
		<u>Added</u>	<u>Found</u>	<u>(ng Co)</u>	<u>(%ng Co)</u>		<u>Area</u>	<u>%Area</u>
A	9	47	41.1	7.26	17.7	6.2	1.04	16.8
B	10	80	81	12.2	15.0	11.6	1.51	13.1
C	10	186	190	21.1	11.1	25.4	2.35	9.25
D	9	280	282	17.4	6.18	35.4	1.61	4.55
E	10	373	370	24.1	6.50	43.0	1.81	4.21
F	10	560	539	79.1	14.7	55.1	5.48	9.94
G	10	600	531	35.1	6.61	54.6	2.60	4.76
H	9	746	774	56.4	7.29	69.4	3.30	4.75
I	8	933	838	37.6	4.48	71.0	1.44	2.30

FIGURE 7

## CALIBRATION CURVE



Retardation factors ( $R_f$ ) for the metals which produced detectable quenching are given in Table 4. Aluminum(III), cadmium(II), magnesium(II), mercury(II), nickel(II), silver(I), tin(IV), and zinc(II) produced no detectable quenching.

A tabulation of the areas obtained for the cobalt peaks with and without various metal ions added is given in Table 4. The mean area was calculated for the four samples which contained only cobalt, and the deviation of each area from this mean is also shown in Table 4. The standard deviation at this concentration (186 parts per million) was found to be 2.3 area units from the earlier statistical data, Table 3. Only four values deviated more than one  $\sigma$  unit from the mean. Of these the area of the cobalt peak with aluminum present deviated more than two  $\sigma$  units. In a Gaussian distribution approximately one third of the values will deviate by more than one  $\sigma$  unit and only about one twentieth will deviate by more than two  $\sigma$  units. The large deviation in the case of aluminum was due to a lowering of the pH since the aluminum ion solution was made up in dilute sulfuric acid to prevent hydrolysis. If the pH of the final cobalt(III) EDTA solution is adjusted between 4 and 6 this deviation is not observed.

### Conclusions

The interference studies show no significant interference from any of the metals tested. Cobalt(III) can compete with other metals for EDTA because it has a significantly larger formation

Table 4

## Interference Studies

<u>Metal Ion Interference</u>	<u>R<sub>f</sub></u> —	<u>Area of Cobalt Peak</u>	<u>Deviation From Mean</u>
None		18.6	+3.6
None	0.54*	22.9	+0.7
None		20.5	-1.7
None		<u>26.9</u>	+4.7
		Mean = 22.2	
Chromium(III)	0.00	22.5	+0.3
Manganese(II)	0.05	20.7	-1.5
Nickel(II)	-----	23.5	+1.3
Zinc(II)	-----	21.0	-1.2
Iron(III)	0.15	22.5	+0.3
Copper(II)	0.28	25.0	+2.8
Cadmium(II)	-----	21.9	-0.3
Silver(I)	-----	18.6	-3.6
Lead(II)	0.29	21.9	-0.3
Mercury(II)	-----	20.1	-2.1
Tin(IV)	-----	20.7	-1.5
Aluminum(III)	-----	17.0	-5.2
Antimony(III)	0.09	21.3	-0.9
Magnesium(II)	-----	25.6	+3.4

\* Average R<sub>f</sub> for these four cobalt peaks

constant,  $\log K_f = 40.7$ , with EDTA than any of the other metals tested (35). A small excess of EDTA is sufficient to ensure complete complexation of all the cobalt(III). The peaks for iron(III), copper(II), and lead(II) are separated completely from the cobalt(III) peak at the concentrations used. However, the iron(III) and copper(II) peaks begin to overlap at higher concentrations. Sources of error in this analysis arise from the measurement of the sample volume when spotting the plate, alignment of the spot with the slit when scanning the plate, and determination of the position and slope of the baseline. The limiting error at high concentrations appears to be that of random sample volume error while at lower concentrations the relative error in measuring the area of the peak increases since baseline errors become more significant.

The major advantage of this method is that the separation and determination are simultaneous. One limitation is that the pH of the sample solution should be adjusted between pH 4 and 7 before it is used since the buffer concentration is low. Higher buffer concentrations increase the diffusion of the cobalt(III) EDTA spots.

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- (35) Flaschka, H. A. and Barnard, A. J., "Comprehensive Analytical Chemistry", Wilson, C. L. and Wilson, D. W., Eds., Elsevier Publishing Corp., (1960) Vol. IB, p297.

### Recommendations

One problem encountered in this study was the non-uniformity of the fluorescent indicator on the thin-layer plates. This is the major cause of random variation in the statistical data, especially at low concentrations. The indicator is in the form of a finely divided solid mixed with the silica gel coating. Brinkmann Instruments Inc. distributes thin-layer plates made by E. Merck (Darmstadt, Germany) which have a slightly more nearly uniform fluorescence. One method which might produce a more uniform fluorescence would be dipping the plate in a solution containing a fluorescent compound which is insoluble in the developing solvent, and then drying the plate before using it. The fluorescent compound could also be applied in the same manner after the thin-layer separation has been completed.

This indirect method may be applied to other metals by using different ligands, different fluorescent indicators, or different coatings and developing solvents. It is also possible that more than one metal could be determined at the same time. A more sensitive and probably more selective direct method should be possible for a metal which will form a fluorescent complex. This would eliminate the need for a fluorescent indicator on the thin-layer plates.

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## VITA

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