APPLICATION OF EUROPIUM-SENSITIZED FLUORESCENCE TO THE DETERMINATION OF SUBSTITUTED 1,3-INDANDIONES

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PREFACE

The purpose of the research detailed in this thesis was to investigate the experimental conditions necessary to increase the fluorescence of weakly fluorescent compounds such that the resulting fluorescence enhancement could be used to develop a sensitive and easily accomplished fluorometric method for the determination of the compounds. The compounds studied were the weakly fluorescent β-carbonyl substituted 1,3-indandiones. These compounds exhibit fluorescence around 500 nm, however the fluorescence is so weak it is only observed in solutions that are 1 X 10^{-1}M or higher. Concentration quenching and inner filter effects make this fluorescence inadequate for quantitative purposes. The method developed here utilizes the formation of a highly fluorescent complex when the 1,3-indandiones are complexed with europium in acetonitrile. This fluorescence is further increased by adding a synergistic agent. The method developed has low limits of detection, a large linear dynamic range and is applicable to the determination of 1,3-indandiones in commercial products. Additionally, the research was to investigate the reasons why the fluorescence is observed and how to obtain the maximum fluorescence. During the course of the research an X-ray

crystal structure was also solved.

The format of the thesis is that each chapter contains its own experimental results and discussion and conclusions sections to allow for a more fluent and easier to read and understand text. Each chapter builds upon the preceding. Chemical structures of the compounds studied are given in Appendix A rather than in the body of the text.

The major portion of the research was performed using a fluorometer that was over twenty years old. Before this research could be started the detector system had to be totally overhauled and a monochromator drive system had to be added. This renovation was started by Mr. Reza Karimi before I joined Dr. Varga's research group. Jointly we completed the overhaul and fine tuned the instrument and the electronics as much as we were able to. I thank Reza for his partnership in keeping the instrument operating during the early stages of this research. I thank Heinz Hall of the physics and chemistry machine shop for his help in the design and construction of the monochromator drive system.

Several times during the course of the research the electronics of the instrument had to be worked on by experienced electronics personnel. I am ever grateful to the physics and chemistry electronics shop personnel for helping me keep the instrument going. Without their assistance this work could not have been completed.

The result was a research instrument that looked old

and worn but could carry its own weight and still be used in research. The instrument was checked at one time during the research and 1 part per billion quinine sulfate could be detected above background, which is comparable to many instruments on the market. However, when the instrument was new, 0.001 part per billion could be detected. Late in the research the power supply for the xenon lamp became inoperable and had to be replaced. Certainly if this work can be performed on this instrument it could be reproduced on any other instrument. Using this instrument has taught me that to do good research one does not need elaborate or expensive instrumentation. What is needed is the ability to think and do the best with what one has. Good research is a reflection on the ability of the investigator and not the equipment.

I thank the Chemistry Department for granting me a teaching assistantship and a Johnson scholarship, the State of Oklahoma for granting me fee waivers, and Conoco Inc. for summer scholarships. Without this financial support I could not have attended graduate school and completed this research. I thank Velsicol Chemical Company and Chempar Chemical Company for the gifts of analytical reference standards used in this research.

I thank Dr. Mottola, Dr. Purdie, and Dr. Chandler for serving on my committee. I also thank Dr. Mottola for allowing me the use of the Perkin-Elmer UV-VIS and the assistance of Mr. Mat Gosnell to obtain the absorption data used

in this research. I thank Dr. Chandler for allowing me the use of the MARQ and FRAMP programs. I thank Dr. Powell of the physics department and his post-doctoral research associate Dr. Suchocki for running the fluorescence lifetimes. I thank Dr. Elizabeth Holt for her help and guidance in solving the X-ray crystal structure and for proofing the chapter describing the X-ray work.

I thank Dr. Varga, my research adviser, for his guidance, support and assistance throughout this research. He allowed me the freedom to pursue this research in my own direction and provided guide posts along the way. For this I am truly thankful. He has helped me to develop into an independent researcher.

I thank the chemistry department faculty at Southwestern Oklahoma State University and the National Science Foundation for allowing me to attend a summer science program for high school students in 1975. It was through this experience that I was exposed to the world of chemistry and all the possibilities that awaited me. I also thank them for making quantitative analysis and laboratory work interesting. For these reasons I decided to pursue analytical chemistry as a vocation.

I thank my parents R.C. and Rita Latimer for their support and raising me to appreciate an education. They nurtured my interest in science and allowed me the freedom to pursue my goals. They taught me that if I work hard enough I can achieve anything no matter what I choose to

do.

Finally, I thank my wife Denise for her support while I attended graduate school. She has sacrificed much so that I could pursue this degree. For this I will be ever thankful to her. Without her love and support this work could not have been completed. I dedicate this work to her and our sons Michael and Jeremy. When things appeared to be bleak and at times difficult to continue, it was my family that kept me going. For this I will be ever indebted to them.

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CHAPTER I

INTRODUCTION

Some Aspects of Fluorescence Spectroscopy

Fluorescence Spectroscopy is one of several methods used to study the interaction of matter with light. Fluorometry specifically deals with one of the modes of energy dissipation that species undergo once the absorption of a quantum of light has occurred. In fluorescence spectroscopy the energy dissipation of interest is the re-emission of light which is of lower energy and longer wavelength than the radiation which was absorbed. There are several books which give an excellent discussion of fluorescence theory, practice and application (1-6). Short discussions on fluorescence can also be found (7-9). The following is a brief overview of the fluorescence process and theory.

Every molecule possesses a series of specifically spaced electronic energy levels (S) which are unique to that molecule. Within each electronic energy level there is a series of vibrational energy sublevels (v). At low temperatures most of the electrons in the molecule will lie

in the lowest vibrational sublevel of the lowest (ground) electronic energy level (S_o). In the ground state of most molecules the electron is spin-paired with another electron and this state is called a singlet state.

Upon absorption (A) of a discrete quantum of energy an electron can be promoted to a vibrational sublevel in an upper (excited) electronic energy level (S_1) in which the electron spins are still paired. The molecule then undergoes a deactivation process to return to the ground state. This deactivation process begins with dissipation of energy as heat by vibrational relaxation (VR) and/or intermolecular collisions (CQ) with other molecules until the electron has reached the lowest vibrational sublevel of the first excited singlet state. At this point several modes of deactivation can occur. The molecule can return to one of the various vibrational sublevels of the ground state by fluorescence emission (F) of a photon of lower energy than that of the absorbed radiation. The molecule can undergo further collisions with other molecules (collisional quenching, CQ) to dissipate energy and return to the ground state. The molecule can undergo internal conversion (IC) in which electronic energy is converted to vibrational energy and lost through vibrational relaxation. The molecule can undergo intersystem crossing (ISC) to a vibrational sublevel of a triplet state (T_1) which involves a change in the spin of the excited electron. From this triplet state the electron can return to the ground state

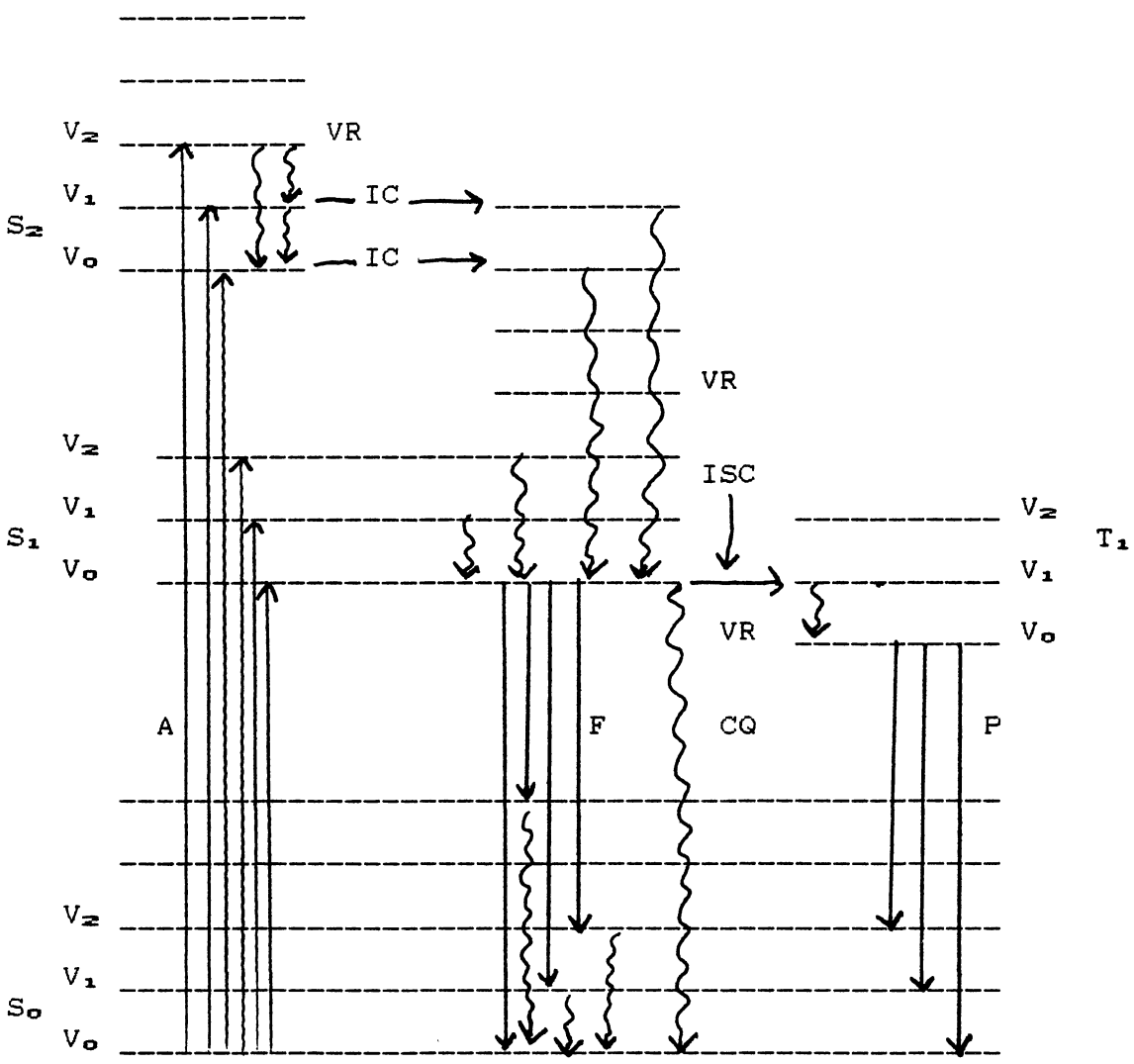


Figure 1. Energy level diagram illustrating the activation and deactivation processes for a typical molecule

by phosphorescence emission (P) of a photon or by collisional quenching.

A measure of how much energy is dissipated in the fluorescence mode of deactivation is the Quantum Yield, ø which is defined as:

ø = Number of Quanta Emitted/Number of Quanta Absorbed.

The quantum yield is a characteristic property of each molecule under specific experimental conditions (temperature, solvent, pH, etc.). As the quantum yield increases the fluorescence emission increases.

To utilize fluorescence emission as an analytical tool for quantitative analysis, a relationship between the fluorescence intensity of the analyte and its concentration must be developed. The Bouger-Beer law of absorption spectroscopy states that the rate of decrease in the radiant power of a beam of light as it passes through an absorbing medium is proportional to the thickness of the absorbing substance, b. The power of the incident beam is given by equation 1.

$$P = E \times \emptyset \qquad (1)$$

Where P is the power of the beam, E is the photon energy in joules per photon, and Ø is the photon flux in photons per sec. per cm^2. The fraction of light transmitted through the medium is given by

$$P/P_o = 10^{-\epsilon bc} \qquad (2)$$

where P_o is the incident power of the light beam on the sample, P is the power of the light emerging from the

sample, ϵ is the molar absorptivity of the absorbing substance, and C is the concentration of the absorbing species in moles per liter. The fraction of light absorbed by the sample is given by

$$1-P/P_o=1 - 10^{-\epsilon bc} \quad (3)$$

which can be rearranged to give

$$P_o - P = P_o (1 - 10^{-\epsilon bc}) \quad (4)$$

The total fluorescence will be equal to the amount of light absorbed multiplied by the quantum yield

$$F_{total} = P_o \phi (1 - 10^{-\epsilon bc}) \quad (5)$$

The intensity of fluorescence which is detected by the instrument is a fraction of the total fluorescence and is given by

$$F_{detected} = k F_{total} \quad (6)$$

where k is an instrument dependent factor based on the instrument geometry, design and electronic considerations. By inserting (6) into (5) the intensity of emission detected is then

$$F_{detected} = k \phi P_o(1 - 10^{-\epsilon bc}) \quad (7)$$

The term

$$1 - 10^{-\epsilon bc} \quad (8)$$

can be expanded into the series expression

$$1-10^{-\epsilon bc} = 2.303\epsilon bC-(2.303\epsilon bC)^2/2!+(2.303\epsilon bC)^3/3!-.. \quad (9)$$

and in dilute solutions where the concentration of the fluorescent analyte is low (where ϵ bC $<$ 0.05) all the terms in (9) except the first are essentially zero and equation (7) reduces to

$$F_{detected} = k \phi P_o(2.303 \varepsilon bC). \quad (10)$$

For quantitative analysis, a plot of $F_{detected}$ versus C is used as a calibration curve. These calibration curves are often linear to four orders of magnitude. This is a much larger range than is normally found in ultraviolet and visible spectrophotometry. This is due to the fluorescence signal being linearly related to the concentration while in spectrophotometry the signal is exponentially related to the concentration.

Fluorometry is able to detect much smaller concentrations of the analyte when compared to spectrophotometry. Limits of detection of 10^{-8} M to 10^{-9}M are not uncommon for conventional fluorometers. Fluorometry is often 10 to 10^3 times more sensitive than other spectroscopic methods. In fluorescence spectroscopy the signal of the very dilute analyte solution is compared to the weak background signal of the blank. In spectrophotometry the signal of the very dilute solution containing the analyte is nearly as large as the signal from the blank solution. It is more difficult in spectrophotometry to accurately detect this small difference in the two large signals than to accurately detect the small difference in the weak signal of the analyte solution and the background of the blank in fluorometry.

Fluorometry is also a very selective tool for qualitative analysis. Only a fraction of all organic molecules exhibit fluorescence and a few metal ions exhibit a native

fluorescence. This allows the determination of a fluorescent analyte in the presence of other absorbing compounds without having to perform a separation on the mixture. This of course assumes that there are no quenching processes which reduce the fluorescence of the analyte in the unknown solution and that there are no unknown synergistic processes which abnormally increase the fluorescence of the analyte.

When more than one fluorescent compound is present in a mixture, selectivity can still be achieved by selecting the appropriate excitation and emission wavelengths such that only the compound of interest is excited and its emission is monitored. There are some cases where the fluorescence properties of two compounds in a mixture are the same with respect to excitation and emission wavelengths. When this occurs there are some procedures which utilize fluorescence to allow the selective determination of one compound in the presence of the other. These procedures make use of the differences in the lifetime of the two compounds. These procedures have been reviewed (10). These methods include time resolved fluorometry, phase resolved fluorometry, selective modulation, total luminescence spectroscopy and synchronous excitation fluorescence.

When the analyte of interest is not fluorescent or only very weakly fluorescent there are steps that can be taken to extend fluorescence spectroscopy to the determina-

tion of these compounds. These include (a) increasing the power of the illumination source, (b) instrument design improvements in optics and electronics, (c) manipulation of the environmental factors such as temperature, solvent, pH, etc., (d) the removal of quenching components, (e) derivatization or functional group transformation or the binding of a highly fluorescent dye to the analyte, and (f) the complexation of metals with chelates which form a fluorescent complex. For a review of derivatization techniques the reader is referred to reference (11).

Factors Affecting the Fluorescence Intensity of an Analyte

The Quantum Yield can also be defined by considering the rates of the several deactivation modes.

$$A^* \longrightarrow A + h\nu \quad \text{Fluorescence} \tag{11}$$

$$A^* \longrightarrow A + \text{heat} \quad \text{IC and CQ} \tag{12}$$

$$A^* \longrightarrow A^3 \quad \text{Intersystem Crossing} \tag{13}$$

The rates of each of the above transitions can be described by the following:

$$\text{Rate}_f = k_f[A^*], \tag{14}$$

$$\text{Rate}_d = k_d[A^*], \tag{15}$$

$$\text{Rate}_x = k_x[A^*]. \tag{16}$$

Where $[A^*]$ is the concentration of the analyte in the excited state. The quantum yield of fluorescence is given by

$$\varphi = k_f /(k_f + k_d + k_x). \tag{17}$$

k_d and k_x are both strongly dependent on the molecular en-

vironment and can be manipulated by adjusting the experimental conditions. The rates of the non-radiative transitions must be decreased in order to achieve the maximum emission from the analyte. Some methods used to reduce these non-radiative transitions are discussed below.

Oxygen Quenching

Oxygen quenching can in some instances cause a large decrease in the fluorescence of a compound (up to as much as 20% when compared to the fluorescence of the compound in the absence of oxygen). A series of mechanisms to account for such a large decrease in intensity has been proposed (12). Consequently there have been a number of proposed procedures to remove oxygen from solutions prior to the taking of the fluorescence measurement. These include purging the solution with an inert gas such as nitrogen for a few minutes (6), subjecting the solution to a series of freeze thaw cycles (13) and chemical scavenging (14,15).

One scavenging procedure employs an oxygen permeable membrane and the ability to generate an oxygen concentration gradient to remove the dissolved oxygen (14). Once the oxygen in the test solution has migrated through the tube it is reacted with Cr(II) which produces an oxygen gradient in the system. The oxygen travels from the area of highest concentration to the area of lowest concentration. However, if the removal of oxygen from around the outside of the tube is the driving force neces-

sary to generate the concentration gradient that the method requires, then it could be possible to remove the oxygen with a stream of dry nitrogen. This would decrease the amount of solutions necessary to perform the deoxygenation and extend the life of the tubing used as the membrane. It could also be that the tubing that is being used for the membrane is acting as a preconcentration vessel which could account for the observation (14) that the fluorescence of the probe decreased during the first few minutes of the experiment and then showed a large increase in fluorescence that was attributed to the removal of oxygen. This preconcentration theory is viable because there is a paper in the literature that outlines the use of tubing of tygon or rubber as a preconcentration vessel (16).

The other procedure that has been published uses zinc metal in the test solution to react with dissolved oxygen to increase the fluorescence of several analytes in solution which are separated using HPLC (15). This method however runs the risk of adding zinc ions to the solution which could quench the fluorescence of other analytes.

Temperature Effects

The temperature of the test solution is also an important consideration in fluorescence spectroscopy. It has been shown that fluorescence intensity reductions of fifty percent is possible if the test solution is warmed ten degrees centigrade (17). The small increase in temperature

can dramatically increase the amount of intermolecular collisions which result in quenching. Temperature increases of a few degrees are possible when the test solution is left in the light path of the fluorometer for just a few minutes causing errors in the fluorescence measurement.

Temperature increases can also decrease the fluorescence of an analyte in solution by inducing intersystem crossing to a triplet level. This occurs when the T_2 energy level is very close in energy to the S_1 energy level. This is most generally found in substituted anthracenes (18,19).

Solvent Considerations

The choice of solvent plays an important part in the fluorescence of an analyte in solution. Dipole dipole, dipole induced dipole, and hydrogen bonding interactions between the analyte and the solvent affect the fluorescence intensity of the analyte. The attraction of the solvent by the analyte in the excited state is reflected in the fluorescence of the analyte and the position of the excitation and emission maxima. When there is a strong interaction there is generally quenching via increased intermolecular collisions. There are examples in the literature which show that the proper choice of solvent is mandatory in order to achieve maximum emission (20, 21). Not only does the intensity of the emission change with each solvent but also the position of the peak maximum and the shape of

the curve (22). For these reasons a choice of solvent must be made which insures that the maximum fluorescence of the analyte is observed.

In order to decrease the solvent-solute interaction and the resulting quenching, there have been reports of using inclusion complexes where the analyte is isolated inside the cavity of a cyclodextrin, crown ether, cryptand or micelle (23-32). In each of these inclusion complexes there must be a match between the internal volume of the cavity and the size of the analyte. Also to be considered is the ability of the analyte to be incorporated into the cavity.

Excitation Source

As can be seen from equation (10), the fluorescence of an analyte is directly proportional to the illuminating power of the lamp. Lasers are being investigated as high intensity sources for fluorometry. Lasers are capable of providing an average power output of 5 watts or more (33). By comparison a 100-W mercury arc lamp at 254 nm provides 4.5 mW, a 50-W deuterium lamp at 230 nm provides 0.9 mW, and a 100-W quartz halogen tungsten lamp at 600 nm provides 17.3 mW of power (34). Detection limits of 10^{-11}M to 10^{-12}M are not uncommon for laser based fluorometry (35).

Laser sources are generally restricted to line illumination however. Therefore one must have a series of lasers in order to have the entire range of absorption

regions covered or only use the laser for special circumstances when a laser of the appropriate wavelength is available. Dye lasers can provide a continuum of 40 to 50 nm but they suffer from a small power loss when compared to the power of the pump laser. For these reasons lasers are very seldom used to obtain excitation spectra.

The laser light is highly collimated permitting focusing of the beam on extremely small samples (36-42). Lasers are finding application in investigating the fluorescence of small volume samples and in the design of fluorometric detectors for liquid chromatographs where the detector volume is as small as possible. High collimation is also responsible for low amounts of stray light when compared to other types of light sources. This high collimation decreases the amount of stray light and background noise considerably.

Solvent scatter of the light is quite intense when a laser is used as the source. For this reason laser techniques are considered to be blank-limited (43). Solvents must be of high purity to reduce the background emission of blank solutions. The application of time-resolved fluorometry in conjunction with laser excitation is used to decrease the background scatter and fluorescence of the solvent and any interferents that may be present in the solution. Time-resolved laser fluorometry is also used in the determination of the components in mixtures where the excitation and emission bands of the compounds in the mix-

ture overlap and there is a difference in their lifetimes (44,45).

Caution must be used in laser fluorometry because of the high power output. Photodecomposition of the analyte is possible when the test solution is subjected to the laser for long periods of time. Also one must be wary of thermal lensing distorting the emission of the solution and taking it out of the focus of the collecting optics of the instrument.

Some Aspects of Qualitative Analysis and Data Reduction in Fluorescence Spectroscopy

Fluorometry is primarily used as a quantitative tool and very seldom as a qualitative tool. This is because not all compounds exhibit fluorescence and because there is at present no correlation charts that correlate the excitation and emission peak positions to a functional group. Infrared spectroscopy, UV-Visible absorption spectrophotometry, NMR, and mass spectroscopy all have correlation charts or a set of empirical rules to interpret spectra and elucidate structures. It would be desirable to have some sort of correlation between fluorescence and structure because fluorescence is able to detect much smaller quantities (of a fluorescing compound) than any of the other above methods which are currently used routinely in structure determination with the exception of mass

spectroscopy.

At first glance one would think that a comparison of the spectrum of an unknown to a library of spectra would be the way to elucidate the structure of a compound. A compendium of library spectra is available at present which would allow an investigator to compare observed fluorescence characteristics to the library and extract some structural information. This entails the point by point comparison of the unknown to each entry in the library. This process can be very time consuming considering the number of data points that make up an excitation or emission spectra. Even utilizing a computer to perform the point by point comparison would require a significant amount of time. For this reason data reduction procedures are being developed (46-52). The procedures systematically analyze the spectrum and break it down to a series of parameters, the number of which is smaller than the total number of points in the excitation and emission spectra.

An early attempt to reduce data for comparison to library entries involved fitting the excitation curve to a polynomial (46). The fitting could be done on single banded spectra such as with quinine with good results but the method failed to fit more complex spectra. The author then resorted to the point by point comparison of the observed spectrum to the library entries.

The identification procedure was speeded up by first comparing the most obvious spectral features visually to

narrow down the library entries which were to be compared to the unknown. The library was limited to chelates of rare earths (46).

A procedure was later published in which the fluorescence spectrum of other compound types was analyzed, again using the most obvious spectral features for identification (47). An extensive compendium of spectra was condensed and cataloged. Each compound was cataloged according to the number of peaks in the excitation and emission spectra, the peak locations, the relative peak intensities, and the width at half height for each peak. To check the comparison and identification procedure some of the library entries were entered as unknowns. A computer program then compared the unknown characteristics to each of the library entries. The comparison results were good with the algorithm returning the correct compound name for the unknown. When the fluorescence spectral characteristics were determined experimentally for a known library entry the algorithm returned the correct identity most of the time, however there were also some other compounds returned as being a possible candidate for the structure. The authors state that when more than one possibility was returned, each of the structures had some similarity of structure. It must be noted that the fluorescence spectra were not corrected in either the library or the experimentally determined spectra. This is probably the reason the computer returned several entries as possible structures.

Alkylated phenols tended to give the algorithm the most trouble in returning the correct identity. However the compounds returned by the program as possible structures were all alkylated phenols or ethers. This was also the case when a compound was entered as an unknown and its fluorescence characteristics were not in the library. The algorithm returned several possible structures and each had some similarity of structure to the unknown. This procedure also shows that it is not necessary to have corrected spectra in order to compare the experimentally obtained spectra to the library. Even though there are differences in instrument design that affect the shape of the fluorescence excitation and emission curves there is still enough similarity to allow comparisons from one instrument to another.

The authors do not indicate how to handle shoulders on major peaks. It would be difficult to assign a width at half height in these instances.

In a later paper the same authors (48) use a Fourier transform procedure to reduce the data that describe the spectra. The first twenty-one pairs of the real and imaginary components of the Fourier transform of the excitation and emission spectra were used to construct the library used for comparisons. When the ring-alkylated phenols were input as unknowns the program correctly identified them.

It was found that when the Fourier transformed data (just the twenty-one pairs) was inverse transformed the

resulting spectrum was essentially an undistorted reconstruction of the original spectrum. It has been shown that in Fourier transformed data, most of the information about the bulk structure of the curve is contained in the first few pairs of the Fourier transform and the details of the fine structure is contained in the remainder of the pairs (49). The authors also state that for single peaked broad-band curves the data could be further reduced by retaining only nine pairs of the Fourier transformed components.

Another method of reducing data and constructing a library consists of fitting the spectrum to a series of bi-gaussian equations (50,52). Each spectrum is fitted with more equations than there are peaks, and there was usually one more equation than there were peaks. This allows the extra equation to fill in and completely fit the observed spectrum. In a later paper the authors reduced the number of equations to fit single peaked fluorescence spectra (51). They indicated that over-fitting these peaks led to extended computer time to fit the peak and that the least square equation used in the fitting equation did not converge when using multiple equations to fit the single peaked spectrum.

The authors also stated that it is probably advantageous to use the most obvious spectral features to narrow down the possibilities for a structure and then use the fitting procedure to identify the unknown from the pos-

sibilities given in the first search.

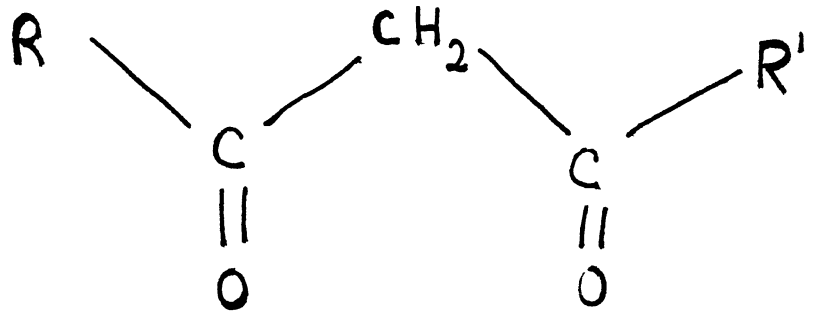
An underlying theme that runs through the above attempts to correlate structure is that each one uses the most obvious spectral features to reduce the fluorescence data and then perform a search based on a second system of data reduction or comparison. Also it is shown that there is no need for a library to contain corrected spectra nor to correct the experimentally obtained spectra.

It is not implied that using fluorescence for qualitative identification is all that should be in the arsenal of the chemist for identification of unknowns. Fluorometry is still in the infant stages of qualitative analysis. Its primary advantage over other methods of qualitative analysis is in its ability to detect much lower concentrations and give good spectra for qualitative analysis.

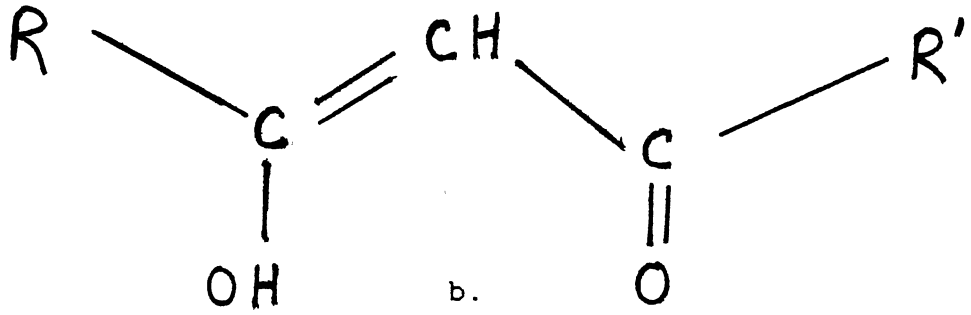
Europium-Sensitized Fluorescence

Europium-sensitized fluorescence (sometimes classified as energy transfer sensitized fluorescence) couples a strongly absorbing but very weakly fluorescing organic compound (called the donor) with the strongly fluorescing europium(III) ion (called the acceptor). An example is the europium(III) ion complexed with an organic compound that has a β-diketone functionality.

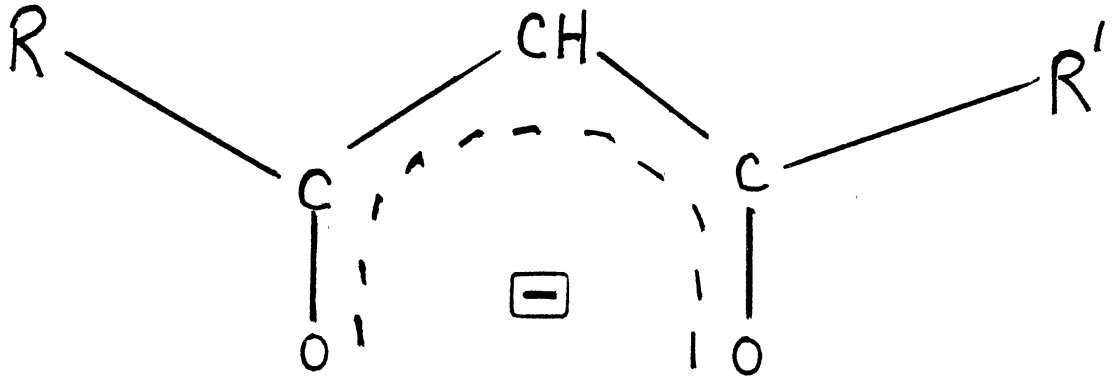
The general structure of a β-diketone is shown in Figure 2. Also shown is the enol form which exists in equilibrium with the keto form. The ratio of keto to enol



a.



b.



c.

Figure 2. Structure of a β-diketone (a.) β-diketone, (b.) enol form (c.) enolate anion formed after removal of the acid proton.

form is dependent upon the R groups (53,54). Also shown in Figure 2 is the enolate anion that is formed upon removal of the acid proton.

The mechanism of europium-sensitized fluorescence is shown in Figure 3. The organic ligand is excited with the appropriate wavelength of light to reach a vibrational sub-level in the first excited singlet state. At this point the organic ligand vibrationally relaxes to the lowest vibrational sublevel and undergoes intersystem crossing to the triplet state where the ligand further releases energy to reach the lowest vibrational sublevel. Energy is then transferred to the europium (III) ion which then emits its characteristic 613 nm emission.

Organic ligands with a carbonyl functional group have been known to undergo intersystem crossing quite easily resulting in their lack of fluorescence or very little fluorescence if at all (6). The ligand is in very close proximity to the europium (III) (approximately 2.3Å). Energy transfer to the europium occurs quite readily if the triplet level of the ligand is just above or very near a resonance level of the metal. This phenomenon was first discovered by Weissman in 1942 (55). Others have investigated the process and proved in fact that the mechanism is a ligand triplet to europium energy transfer (56-59). The fluorescence intensity of these complexes is quite high in some cases. The approximate triplet level of some ligands are given in Appendix C. The levels were placed by

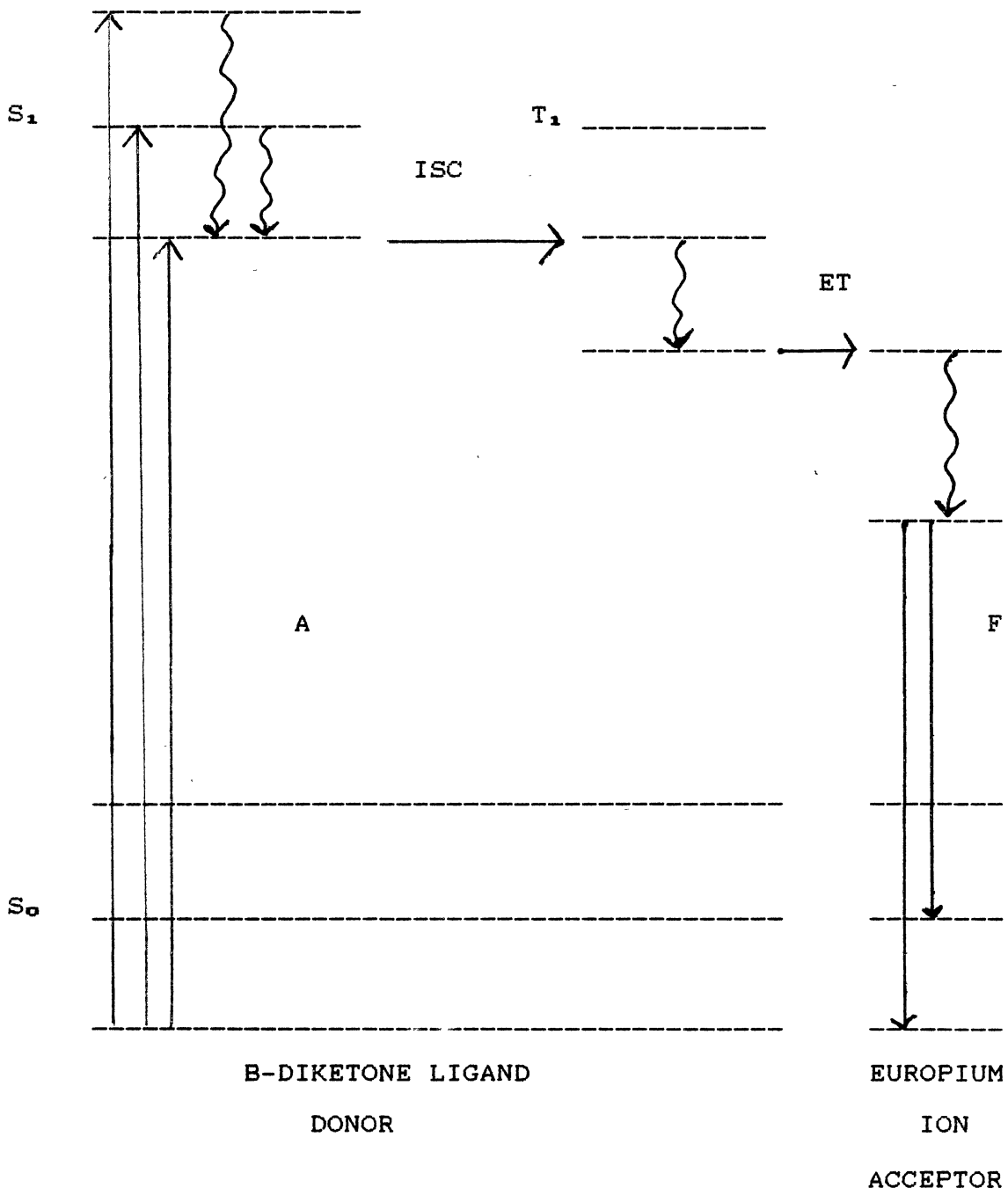


Figure 3. Energy transfer process in europium-sensitized fluorescence.

the presence or absence of rare earth fluorescence upon complexation with the ligands.

The 613 nm europium emission is from the $^5D_0$ to $^7F_2$ 4f energy level of the Eu^{3+}. Selection rules indicate that $J \leq 6$ for a transition to be allowed. However when either the initial of final J value is 0 then Δ J will be 2, 4 or 6 for the transition to be highly probable (67). For europium (III), J initial = 0 and Δ J = 2 for this transition. The emission is very strong and line-like. This line-like emission is due to the 4f levels being buried deep within the atom and the broadening effect of ligand vibrations is minimized.

Other workers have shown that the europium ion can accommodate as many as eight oxygens in its outer coordination sphere and that the stoichiometry of the complex is usually three bidentate ligands to one europium with the other coordination sites occupied by oxygen atoms from water molecules (60). The very intense emission observed is due to the energy transfer from three absorbing ligands to one europium. The amount of energy can be quite large especially when the molar absorptivity of the ligand is very large. Another aspect that makes the emission quite intense is that the emission of the europium is narrow and almost line-like in appearance. This acts to channel the broad absorption of the ligand into a very narrow emission band. Ligands are being specifically designed to have very large molar absorptivity, large broad banded absorption,

and to match the triplet energy level of the ligand to the resonance energy level of the europium (61).

As stated above the ligands generally provide six oxygens to the europium which leaves two coordination sites still available. The coordination sites are usually occupied by water molecules which are either carried along in the solvent or picked up from the atmosphere (62). In some cases a nitrogen from an organic base such as pyridine will occupy these extra sites (63). It has been found that when these extra sites on the europium are occupied by oxygen atoms from a trialkyl phosphine the emission of the complex increases dramatically (60,64). This synergistic effect of increasing the fluorescence emission of the complex is due to the added rigidity of the complex when there are trialkyl phosphine oxide molecules in the outer coordination sphere and the removal of the small water molecules that can dissipate energy through molecular vibrations.

This process has been widely used in the determination of europium. Detection limits of 10^{-11}M for europium are possible (65). Only recently has this process been used in the determination of the ligand (66). Tetracyclines are organic molecules that contain a β-diketone structure and are able to complex europium (66). The tetracyclines are themselves fluorescent, however upon complexation with the europium the energy transfer mechanism produces quite intense fluorescence. Detection limits for the tetracyclines are on the order of 10^{-9}M which is one order of magnitude

lower than any other fluorescence method for their determination. The linear dynamic range for the determination is four orders of magnitude from 10^{-8}M to 10^{-4}M. This work was done in aqueous solution buffered at pH 7.2. The authors did not investigate the use of nonaqueous solvents which may increase the emission further and lower the detection limit. Furthermore, the stoichiometry of the complex was determined to be 1:1. In nonaqueous solution perhaps the stoichiometry could be changed to 2:1 or 3:1 and increase the emission further.

Another aspect of europium sensitized fluorescence that makes it attractive for the determination of important ligands which have the β-diketone functionally is that it is most fluorescent when complexed with β-diketones. Complexes with other ligands do not exhibit the intense emission as is observed with β-diketones. Also the emission of the europium (III) at 613 nm is spectrally removed from where other compounds fluoresce. This allows the determination of the ligand in the presence of other fluorescing compounds without having to perform a separation on the mixture. To date the only application of europium sensitized fluorescence to the determination of the ligand is that of the determination of tetracyclines (66). Clearly there is much work to be done to identify other ligands of importance which have a β-diketone functionality which may be determined by utilizing europium sensitized fluorescence.

Some Final Introductory Comments on Fluorescence Spectroscopy

Fluorometry is a rather simple analytical tool yet is quite complex. Simple when all the analyst has to do is insert a cuvet containing the analyte into the cell compartment of the instrument and record the fluorescence on a strip of chart paper, yet complex when one must be mindful of all the possible interactions that can affect the fluorescence of the analyte and in the long run affect the outcome of the experiment. Fluorescence is also complex when trying to elucidate the structure of an unknown from its fluorescence spectrum.

When designing an analytical procedure that relies on the measurement of a fluorescence signal, the analyst must be aware of all the possible things that can affect the fluorescence of the analyte or at least be suspect of interactions that can affect the measurement. As in all of science the experiment is only as good as the experimentalist regardless of the type of instrumentation or technology available.

Literature Survey of the Determination of Diphacinone, Chlorophacinone, and Pindone

Diphacinone (2-diphenylacetyl-1,3-indandione),

chlorophacinone [2-(2-p-chlorophenyl)phenyl-1,3-indandione], and pindone (2-pivaloyl-1,3-indandione) are substituted 1,3-indandiones which are the active ingredients in some commercial rodenticides. LD_{50} values of 2.31 mg/kg for diphacinone, 20.5 mg/kg for chlorophacinone and 50 mg/kg for pindone for rats and mice have been determined (68-70). They each have a β-diketone structure and spectroscopic and x-ray crystallographic evidence indicates that they exist predominantly in the enol form (54,71,72). They are available for use as tracking powders, grain mix baits, paraffinized pellets, wax bait blocks or cubes, and mineral oil concentrates. Typical concentrations of these poisons in the commercial products range from 0.005% to 2.0%.

Very sensitive methods for the determination of these compounds is necessary for several reasons. The manufacturer needs a quality control procedure to insure that the content of the active ingredient in the bait that is sold to the public is correct. It is necessary to have a sensitive method of determination in order to follow the metabolism of the poison in the rodent or other animals that may ingest the poison, to have a sensitive method in order to follow the poison down the food chain and to have a sensitive method for use in forensic and clinical investigations.

Most methods published rely on a three step procedure for the determination of these compounds in a wide variety

of samples. These steps are solvent extraction, chromatographic separation and absorption spectroscopy for the quantitative determination. Most of the published methods usually require from two to six hours for the extraction, twelve minutes to several hours for the chromatographic separation and spectroscopic determination.

Early attempts to determine the diphacinone and pindone content of baits utilized extraction of the bait with ether for four hours (73). The extract solution is evaporated to a small volume and extracted with a 1% sodium pyrophosphate. In the pyrophosphate solution the indandiones are converted to the sodium salt. The absorption of the solution is then read at an appropriate wavelength and compared to a series of standards. This method suffers from interferents which are also extracted and which have an absorption at the same wavelength.

In an attempt to reduce the background interference in the determination of diphacinone in biological materials, a phosphorescence method was developed (74). Since diphacinone undergoes intersystem crossing to the triplet state so readily, it does have some phosphorescence emission and very little fluorescence emission. In ethanol diphacinone has an absorption maximum at 295 nm and a phosphorescence maximum at 475 nm. The resulting procedure gives a limit of detection of 1 mg L^{-1}.

A gas chromatographic procedure for the determination of diphacinone and chlorophacinone in baits and biological

materials has been developed (75). In this procedure the material to be analyzed is mixed with sodium sulfate and freeze dried. It is then extracted by shaking with acidified acetone containing a small amount of ascorbic acid for ten minutes. The extract is cleaned up by partitioning between sodium hydroxide and methylene chloride. The methylene phase which contains the active ingredient is evaporated to dryness and the residue is reacted with chromium trioxide to produce benzophenone and p-chlorobenzophenone which is extracted into hexane and cleaned up by washing with water. The hexane solution was evaporated to a small volume and the derivatives separated by gas chromatography and detected with an electron capture detector. The recovery from spiked samples ranged from 50% to 77%.

The authors also report that the sample can be partitioned between acetonitrile and hexane after the acetone extraction. The chlorophacinone and diphacinone are contained in the acetonitrile fraction and analyzed by spectrophotometry measuring the absorbance at 325 nm.

This procedure is long and depends on several extraction and cleanup steps and a derivatization step which can result in the loss of sample during the determination. The efficiency of the conversion of chlorophacinone and diphacinone to benzophenone and chlorobenzophenone was found to be inversely proportional to the diphacinone and chlorophacinone content of the sample.

A collection of extraction and determination procedures has been compiled which outline in detail the steps for the determination of several substituted 1,3 indandiones and other rodenticides (76). This publication describes many multi-step extractions and chromatographic separations. The publication however does not report any experimental results as to the reliability of the procedures, pitfalls, interferences, or expected error in the determinations.

A simple procedure for the determination of substituted indandiones in baits and biological materials utilizing high pressure liquid chromatography has been reported (77). The indandione is extracted from the sample with acetonitrile which contains 4% potassium chloride. The sample is shaken with the extraction solvent for two hours followed by centrifugation and cleanup by extraction with iso-octane which is discarded. An aliquot of the acetonitrile is taken and separated on a ten centimeter RP-18 column with a gradient elution program. The solvents are acetonitrile, 1.5% acetic acid adjusted to pH 4.7 with ammonium hydroxide, and water adjusted to pH 10 with ammonium hydroxide. Detection is made by monitoring the ultraviolet absorption at 283 nm. Recovery from commercial baits ranged from 100% to 86%.

Another high pressure liquid chromatography procedure for the determination of chlorophacinone in mice that have been poisoned has been reported (78). In this procedure

the whole mouse is homogenized and freeze-dried. A sample is then taken and extracted with acetonitrile. The extract is concentrated by evaporation and cleaned up on a Florisil column by elution of the impurities with dichloromethane followed by acetonitrile. The chlorophacinone is then eluted with methanol. The methanol fraction is evaporated to concentrate the solution and injected onto a lichrosorb NH_2 column and eluted with a solvent made of 80/20 acetonitrile/water. Detection is made by monitoring the absorption at 254 nm. The chromatograms are quite complex and the chlorophacinone peak is not well resolved from other eluting compounds. The reported linear dynamic range is from 0-80 ng. Spiked samples showed recovery of greater than 95%.

The chlorophacinone and diphacinone content of paraffinized baits has been determined by extraction with glacial acetic acid followed by separation on a Partisil PXS ODS column (79). The mobile phase consisted of a mixture of glacial acetic acid-tetrahydrofuran-water (14:2:9). Detection was made by monitoring the absorption at 288 nm. Calibration plots for both chlorophacinone and diphacinone were linear from 1 to 40 mg L^{-1}. The average recovery from spiked samples was 96%.

A paired ion high performance liquid chromatography separation has been published in which the diphacinone in a bait concentrate is extracted by shaking with methanol for thirty minutes (80). An aliquot of the extract is mixed

with a pairing ion buffer and separated on a Partisil 10 ODS column. The mobile phase was a mixture of methanol and an aqueous solution of sodium dihydrogen orthophosphate and tetrabutylammonium hydroxide with the pH adjusted to 7.5 with sodium hydroxide. Detection was made by monitoring the absorption at 286 nm. The recovery from spiked samples ranged from 90 to 95%. Recovery decreased with decreasing diphacinone concentrations.

The Environmental Protection Agency has also published a procedure for the determination of diphacinone (81) and chlorophacinone (82) in technical samples, but the report gives no details about either the linear range of the procedure or the limit of detection. The procedure also gives no details of how to prepare bait samples for analysis.

A very complex extraction and multiple step chromatographic separation and determination of chlorophacinone in animal tissues has been published (83,84). The freeze dried tissue is extracted with acetonitrile or it is homogenized with either acetone or a 1:1 mixture of chloroform and acetone. The extract is cleaned up by passing it through a column packed with Bio-Beads SX-3. This gel permeation chromatography is not efficient enough to separate the chlorophacinone from the fats and lipids which are also extracted. Further cleanup is made by passing the extract through a Sep-Pak cartridge of either silica or C-18 reversed phase. Dichloromethane acidified with formic acid then elutes the chlorophacinone

from the cartridge. The chlorophacinone was further separated from other compounds by separation on a C_{18} column. Detection was made by monitoring the UV spectrum taken on line with a linear diode array detection system. The limit of detection at the detector is approximately 200 pg and the response was linear up to 1 ug.

Diphacinone and its metabolites have been extracted from biological material and separated on silica gel G TLC plates (85). in this study the diphacinone was tagged with ^{14}C. The diphacinone and its metabolites are extracted from liquid samples with ethyl acetate and from solid samples with methanol. Solids were further extracted by refluxing with a solution of boric acid at pH 9.0. This solution was then extracted with methanol. Tissue and organ samples were extracted with methanol. The methanol was evaporated and the solid residue that remained was partitioned between water and dichloromethane. The dichloromethane fraction was then dried over anhydrous sodium sulfate. Extract solutions and wash solutions were radioassayed. An aliquot of the dichloromethane solution was evaporated to dryness and the residue taken up in acetonitrile for TLC separation. By isolating the radioactive spots on the TLC plate the metabolites were identified by mass spectroscopy. Non radioactive spots were located by viewing under UV light. The metabolites were identified by mass spectroscopy. It was found that 20% of the diphacinone is eliminated from treated rats and mice

unchanged. The metabolites isolated were hydroxylated diphacinone compounds. Each metabolite retained its B-diketone functionality with the hydroxides being added to various places on the aromatic rings.

A manufacturers procedure for the determination of chlorophacinone is available (86). Mineral oils, tracking powders, grain baits, pellets, and wax bait block samples are extracted with ethyl acetate by ultrasound for 2 hours. To insure that the extraction is complete a second extraction is performed on the sample. The ethyl acetate extracts are combined and filtered to remove any suspended particles in the solution. The solvent is evaporated almost to dryness with a rotary evaporator, then transferred to a volumetric flask for final evaporation to dryness. The oily residue is then taken up in a small portion of a chromatographic mobile phase. The mobile phase is a mixture of methanol, water, and 0.01M disodium phosphate (65%:15%:20%).

The sample is chromatographed on a silica column grafted with a NUCLEOSIL 5u column 15cm in length. Detection is made by monitoring the absorption at 286 nm. The technical bulletin gives no details about the extraction efficiency, the limits of detection, or any problems encountered with the determination.

Impetus for the Research

In each of the procedures discussed above for the

determination of each of the substituted indandiones, one notes that they are all long processes requiring several cleanup steps after the extraction or a chromatographic procedure to separate the indandione before the spectrophotometric determination. It is desirable to develop a procedure that could eliminate the cleanup steps so that the analysis time is cut down and eliminate steps in which some of the sample is lost due to inefficiencies in the cleanup procedure. It is also desirable to eliminate the chromatographic separation if possible in the analysis of less complex samples such as in oil concentrates and tracking powders. It is also desirable to develop some type of identification procedure based on the fluorescence properties of the compounds to be applied when only small amounts of the compounds are available.

As noted earlier these substituted 1,3-indandiones that have a β-carbonyl exist primarily in the enol form. It is also known that each of these compounds form alkali metal salts which indicates that they can be made to give up their acid proton to form an enolate anion. Enolate anions are ligands that are known to form complexes with the europium (III) ion. These complexes are known to be highly fluorescent. This makes the β-carbonyl substituted 1,3-indandiones prime candidates for the application of europium-sensitized fluorescence to their determination. These compounds are of analytical interest as evidenced by the number of papers geared toward their extraction and

determination.

These considerations provide the background for the experimental sections that follow. The goal will be the development of new methods of determination for β-carbonyl substituted 1,3-indandiones that are simpler, faster, and more conservative in the handling of samples to reduce loss of the analyte prior to the determination step. The method should also have a lower limit of detection and longer linear dynamic range than those published. The method developed should also be free from interferences such that the analysis can be performed without having to perform a separation of the analyte from the interfering compounds. Furthermore the method should have a high degree of reproducibility and the ability to provide qualitative information as to which indandione is present.

CHAPTER II

EUROPIUM-SENSITIZED FLUOROMETRIC DETERMINATION OF CHLOROPHACINONE, DIPHACINONE AND PINDONE STANDARD SOLUTIONS

As discussed in Chapter I, there is a need for a sensitive, quick and inexpensive method for the determination of β-carbonyl substituted 1,3-indandiones that is free from interferences. Described here is the application of Europium-Sensitized fluorescence to the determination of these compounds, the optimization of the conditions for the fluorescence, and control of the stoichiometry.

Experimental

Apparatus

All fluorescence measurements were made using a Farrand Spectrofluorometer (Farrand Optical Co., New York) which has been described previously (46). A type 117 synchronous motor (Cramer Controls Corp., Centerbrook, CN) was added to the fluorometer to drive the excitation and emission monochromators. The source is a Hanovia 150W xenon arc lamp and the detector is an RCA type 4840 PMT

(RCA, Lancaster, PA). High voltage was supplied to the PMT by a Model 204 High Voltage Power Supply (Pacific Precision Instruments, Concord, CA). The output of the PMT was amplified by a Model 417 High Speed Picoammeter (Keithly Instruments, Cleveland, OH) and recorded on a strip chart recorder. Absorption measurements were made with a Perkin-Elmer UV/VIS Lambda Array model 3840 (Perkin-Elmer, Inc., Norwalk, CT). Cuvets for fluorescence and absorbance measurements were all quartz with 1 cm path length (Scientific Cell Company, New York) including one 8 uL quartz high pressure micro flow cell (NSG Precision Cells, Hicksville, NY). Solutions were mixed using an ultrasonic bath (Cole-Parmer, Chicago, IL). A mini-pulse 2 peristaltic pump (Gilson, Middleton, WI) was used as described in the procedure section.

Chemicals

Acetonitrile, acetone, and chloroform (Fisher, certified ACS), pyridine (Fisher, technical) and concentrated nitric acid (Fisher, reagent ACS) were used as received, The water used was deionized and further purified by distillation in an all glass still with a quartz immersion heater. Pindone 97% minimum purity (Fluka, Hauppauge, NY), diphacinone 99.2% (Velsicol Chemical Corporation, Chicago, IL) and chlorophacinone 99.35% (Chempar Chemical Company, New York) were used as standards. Europium oxide 99.99% (Pfaltz and Bauer, Stamford, CN) and trioctylphosphine

oxide (Eastman Organics, Rochester, NY) were used as received.

Procedure

Reagent Preparation. A stock solution of 0.0100M $Eu(NO_3)_3$ in acetonitrile was prepared by dissolving 0.4400 g of the oxide in approximately 3 mL concentrated nitric acid and 5 mL water. The solution was warmed gently on a hot plate and the solution was quantitatively transferred to a 250 mL volumetric flask and diluted to the mark with acetonitrile.

A 0.036M stock solution of trioctylphosphine oxide was prepared by dissolving 7.02 g of the reagent in 100 mL of acetonitrile with gentle heating on a hot plate, Once the reagent was dissolved it was quantitatively transferred to a 500 mL volumetric and diluted to the mark with acetonitrile.

Stock solutions of diphacinone, chlorophacinone and pindone were prepared by dissolving a weighed amount of the standard (0.16 to 0.20 g) in 50 mL of acetonitrile using ultrasound to agitate the solution. The solution was quantitatively transferred to a 250 mL volumetric flask and diluted to volume with acetonitrile.

Less concentrated solutions and mixtures of the ligands and europium were made by serial dilution of these stock solutions.

Fluorometric Titrations. Titrations were performed using a class A buret. A measured amount of the ligand was placed in a 100 mL beaker. To the ligand was added 1 mL of the trioctylphosphine oxide reagent, 0.5 mL pyridine and the total volume adjusted to approximately 50 mL with acetonitrile. The solution was stirred during the titration, and the peristaltic pump was used to circulate the solution from the beaker to the fluorometer through the micro flow cell. Europium was added to the ligand from the buret, and the fluorescence was measured and allowed to stabilize before the next addition. Titrations of the europium with the ligand were performed in a like manner by placing a measured amount of the europium in the beaker along with the trioctylphosphine oxide and the pyridine. The ligand was added from the buret and the fluorescence measured.

Results and Discussion

Optimization of Fluorescence

The ligands themselves are only weakly fluorescent with the broad band emission centered around 520 nm. This fluorescence is not analytically useful due to concentration quenching and inner filter effects. Upon complexation with europium a very intense fluorescence is observed. The excitation and emission spectra for the diphacinone-europium complex are shown in Figures 4 and 5

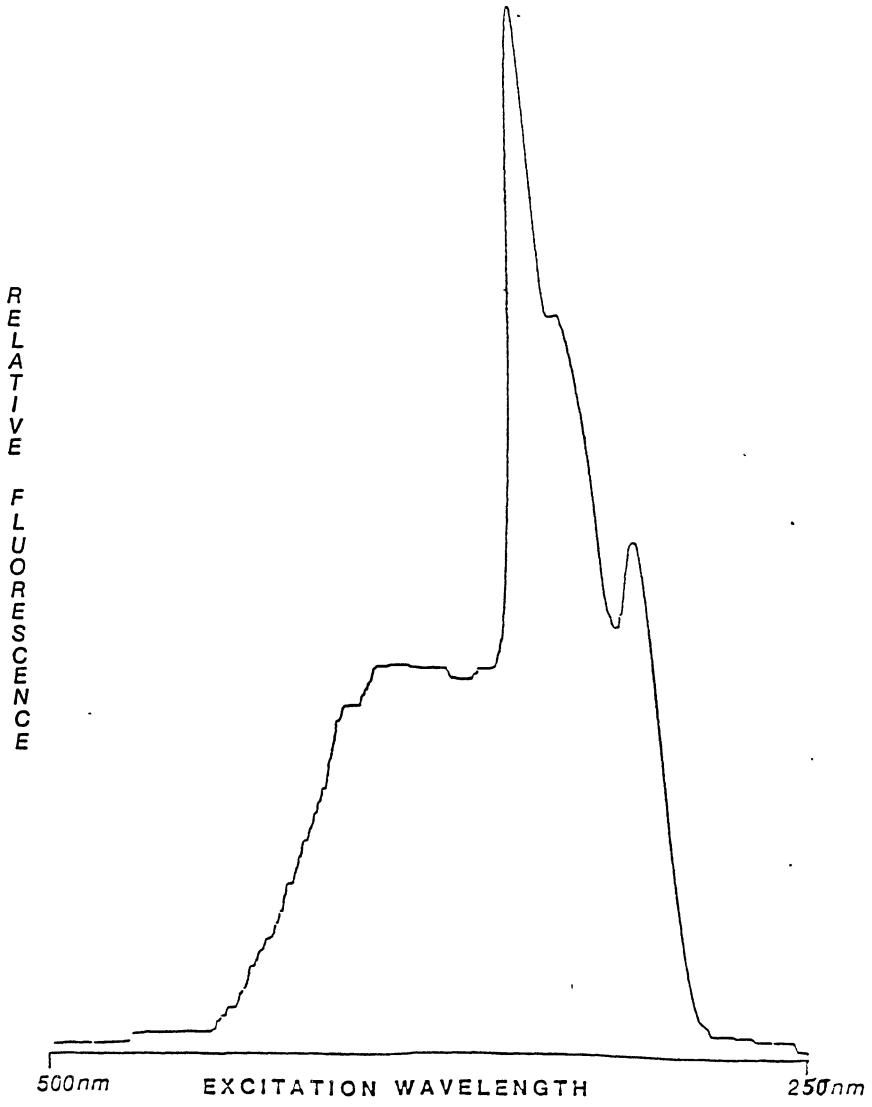


Figure 4. Excitation spectrum of the europium-diphacinone complex. Conditions: 7.88×10^{-6} M diphacinone, 1×10^{-4} M europium, 7.2×10^{-4} M trioctylphosphine oxide, 1% pyridine in acetonitrile. Emission wavelength 613 nm. Excitation max. 328 nm.

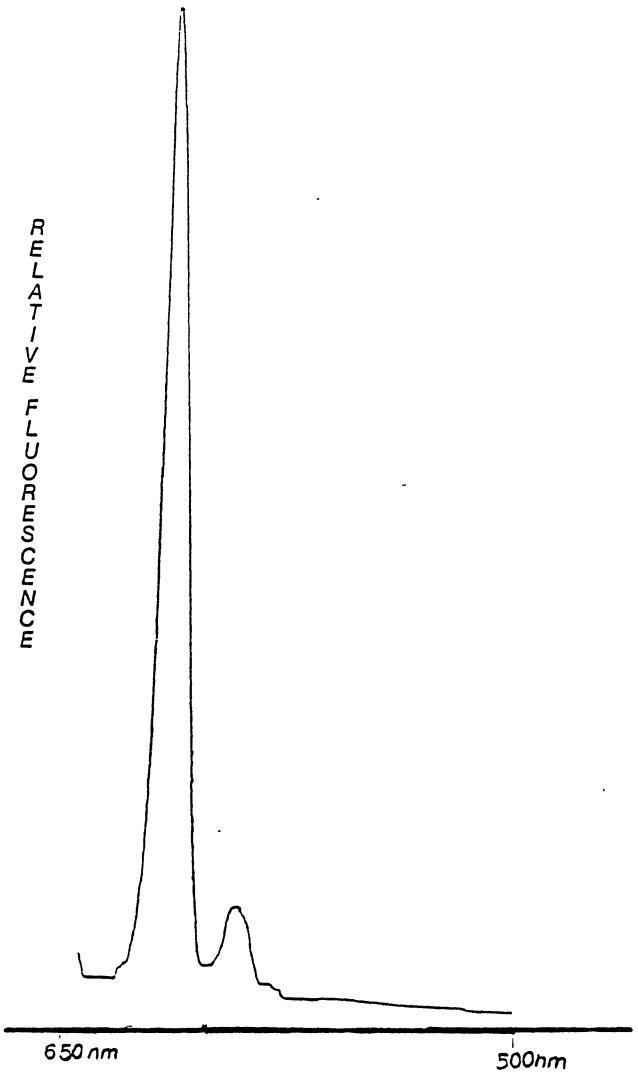


Figure 5. Emission spectrum of the europium-diphacinone complex. Conditions are the same as Figure 4. Excitation 328 nm. Emission max. 613 nm.

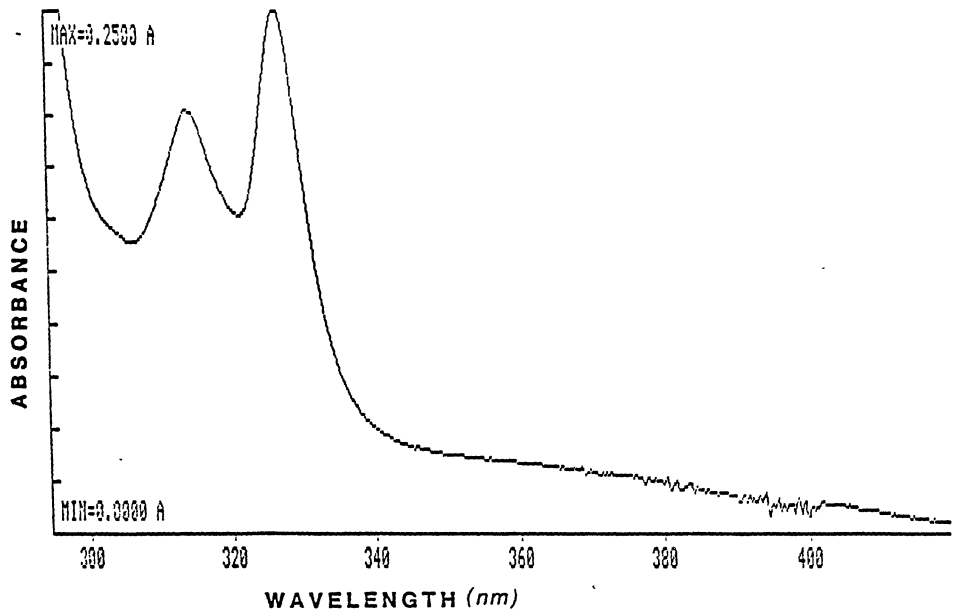


Figure 6. Absorption spectrum of the europium-diphacinone complex. Conditions: 1.58×10^{-5}M diphacinone, 1×10^{-4}M europium, 7.2×10^{-4}M trioctylphosphine oxide, 1% pyridine in acetonitrile.

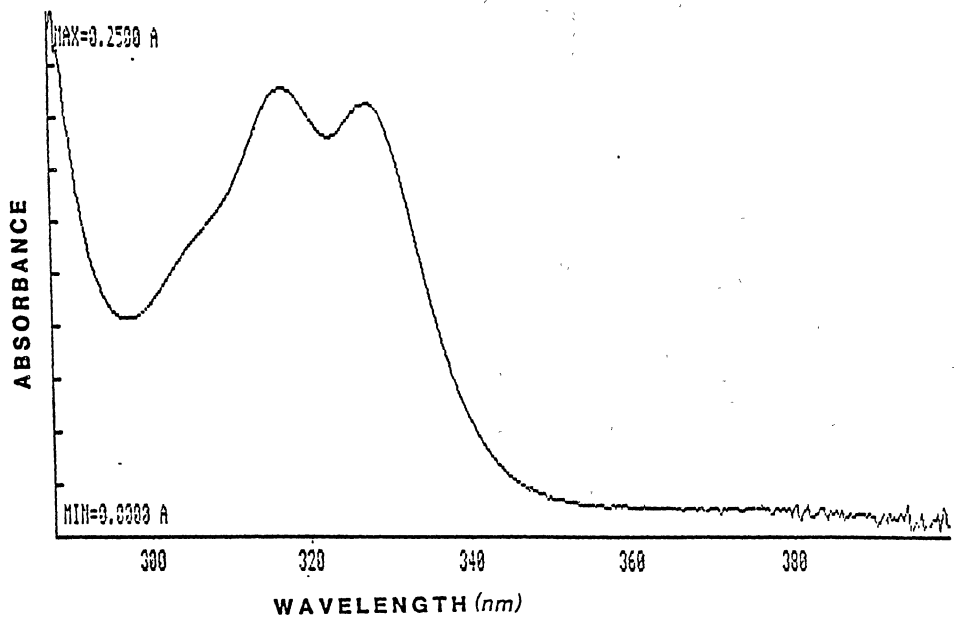


Figure 7. Absorption spectrum of diphacinone. Same conditions as Figure 6 except the sample has no europium.

and the absorption spectrum is shown in Figure 6. The absorption spectrum of the uncomplexed ligand is shown in Figure 7. The maxima occur at 328 nm excitation and 613 nm emission. These figures suggest that the broad band of excitation energies is channeled into the line-like emission of the europium accounting for the low limits of detection that are observed. The absorption maxima shift and there is a slight increase in the molar absorptivity upon complexation. The absorption spectrum shows an absorption at 286 nm which is not capable of exciting fluorescence. This absorption (286 nm) is probably a charge transfer absorption and accounts for the absence of europium emission. The shape of the excitation band is broad and characteristic of an organic ligand and the emission is the characteristic line-like emission of the 4f electronic energy levels of the europium (III) ion. The excitation and emission spectra of the chlorophacinone and pindone complexes are the same and the fluorescence characteristics of each complex is summarized in Table I. The excitation spectra are shown in Figures 8 and 9.

The optimum europium concentration of 1 X 10^{-4}M for fluorescence was found by monitoring the fluorescence of a 5 X 10^{-5}M diphacinone solution containing 1% pyridine and 3.6 X 10^{-4}M trioctylphosphine oxide and varying the europium concentration. The fluorescence increased with increasing europium concentration from 10^{-6}M to 10^{-4}M. Above 10^{-4}M europium a heavy precipitate formed decreasing

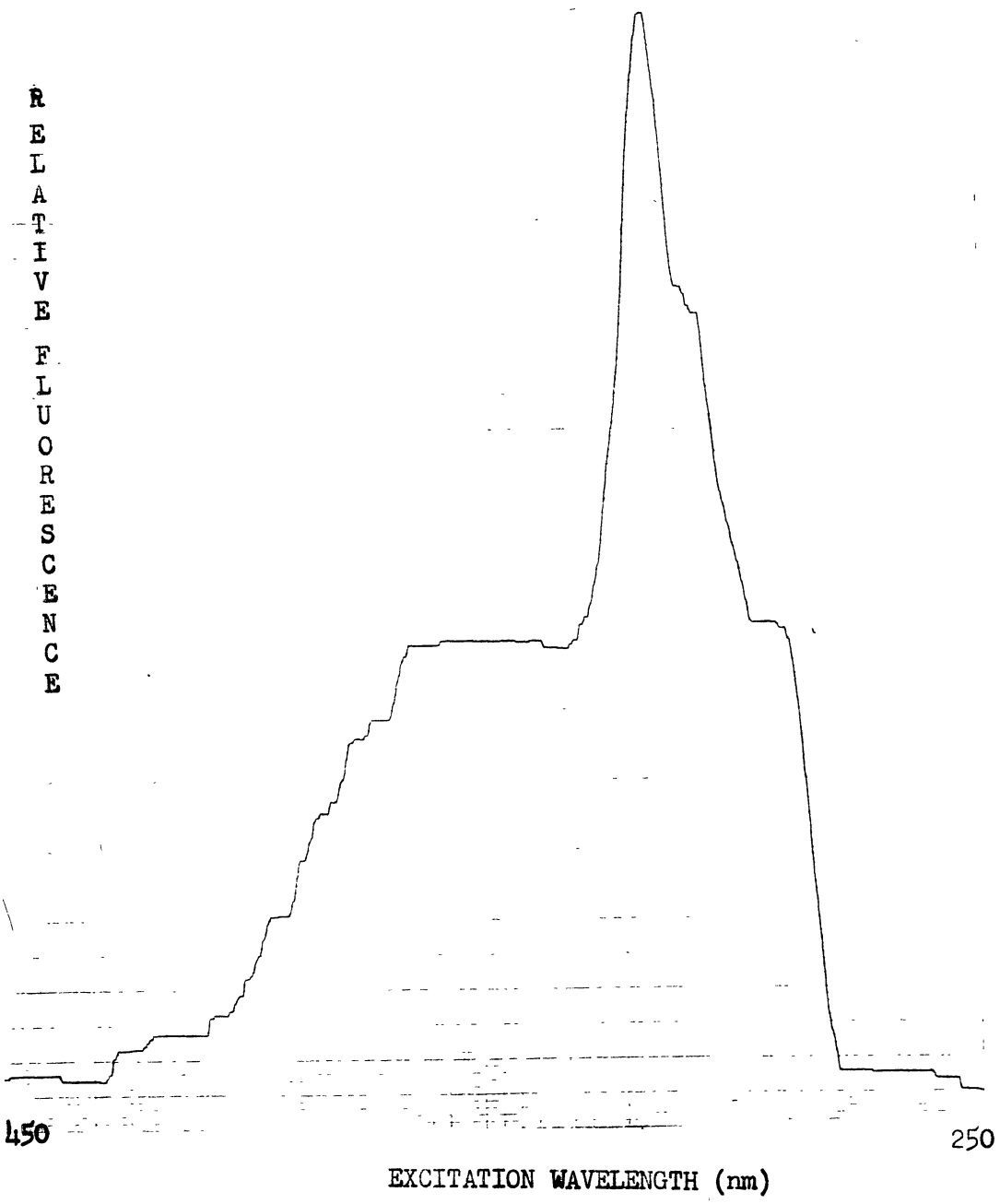


Figure 8. Excitation spectrum of the chlorophacinone-europium complex. 3.46×10^{-5} M chlorophacinone, other conditions are the same as Figure 4. Excitation max. 328 nm. Emission wavelength 613 nm.

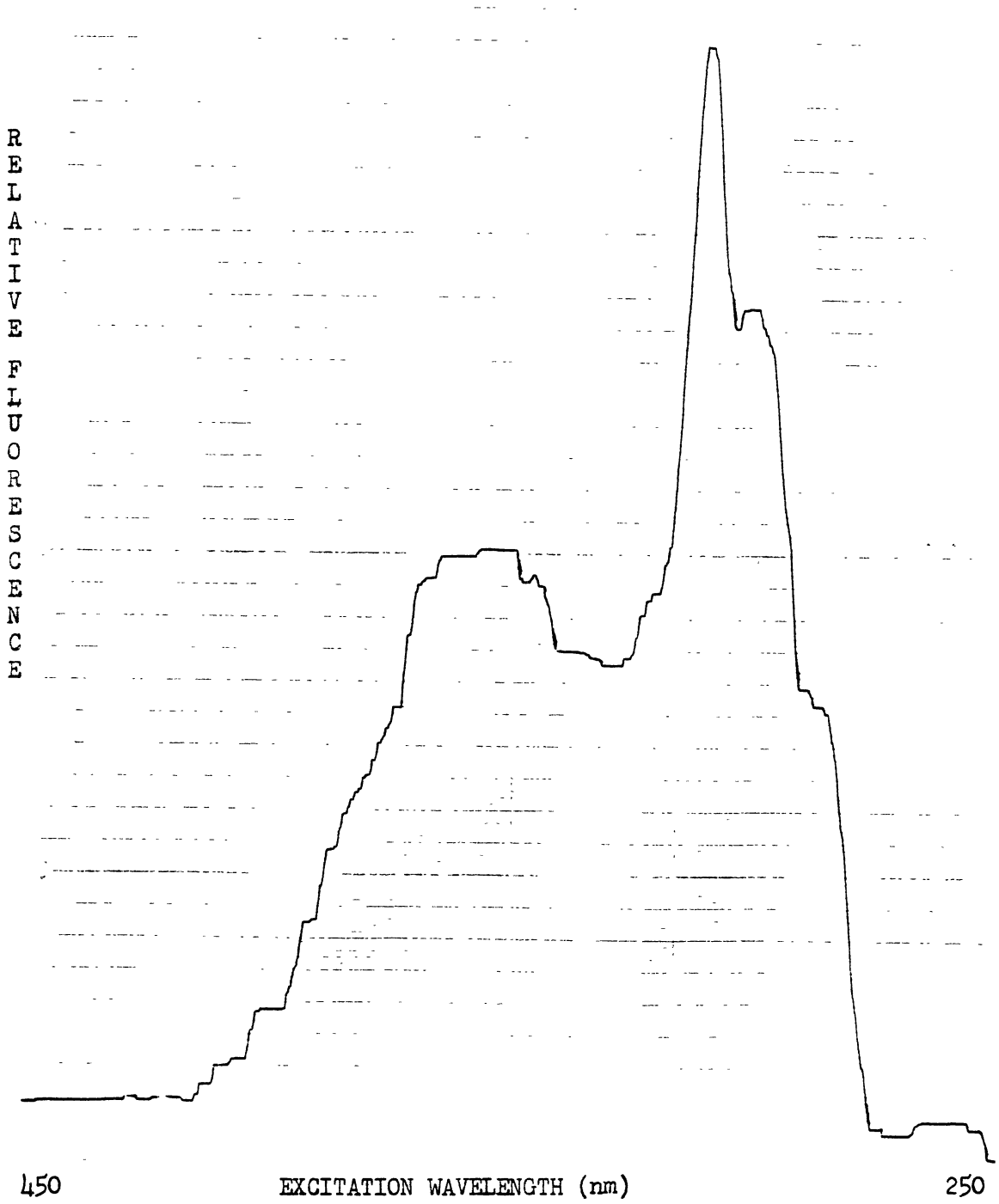


Figure 9. Excitation spectrum of the pindone-europium complex. 5.24×10^{-5} M pindone, other conditions the same as Figure 4. Excitation mas. 328 nm. Emission wavelength 613 nm.

TABLE I

FLUORESCENCE EXCITATION CHARACTERISTICS OF THE EUROPIUM COMPLEXES

Ligand	Excitation Wavelength (nm) Relative Fluorescence Intensity[a]			
	292	318	328	365
Chlorophacinone	0.45	0.72	1.00	0.48
	297	318	328	378
Diphacinone	0.45	0.72	1.00	0.39
	301	316	328	382
Pindone	0.39	0.76	1.00	0.54

a. Emission wavelength 613 nm.

TABLE II

LIMITS OF DETECTION AND LINEAR DYNAMIC RANGES OF THE CALIBRATION CURVES

Ligand	Limit of Detection (M)		Linear Dynamic Range (M)			
	Fluor.	UV-Abs	Fluor.		UV-Abs	
Diphacinone	$7X10^{-9}$	$2X10^{-7}$	$1X10^{-7}$	$8X10^{-5}$	$8X10^{-7}$	$8X10^{-5}$
Chlorophacinone	$3X10^{-8}$	$1X10^{-6}$	$1X10^{-7}$	$8x10^{-5}$	$2X10^{-6}$	$4X10^{-5}$
Pindone	$8X10^{-7}$	$5X10^{-7}$	$5X10^{-6}$	$5X10^{-5}$	$3X10^{-6}$	$7X10^{-5}$

the fluorescence of the solution.

The optimum trioctylphosphine oxide concentration was found to be 7.2 X 10^{-4}M in a like manner. Trioctylphosphine oxide in this concentration increases the fluorescence of the complex approximately twenty times. Above this concentration there was no further increase in the fluorescence of the solution.

The optimum pyridine concentration was found to be 1%. There must be a small amount of base present in order to remove the acid proton of the ligands to form the enolate anion that is necessary for complexation of the europium. In absence of pyridine the fluorescence of the complex is not observed. Above 1% pyridine the fluorescence tended to decrease slightly.

Using the collective optimum conditions, the limits of detection and linear dynamic range for each ligand was found. These results are summarized in Table II along with the corresponding values found using the UV-absorption method (73). The upper end of the fluorometric calibration curve is limited due to inner filter effects and concentration quenching. Working curves for diphacinone and chlorophacinone in the range from 10^{-6}M to 10^{-5}M had correlation coefficients of 0.997 or better. The method is blank-limited due to the weak fluorescence of the uncomplexed europium. The limit of detection was taken as the concentration of ligand necessary to give a signal equal to the blank plus three standard deviations for the fluores-

cence method and the limit of detection for the absorption method was found by taking three times the standard deviation of the blank divided by the slope of the calibration curve. The shape of the fluorescence calibration curve of the complexes between the limit of detection and the lower limit of linearity is convex. This curvature is attributed to the age of the instrument, the deterioration of the optics and light leaks in the monochromator portion of the instrument.

The limits of detection for the fluorometric procedure are an order of magnitude lower than those obtained using the UV absorption method for diphacinone and chlorophacinone and only comparable for pindone. This can be explained by examination of the quantum yield and molar absorptivity of the complexes. Quantum yield determinations (measured relative to anthracene) indicate that the quantum yield for diphacinone and chlorophacinone complexes is 10% compared to 0.75% for the pindone complex. This is probably due to the lack of rigidity in the pindone complex, its lesser amount of aromatic character, and its ability to dissipate the absorbed energy through vibrational relaxation before fluorescence can occur. The molar absorptivity of the pindone complex (5.02×10^{3} L mole^{-1} cm^{-1}) is much lower than that of the other two complexes (1.78×10^{4} L mole^{-1} cm^{-1} for the diphacinone complex and 1.01×10^{4} L mole^{-1} cm^{-1} for the chlorophacinone complex at 328 nm.)

Stoichiometry

The stoichiometry of the complexes appears to be EuL_2 under the conditions of the titrations (L representing the indandione ligand). Typical titration curves are shown in Figures 10 and 11. The breaks in the titration curves for all three compounds are indicative of the EuL_2 stoichiometry. X-ray crystallographic studies have indicated that the coordination number of the europium is eight (62, 63, 87). In these structures there are three β-diketone ligands occupying six of the coordination sites and the other two sites are occupied by oxygen atoms from water molecules or some other oxygen containing molecule or the nitrogen atom from an organic base such as pyridine. It is believed that the high concentration of trioctylphosphine oxide and pyridine in the titration solutions is causing the exclusion of the third ligand. Had the stoichiometry of the complex been EuL_3 with the addition of the third ligand not increasing the fluorescence of the complex, the maximum fluorescence in the titration of the ligands with europium would have occurred at a metal to ligand ratio of 1:3.

Conclusions

It has been shown that europium-sensitized fluorescence is applicable to the determination of some substituted 1,3-indandiones. The procedure is simple, fast,

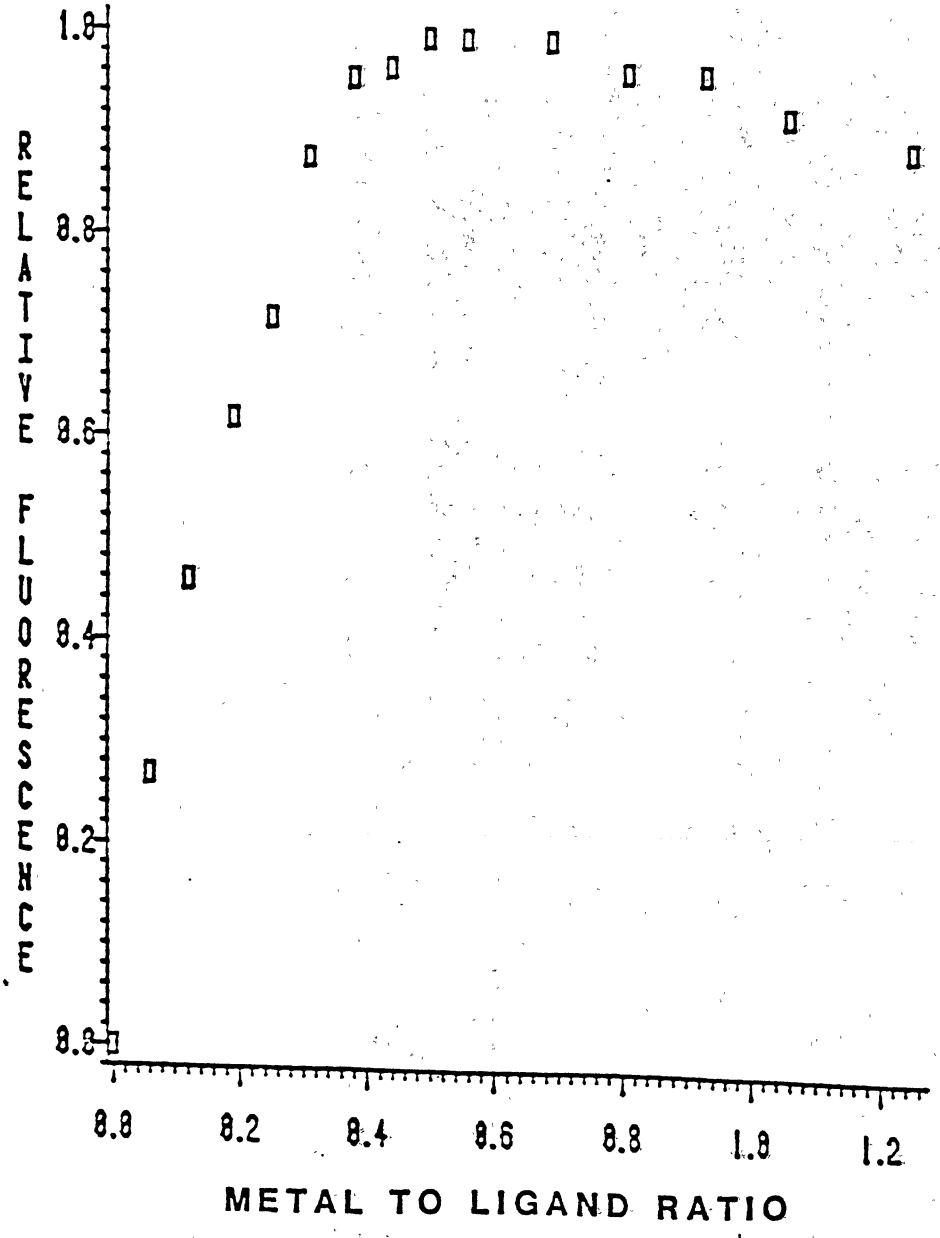


Figure 10. Fluorometric titration of 8×10^{-4} mmole chlorophacinone with 1.0×10^{-4} M europium. Conditions of the titration are given in the text.

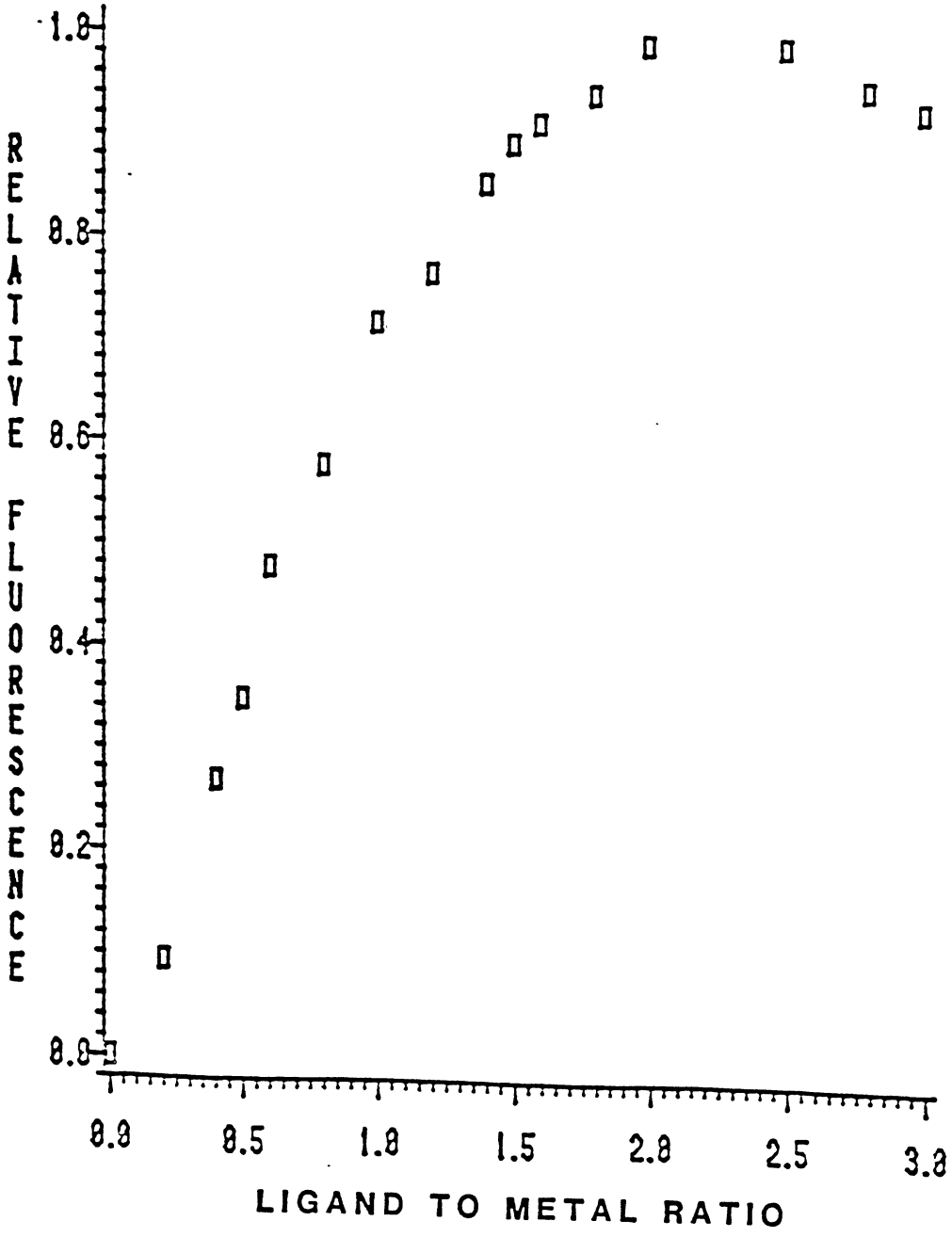


Figure 11. Fluorometric titration of 2 X 10^{-4} mmole of europium with 8.0 X 10^{-5} M chlorophacinone. conditions of the titration are given in the text.

and has low limits of detection. The linear dynamic range is shortened by the convex shape of the calibration curve. Perhaps the use of an instrument that is in better condition could eliminate the curvature and extend the linear dynamic range.

CHAPTER III

APPLICATION OF EUROPIUM-SENSITIZED FLUORESCENCE TO THE DETERMINATION OF DIPHACINONE AND CHLOROPHACINONE IN COMMERCIAL PRODUCTS

As discussed in Chapter I there are many published procedures for the determination of substituted 1,3-indandiones in commercial products and biological media. In each of the methods there is an extraction step, a chromatographic separation step, and a spectrophotometric determination step. These procedures can become long and time consuming. This chapter describes the development of a procedure for the determination of diphacinone and chlorophacinone in some commercial products.

EXPERIMENTAL

Apparatus, Chemicals and Commercial Products

The experimental apparatus and chemicals used were described in the previous chapter. In addition a wrist-action shaker (Burrell Corporation, Pittsburgh, PA) was used. The commercial products containing chlorophacinone were 0.28% mineral oil concentrate, 2.0% and 0.1% tracking powders and 0.005% pellets (Chempar Chemical Co. New York,

NY). The commercial products containing diphacinone were 0.005% pellets and 0.005% grain-mix baits. These were purchased at a local farm supply store.

Extraction Procedures

0.28% chlorophacinone mineral oil concentrates. 0.25 g of the oil was placed in a 25.0 mL volumetric flask, covered with 10 mL of acetonitrile and agitated with ultrasound for 45 minutes. The solution was diluted to the mark with acetonitrile and the oil allowed to separate and form a layer on the bottom of the flask. A 1.0 mL aliquot of the acetonitrile fraction was transferred to another 25 mL volumetric flask where 0.25 mL of the 0.01M europium reagent, 0.5 mL of the 0.036M trioctylphosphine oxide reagent and 0.25 mL of pyridine were added followed by dilution to the mark with acetonitrile.

2.0% chlorophacinone tracking powder. 0.10 g of the tracking powder was placed in an 18 mm X 125 mm test tube, covered with 5 mL of acetonitrile and stoppered. The solution was agitated 45 minutes with ultrasound then centrifuged 15 minutes at 1550 rpm. The supernate was transferred to a 50.0 mL volumetric flask, the sample was extracted a second time for 10 minutes, centrifuged and the supernate added to the first. The combined extracts were diluted to 50.0 mL with acetonitrile and a 1.0 mL aliquot was transferred to a 25.0 mL volumetric flask where the

europium, trioctylphosphine oxide, and pyridine were added and the solution diluted to the mark with acetonitrile.

0.10% chlorophacinone tracking powder. 0.10 g of the powder was placed in a test tube and extracted in the same manner as the 2.0% tracking powder. The combined extracts were transferred to a 25.0 mL volumetric flask and diluted to volume. A 5.0 mL aliquot was transferred to a second 25.0 mL volumetric flask where the europium, trioctylphosphine oxide and pyridine were added.

0.005% chlorophacinone and diphacinone pellets and grain mixed baits. Approximately 10 g of the bait was ground to a powder, 5.0 g placed in a soxhlet thimble and extracted four hours using a ternary solvent mixture of acetonitrile, acetone, and chloroform (2:1:1 by volume). After the extraction was complete, the volume of the extract solution was evaporated to less than 25 mL with a rotary evaporator, quantitatively transferred to a 50.0 mL volumetric flask and diluted to volume with acetonitrile. In the event a precipitate formed upon volume reduction, the solution was filtered, the precipitate washed with acetonitrile and the filtrate and washings transferred to the 50.0 mL volumetric flask for dilution. 5.0 mL aliquots were placed in a series of 25.0 mL volumetric flasks for standard addition analysis. Known amounts of the standards were added to the sample extracts along with the europium, trioctylphosphine oxide, and pyridine.

The fluorescence of all solutions was measured at 328 nm excitation and 613 nm emission. Calibration curves were made from serial dilutions of the known standard stock solutions. Blanks were made by mixing an aliquot of the extract with the trioctylphosphine oxide and pyridine in the above proportions. Blanks did not contain europium.

Results and Discussion

Table III shows the results of the analyses of the commercial samples. These results show that it was possible to determine the active ingredient content of oils, tracking powders, grain mix baits, and pelletized baits without an extensive cleanup step or chromatographic separation step. The precision may seem large at first, but this was attributed to the small sample size and the inhomogeneity of the samples. Agreement from day to day was good for the procedure. Acetonitrile is able to extract the chlorophacinone from the oil and tracking powders quickly and without interferences. The excitation and emission spectra of the extracts indicate there are no other fluorescing compounds extracted.

The ternary solvent used in the determination of diphacinone and chlorophacinone in pellets and grain mixed baits was found to be the most compatible between the extraction step and the determination step. When acetonitrile was used as the extraction solvent the percent active ingredient found was much lower than the labeled

TABLE III

DETERMINATION OF CHLOROPHACINONE AND DIPHACINONE IN COMMERCIAL PRODUCTS*

Product	% Found (Day 1)	% Found (Day 2)	% Found (Day2)
Chlorophacinone			
0.28% Oil Conc.	0.28±0.01	0.28±0.02	
2.0% Track Powd.	1.97±0.06	1.98±0.04	
0.1% Track Powd.	0.099±0.009	0.097±0.002	
0.005% Pellets	0.0063±0.0001	0.0061±0.0002	
Diphacinone			
0.005% Pellets	0.0034±0.0001	0.0030±0.0002	
0.005% Grain Mix	0.0046±0.0002	0.0046±0.0002	0.0046±0.0005

* Shown are the standard deviations of triplicate determinations

values. Simple shaking of the bait samples or agitation with ultrasound using acetonitrile resulted in incomplete extraction. Attempts to use multiple extractions over six hours at room temperature with fresh solvent did not improve the extraction efficiency. Soxhlet extraction for up to eight hours with acetonitrile did not improve the extraction.

Other solvents were found to either cause heavy turbidity when the europium reagent was added to the extract or the fluorescence of the complex was quenched when compared to the fluorescence observed in acetonitrile. Table IV lists the various solvents that were screened for possible use as extraction solvents. Screening consisted of using the listed solvent as the solvent for fluorescence. If it was observed that the solvent caused total fluorescence quenching or the formation of a precipitate upon addition of the europium reagent, the solvent was no longer considered. If the fluorescence was only slightly quenched a small sample of grain mixed bait (1 g) was shaken with 5 mL of the solvent using the wrist action shaker. The solvent extract was removed and 1 mL was placed in a 25 mL volumetric flask where the europium, trioctylphosphine oxide and the pyridine were added. If a heavy precipitate formed or if the fluorescence was less than the fluorescence obtained when acetonitrile was used as the extraction solvent the solvent was no longer considered to be useful.

The ternary solvent system gave good recovery of the

TABLE IV

SOLVENTS SCREENED FOR EXTRACTION

Solvent	Reason For Rejection
Acetone	Quenching
Acetone/Chloroform (1:1)	Precipitate Formation
Acetonitrile/Acetone (1:1)	Slight Quenching
Acetonitrile/Chloroform (1:1)	Slight Quenching
Acetonitrile/4%KCl(9:1)	Quenching
Benzene	Quenching
Chloroform	Quenching
Cyclohexane	Precipitate Formation
Diethyl Ether	Precipitate Formation
Dioxane	Precipitate Formation
Ethanol	Quenching
Hexane	Precipitate Formation
Isopropyl Alcohol	Quenching
Methyl Ethyl Ketone	Quenching
Petroleum Ether	Precipitate Formation
Tetrahydrofuran	Precipitate Formation
Toluene	Quenching

active ingredient, very little turbidity in the determination step and did not quench the fluorescence appreciably. The slight turbidity formed is the reason for utilizing the standard addition procedure in the analyses. Simple shaking of the bait with this solvent did not completely extract the active ingredient from the baits. Multiple extractions at room temperature with fresh solvent did not improve the efficiency of the extraction. For these reasons soxhlet extraction was employed which gave good results. Examination of the excitation and emission spectra of the extracts also indicated that no other fluorescing compound was extracted from these baits.

Conclusions

It has been shown that europium-sensitized fluorescence can be used in the determination of the active ingredient content of commercial products that contain diphacinone and chlorophacinone. The procedure is fast, simple and requires no chromatographic separation step or extract cleanup step.

CHAPTER IV

FEASIBILITY OF POST COLUMN COMPLEXATION

In some instances it may be necessary to perform a separation on a sample in order to accurately determine the amount of substituted indandione that is present. This would occur when the sample forms a heavy precipitate upon the addition of the europium reagent or the sample may contain two or more of the indandiones or the identification of the indandione may not be known and the chromatographic retention time could be used to identify the indandione that is present.

This chapter describes a post-column complexation apparatus that could be utilized in conjunction with liquid chromatography for the fluorometric determination of indandiones. The principles described here could also be used to develop a flow injection apparatus for the routine determination of indandiones. This is desirable because flow injection analysis has many attributes that make it a good procedure for analysis (88).

Experimental

Apparatus and Chemicals.

The fluorometer and chemicals have been described in the previous chapters. The post column complexation apparatus is shown in Figure 5 and was constructed using a 500 mL suction flask as a carrier reservoir. The flask was stoppered and fitted with a glass tube (6.0 mm o.d., 3.0 mm i.d.) which was connected to the center portion of a Swagelok T. Nitrogen gas supplied pressure to the reservoir to pump the carrier solution. A rubber septum was placed in one end of the T to act as an injection port. To the other end of the T was affixed a glass tube which was tapered on one end to fit a 3.0 mm o.d., 1.5 mm i.d. teflon tube, 64 cm in length which was attached to the micro-flow cell placed in the fluorometer.

The carrier solution was a mixture containing 1 X 10^{-4}M europium, 7.4 X 10^{-4}M trioctylphosphine oxide, and 1% pyridine in acetonitrile. The flow rate was adjusted to 2 mL per minute by adjustment of the nitrogen pressure. Diphacinone was injected into the flowing reagent stream with a 50 uL gas tight syringe. The fluorescence of the complex was monitored at 328 nm excitation and 613 nm emission. The apparatus is diagrammed in Figure 12.

Results and Discussion

Figure 13 shows the response of the fluorometer to

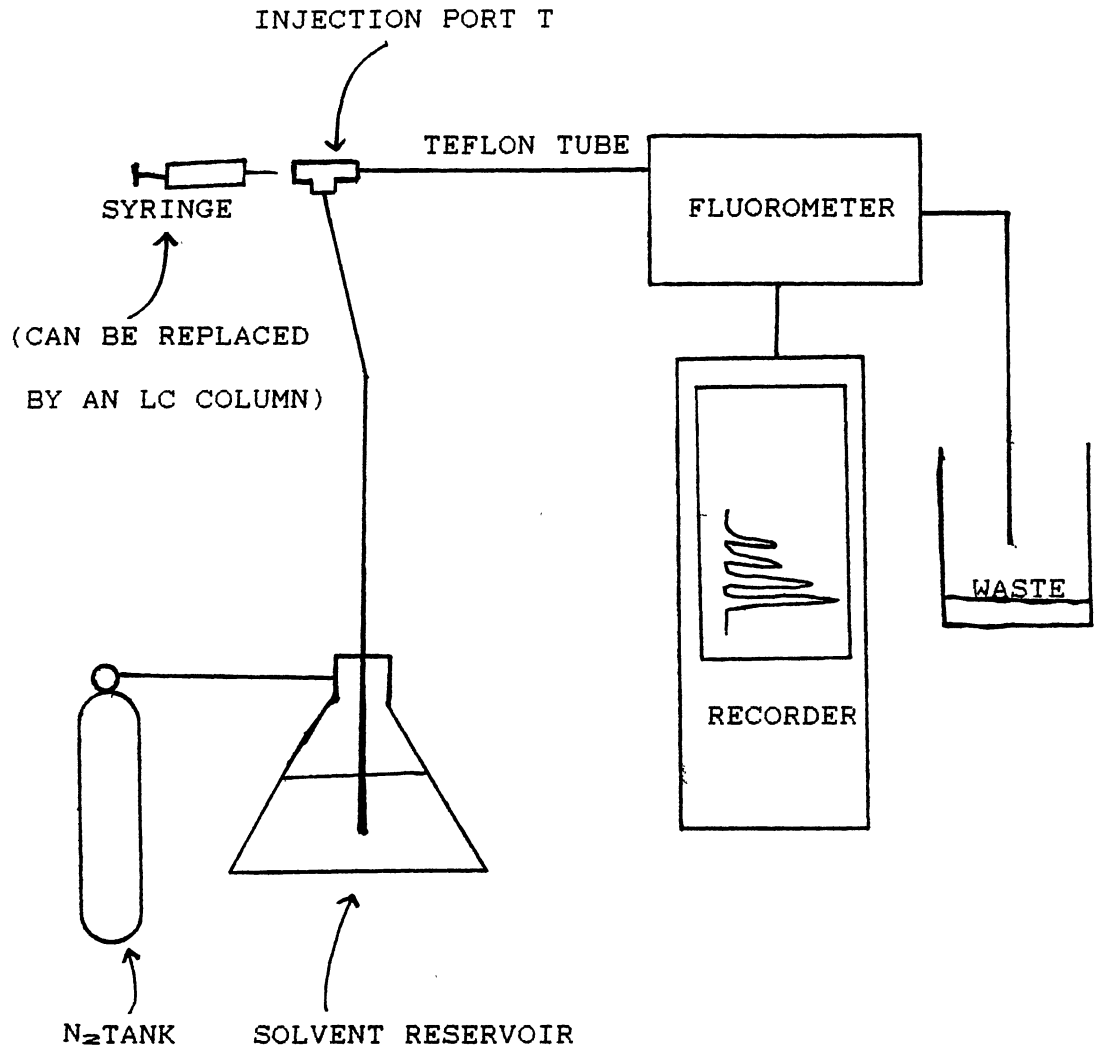


Figure 12. Apparatus for the post-column complexation experiment.

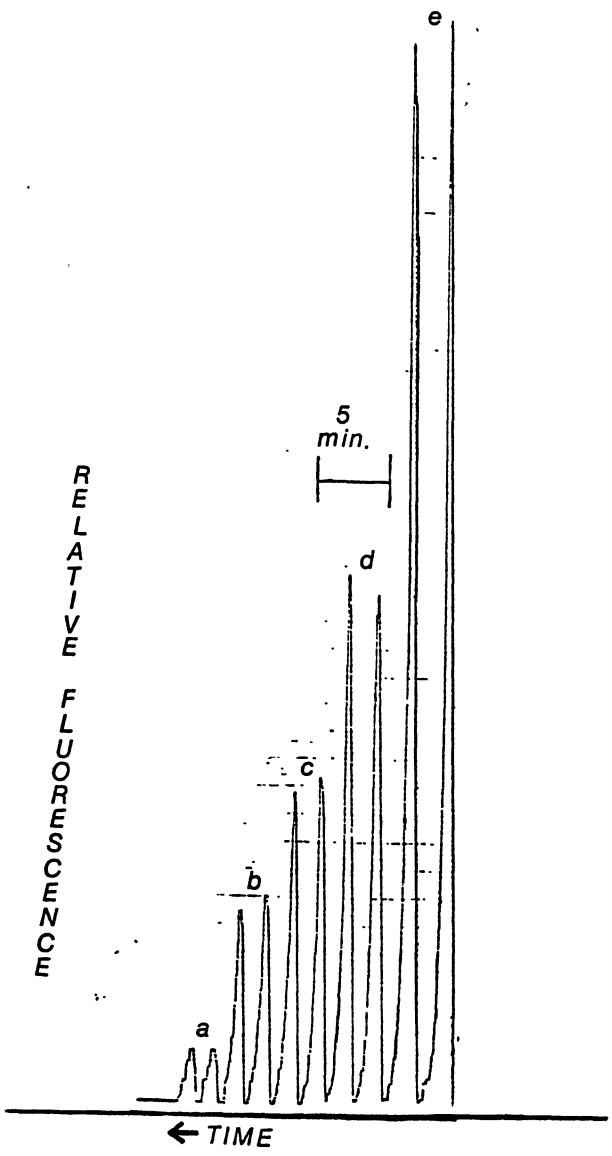


Figure 13. Response of the fluorometer to various amounts of diphacinone injected in the LC post column complexation experiment. a. 4.7 ug; b. 9.5 ug; c. 14 ug; d. 19 ug; e. 23 ug.

various amounts of diphacinone. The system and the experimental conditions are not optimized but the results show that post column complexation is possible and that it may be coupled with liquid chromatography when there must be a separation before the determination step.

The results also show that the procedure is applicable to flow injection analysis. The sample throughput is three samples per five minutes and the consumption of reagents is low. For the system to be totally optimized the reagent concentrations, flow rate, mixing tube length, and flow cell volume will have to be investigated.

CHAPTER V

SOME QUALITATIVE ASPECTS AND DATA REDUCTION

As discussed previously in Chapter I, there is little application of fluorescence spectroscopy to qualitative analysis due to the lack of any correlation charts or sets of empirical rules to correlate the structure of a molecule or complex to its excitation and emission spectra. Fluorescence spectroscopy would however be a powerful qualitative tool due to the low detection limits often observed. At present qualitative analysis from fluorescence spectroscopic data is limited to the comparison of the experimentally observed spectra to a library of spectra of known compounds. When there is a large degree of similarity between the observed spectra and a library entry (or entries), this gives the chemist a good starting point for identification of the unknown.

This chapter deals with the qualitative information that can be obtained when the excitation spectrum of a B-diketone europium complex is examined. This chapter also presents a data reduction program that could be useful in condensing the large amount of data that is obtained in fluorescence spectroscopy. Data reduction is useful in the

development of a fluorescence spectra library to decrease the amount of time required for searches and to decrease the amount of memory required to store the library entries.

The data reduction presented here is based on fitting the excitation spectrum to a series of exponential equations (50-52). The driver program is listed in Appendix B. The library subroutines used for fitting the spectra to the equations and the plotting subroutines have been described elsewhere (89).

Experimental

Apparatus and Chemicals.

The fluorometer and the reagents have all been described in the previous chapters. Other chemicals used in this section were tetracycline (Fluka), oxytetracycline and chlorotetracycline (Sigma, St. Louis, MO), FOD (Aldrich, Milwaukee, WI), dibenzoylmethane, benzoylacetone, and thenoyltrifluoroacetone, (Eastman, Rochester, NY). All were used as received.

Procedure.

Fluorescence spectra of all the β-diketone ligands complexed with europium were obtained using the optimum conditions with respect to europium, trioctylphosphine oxide, and pyridine found in Chapter II. Excitation spectra of the indandione complexes were converted to digital form from the

strip chart recording manually by reading the intensity every 8.5 nm. The digitized spectra was then input into the computer program listed in Appendix B.

RESULTS AND DISCUSSION

All of the europium complexes have the same emission spectra. The emission spectra show the weak emission at 593 nm and the very intense emission at 613 nm. Table V lists the excitation characteristics for the non-indandione ligands upon complexation with europium. Each of the excitation bands are broad and contain only one peak. It is obvious upon comparison with Table I, Chapter I, that the excitation spectra of the indandiones are significantly different from the other ligands investigated here. By examination of the excitation spectra it appears possible to differentiate between one of the substituted indandiones and the other compounds.

However, it is not as easy to differentiate between the indandiones. They each have four distinct peaks in the excitation bands, and the peaks occur at approximately the same wavelengths with approximately the same intensity. For these reasons the data reduction and fitting program was developed in an attempt to identify the indandione from its excitation spectrum.

Each excitation spectrum was fitted to a series of exponential equations. The exponential equation has the form

$$I_{\lambda} = \sum_{i}^{\hat{}} I_{\lambda\ max\,i} \times \exp[x_j(\lambda - \lambda_{max\,i})^2] \qquad (18)$$

TABLE V

EXCITATION CHARACTERISTICS OF SOME NON-INDANDIONE LIGANDS COMPLEXED WITH EUROPIUM

Ligand	Excitation Max* (nm)
Thenoyltrifluoroacetone	362
Dibenzoylmethane	389
Benzoylacetone	385
Tetracycline	410
Oxytetracycline	408
Chlorotetracycline	410
Fod[a]	320

* All emission bands are broad bands with only one peak in the excitation spectrum. Conditions: 1 X 10^{-4}M, europium, 7.2 X 10^{-4}M trioctylphosphine oxide, 1% pyridine in acetonitrile. Emission wavelength, 613 nm.

a 2,2-dimethyl-6,6,7,7,8,8,8-heptafluoro-3,5-octanedione

where I_{λ} is the intensity of fluorescence at wavelength λ, $I_{\lambda max_i}$ is the intensity of fluorescence at the peak, λ_{max_i} and x_j is a peak shape parameter. Each peak is described by two of these exponential equations such that there is a peak shape parameter $x_j(j=i)$ that describes the peak for $\lambda < \lambda_{max_i}$, and a peak shape parameter x_j $(j=i+n)$ that describes the peak for $\lambda > \lambda_{max_i}$. The total excitation spectrum is then described by the sum of the exponentials for all peaks, n.

The program finds the peak shape parameters that best fits the digitized excitation spectrum when given the digitized spectrum, the locations of the peaks and the intensity of fluorescence at each peak. The total number of parameters that can describe the excitation spectrum is four times the number of peaks. For the indandiones with four peaks the spectrum can be described with 16 parameters, (the eight peak shape parameters, the four excitation wavelengths of the peaks, and the four fluorescence intensities at the peaks). This results in a substantial amount of data reduction when one considers that the excitation spectra span 250 nm or 250 wavelength intensity pairs.

Figure 14 shows an experimentally observed excitation spectrum for diphacinone and Figure 15 shows the digitized excitation spectrum and Figure 16 shows the fitted spectrum. The figures show that the excitation spectrum can be digitized and fitted with some distortion. Table VI shows the variation in the peak shape parameters for the diphacinone complex for several concentrations of the

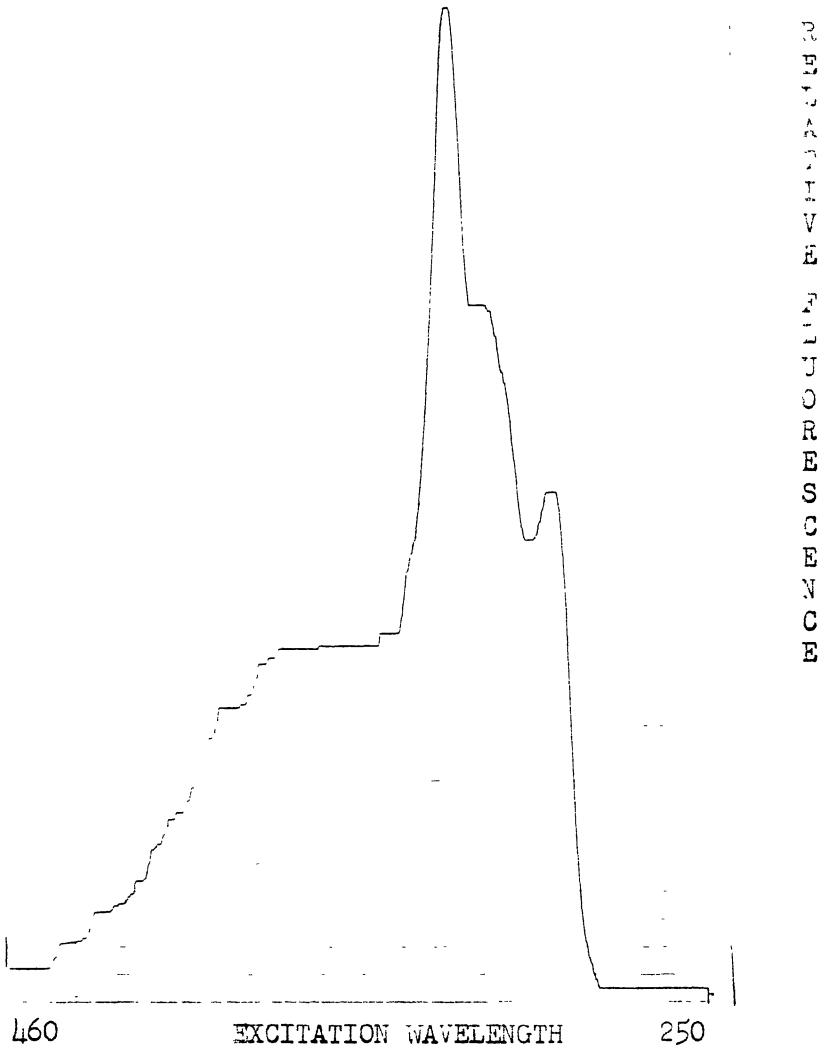


Figure 14. Excitation spectrum of diphacinone europium complex used to test the fitting program.

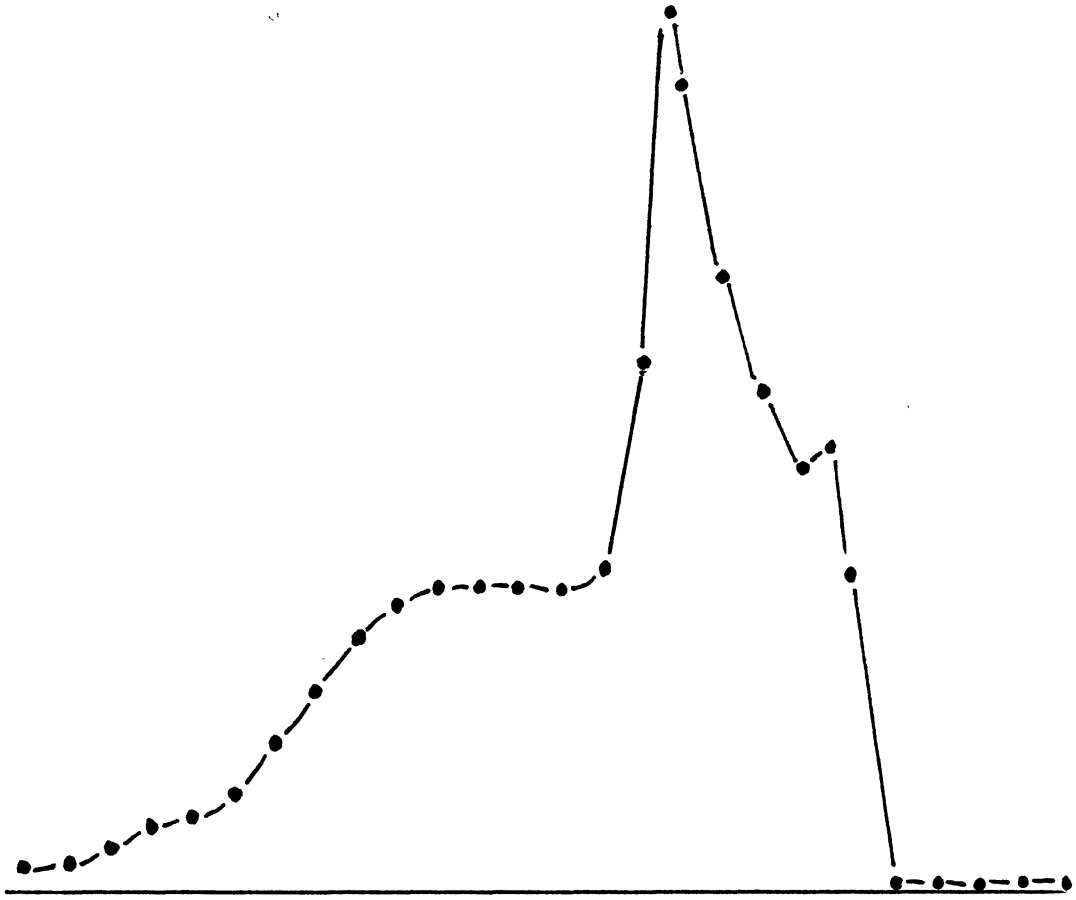


Figure 15. The digitized spectrum

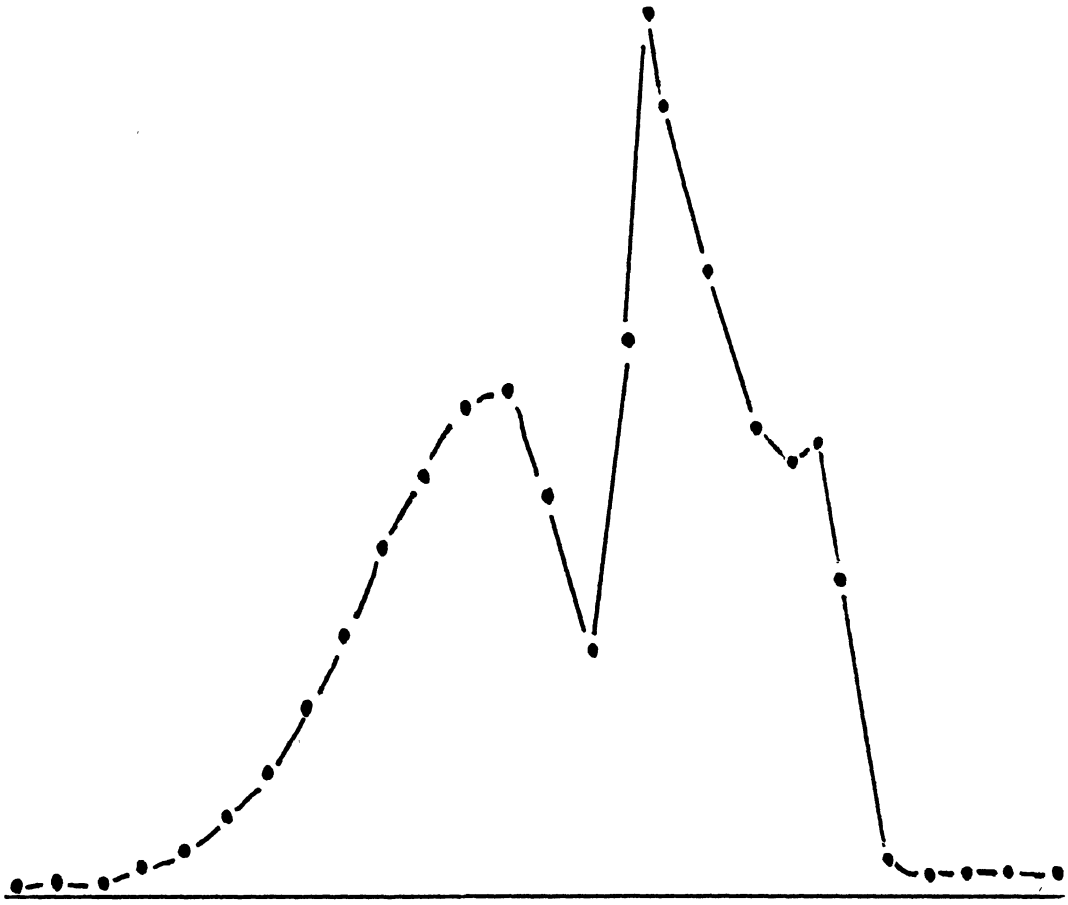


Figure 16. The fitted spectrum

TABLE VI

PEAK SHAPE PARAMETERS

Concentration (M)	X1	X2	X3	X4	X5	X6	X7	X8
Chlorophacinone								
1.28×10^{-6}	-0.00935	-0.189	-0.0144	-0.000428	-0.0173	-0.00719	-0.113	-0.000836
1.60×10^{-6}	-0.00390	-0.157	-0.0194	-0.000316	-0.0120	-0.00542	-0.203	-0.000950
6.40×10^{-6}	-0.00828	-0.179	-0.0114	-0.000454	-0.0284	-0.00755	-0.113	-0.000927
9.60×10^{-6}	-0.01856	-1.101	-0.0126	-0.001025	-0.0225	-0.00597	-0.473	-0.000487
Average	-0.01002	-0.406	-0.0144	-0.000556	-0.0200	-0.00653	-0.226	-0.000800
Diphacinone								
1.58×10^{-6}	-0.00864	-0.024	-0.00810	-0.000519	-0.0162	-0.01310	-0.018	-0.000745
3.15×10^{-6}	-0.00707	-0.191	-0.00519	-0.000489	-0.0417	-0.00915	-0.235	-0.000819
4.73×10^{-6}	-0.00819	-0.306	-0.01258	-0.000557	-0.0124	-0.00886	-0.137	-0.000526
6.30×10^{-6}	-0.01688	-0.138	-0.00734	-0.000543	-0.0169	-0.00708	-0.198	-0.000647
7.88×10^{-6}	-0.00895	-0.364	-0.01008	-0.000499	-0.0124	-0.00868	-0.130	-0.000576
Average	-0.00995	-0.205	-0.00866	-0.000521	-0.0199	-0.00937	-0.144	-0.000663
Pindone								
2.96×10^{-5}	-0.0131	-0.309	-0.01253	-0.000385	-0.00299	-0.00982	-0.0945	-0.000526
4.40×10^{-5}	-0.0103	-0.108	-0.00555	-0.000474	-0.00323	-0.01006	-0.0748	-0.000649
5.90×10^{-5}	-0.0124	-0.222	-0.01643	-0.000578	-0.00363	-0.00996	-0.1295	-0.000446
8.87×10^{-5}	-0.0128	-0.148	-0.00494	-0.000464	-0.00486	-0.01305	-0.0783	-0.000672
Average	-0.0122	-0.197	-0.00986	-0.000475	-0.00368	-0.01072	-0.0943	-0.000573

ligand. The variation in the values is possibly due to the method of digitization of the spectra. The gearing mechanism of the fluorometer has deteriorated somewhat with age and the scan rate fluctuates slightly while the spectrum is being recorded adding a small error to the wavelength values. However the results do show that the peak shape parameters are relatively consistent from one concentration to another.

Table VI also shows the variation of the peak shape parameters of the chlorophacinone and pindone complexes. These results show that examination of the peak shape parameters in an attempt to identify the indandione would be difficult. This is because the excitation spectra are so similar and have the same general shape. Perhaps if a better method of digitization were available and a fluorometer which has a more stable wavelength drive were used, the use of the peak shape parameters for identification could be decisive.

CONCLUSIONS

It has been shown that the excitation spectra of the indandione europium complexes are different from other β-diketone europium complexes and that this information can be useful in qualitative analysis. It has also been shown that the excitation spectra of the indandione europium complexes can be adequately fitted to a series of exponential equations for data reduction purposes. This is useful in build-

ing a library of excitation spectra that would require less memory than storing the total excitation spectra. The peak shape parameters used in the fitting can not be used to differentiate between the indandiones due to the high degree of similarity of the spectra.

CHAPTER VI

SOME THEORETICAL IMPLICATIONS

This chapter deals with the study of other lanthanides when complexed with the 1,3-indandiones, the structure of the indandione and the resulting fluorescence, and the investigation of other β-keto compounds in an effort to extend the work to other types of compounds.

EXPERIMENTAL

Apparatus and Chemicals

The fluorometer and spectrophotometer have been described. The lanthanide chloride hexahydrates used were those that were used and described in a previous study (46). These included the chlorides of samarium, terbium, gadolinium, praseodymium, neodymium, dysprosium, and erbium. β-keto compounds used were 2-diphenylacetyl-1,3-indandione-1-hydrazone, 1,3-indandione, curcumin, 2-phenyl-1,3-indandione (Aldrich, Milwaukee, WI). Acetone, benzophenone, acetophenone, vanillin, benzaldehyde, acetoacetanilide, o-acetoacetanisidide, o-acetoacetotoluidide (Fisher) were used as received. Aldehyde and ketone derivatives of 2-diphenylacetyl-1,3-indandione-1-hydrazone were prepared ac-

cording to the published procedure (90). Ethyl acetoacetate was synthesized according to the published procedure (91). 2-acetyl-1,3-indandione and 2-benzoyl-1,3-indandione were synthesized according to the published procedure (92).

Procedure

Stock solutions of the lanthanide chlorides in acetonitrile were prepared by dissolving a weighed amount in a mixture of 0.5 mL of water and 0.5 mL ethanol with slight heating on a hot plate, then quantitatively transferring the solution to a 50 mL volumetric flask and diluting to the mark with acetonitrile. Mixtures of the lanthanides and diphacinone, chlorophacinone, and pindone were prepared by mixing the ligand with the lanthanide and adding pyridine and trioctylphosphine oxide in the optimum proportions described in Chapter II followed by dilution to volume with acetonitrile.

Acetonitrile solutions of the β-keto compounds were prepared by dissolving a weighed amount in 5 mL of acetonitrile followed by quantitative transfer to a 50 mL volumetric flask and dilution to the mark. Mixtures of the β-keto compounds and europium in acetonitrile were prepared in the presence of the trioctylphosphine oxide and pyridine.

The hydrazone derivatives were dissolved in a small amount of acetonitrile and europium, pyridine and trioctylphosphine oxide were added.

Results and Discussion

Lanthanide Ion Fluorescence

Only the very weak fluorescence of the samarium-diphacinone complex is observed. The samarium emission is observed at 567 nm, 605 nm and 651 nm and is shown in Figure 17. The broad band is the weak fluorescence of diphacinone. No fluorescence was observed from any of the other lanthanides, nor was any fluorescence observed from the complexes of pindone and chlorophacinone. This indicates that the triplet level of these ligands lies below the principal fluorescence resonance levels of the lanthanides and that the triplet level of the ligands lie in very close proximity to the resonance level of the europium(III) ion.

β-keto Esters, Amides and Ketones

No fluorescence is observed from the europium B-keto ester and amide complexes. These compounds are converted to the enol form upon addition of base and are known to complex metal ions (91, 93), however the esters and amides used here do not have a chromophore adjacent to the complexation site which may account for the absence of fluorescence. These compounds were investigated due to their commercial availability.

2-diphenylacetyl-1,3-indandione-1-hydrazone, the aldehyde and ketone derivatives and curcumin are β-diketone compounds that are fluorescent. Upon mixing these compounds

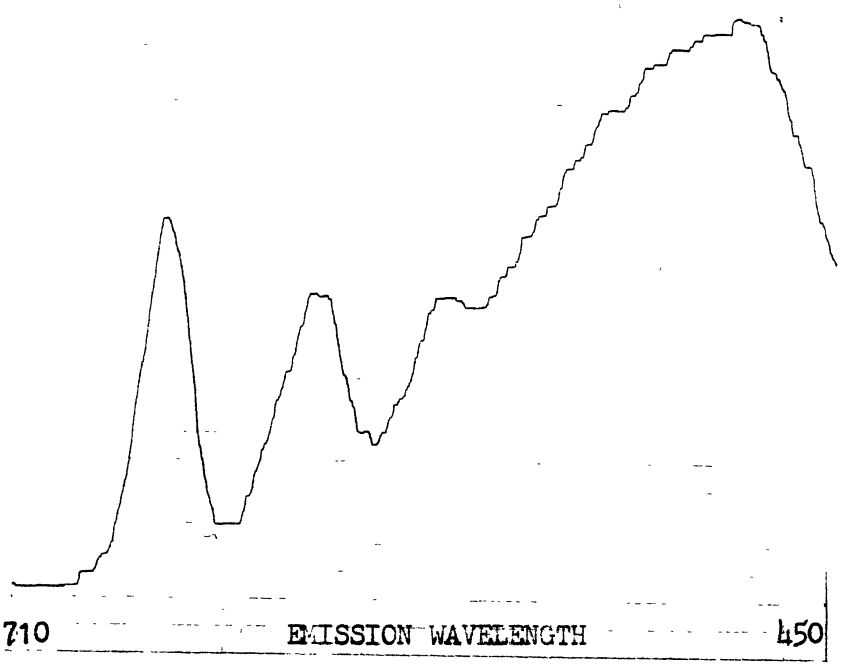


Figure 17. Emission of the samarium-diphacinone complex. 10^{-4} M in samarium and diphacinone in acetonitrile.

with europium, only the fluorescence of the ligand is observed. No emission from the europium is observed. This indicates that either there is no energy available to be transferred to the europium because all the absorbed energy is dissipated by the fluorescence of the ligand or that there is no complex formed.

Geometry of the Indandione Enolate

When 1,3-indandione and 2-phenyl-1,3-indandione are mixed with europium under the optimum conditions described in Chapter II, no europium fluorescence is observed. These ligands form trans enolates (54) and the necessary geometry of the enolate which points the oxygen atoms toward the europium is not present and a complex is not formed. This can possibly account for the absence of the europium fluorescence.

2-Benzoyl and 2-Acety-1,3-Indandiones

When these compounds are mixed with europium under the optimum conditions, europium fluorescence is observed. The excitation spectra for these compounds are shown in Figures 18 and 19. The spectrum of the acetyl derivative is very similar to the excitation spectra of the diphacinone, chlorophacinone and pindone complexes. The spectrum of the benzoyl derivative is different in that it does not have the strong, sharp peaks around 300 to 328 nm. This compound, however is the only one of the group that does not have a

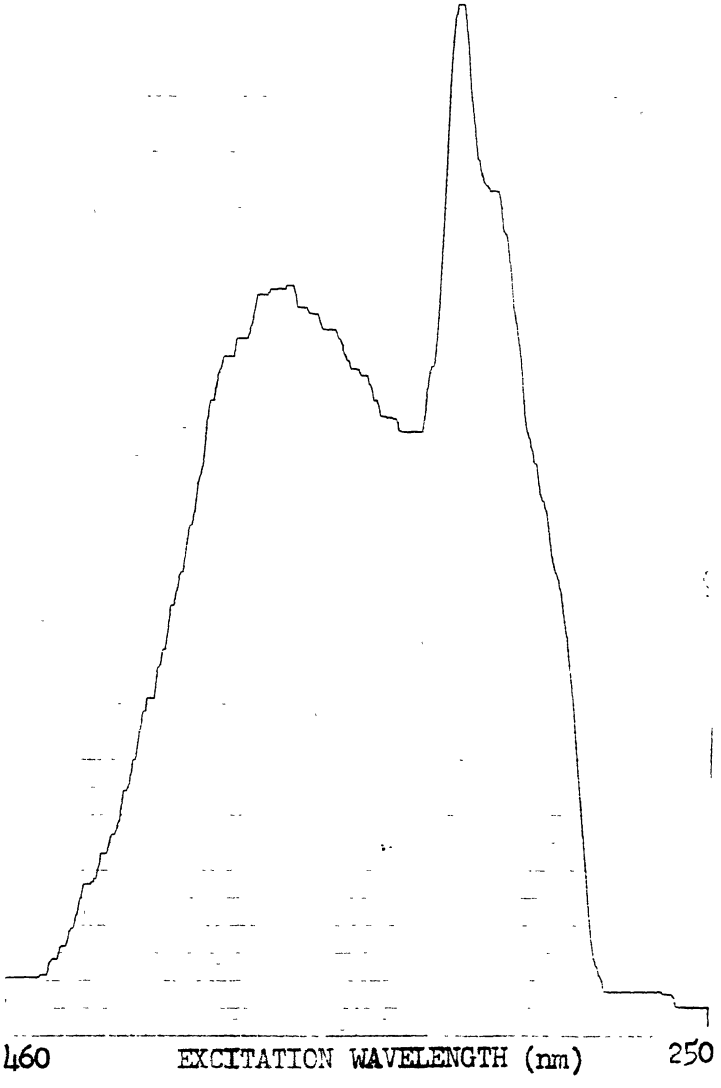


Figure 18. Excitation spectrum of the 2-acetyl-1,3-indandione-europium complex. Emission wavelength 613 nm.

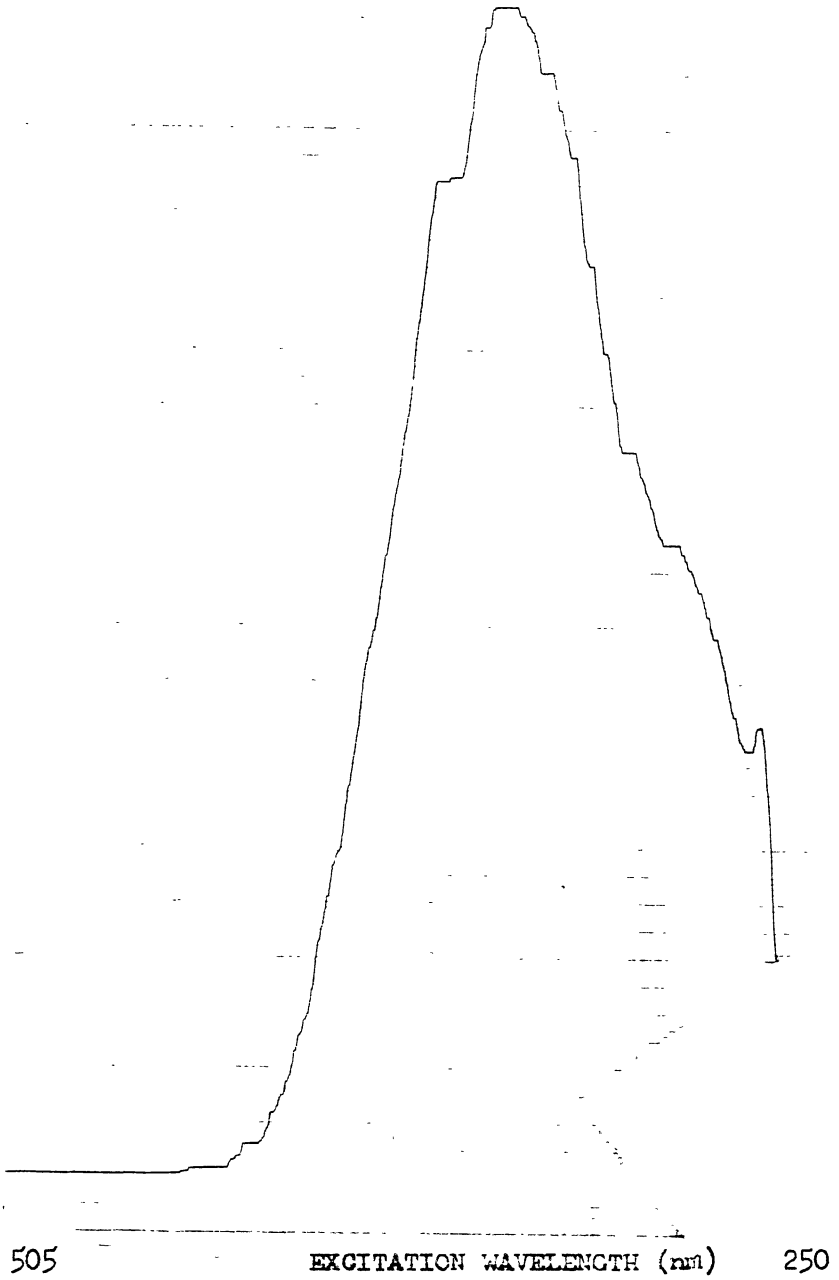


Figure 19. Excitation spectrum of the 2-benzoyl-1,3-indandione europium complex. Emission wavelength 613 nm.

tetrahedral carbon in the 2-substitution position. This may be the reason for the difference in the shape of the excitation spectrum. The quantum yield of the benzoyl and acetyl complexes were determined to be 3% relative to anthracene. The quantum yield of these compounds was expected to be greater than that of the pindone complex and less than that of the diphacinone and chlorophacinone complexes due to the rigidity of these complexes being intermediate between the pindone and diphacinone and chlorophacinone complexes. The molar absorptivity of the benzoyl complex is 6.0×10^3 L $mole^{-1}$ cm^{-1} and the molar absorptivity of the acetyl complex is 1.2×10^3 L $mole^{-1}$ cm^{-1} which was expected for the same reasons.

CONCLUSIONS

It has been shown that the triplet levels of the β-carbonyl-1,3-indandiones lie very close in proximity to the resonance levels of the europium and to no other lanthanide except perhaps samarium. The geometry of the enolate is important in producing the europium fluorescence. Another important aspect is that when the ligand exhibits its own native fluorescence either there is no energy available for transfer to the europium ion for subsequent europium emission, or the triplet level of the ligand is slightly below the fluorescence level of the metal ion.

CHAPTER VII

FLUORESCENCE LIFETIMES

The previous chapters have shown that qualitative analysis based on the shape of the excitation spectrum would be difficult. In the case of a mixture of the indandiones it would be next to impossible to make a determination without performing a separation on the sample because the excitation bands overlap almost completely. This chapter presents the fluorescence lifetimes of the three complexes in another attempt to gain some qualitative information about the identity of the indandione from the fluorescence data.

The laser system described here could also be used to lower the detection limits for the compounds. Recalling from equation 10 in Chapter I, the fluorescence detected by the instrument is directly proportional to the power of the illuminating source. The nitrogen laser used here is capable of a peak power output of 100 KW at 337 nm. This is more power than the 150 watt Xenon arc lamp. Since the method is blank-limited under steady state fluorescence conditions, using time discrimination of pulsed excitation and allowing the detector to sample the emission at some time after the excitation could make the determination

limited by the random noise of the electronics of the instrument. In the case of a mixture of the ligands, it may be possible to determine each compound if the lifetimes are different.

The ligands have a very strong absorption at 337 nm where the nitrogen laser emits. This is a case where there is a very good match between the excitation band of the analyte and the output of the laser. In some cases the laser output does not match an excitation band and the advantage of using the high power of the laser is offset by a small absorption of the analyte at the wavelength of the laser excitation.

Experimental

Apparatus

The laser system used here belongs to the research group of Dr. Powell of the Oklahoma State University Physics Department. The system consists of a Molectron model UV-14 nitrogen laser (Molectron Corp. Sunnyvale, CA). The laser beam was focused upon the sample in a quartz cuvet and the right angle fluorescence was focused upon the entrance slit of a Spex 1400 spectrophotometer (Spex Industries, Metuchen, NJ) set at 614 nm. The detector was an RCA C31034 photomultiplier tube. The output was fed to a Princeton Applied Research model 4202 signal averager (Princeton, NJ). The laser trigger was applied to the sig-

nal averager and the fluorescence rise and decay was monitored on an oscilloscope. After obtaining the fluorescence decay curve, the data was recorded with a strip chart recorder.

Results and Discussion

The fluorescence decay curves for the europium complexes of pindone, diphacinone, and chlorophacinone are shown in Figures 20, 21, and 22 respectfully. A plot of ln I vs. time (6) yielded the fluorescence lifetime of each complex. The lifetimes are 786 μsec for the chlorophacinone complex, 738 μsec for the diphacinone complex, and 306 μsec for the pindone complex. These values compare to the lifetime of the europium-thenoyltrifluoroacetone complex lifetime of 420 μsec (45) and 20 μsec for the tetracycline complex in water (94).

On the basis of lifetimes it might be possible to identify pindone from its short lifetime. It may be difficult to differentiate diphacinone from chlorophacinone. Since the lifetime was calculated from only one determination, the reproducibility of the measurement or the error associated with the measurement is not known.

Using the difference in lifetimes it appears that mixtures could be analyzed without having to perform a separation. Time did not allow a more detailed study of the possible applications of lifetime discrimination, nor a

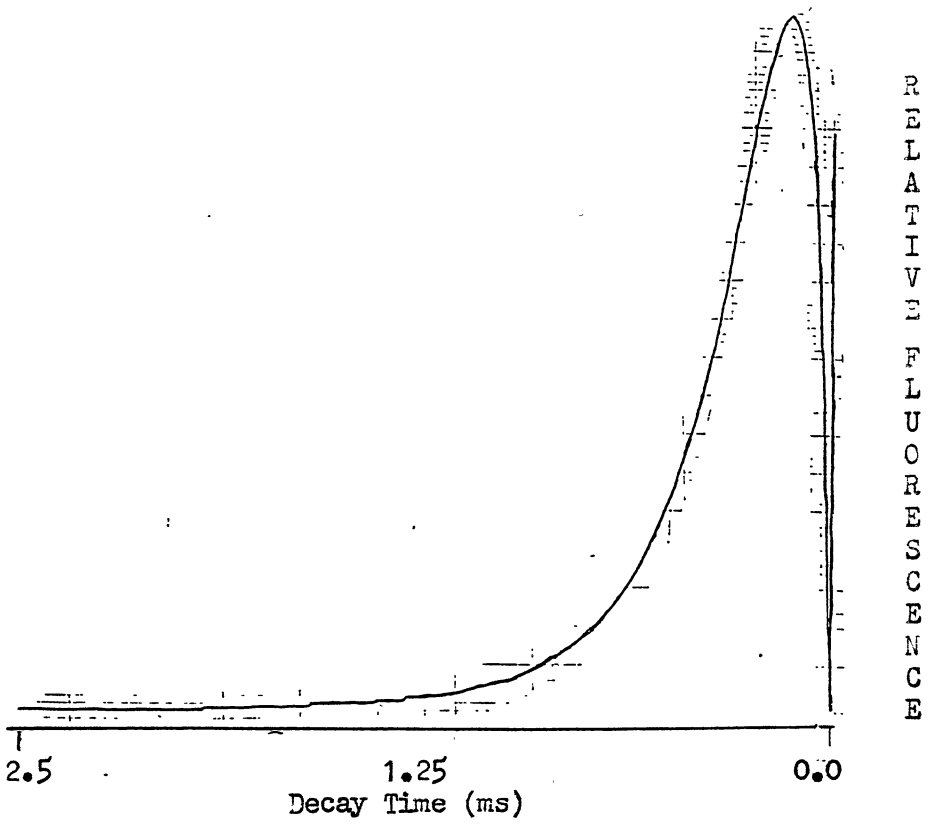


Figure 20. Fluorescence decay of 1.0×10^{-5} M pindone. Europium, trioctylphosphine oxide, and pyridine concentrations are the optimum given in the text, Chapter II, in acetonitrile.

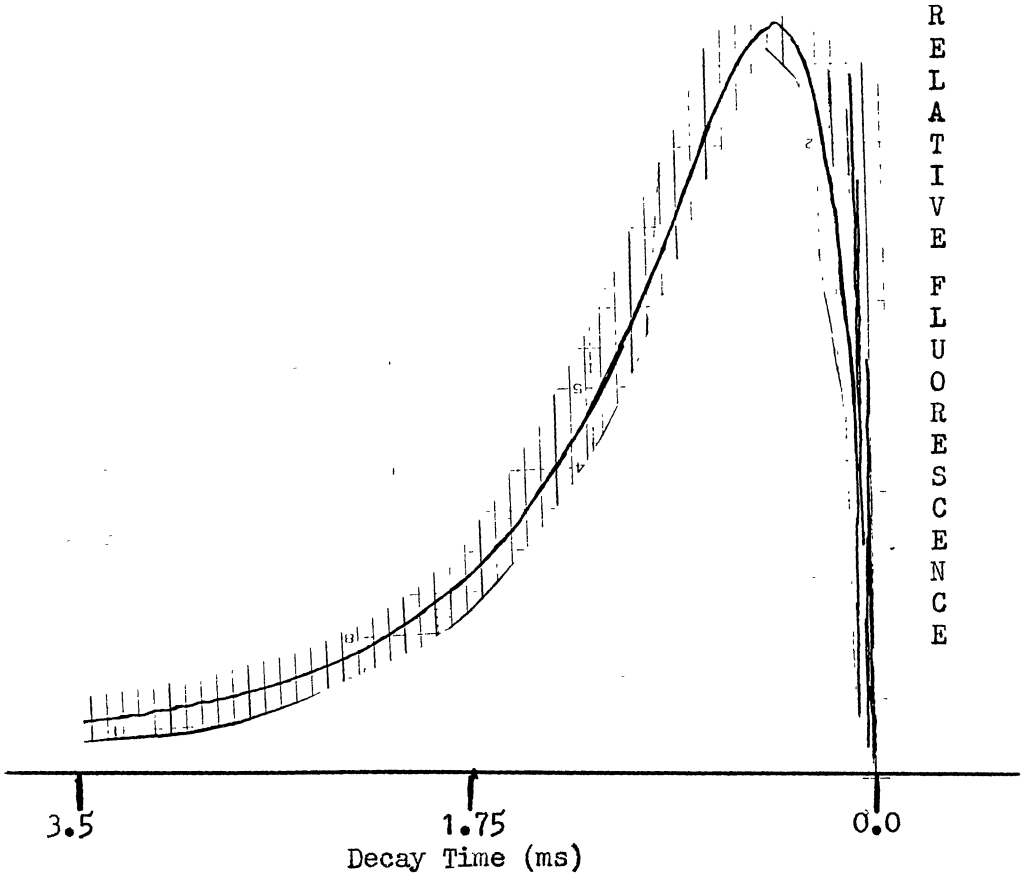


Figure 21. Fluorescence decay of 1.50×10^{-6} M diphacinone. Europium, trioctylphosphine oxide, and pyridine concentrations are the optimum given in the text, Chapter II, in acetonitrile.

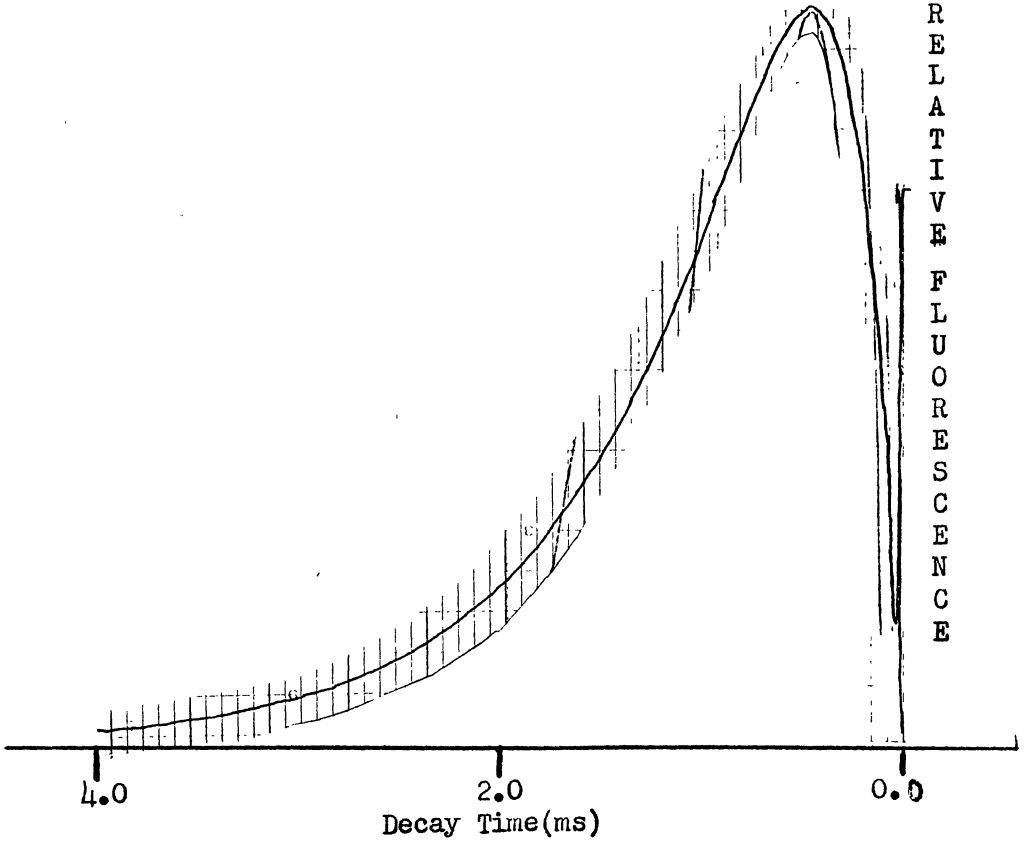


Figure 22. Fluorescence decay of 1.60×10^{-6} M chlorophacinone. Europium, trioctylphosphine oxide, and pyridine concentrations are the optimum given in the text, Chapter II, in acetonitrile.

study of how the limit of detection for each compound would be affected utilizing lifetime discrimination.

CHAPTER VIII

INVESTIGATION OF THE FLUORESCENCE IN AQUEOUS SOLVENT

The fluorescence of europium in water is weak and usually not analytically useful. One exception to this is the fluorescence of the tetracycline complexes (66, 94) and the authors point out that the efficiency of the energy transfer from the tetracycline to the europium is strongly influenced by the solvent and upon the ionization state of the ligand. The fluorescence lifetimes of the complex change with the pH of the solution. The life time at pH 2.5 is 1 μsec and 20 μsec at pH 7.

Another investigation into the effect of water on the emission of europium after direct excitation (95) indicated that water molecules can efficiently dissipate the absorbed energy through vibrational interactions. It was found that the emission increased upon substituting D_2O as the solvent. The emission was also found to increase in water by adding acetate ions which displace the water in the coordination sphere. The same effect was also observed for terbium (III). In order to observe the emission from the direct excitation the europium and terbium concentrations were 1M.

Emission from europium in aqueous solvents is rather weak and the strong emission observed from the tetracycline complexes is the exception rather than the rule. Terbium fluorescence is also observed in aqueous solution and in some cases is quite strong, as when complexed with sulfosalicylic acid, SSA (96). In this study EDTA was used to fill in the other coordination sites of the terbium ion at pH 10 to 11. The stoichiometry of the complex was determined to be 1:1:1 Tb:SSA:EDTA. The corresponding complex with europium however did not fluoresce and this was attributed to the mis-match of the SSA triplet level and the resonance levels of the europium.

In another study (97), the europium emission decay curve upon complexation with 1,10-phenanthroline showed two distinct regions. The shorter lived emission was due to the solvated europium that was produced by protonation of the ligand and then exchange of the ligand for a molecule of water. The hydrated europium then dissipated the absorbed energy through the vibrations of the water before much fluorescence could occur. The longer lived emission was from the complex without ligand exchange occurring. The exchange in the case of terbium was also observed upon complexation with SSA. This was evidenced again from lifetime data that indicated there were two species present that were emitting. The shorter lived species was attributed to the hydrated terbium and the longer lived to the terbium complexed with SSA. Others have found that the

rate of exchange of ligands for water decreases as the atomic number of the lanthanide increases (98) and that the stability constant of complexation increases as the atomic number of the lanthanide increases. This is seen in the formation constants of the europium and terbium complexes of sulfosalicylic acid (99). The formation constant of the EuSSA complex is 6.2×10^6 and the formation constant of the TbSSA complex is 8.9×10^6.

The fluorescence of the tetracycline complex in water may be strong due to the shear size of the ligand. The ligand may be so large that the molecule cannot vacate the coordination sphere of the europium to allow the addition of another two water molecules. Also the tetracycline ligand has several other sites in which protonation can occur and still allow the tetracycline molecule to retain its enolate structure for complexation and energy transfer fluorescence. It would be interesting to see if the fluorescence of the tetracycline-europium complex would increase if an additional complexing agent such as EDTA were added to fill the outer coordination sphere and exclude water. Allowing water in the coordination sphere may be why the lifetime of the tetracycline complexes are so short. However, short lifetimes favor strong fluorescence by not allowing time for vibrational energy dissipation.

The fluorescence of the diphacinone-europium complex is observed in water but is weaker than that of the complex in acetonitrile. This chapter describes the conditions

necessary to observe the fluorescence and the stoichiometry of the complex.

Experimental

Apparatus, Chemicals, and Procedure

The fluorometer has been described as well as a large majority of the chemicals used here. Additionally, an Orion Research pH meter equipped with a silver-silver-chloride pH electrode (Corning) and a saturated calomel electrode (Coleman) were used to measure the pH of the test solutions. Primary analytical standard THAM, (trishydroxymethylaminomethane, Fisher), concentrated nitric acid and 6N sodium hydroxide were used to buffer and adjust the pH of the solutions.

An aqueous solution of diphacinone was prepared by dissolving 0.0136 g in 50 mL of ethanol with heat and then transferring the solution to a 500 mL volumetric flask and diluting to volume with water. During the addition of water the diphacinone began to precipitate. 0.15 N NaOH was added drop wise until the diphacinone returned to solution. A stock solution of europium (III) in water was prepared by dissolving 1.254 g of the oxide in 10 mL of concentrated nitric acid and 10 mL of water. The resulting solution was transferred to a 500 mL volumetric flask and diluted to the mark with water.

Results and Discussion

The fluorescence excitation spectra of the europium diphacinone complex at pH 7.2 is shown in Figure 23. The strong band at 313 nm is due to solvent scatter. The excitation peaks occur at 341 nm and 379 nm. The emission spectra is essentially the same as the emission spectra of the complex in acetonitrile.

The optimum pH for fluorescence was found by placing 12.5 mL of the diphacinone stock solution in a 250 mL beaker along with 17.5 mL of the europium solution and .1 g of THAM. Water was added to bring the total volume to approximately 80 mL. The pH was adjusted first to pH 1 by the drop wise addition of concentrated nitric acid. The pH was raised by the addition of 6N sodium hydroxide. Aliquots of the solution were placed in a cuvet at various pH values, the excitation spectrum was scanned and then the aliquot was returned to the beaker to minimize loss of the ligand and europium. The maximum fluorescence intensity of each solution occurred at 341 nm excitation. The optimum pH for the fluorescence was found to be 7.2 (see Figure 24). Above this pH the solution turned cloudy and the fluorescence decreased.

The stoichiometry of the complex was determined to be 1:1 by making a series of solutions that were 1.60 X 10^{-5}M in diphacinone and varying in europium concentration. All solutions were buffered to pH 7.2 by the addition of 0.01M

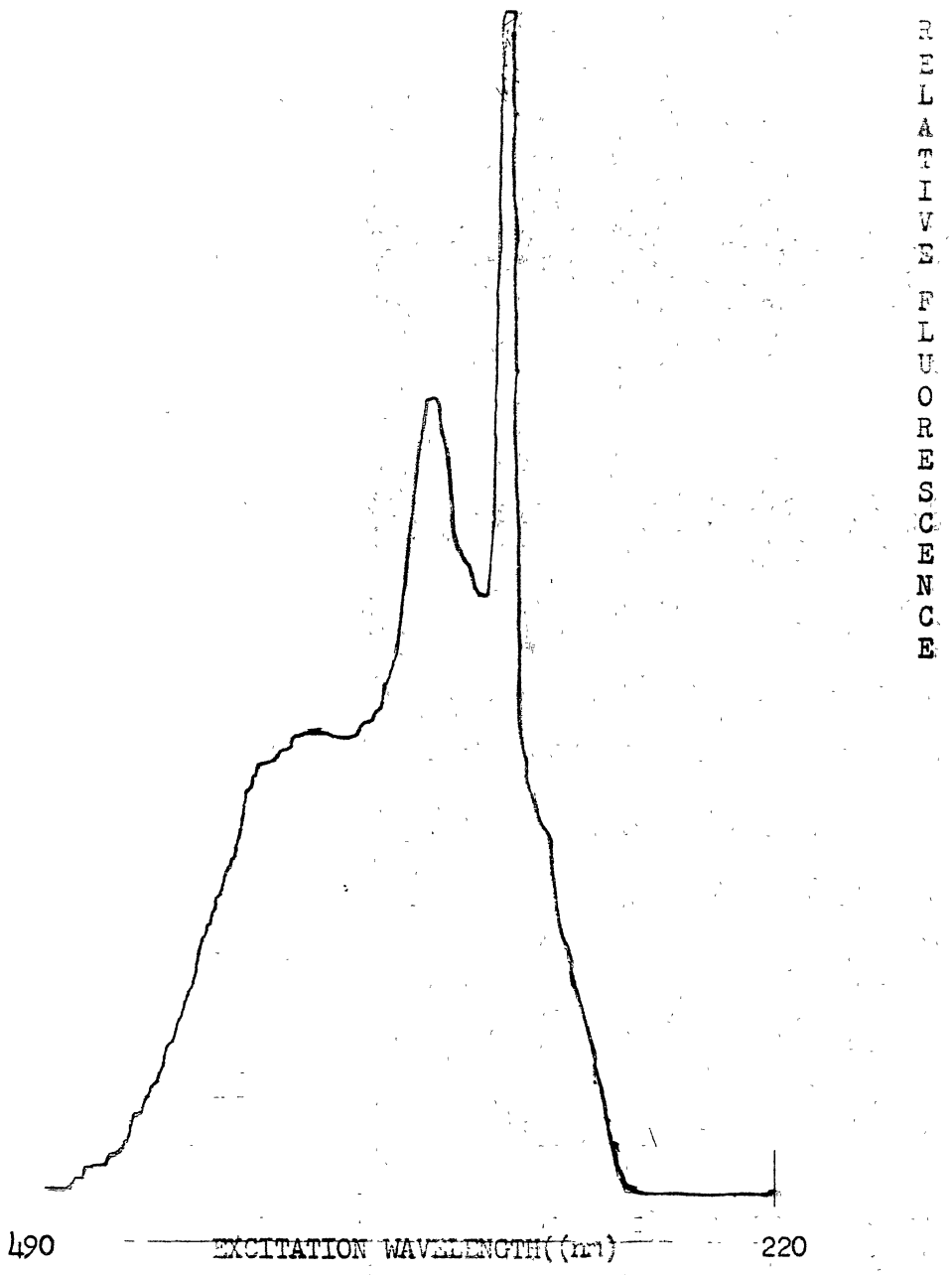


Figure 23. Excitation spectrum of the diphacinone-europium complex in water. pH, 7.2, diphacinone, 6.4 X 10^{-6}M, europium, 1 X 10^{-4}M. Emission wavelength 613 nm.

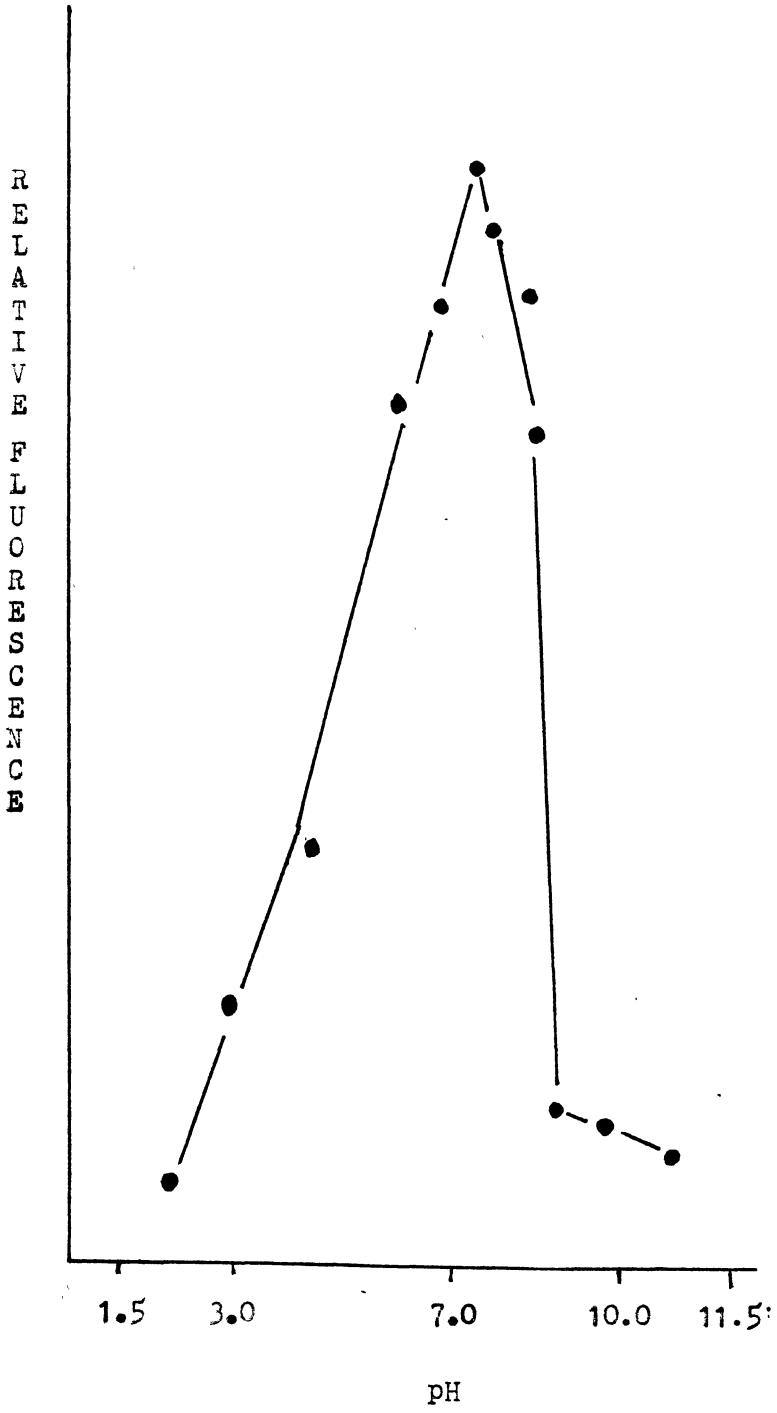


Figure 24. Effect of pH on the fluorescence of the diphacinone-europium complex in water. Conditions are given in the text.

THAM that had been adjusted to pH 7.2. The maximum fluorescence was found at a stoichiometry of 1:1 (see Figure 25). This is the same stoichiometry as observed for the europium tetracycline complex (66). Apparently the excess of water and the absence of any agent such as trioctylphosphine oxide or EDTA is precluding the addition of the second ligand as observed in acetonitrile.

The optimum europium concentration was found by adding varying amounts of europium to solutions that were 1 X 10^{-6}M in diphacinone at pH 7.2. The fluorescence increased as the europium concentrations increased from 10^{-7}M to 10^{-4}M. Above this concentration the solutions started appearing cloudy and the fluorescence tended to decrease slightly. For this reason the optimum europium concentration was taken to be 10^{-4}M.

Using these optimum conditions (pH and europium concentration) the calibration curve for diphacinone was found to be linear from 3 X 10^{-7}M to 2 X 10^{-5}M and the limit of detection was found to be 1 X 10^{-7}M. These parameters are not as good as those found for diphacinone in acetonitrile. This is probably due to the dissipation of energy by water molecules in the coordination sphere of the europium in the aqueous solvent. Perhaps the addition of EDTA could improve these results. The fluorescence may also be limited by having only one ligand pumping the europium ion in water and two ligands pumping the ion in acetonitrile. The fluorescence calibration curve is also

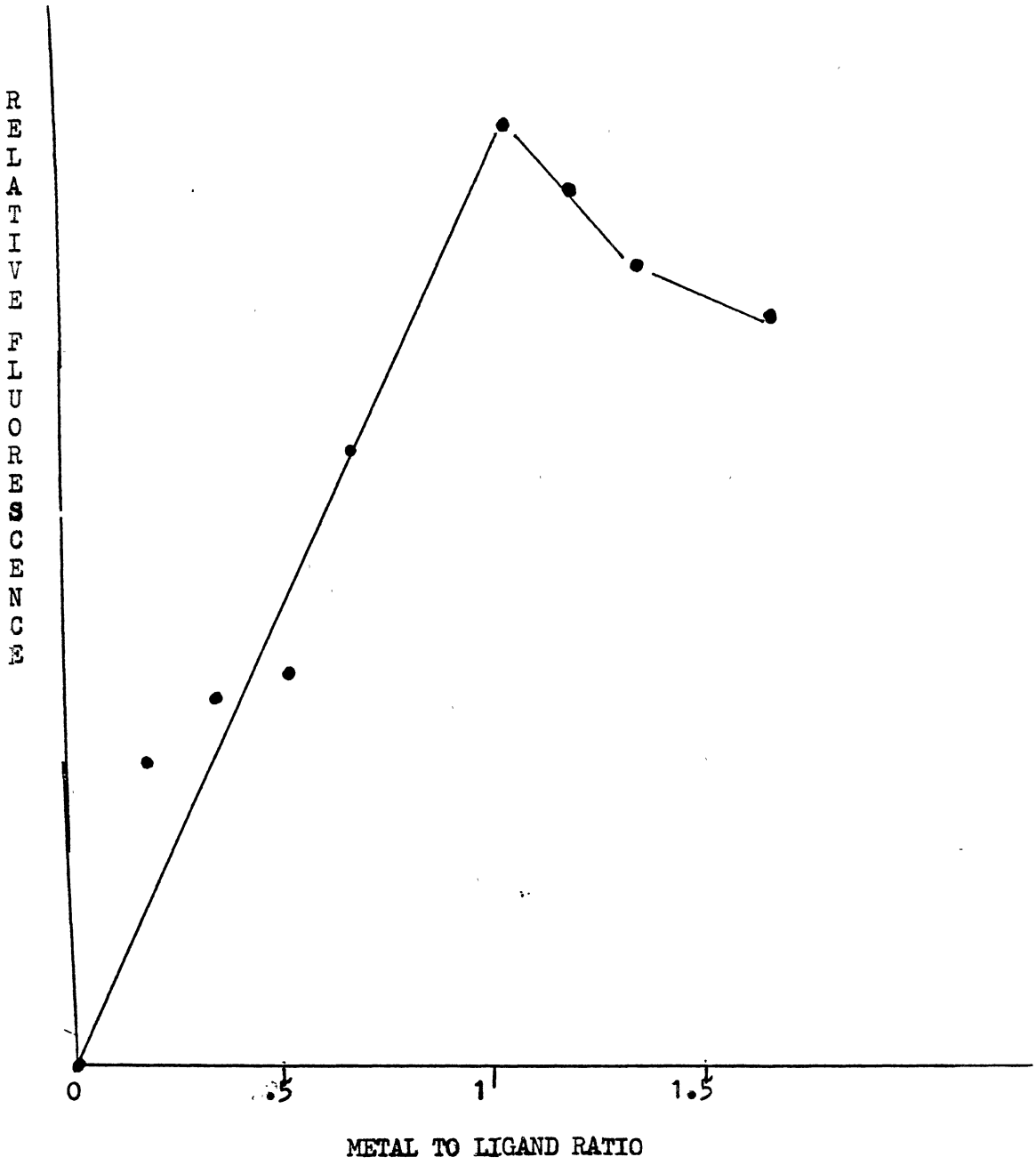


Figure 25. Stoichiometry of the diphacinone-europium complex in water at pH 7.2

limited due to the strong scatter band of the solvent being very close to the excitation maximum of the ligand (see Figure 23).

Conclusions

It has been shown that the fluorescence of the diphacinone complex is observed in water but the emission is weaker than that observed in acetonitrile. It would however, be advantageous to work in water to save on the expense of using acetonitrile. Also, working in aqueous solution dispenses of the problem of disposal of the spent organic solvent. (Several attempts were made to recycle the spent acetonitrile used in the previous chapters however the solvent could never be sufficiently purified and the fluorescence of the complex in the recycled acetonitrile was 50% lower than the fluorescence in fresh acetonitrile).

Another difficulty that would be encountered in using water as the working solvent would be in adapting the determination step to be compatible with the extraction step in the analysis of commercial samples and biological materials. This could possibly be accomplished by using liquid chromatography coupled with the post-column complexation apparatus described in a previous chapter. In the literature survey of the methods of determination of diphacinone, the extraction step was usually performed using an organic solvent followed by liquid chromatography

using an aqueous solvent or a mixture of water and an organic solvent.

CHAPTER IX

X-RAY CRYSTAL STRUCTURE OF THE PINDONE-EUROPIUM COMPLEX

As shown in Chapter II, the apparent stoichiometry of the indandione-europium complex in solution is EuL_2, where L is the indandione. This stoichiometry may be attributed to the large amount of trioctylphosphine oxide and pyridine in the titration solution. An X-ray study was undertaken to determine the stoichiometry of the complexes in the absence of trioctylphosphine oxide.

Pindone exists predominantly in the enol form and is known to form complexes with alkali metal ions. This work shows that the ligand is also capable of forming a stable complex with the europium (III) ion. (The other two ligands, diphacinone and chlorophacinone, form complexes which crystallize but are not ordered and the single crystal structures could not be determined.)

Europium(III) has been observed to form fluorescent complexes with other β-diketones and four of these have been examined by single crystal X-ray analysis (62, 63, 87, 100). In these structures (with one exception (100)) the coordination number of the europium is eight. Six of the sites are occupied by oxygen donor atoms of three B-

diketone ligands and the other two sites are occupied by oxygen atoms from water molecules or some other oxygen containing molecule such as dimethyl formamide, or the nitrogen atom of an organic base such as pyridine. In each of these structures square antiprism geometry of ligated atoms about the europium is observed .

Experimental

Synthesis.

0.176 g of europium oxide (1 mmole of europium) was placed in a 100 mL beaker and dissolved by drop wise addition of 3 mL concentrated nitric acid, followed by the addition of 5 mL of water. The solution was warmed on a hot plate until the oxide was totally reacted and the solution was further heated to evaporate the volume to approximately half of its original volume. To the resulting solution, 50 mL of absolute ethanol was added.

0.921 g of pindone (4 mmole) was dissolved in 100 mL of absolute ethanol using slight heat from a hot plate with stirring. Once the pindone had dissolved, the ethanolic solution of europium was added followed by the addition of 1 mL of pyridine. Upon addition of the pyridine the solution turned dark red and a check of the solution under an ultraviolet lamp yielded the characteristic fluorescence of europium. The solution was then set aside and covered with a watch glass to allow crystallization to proceed.

After three weeks, a dark red tar formed on the bottom of the beaker which was covered with a bright yellow powder. Illumination of the solid mass with ultraviolet light indicated the tar to be the europium containing complex and the bright yellow crystals to be the uncomplexed ligand. The yellow crystals were scraped off of the tar and the tar was dissolved in absolute ethanol with slight heating. The solution was set aside in a beaker and covered with a watch glass to allow crystals to grow.

After three weeks, suitable crystals for x-ray crystallographic study were present. The crystals were yellow in color, and when illuminated with ultraviolet light, they fluoresced red.

Crystallographic.

A crystal was mounted on a Syntex P3 automated diffractometer. Unit cell dimensions (Table VII) were determined by least square refinement of the best angular positions for fifteen independent reflections ($2\theta > 15^{\circ}$) during normal alignment procedures using molybdenum radiation (λ=0.71069A). Data, (10649 points), were collected at room temperature using a variable scan rate, a θ -2θ scan mode and a scan width of 1.2° below K_{α_1} and 1.2° above K_{α_2} to a maximum 2θ value of 60.0°. Backgrounds were measured at each side of the scan for a combined time equal to the total scan time. The intensities of three standard reflections were remeasured after every 97 reflections and

TABLE VII

Crystal Data For The Pindone-Europium Complex

Formula	$Eu(C_{14}H_{13}O_3)_3 \cdot 2H_2O \cdot C_{14}H_{14}O_3$ $[EuC_{56}H_{57}O_{14}]$
MWT	1106.02 g mole^{-1}
a	15.294(7) Å
b	26.405(8) Å
c	13.257(3) Å
	90.0°
	92.27(3)°
	90.00°
V	5329(3) Å^3
F(000)	2272
μMoK	12.333 cm^{-1}
λ MoK	0.71069 Å
D_{calc}	1.373 g cm^{-3}
Z	4
Obs. Refl.	7043
R/R_w	5.2%/7.7%
Space group	$P2_1/n$

as the intensities of these reflections showed less than 6% variation, corrections for decomposition were deemed unnecessary. Data were corrected for Lorentz, polarization and background effects. After removal of redundant and space group forbidden data, 7043 points were considered observed ($I>3.0\sigma(I)$). The structure was solved using a Patterson synthesis to locate the heavy atom position. Successive cycles of least squares refinement followed by difference Fourier synthesis allowed location of the remainder of the nonhydrogen atoms.

Refinement of scale factor, positional and anisotropic thermal parameters for all nonhydrogen atoms was carried out to convergence. Hydrogen positions for those hydrogen atoms bonded to atoms of the aromatic rings were calculated. Hydrogen atom positions and assigned isotropic thermal parameters were included in the final cycles of refinement (101) but were held fixed. Scattering factors were taken from Cromer and Mann (102). Anomalous dispersion corrections were made for europium (103). Unit weights were used until the final cycles of refinement when a weight = $1/\sigma F$ was introduced. R_w = 7.7%.

The final cycle of refinement - [function minimized $(/F_o/ - /F_c/)^2$] led to a final agreement factor, R = 5.2%, $R=(\Sigma||F_o| - |F_c||/\Sigma|F_o|)$ X 100.

Results and Discussion

The crystals isolated had the stoichiometry EuL_3 $2H_2O$ L. The fourth ligand was found to be included in the cell and not involved in bonding to the europium ion. The water associated with the complex is probably due to the use of non-dry reagents and exposure to the atmosphere of the mother liquor from which the crystals grew. The structure of the complex is shown in Figure 26. Figure 27 shows the structure of the complex with the noncoordinated ligand removed for clarity. Figure 28 shows the coordination sphere about the europium and illustrates the square antiprism geometry that is observed.

A similar procedure to produce (46) crystals of europium complexed with dibenzoylmethane, thenoyltrifluoroacetone, and benzoylacetone yielded complexes of the form EuL_4 HP where L is the β-diketone ligand and HP is the piperidinium cation . This stoichiometry was deduced from elemental analysis of the europium thenoyltrifluoroacetone complex and various other lanthanides complexed with these ligands. It is possible that the stoichiometry of the complexes was ML_3 $2H_2O$ HP L and the presence of the two water molecules were not deduced from the elemental analysis.

In another study (62) the crystal structure of the thenoyltrifluoroacetone europium complex shows the stoichiometry to be of the form EuL_3 $2H_2O$. Clearly there

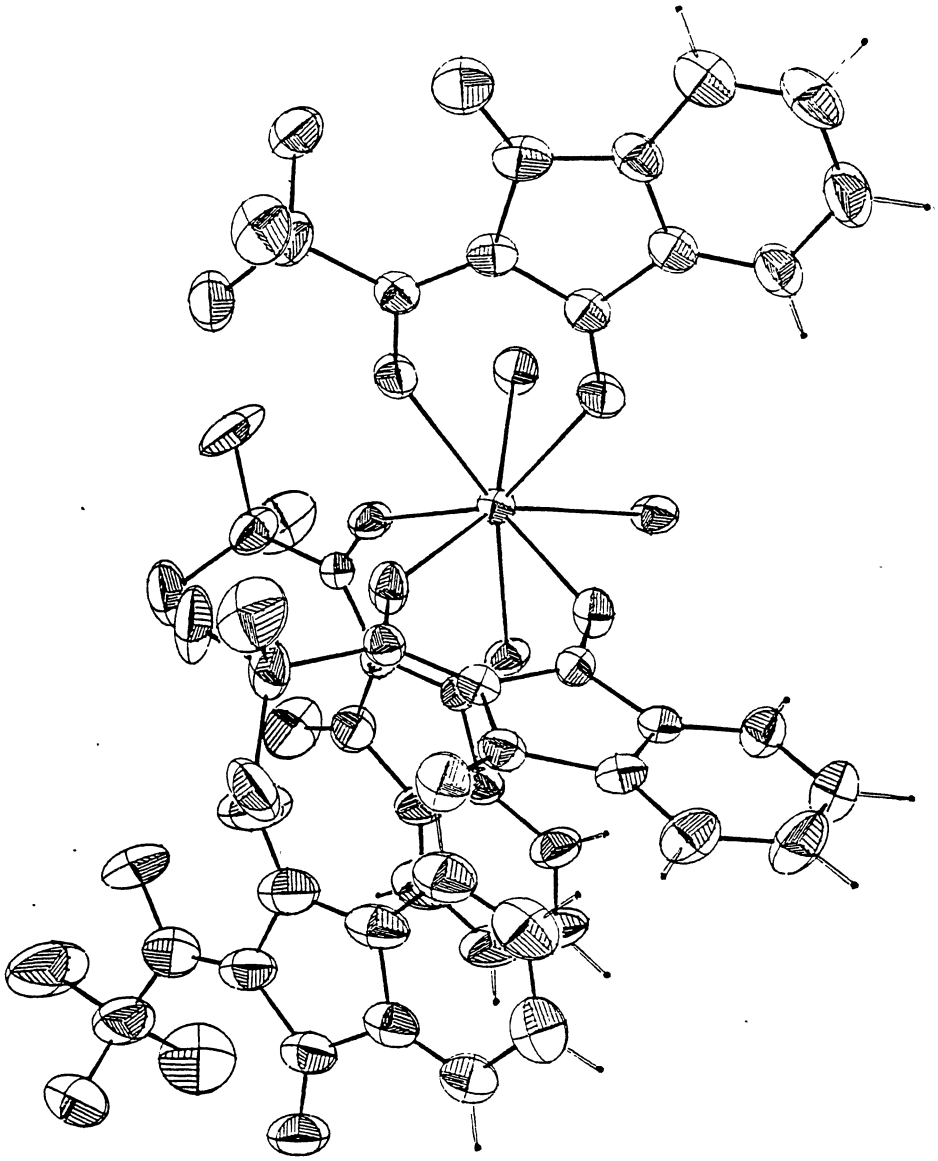


Figure 26. Projection view of the pindone-europium Complex.

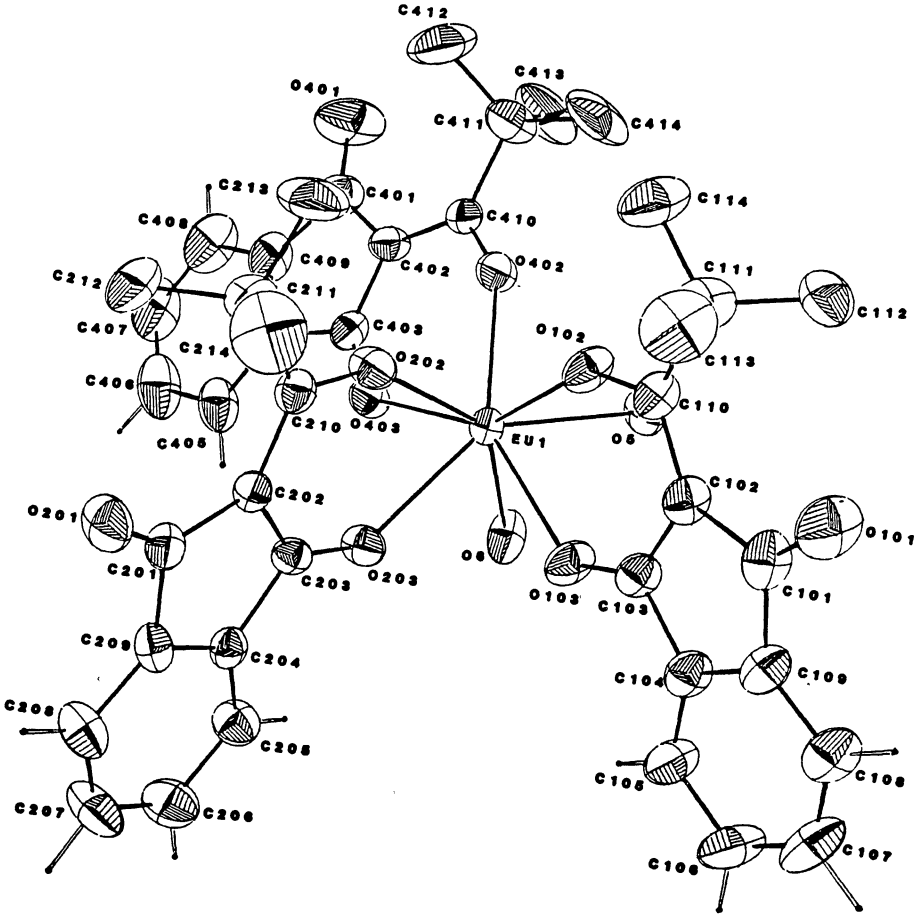


Figure 27. View of the pindone-europium complex with the uncoordinated ligand not shown.

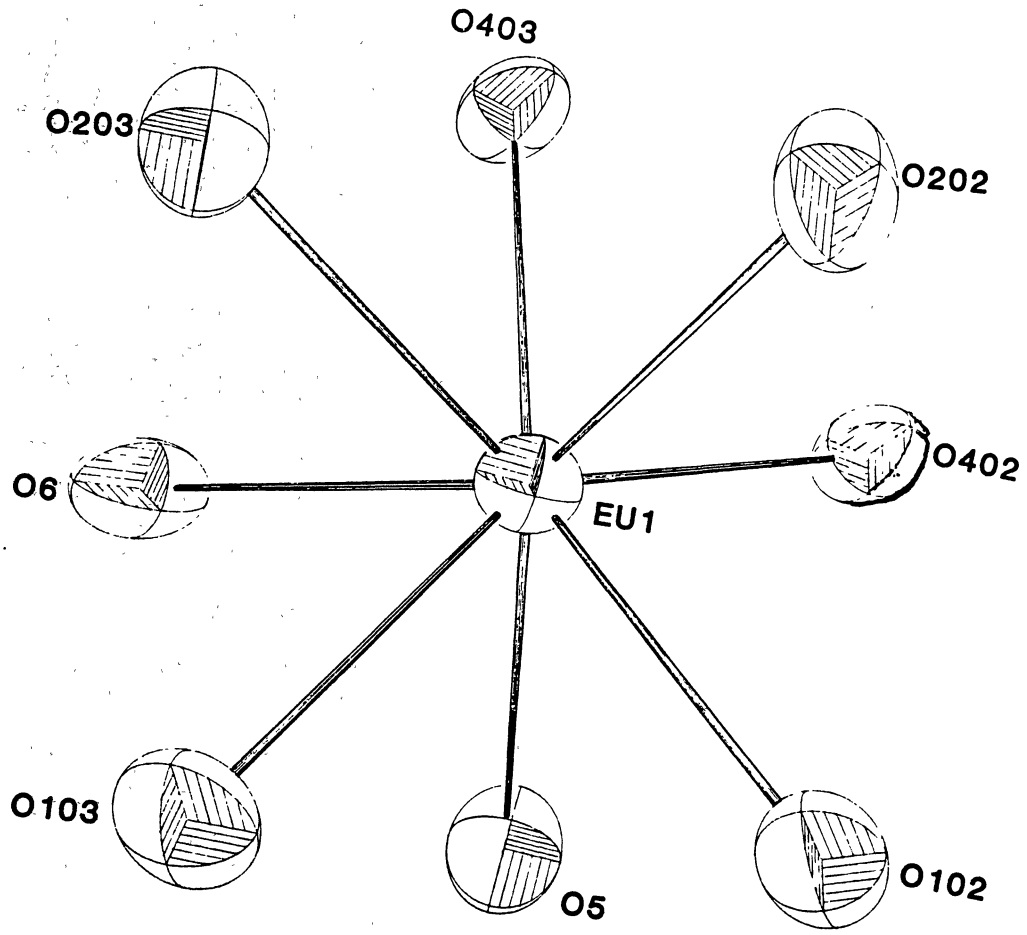


Figure 28. Coordination sphere about the central europium ion in the pindone-europium complex.

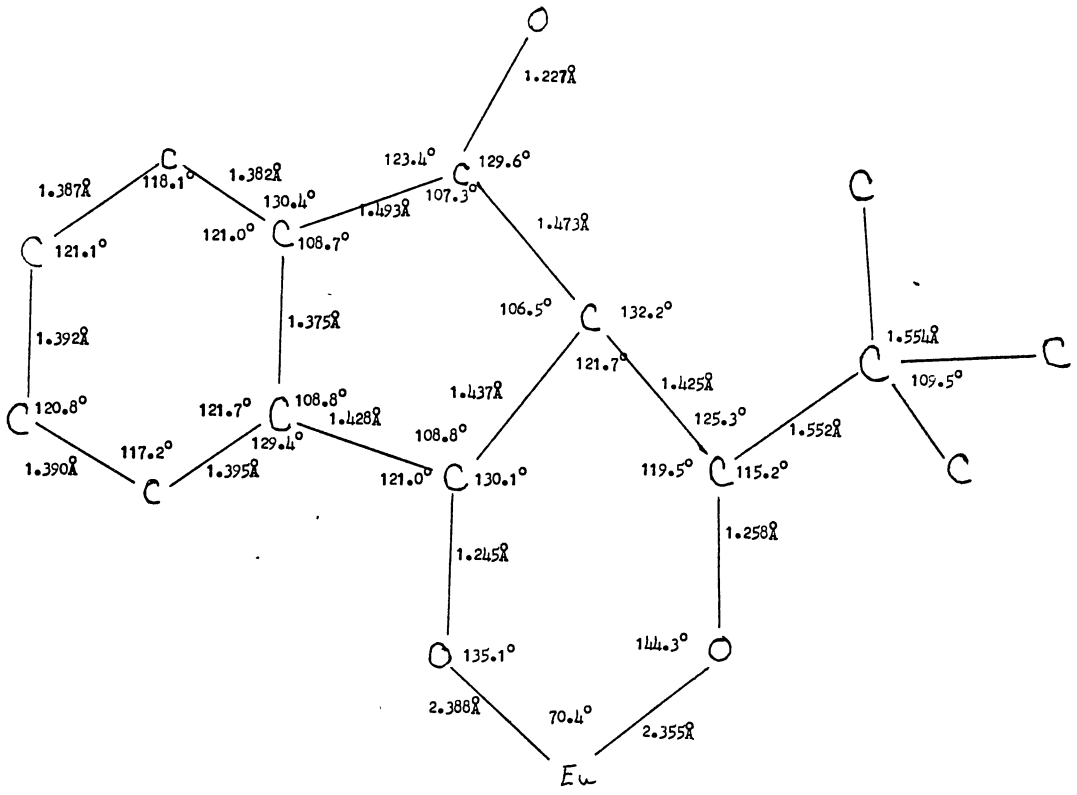


Figure 29. Average bond angles and distances of the coordinated pindone ligands and the europium ion.

are many different possibilities for the empirical formula depending on the procedure used to synthesize the crystals.

The average bond distances and angles for the coordinated ligands and those involving europium are shown in Figure 29. Table VIII lists comparison bond distances and Table IX lists comparison bond angles for the enolate portion of some published europium β-diketone structures. There are no large discrepancies between the bond distances and bond angles reported here and those of previously reported structures.

It is observed that the Eu-O and Eu-N distances of the non-β-diketone molecules (water, pyridine, dimethyl formamide) show a considerable range of values. Eu-O distances vary from 2.46Å to 2.53Å and the Eu-N distances are 2.60Å to 2.65Å. It appears that this distinct difference in Eu-N and Eu-O bonding distances could be used to discriminate between non-β-diketone ligand oxygen atoms and nitrogen atoms in structures where there is some question about the identity of the atom.

The bond angles for all the atoms in the structure are given in Table X and the bond distances are given in Table XI. The positional coordinates of all the atoms are given in Table XII. Anisotropic thermal parameters are given in Table XIII and are of the form

$$\exp[-2\pi^2(U_{11}h^2a^{*2}+U_{22}k^2b^{*2}+U_{33}l^2c^{*2}+2U_{12}hka^*b^*+ 2U_{13}hla^*c^*+2U_{23}klb^*c^*)] \times 10^3 \qquad (19)$$

The uncoordinated ligand in this structure shows the

Table VIII

Comparison Bond Distances (Å) for Various Europium-B-diketone Complexes

Complex (Ref.)	Eu-O Ketone	Eu-O Non-Ligand	Eu-N Non-Ligand	O-C Enolate	C-C Enolate
$Eu(TTA)_3 2H_2O$ (62)	2.42	2.53		1.27	1.42
$Eu(DPM)_3$ 2Pyridine (63)	2.35		2.65	1.25	1.38
Eu(DPM) Quin. (100)	2.33		2.63	1.27	1.39
Eu(THD) 2DMF (87)	2.36	2.45		1.39	1.38
This Work	2.37	2.46		1.25	1.43

Table IX

Comparison Bond Angles (°) for Various Europium-B-Diketones

Complex	O-Eu-O	Eu-O-C	O-C-C' C'Enolate	O-C-C' C'External	C-C-C Enolate
$Eu(TTA)_3$ $2H_2O$	73				
$Eu(DPM)_3$ 2Pyridine	71	136	124	115	125
$Eu(DPM)_3$ Quin.	72	134	124	115	126
$Eu(THD)_3$ 2DMF	70	136	125	114	122
This Work	70	134 144	119 130	115 121	121

TABLE X

BOND ANGLES FOR Eu C_{56} H_{57} O_{14}

ATOM ONE	ATOM TWO	ATOM THREE	ANGLE(°)	ATOM ONE	ATOM TWO	ATOM THREE	ANGLE(°)
O5	EU1	O102	75.5(2)	O103	C103	C104	121.3(7)
O5	EU1	O103	77.4(2)	O201	C201	C202	130.2(8)
O5	EU1	O202	146.2(2)	O201	C201	C209	122.6(8)
O5	EU1	O203	140.8(2)	O202	C210	C202	119.6(7)
O5	EU1	O402	75.8(2)	O202	C210	C211	113.6(7)
O5	EU1	O403	113.0(2)	O203	C203	C202	130.9(7)
O6	EU1	O102	135.2(2)	O203	C203	C204	121.0(7)
O6	EU1	O103	74.5(2)	O301	C301	C302	127.6(10)
O6	EU1	O202	143.7(2)	O301	C301	C309	123.0(9)
O6	EU1	O203	76.1(2)	O302	C310	C302	117.1(10)
O6	EU1	O402	113.6(2)	O302	C310	C311	116.0(10)
O6	EU1	O403	72.8(2)	O303	C303	C302	124.7(11)
O102	EU1	O103	70.6(2)	O303	C303	C304	126.2(10)
O102	EU1	O202	74.7(2)	O401	C401	C402	130.3(9)
O102	EU1	O203	119.4(2)	O401	C401	C409	123.3(9)
O102	EU1	O402	83.8(2)	O402	C410	C402	119.3(7)
O102	EU1	O403	149.4(2)	O402	C410	C411	117.0(7)
O103	EU1	O202	107.0(2)	O403	C403	C402	129.8(7)
O103	EU1	O203	74.9(2)	O403	C403	C404	120.8(7)
O103	EU1	O402	146.7(2)	C101	C102	C103	105.1(7)
O103	EU1	O403	139.0(2)	C101	C102	C110	132.7(8)
O202	EU1	O203	69.7(2)	C101	C109	C104	108.1(8)
O202	EU1	O402	85.4(2)	C101	C109	C108	130.1(8)
O202	EU1	O403	86.0(2)	C102	C101	C109	108.1(7)
O203	EU1	O402	137.8(2)	C102	C103	C104	109.0(7)
O203	EU1	O403	73.6(2)	C102	C110	C111	125.4(7)
O402	EU1	O403	71.0(2)	C103	C102	C110	122.1(7)
EU1	O102	C110	143.7(6)	C103	C104	C105	129.9(8)
EU1	0103	C103	133.9(6)	C103	C104	C109	109.4(8)
EU1	O202	C210	144.6(5)	C104	C105	C106	117.8(9)
EU1	O203	C203	138.2(5)	C104	C109	C108	121.8(9)
EU1	O402	C410	144.6(5)	C105	C104	C109	120.6(8)
EU1	0403	C403	133.2(5)	C105	C106	C107	120.6(10)
O101	C101	C102	127.8(9)	C106	C107	C108	120.5(10)
O101	C101	C109	124.2(8)	C107	C108	C109	118.6(9)
O102	C110	C102	119.6(8)	C110	C111	C112	109.5(8)
O102	C110	C111	115.0(7)	C110	C111	C113	106.8(8)
O103	C103	C102	129.6(7)	C110	C111	C114	109.7(8)
C112	C111	C113	111.5(8)	C303	C304	C309	109.8(9)
C112	C111	C114	110.1(8)	C304	C305	C306	121.1(10)

TABLE X (Continued)

C113	C111	C114	109.1(9)	C304	C309	C308	122.8(9)
C201	C202	C203	107.6(7)	C305	C304	C309	118.8(10)
C201	C202	C210	132.4(7)	C305	C306	C307	118.9(10)
C201	C209	C204	108.2(7)	C306	C307	C308	121.3(10)
C201	C209	C208	131.7(8)	C307	C308	C309	117.2(8)
C202	C201	C209	107.1(7)	C310	C311	C312	107.2(11)
C202	C203	C204	108.0(6)	C310	C311	C313	108.6(10)
C202	C210	C211	126.7(7)	C310	C311	C314	108.7(11)
C203	C202	C210	120.0(7)	C312	C311	C313	109.6(12)
C203	C204	C205	128.8(8)	C312	C311	C314	111.8(10)
C203	C204	C209	108.9(7)	C313	C311	C314	110.9(13)
C204	C205	C206	118.1(8)	C401	C402	C403	106.4(7)
C204	C209	C208	120.1(8)	C401	C402	C410	131.6(8)
C205	C204	C209	122.3(8)	C401	C409	C404	109.7(8)
C205	C206	C207	119.8(9)	C401	C409	C408	129.3(10)
C206	C207	C208	121.4(9)	C402	C401	C409	106.6(8)
C207	C208	C209	118.3(9)	C402	C403	C404	109.3(7)
C210	C211	C212	110.6(8)	C402	C410	C411	123.7(7)
C210	C211	C213	110.3(8)	C403	C402	C410	122.9(7)
C210	C211	C214	105.2(8)	C403	C404	C405	129.6(8)
C212	C211	C213	110.0(8)	C403	C404	C409	108.0(8)
C212	C211	C214	109.9(9)	C404	C405	C406	115.8(9)
C213	C211	C214	110.8(9)	C404	C409	C408	121.0(10)
C301	C302	C303	105.0(9)	C405	C404	C409	122.3(8)
C301	C302	C310	135.2(9)	C405	C406	C407	122.1(10)
C301	C309	C304	106.7(9)	C406	C407	C408	121.4(10)
C301	C309	C308	130.5(8)	C407	C408	C409	117.3(10)
C302	C301	C309	109.4(8)	C410	C411	C412	108.5(8)
C302	C303	C304	109.1(8)	C410	C411	C413	109.2(8)
C302	C310	C311	126.9(10)	C410	C411	C414	107.3(8)
C303	C302	C310	119.8(9)	C412	C411	C413	114.6(9)
C303	C304	C305	131.5(9)	C412	C411	C414	108.7(9)
				C413	C411	C414	108.3(9)

TABLE XI

BOND DISTANCES FOR EU C_{56} H_{57} O_{14}

ATOM ONE	ATOM TWO	DIST(A)	ATOM ONE	ATOM TWO	DIST(A)
EU1	O5	2.461(6)	C202	C203	1.448(11)
EU1	O6	2.462(6)	C202	C210	1.426(11)
EU1	O102	2.380(6)	C203	C204	1.485(11)
EU1	O103	3.384(6)	C204	C205	1.389(12)
EU1	O202	2.368(6)	C204	C209	1.390(12)
EU1	O203	2.391(6)	C205	C206	1.393(13)
EU1	O402	2.316(6)	C206	C207	1.407(16)
EU1	O403	2.389(5)	C207	C208	1.374(14)
O101	C101	1.234(11)	C208	C209	1.387(13)
O102	C110	1.252(10)	C210	C211	1.560(12)
O103	C103	1.249(10)	C211	C212	1.560(15)
O201	C201	1.217(10)	C211	C213	1.544(14)
O202	C210	1.263(10)	C211	C214	1.569(15)
O203	C203	1.236(10)	C301	C302	1.469(14)
O301	C301	1.239(11)	C301	C309	1.509(15)
O302	C310	1.316(14)	C302	C303	1.469(14)
O303	C303	1.253(13)	C302	C310	1.375(16)
O401	C401	1.230(13)	C303	C304	1.477(17)
O402	C410	1.260(10)	C304	C305	1.383(16)
O403	C403	1.250(10)	C305	C306	1.358(17)
C101	C102	1.472(12)	C306	C307	1.400(16)
C101	C109	1.481(14)	C307	C308	1.412(16)
C102	C103	1.448(12)	C308	C309	1.349(15)
C102	C110	1.419(12)	C310	C311	1.537(16)
C103	C104	1.460(12)	C311	C312	1.549(20)
C104	C105	1.389(13)	C311	C313	1.556(19)
C104	C109	1.370(12)	C311	C314	1.540(21)
C105	C106	1.392(14)	C401	C402	1.490(12)
C106	C107	1.399(15)	C401	C409	1.491(14)
C107	C108	1.370(15)	C402	C403	1.420(12)
C108	C109	1.376(13)	C402	C410	1.431(11)
C110	C111	1.562(13)	C403	C404	1.500(11)
C111	C112	1.555(14)	C404	C405	1.406(13)
C111	C113	1.576(14)	C404	C409	1.366(14)
C111	C114	1.516(15)	C405	C406	1.386(14)
C201	C202	1.457(11)	C406	C407	1.370(18)
C201	C209	1.508(12)	C407	C408	1.391(17)
C408	C409	1.384(14)	C411	C413	1.548(17)
C410	C411	1.534(12)	C411	C414	1.568(16)

TABLE XII

POSITIONAL COORDINATES OF THE ATOMS

ATOM	X(SIG(X))	Y(SIG(Y))	Z(SIG(Z))
EU1	0.18203(2)	0.19025(1)	0.47525(3)
O5	0.2628(4)	0.1693(2)	0.3239(4)
O6	0.3057(4)	0.2491(2)	0.4590(4)
O101	-0.0730(5)	0.2124(3)	0.0937(5)
O102	0.0749(4)	0.1542(2)	0.3618(4)
O103	0.1316(4)	0.2531(2)	0.3575(4)
O201	-0.1167(4)	0.2618(2)	0.7565(5)
O202	0.0626(4)	0.1696(2)	0.5759(4)
O203	0.1438(3)	0.2613(2)	0.5762(4)
O301	0.2487(5)	0.1051(3)	1.1600(5)
O302	0.2113(6)	-0.0015(3)	0.8997(6)
O303	0.1657(6)	0.0795(4)	0.8170(5)
O401	0.3915(6)	0.0271(3)	0.7338(6)
O402	0.2245(4)	0.1069(2)	0.5001(4)
O403	0.2702(4)	0.1845(2)	0.6276(4)
C101	-0.0167(6)	0.2330(4)	0.1494(6)
C102	0.0298(6)	0.2111(3)	0.2383(6)
C103	0.0823(5)	0.2522(3)	0.2801(6)
C104	0.0728(6)	0.2963(3)	0.2142(6)
C105	0.1124(7)	0.3436(4)	0.2214(7)
C106	0.0918(7)	0.3789(4)	0.1467(8)
C107	0.0337(7)	0.3667(4)	0.0660(8)
C108	-0.0050(6)	0.3200(4)	0.0607(7)
C109	0.0148(6)	0.2855(4)	0.1359(6)
C110	0.0285(5)	0.1625(3)	0.2836(6)
C111	-0.0277(6)	0.1165(4)	0.2448(7)
C112	-0.0060(7)	0.1048(4)	0.1334(7)
C113	-0.1265(6)	0.1319(5)	0.2542(9)
C114	-0.0081(7)	0.0706(4)	0.3106(8)
C201	-0.0459(5)	0.2709(3)	0.7217(6)
C202	0.0108(5)	0.2394(3)	0.6623(5)
C203	0.0834(5)	0.2704(3)	0.6328(5)
C204	0.0745(5)	0.3210(3)	0.6800(6)
C205	0.1289(6)	0.3621(3)	0.6756(7)
C206	0.1043(7)	0.4066(4)	0.7234(8)
C207	0.0250(7)	0.4083(4)	0.7734(8)
C208	-0.0279(6)	0.3665(4)	0.7795(7)
C209	-0.0032(6)	0.3224(3)	0.7310(6)
C210	0.0069(5)	0.1873(3)	0.6343(6)
C211	-0.0589(6)	0.1469(4)	0.6716(7)
C212	-0.0631(8)	0.1482(4)	0.7888(8)
C213	-0.0323(8)	0.0936(4)	0.6364(9)
C214	-0.1500(7)	0.1625(5)	0.6223(10)
C301	0.2272(6)	0.1112(4)	1.0696(7)
C302	0.2164(7)	0.0723(4)	0.9911(7)
C303	0.1849(7)	0.0998(5)	0.9005(7)
C304	0.1804(6)	0.1546(4)	0.9234(7)
C305	0.1586(7)	0.1950(4)	0.8618(8)
C306	0.1597(8)	0.2428(5)	0.9021(9)
C307	0.1857(7)	0.2505(4)	1.0037(9)
C308	0.2100(7)	0.2092(4)	1.0664(7)

TABLE XII(Continued)

C309	0.2062(6)	0.1626(4)	1.0245(7)
C310	0.2295(8)	0.0209(5)	0.9866(8)
C311	0.2636(10)	-0.0137(4)	1.0729(9)
C312	0.1944(10)	-0.0130(5)	1.1552(9)
C313	0.2725(13)	-0.0686(5)	1.0312(10)
C314	0.3528(9)	0.0066(6)	1.1130(11)
C401	0.3677(7)	0.0715(4)	0.7260(7)
C402	0.3140(6)	0.0968(3)	0.6444(6)
C403	0.3085(5)	0.1485(3)	0.6722(6)
C404	0.3575(6)	0.1567(3)	0.7710(6)
C405	0.3703(7)	0.2013(4)	0.8275(7)
C406	0.4180(7)	0.1961(5)	0.9180(8)
C407	0.4516(8)	0.1504(5)	0.9496(8)
C408	0.4399(8)	0.1069(5)	0.8920(8)
C409	0.3918(6)	0.1113(4)	0.8019(7)
C410	0.2704(5)	0.0774(3)	0.5551(6)
C411	0.2759(7)	0.0220(4)	0.5213(7)
C412	0.2319(9)	-0.0118(4)	0.6007(10)
C413	0.3724(9)	0.0085(5)	0.5033(10)
C414	0.2226(10)	0.0174(4)	0.4181(9)
H15	0.1536	0.3518	0.2787
H16	0.1186	0.4136	0.1507
H17	0.0200	0.3919	0.0125
H18	-0.0454	0.3111	0.0032
H25	0.1845	0.3601	0.6402
H26	0.1416	0.4371	0.7213
H27	0.0069	0.4404	0.8045
H28	-0.0823	0.3677	0.8173
H35	0.1425	0.1892	0.7894
H36	0.1427	0.2727	0.8595
H37	0.1866	0.2860	1.0326
H38	0.2291	0.2138	1.1380
H45	0.3465	0.2348	0.8037
H46	0.4293	0.2262	0.9613
H47	0.4855	0.1480	1.0150
H48	0.4651	0.0727	0.9146

TABLE XIII

ANISOTROPIC THERMAL PARAMETERS

ATOM	U11	U22	U33	U12	U13	U23
EU1	21.7(2)	30.3(1)	24.6(2)	1.4(1)	0.3(1)	1.1(1)
O5	48(4)	45(3)	43(3)	-5(3)	21(2)	-8(2)
O6	35(3)	67(4)	31(3)	-19(3)	-2(2)	0(2)
O101	55(4)	68(4)	66(4)	-3(3)	-29(3)	12(3)
O102	39(4)	45(4)	41(3)	-10(2)	-13(2)	13(2)
O103	44(4)	43(3)	41(3)	0(2)	-15(2)	11(2)
O201	41(4)	52(4)	59(4)	-3(3)	22(3)	-9(3)
O202	38(3)	34(3)	53(3)	-7(2)	20(2)	-6(2)
O203	29(3)	33(3)	47(3)	0(2)	14(2)	-2(2)
O301	91(6)	71(5)	34(3)	4(4)	-5(3)	-2(3)
O302	144(8)	91(6)	51(4)	-20(5)	0(4)	-34(4)
O303	99(6)	125(7)	46(4)	-13(5)	-11(4)	-17(4)
O401	122(7)	49(5)	94(6)	13(4)	-61(5)	15(4)
O402	49(4)	33(3)	32(3)	4(2)	-11(2)	0(2)
O403	42(3)	34(3)	32(3)	6(2)	-12(2)	-3(2)
C101	27(5)	62(6)	40(5)	7(4)	-10(3)	2(4)
C102	29(5)	44(5)	37(4)	8(4)	-9(3)	4(3)
C103	31(5)	37(5)	37(4)	6(3)	-3(3)	4(3)
C104	36(5)	43(5)	40(5)	8(3)	-3(3)	6(3)
C105	59(7)	44(6)	52(6)	-2(4)	-4(4)	15(4)
C106	75(8)	40(6)	78(7)	-2(5)	-11(5)	23(5)
C107	64(7)	67(7)	63(7)	11(5)	-6(5)	34(5)
C108	31(5)	59(6)	55(6)	17(4)	-1(4)	13(4)
C109	41(5)	46(5)	42(5)	3(4)	-1(3)	11(4)
C110	26(5)	43(5)	36(4)	0(3)	-2(3)	6(3)
C111	38(6)	51(6)	63(6)	-14(4)	-17(4)	4(4)
C112	68(7)	52(6)	54(6)	-6(5)	-8(5)	-11(4)
C113	28(6)	86(8)	88(8)	-15(5)	-7(5)	7(6)
C114	74(8)	54(7)	76(7)	-24(6)	-30(6)	24(5)
C201	38(5)	39(5)	30(4)	0(3)	6(3)	0(3)
C202	24(4)	31(4)	28(4)	6(3)	2(3)	2(3)
C203	29(4)	27(4)	31(4)	3(3)	-3(3)	0(3)
C204	32(5)	35(5)	25(4)	1(3)	-1(3)	1(3)
C205	39(5)	40(5)	47(5)	-5(4)	2(4)	-5(3)
C206	55(6)	39(6)	67(6)	-8(4)	0(4)	-9(4)
C207	66(7)	46(6)	73(7)	0(5)	11(5)	-29(5)
C208	44(6)	52(6)	53(5)	5(4)	7(4)	-17(4)
C209	35(5)	43(5)	28(4)	3(3)	0(3)	-6(3)
C210	29(4)	37(4)	32(4)	-3(3)	5(3)	1(3)
C211	34(5)	46(6)	64(6)	-15(4)	18(4)	-5(4)
C212	87(8)	59(7)	64(7)	-11(6)	35(6)	16(5)
C213	83(8)	32(6)	112(9)	-21(5)	36(7)	-12(5)
C214	38(7)	109(10)	97(9)	-18(6)	0(6)	-11(7)
C301	46(6)	73(7)	36(5)	0(5)	5(4)	-1(4)
C302	59(7)	73(8)	38(5)	-1(5)	6(4)	-8(5)
C303	58(7)	87(8)	43(5)	-6(6)	1(4)	-5(5)
C304	40(6)	93(8)	37(5)	1(5)	0(4)	5(5)
C305	60(7)	84(9)	47(6)	3(6)	-5(4)	8(5)
C306	56(8)	86(9)	70(7)	10(6)	-7(5)	21(6)
C307	48(7)	69(8)	81(7)	1(5)	9(5)	7(6)
C308	55(7)	64(7)	47(6)	6(5)	0(4)	4(4)
C309	32(5)	74(7)	39(5)	-5(4)	2(4)	4(4)
C310	82(8)	73(9)	56(7)	-5(6)	19(5)	-10(5)
C311	151(13)	55(7)	54(7)	3(8)	11(7)	-12(5)

TABLE XIII (Continued)

C312	149(12)	88(10)	58(7)	-16(8)	28(7)	5(6)
C313	277(19)	64(9)	75(9)	25(11)	0(10)	-21(7)
C314	90(10)	118(12)	109(11)	24(9)	-17(8)	11(9)
C401	59(7)	43(6)	50(5)	0(5)	-13(4)	8(4)
C402	38(5)	27(4)	38(4)	0(3)	-6(3)	1(3)
C403	33(5)	37(5)	29(4)	-8(3)	-5(3)	3(3)
C404	33(5)	50(6)	30(4)	-5(4)	-3(3)	1(3)
C405	46(6)	71(7)	39(5)	-16(5)	0(4)	-13(4)
C406	59(7)	106(10)	42(5)	-13(6)	-12(4)	-20(5)
C407	77(9)	113(11)	40(6)	-14(7)	-31(5)	-3(6)
C408	68(8)	98(9)	51(6)	6(7)	-29(5)	21(6)
C409	51(6)	67(7)	35(5)	-9(5)	-16(4)	2(4)
C410	39(5)	27(4)	29(4)	-4(3)	0(3)	1(3)
C411	60(6)	37(5)	56(6)	6(4)	-19(4)	-7(4)
C412	109(11)	49(7)	94(9)	-30(7)	-8(7)	25(6)
C413	87(9)	64(8)	103(9)	42(7)	-8(7)	-38(7)
C414	140(13)	49(7)	76(8)	9(7)	-62(8)	-29(6)

Anisotropic thermal parameters are in the form:

$$\exp(-2\pi^2(U_{11}h^2a^{*2}+U_{22}k^2b^{*2}+U_{33}l^2c^{*2}+2U_{12}hka^*b^*+ 2U_{13}hla^*c^*+2U_{23}klb^*c^*)) \times 10^3$$

same bond distances and angles as those reported elsewhere for the uncomplexed pindone molecule (72). This indicates that the uncoordinated ligand here is present in the enol form. This is also evident from examination of the C-O distances of the uncomplexed ligand. Even though the enol hydrogen was not located, the O302-C310 distance of 1.32(1)Å is indicative of a C-OH bond distance, and the C302-C310 bond distance of 1.38(2)Å is indicative of a C=C bond.

CHAPTER X

SUMMARY AND CONCLUSIONS

This study has shown that europium-sensitized fluorescence can be used in the determination of diphacinone, chlorophacinone and pindone and may be useful in the determination of other similar compounds such as 2-benzoyl-1,3-indandione and 2-acetyl-1,3-indandione. This work has developed a procedure for the fluorometric determination of these compounds in commercial products. This is desirable because fluorometric procedures generally have lower limits of detection and larger linear dynamic ranges than spectrophotometric methods. The limits of detection utilizing this fluorescence procedure are lower than the UV-VIS method for diphacinone and chlorophacinone and comparable for pindone.

A new procedure has been developed for the determination of diphacinone and chlorophacinone in commercial products which does not require a chromatographic separation step. This reduces the amount of time necessary to complete the analysis. The procedure is relatively free from interferences. In instances when a chromatographic separation step is necessary, a post-column complexation apparatus has been described. The apparatus can also be used to

develop a flow injection analysis system for routine analysis.

Some qualitative information can be obtained from examination of the excitation spectra. The substituted indandiones have a unique excitation spectra and can be differentiated from other β-diketone ligands that exhibit europium-sensitized fluorescence. However discrimination among the indandiones themselves would be difficult based strictly on the shape of the excitation spectra.

The amount of data necessary to describe the excitation spectra of the indandiones can be greatly reduced using the fitting program that has been written. This may be useful in the identification of the indandione and in producing a library of spectra for the qualitative analysis of unknowns by storing only the peak shape parameters and the locations and peak intensities of the excitation spectra. The problem with the procedure lies in the manual digitization of the spectra. This was also noted in an earlier study (46) and the conclusion now as then is that for the data reduction to be feasible in routine qualitative analysis there has to be a direct digital readout of the spectrum. Also, the reliability of the instrument to give a constant scan rate must be upgraded. There are instruments on the market that are micro-processor controlled and perhaps the results obtained with one of these instruments would have less error.

Some theoretical aspects of the fluorescence have been investigated. The stoichiometry of the complexes is EuL_2 in

acetonitrile in the presence of trioctylphosphine oxide and EuL in water at pH 7.2. The fluorescence in water is weaker than that observed in acetonitrile. A subject of future investigation would be to add another complexing molecule, such as EDTA to the aqueous complex in an attempt to exclude water from the coordination sphere and increase the emission from the complex. The triplet level of the ligands must be equal to or slightly greater than the resonance level of the lanthanide before the sensitized fluorescence is observed. The fluorescence of the complexes can be increased using the synergistic agent trioctylphosphine oxide. There are other trialkylphosphine oxides available and a future study should investigate which of these, if any, could further increase the fluorescence.

The geometry of the enolate anion is important. Apparently the trans enolates are not capable of producing the sensitized fluorescence because no complex is formed with these ligands. The ligand also must not dissipate the absorbed energy by native fluorescence in order to observe the europium fluorescence. This requirement probably is related to the energy level match referred to above.

The X-ray crystal structure of the europium-pindone complex shows bond angles and distances about the europium and the enolate that are very similar to other reported structures. The square-antiprism geometry about the central europium ion which is observed is common to europium-B-diketone structures and the coordination number of the

europium is eight which is also common. The included ligand which is not involved in bonding to the europium ion is present in the enolate form which is consistent with the structure reported for uncomplexed pindone. Attempts to obtain the structure of the diphacinone and chlorophacinone europium complexes were unsuccessful because the crystals that grew were disordered and decomposed slightly upon standing.

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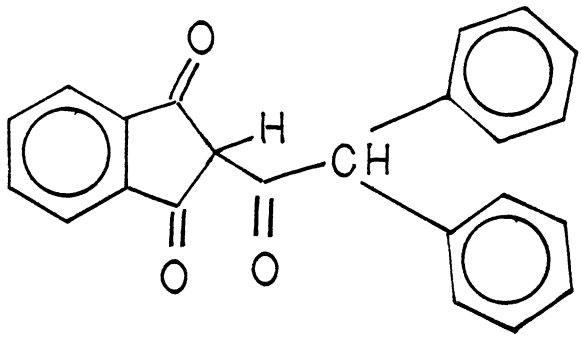
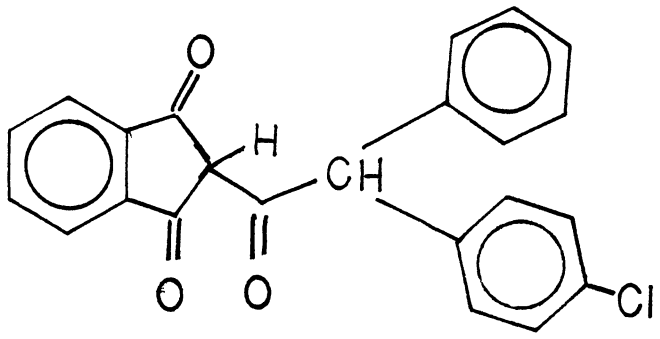
APPENDIX A

MOLECULAR STRUCTURES

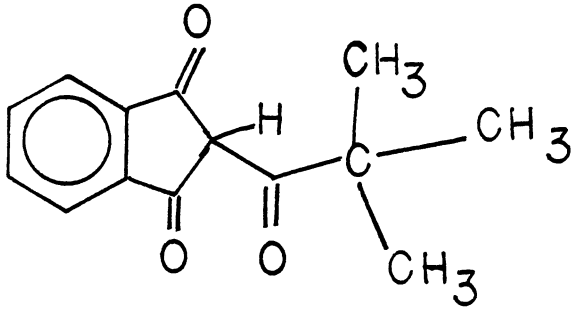
Diphacinone



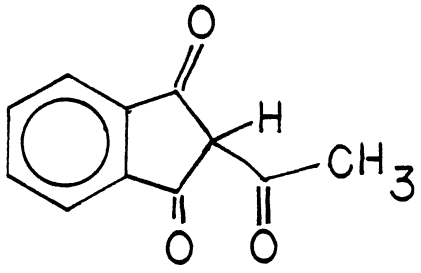
Chlorophacinone



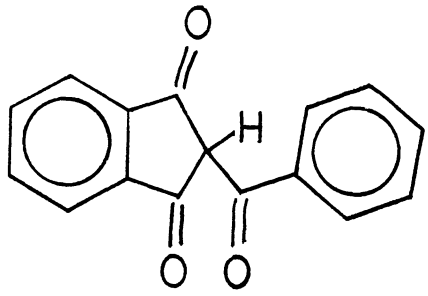
Pindone



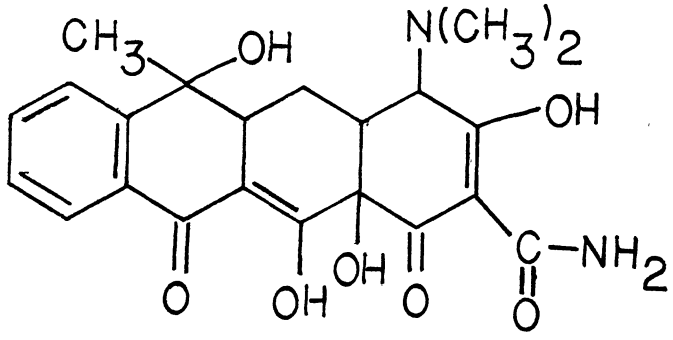
2-Acetyl-1,3-indandione



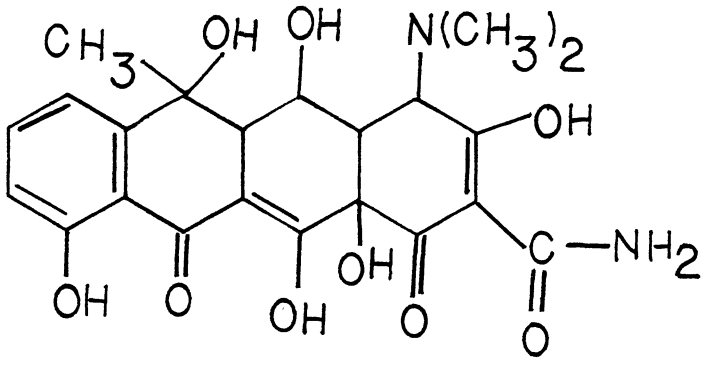
2-Benzoyl-1,3-indandione



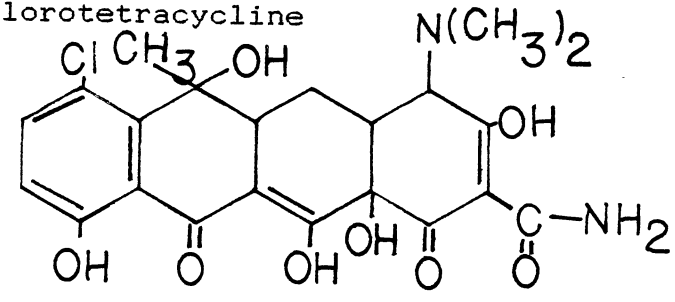
Tetracycline



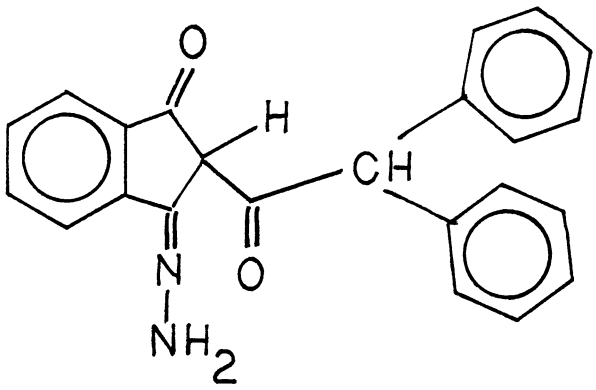
Oxytetracycline



Chlorotetracycline

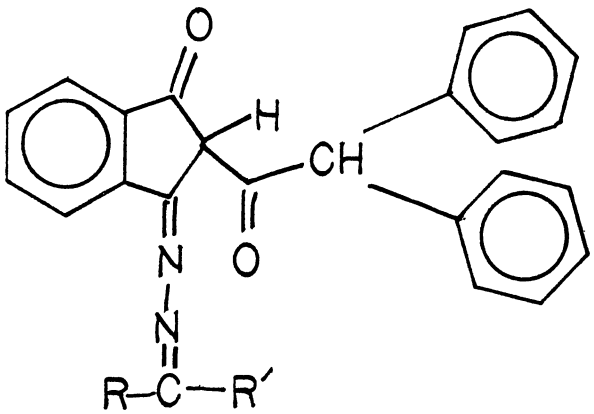


2-Diphenylacetyl-1,3-indandione-1-hydrazone



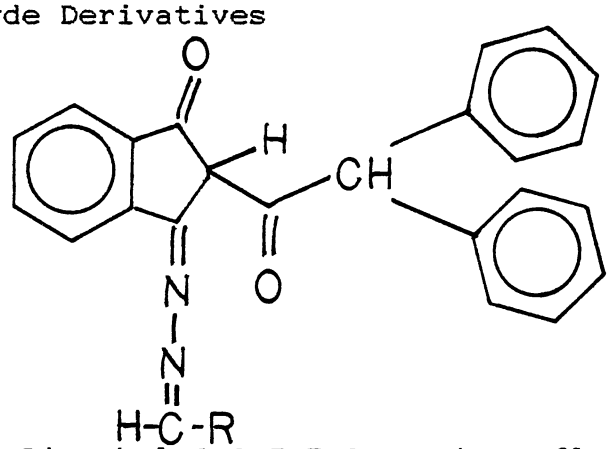
2-Diphenylacetyl-1,3-indandione-1-hydrazone

Ketone Derivatives

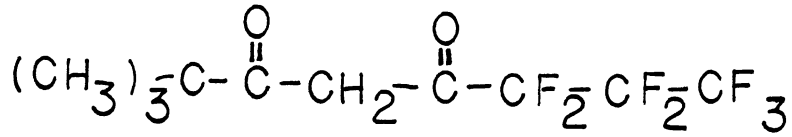


2-Diphenylacetyl-1,3-indandione-1-hydrazone

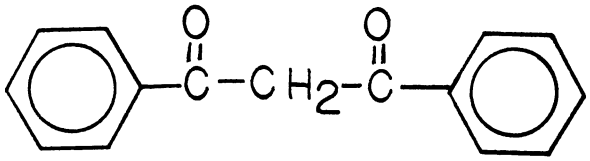
Aldehyde Derivatives



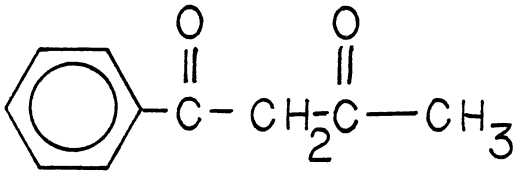
FOD, 2,2-Dimethyl-6,6,7,7,8,8,8-heptafluoro-3,5-octanedione



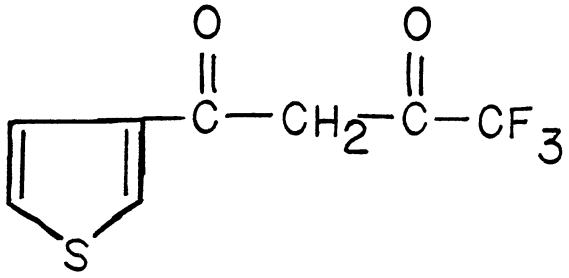
Dibenzoylmethane



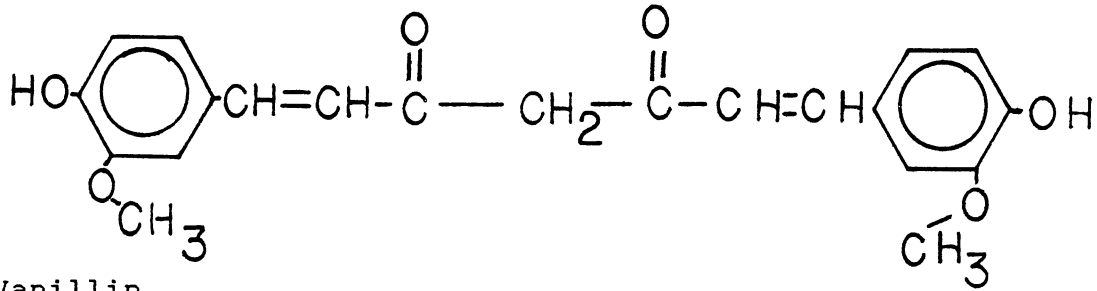
Benzoylacetone



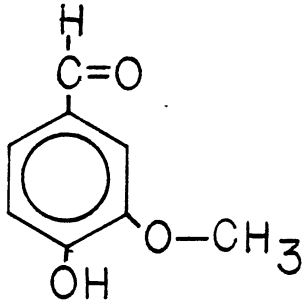
Thenoyltrifluoroacetone



Curcumin



Vanillin



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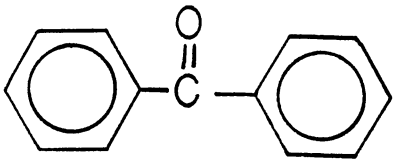
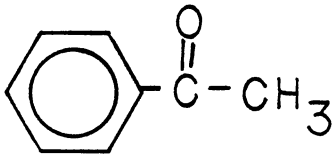
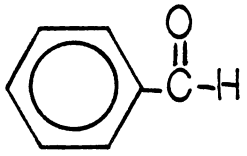


Acetophenone



Benzaldehyde



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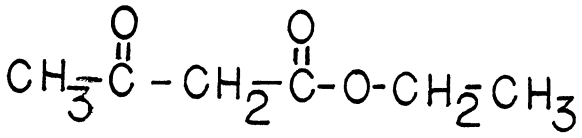

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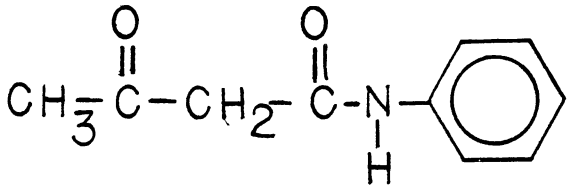
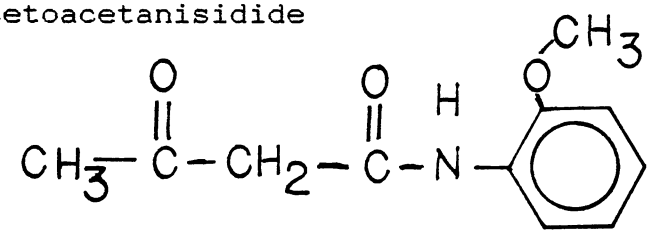


o-Acetoacetanisidide



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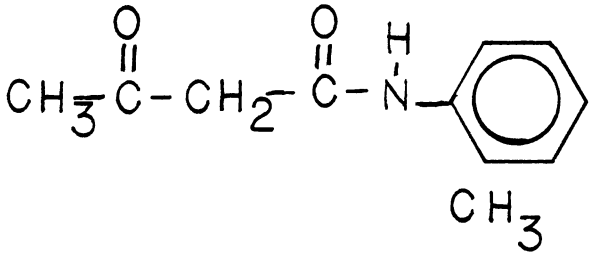
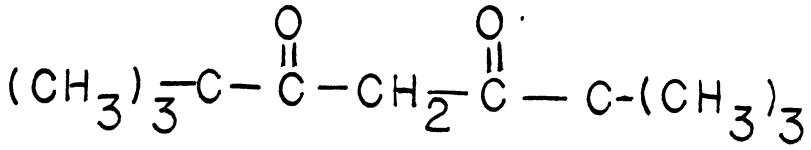


DPM, THD, 2,2,6,6-tetramethylheptane-3,5-dione



APPENDIX B

DATA REDUCTION AND FITTING PROGRAM

```
C
C
C     THIS IS THE DRIVER PROGRAM THAT FITS THE EXCITATION
C     SPECTRA OF A EU B-DIKETONE TO A SERIES OF
C     EXPONENTIALS FOR DATA REDUCTION
C     AND IDENTIFICATION USING THE MARQ LIBRARY ROUTINES.
C
C
C     SUBROUTINE CALC IS THE SUBROUTINE WHICH USES THE X
C     PARAMETERS SUPPLIED BY THE MARQ ROUTINES TO CALCULATE
C     THE FITTED SPECTRUM.
C     THE MARQ ROUTINES ARE LIBRARY ROUTINES USED TO FIT
C     THE DATA TO THE EQUATIONS.
C
C
      EXTERNAL CALC
C
      DOUBLE PRECISION XMAX, XMIN, DELTX, DELMN, ERR, FOBJ,
     *FLAMB, FNU, RELDF, RELMN, RZERO, HUGE, X,
     *FIT, FITSV, Y, YSIG, P, PH, PK, EX, AMAX
      COMMON/CDAT/ FIT(300), Y(300), YSIG(300), NPTS
      COMMON/CSTEP/ X(20), XMAX(20), XMIN(20),DELTA(20),
     *DELMN(20), ERR(20,21), FOBJ, NV, NTRAC, MATRX,
     *MASK(20), NFMAX, NFLAT, JVARY, NXTRA, KFLAG, NOREP,
     *KERFL, KW
      COMMON/NLLS4/ FKAMB, FNU, RELDF, RELMN, METHD, KALP,
      KORDF, MAXIT, LEQU, MXSUB, MXPUD
```

```
      COMMON/PARAM/ EX(300), PK(20), PH(20), NPKS, NPS
      DIMENSION EXW(300), FI(300), NAME(9)
C
C
C     IN=UNIT NUMBER OF THE INPUT DEVICE
C     KW=UNIT NUMBER OF THE OUTPUT DEVICE
C
      IN=5
      KW=6
      WRITE(KW,400)
400   FORMAT('0','WHAT IS THE LIGAND NAME AND ITS CONC')
      READ(IN,401)(NAME(I),I=1,9)
401   FORMAT(9A4)
      WRITE(KW,402)(NAME(I),I=1,9)
402   FORMAT('0','THIS FIT IS FOR   ',1X,9A4)
C
C     SET SEVERAL VALUES FOR THE MARQ ROUTINES
C
      CALL STSET
C
C     DATA SECTION
C     NPKS=NUMBER OF PEAKS IN THE EXCITATION SPECTRUM
C     NPTS=NUMBER OF DATA POINTS AS WAVELENGTH, INTENSITY
C     PAIRS
C     PK=PEAK LOCATION OF EACK PEAK IN NANOMETERS
C     PH=INTENSITY OF EACH PEAK
C
```

```
C     READ IN THE PEAK PARAMETERS
C
      WRITE(KW,290)
290   FORMAT('0','ENTER THE NUMBER OF PEAKS AND THE NUMBER
     *OF DATA POINTS')
      WRITE(KW,390)
390   FORMAT(1X,'AS XX,XX')
      READ(IN,255) NPKS, NPTS
255   FORMAT(I2,1X,I2)
      WRITE(KW,256)NPKS,NPTS
256   FORMAT('0','NPKS= ',I2,1X,'  NPTS= ',I2)
C
C     SET THE NUMBER OF VARIABLES TO FIT THE
C     SPECTRUM AS 2 TIMES THE NUMBER OF PEAKS
C     NV=THE NUMBER OF PARAMETERS TO BE FIT
C
      NPS=NPTS
      NV=NPKS*2
C
C     SET THE PEAK LOCATIONS AND INTENSITIES
C
      WRITE(KW,291)
291   FORMAT('0','ENTER THE DATA FOR EACK PEAK AS
     *WAVE,INT')
      WRITE(KW,391)
391   FORMAT(1X,'AS XXX.X,XXX.X')
      WRITE(KW,700)
```

```
700   FORMAT(1X,'THIS DATA WILL BE NORMALIZED')
      AMAX=0.00
      DO 45 I=1,NPK
      READ(IN,260)PK(I),PH(I)
45    IF(PH(I).GT.AMAX) AMAX=PH(I)
260   FORMAT(F5.1,1X,F5.1)
      WRITE(KW,262)
262   FORMAT('0','PK     PH')
C
C     NORMALIZE THE DATA
C
      DO 47 I=1,NPKS
47    PH(I)=PH(I)/AMAX
      DO 77 I=1,NPKS
77    WRITE(KW,261)PK(I),PH(I)
261   FORMAT(1X,F5.1,3X,F5.3)
C
C     INITIALIZE THE PEAK SHAPE PATAMERERS X
C
      DO 30 I=1,NV
      XMAX(I)=0.
30    X(I)=-1.D-2
      WRITE(KW,294)
294   FORMAT('0','ENTER THE DIGITIZED SPECTRUM AS ')
      WRITE(KW,295)
295   FORMAT(1X,'WAVE,INT AS XXX.X,XXX.X')
C
```

```
C     ENTER THE DIGITIZED SPECTRUM AND SET THE ESTIMATED
C     ERROR FOR EACH INTENSITY
C
      DO 10 I=1,NPTS
      YSIG(I)=1./AMAX
10    READ(IN,100)EX(I),Y(I)
100   FORMAT(F5.1,1X,F5.1)
      WRITE(KW,250)
250   FORMAT('0','EX WAVE     INT')
      DO 50 I=1,NPTS
      EXW(I)=EX(I)
      Y(I)=Y(I)/AMAX
      FI(I)=Y(I)
50    WRITE(KW,200)EX(I),Y(I)
200   FORMAT(1X,F5.1,4X,F5.3)
C
C     PLOT THE DIGITIZED SPECTRUM USING LIBRARY ROUTINE
C     FRAMP
C
      WRITE(KW,300)
300   FORMAT('1','PLOT OF THE DIGITIZED SPECTRUM')
      CALL FRAMP(EXW,FI,NPTS,KW)
C
C     START THE FIT PROCEDURE USING THE LIBRARY ROUTINES
C
      CALL STEPT(CALC)
C
```

```
C     THE FIT IS COMPLETED.  PLOT THE FITTED SPECTRUM
C
      DO 20 I=1,NPTS
      EXW(I)=EX(I)
      FI(I)=FIT(I)
20    CONTINUE
      WRITE(KW,301)
301   FORMAT('1','PLOT OF THE FITTED SPECTRUM')
      CALL FRAMP(EXW,FI,NPTS,KW)
      STOP
      END
C
C
C
C     SUBROUTINE CALC THAT CALCULATES THE FITTED SPECTRUM
C     UPON RECIEVING THE FIT PARAMETERS FROM THE MARQ
C     ROUTINES
C
      SUBROUTINE CALC(FIT)
      DOUBLE PRECISION XMAX, XMIN, DELTX, ERR, FOBJ, FLAMB,
     *FNU, RELDF, RELMN, RZERO, HUGE, X, FIT, W, PH, EX,
     *PK, DELMN
      DIMENSION FIT(300)
      COMMON/CSTEP/ X(20), XMAX(20), XMIN(20),DELTA(20),
     *DELMN(20), ERR(20,21), FOBJ, NV, NTRAC, MATRX,
     *MASK(20), NFMAX, NFLAT, JVARY, NXTRA, KFLAG, NOREP,
     *KERFL, KW
```

```
      COMMON/PARAM/ EX(300), PK(20), PH(20), NPKS, NPS
      NPTS=NPS
      DO 20 J=1,NPTS
      FIT(J)=0.00
      DO 10 I=1,NPKS
      IP=I+NPKS
      W=(EX(J)-PK(I))**2
      IF(EX(J).GT.PK(I))W=W*X(I)
      IF(EX(J).LE.PK(I))W=W*X(IP)
      IF(W.LE.-10.)GO TO 11
      W=DEXP(W)
      GO TO 12
11    W=0.0
12    W=PH(I)*W
10    FIT(J)=FIT(J)+W
20    CONTINUE
      RETURN
      END
```

APPENDIX C

APPROXIMATE TRIPLET LEVELS OF SOME LIGANDS

cm^{-1} X 10^{-3}	Lanthanide Ion	Ligand	Ref
		Thenoyltrifluoroacetone	e
22		Benzoylacetone	a,b,e
	Dy^{+3}_____	Tribenzoylmethane	b
		Dibenzoylmethane	a,b
	Tb^{+3}_____	Sulfosalicyclic acid	d
20			
		Dibenzoylmethane	e
		2-Methyl-8-Hydroxyquinoline	c
18		o-Hydroxybenzophenone	a
	Sm^{+3}_____	Diphacinone	f
		8-Hydroxyquinoline	a
	Eu^{+3}_____	Pindone, Chlorophacinone	f
16			

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