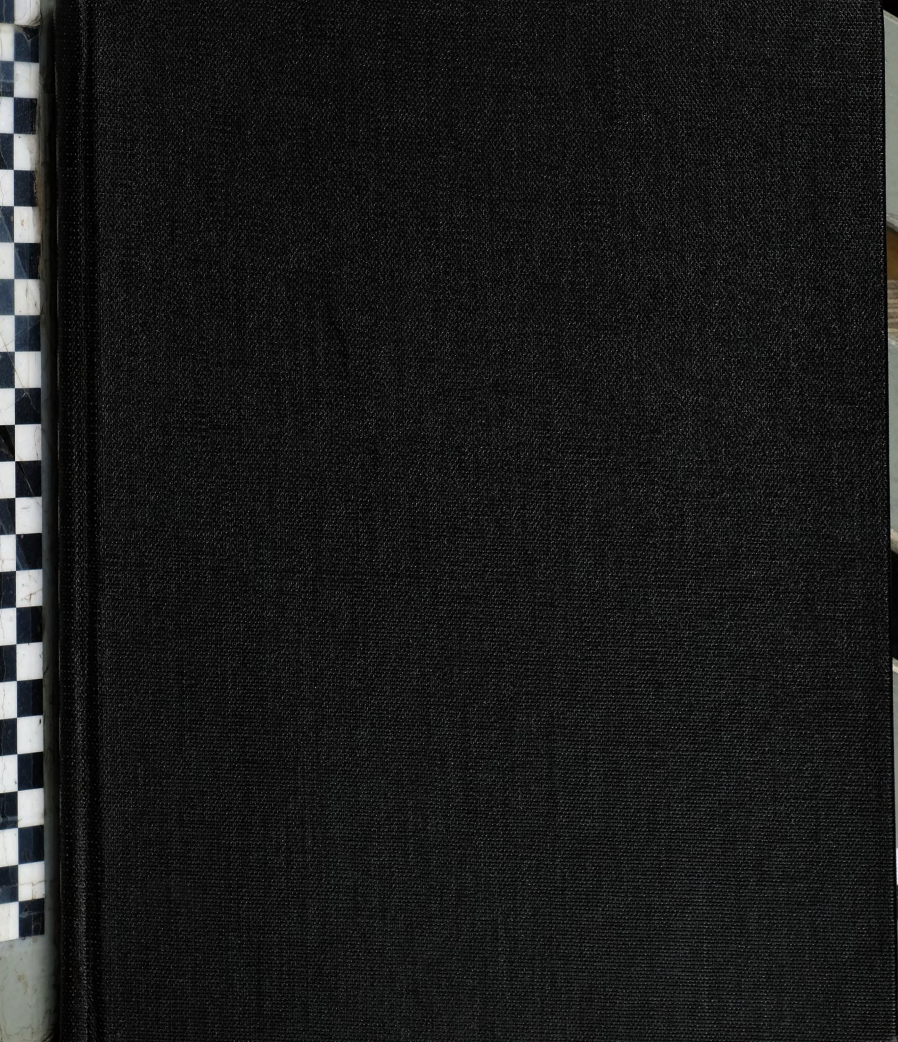






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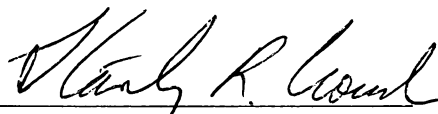
Room-temperature Phosphorescence Study of Poly-
nuclear Aromatic Hydrocarbon Compounds in
Organized Media

presented by

Hai-Dong Kim

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Chemistry


Major professor

Date September 12, 1989

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THE HYDROGRAPHIC SURVEY OF THE

ROOM-TEMPERATURE PHOSPHORESCENCE STUDY OF POLYNUCLEAR
AROMATIC HYDROCARBON COMPOUNDS IN ORGANIZED MEDIA

By

Hai-Dong Kim

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Chemistry

1989

ABSTRACT

ROOM-TEMPERATURE PHOSPHORESCENCE STUDY OF POLYNUCLEAR
AROMATIC HYDROCARBON COMPOUNDS IN ORGANIZED MEDIA

By

Hai-Dong Kim

Room-temperature phosphorescence spectrometry (RTP) is a very convenient method compared to conventional low-temperature phosphorimetry. Although the importance of RTP has been realized in the analytical world over the last two decades, it still needs a number of improvements to be used in routine chemical analysis. This research was intended to develop a new RTP methodology using a synthetic enzyme model compound, $N,N,N',N',N'',N'',N''',N'''$ -octamethyl-2,11,20,29-tetraaza[3.3.3.3]paracyclophanetetraammonium tetrafluoroborate (methyl-APC).

In the first part of the research, a computer-controlled versatile luminescence spectrometer was developed with completely menu-driven software for the control of the instrument, data acquisition, and data manipulation on a personal computer. The use of a disk-type chopper gave a number of advantages over the conventional rotating-can type

phosphoroscope. Results are presented to show the versatility of the developed instrument.

A new decay kinetic model of micellar stabilized RTP (MS-RTP) is proposed based on triplet quenching on the micellar surface. The dependence of MS-RTP lifetimes on environmental conditions was investigated. It was found that heavy atoms were the major contributor in MS-RTP lifetimes. Also, solution temperature and the method of deoxygenation affect observed lifetimes significantly.

The potential analytical applicability of methyl-APC in RTP was examined. Methyl-APC showed a very strong binding ability toward anionic and neutral compounds, but did not induce RTP when used alone. The addition of pre-micellar concentrations of surfactant effectively covered both open ends of the APC-guest inclusion complex and, thereby, induced RTP. Methyl-APC also showed more selectivity and sensitivity than cyclodextrins due to the hydrophobic and electrostatic interactions involved.



ACKNOWLEDGEMENTS

It took a long time to realize the true meaning of "That's the way of science " mentally and physically. Whenever I sank down hopelessly during my research, other fellows in the Lab tried to persuade me how science is difficult by saying "That's the way of science.". At that time I had no answer for that, but now, I have an answer for that. "But it's worth to try."

I would like to thank sincerely Dr. Stanley R. Crouch and Dr. Matthew J. Zabik for their warm advice and guidance throughout my research. Both of you really inspired me to complete my work. When I joined the Crouch group, there were already eighteen people hanging around to make me dizzy. I was really surprised to see Stan's ability to manage each kid including me. Dr. Zabik's Lab was more spacious compared to the Lab in chemistry. Thanks to Dr. Zabik's special attention, I could complete my research in his Lab.

I would like to thank to all group members of both groups. These include, Peter, Jimmy, Mayda, Steve, Larry, Pavlos, Kris, Susan, Mary Ann, Gamal, Glen, Ines, Salah, and everyone else who shared the same adviser. I also thank Dr. Leavitt and his group members who helped me in many ways during my research.

Dr. Nelson Herron and Dr. Paul Kraus were the old fellows who deserve my deep acknowledgement. We were the same funny



guys who tried to catch the tough fruit, RTP. Also, we were the same guys who shared the same bitterness and fragrance of that fruit. I still remember a loud shouting session when Nelson exchanged punches with the famous, old instrument.

A special thanks is reserved for my family: my wife, Sook; our pretty daughter, Suyoun; our baby son, Junyoung; and my parents who prayed and waited patiently for my success.

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CHAPTER I

INTRODUCTION

Luminescence spectrometric methods are among the most popular analytical methods. Luminescence spectrometric methods allow a greater sensitivity and selectivity in chemical analysis than absorption spectrometry. Also, luminescence spectrometry provides a lower limit of detection and a larger linear dynamic range compared to the absorption spectrometry.

Room-temperature phosphorimetry (RTP) is quite different from the classic low temperature phosphorescence technique which is typically performed in glass matrices at liquid nitrogen temperature (1). Since the discovery of RTP, numerous techniques have been developed to induce RTP from various molecules. These include solid-state RTP (2), micellar-stabilized RTP (3), sensitized RTP (4), cyclodextrin enhanced RTP (5), and colloidal or microcrystalline RTP (6). The probability for observing RTP (especially in solutions) is enhanced in a rigid molecular environment due to reduced quenching by oxygen or other impurities and in the presence of a heavy atom due to an increase in the rate of intersystem crossing.

Photophysical and photochemical properties of organic molecules included in the cavity of cyclodextrins (CDs) have

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been of considerable interest in the past decade (7). Cyclodextrins form complexes with hydrophobic organic and organometallic molecules in aqueous solution. Although there is no direct proof for a fixation of the guest molecules within the void space of the cyclodextrin, the complexes are usually regarded as inclusion compounds, host-guest compounds, in which hydrogen bonding, van der Waals forces, and hydrophobic interactions are the main binding forces (8). When lumiphores are included inside the cyclodextrin molecules, the resulting RTP shows enhanced intensity and lifetime because the cyclodextrins protect the lumiphores from quenchers (9).

The synthetic macrocyclic enzyme model compounds, azaparacyclophanes (APCs) can act as inclusion hosts capable of molecular organization by forming complexes with a variety of hydrophobic molecules (10). Water-soluble azaparacyclophane, N,N,N',N',N'',N'',N''',N'''-octamethyl-2,11,20,29-tetraaza[3.3.3.3]paracyclophanetetraammonium tetrafluoro-borate (mehtyl-APC), is an excellent inclusion host toward certain organic substrates. A unique substrate specificity was observed due to its cavity size and functionality (11). The macrocyclic cavity is surrounded by the wall which is formed by four benzene rings and four quarternary ammonium residues around the macrocyclic ring.

The objectives of this research are divided into three parts. The first objective of this research was to develop a versatile computer-controlled luminescence instrument for

measuring fluorescence, RTP, and RTP lifetimes. The second objective was to study the decay kinetics for micellar stabilized RTP in order to understand the mechanism of triplet quenching in micellar solution. The last objective of this research was to develop a new RTP method using a synthetic enzyme model compound, methyl-APC. This dissertation presents work performed mainly on these three projects.

The entire dissertation is divided into eight chapters plus appendix. Following introductory remarks in this chapter, the fundamental background of molecular luminescence is described in chapter II. This chapter is divided into four main categories: the absorption and emission of light; the effect of molecular structure; the effect of medium condition; and, quenching of luminescence. This background is very important for understanding the RTP phenomenon.

In chapter III, recent developments in RTP are reviewed. Various conditions necessary for the observation of RTP are discussed first. A brief historical survey of RTP and various methods for RTP measurements developed so far are described in the following part. The last two parts discuss the essential requirements to observe RTP in solution, deoxygenation methods and the heavy atom effect.

Applications of macrocyclic compounds in chemical analysis are discussed in chapter IV. The host-guest molecular inclusion phenomenon is discussed first. The general



overview of synthetic enzyme model compounds, azaparcyclophanes, in host-guest chemistry and their applications are presented in the second part. The last part reviews various macrocyclic compounds currently used in chemical analysis.

The development of a computerized RTP instrument for this research is described in chapter V. This chapter is divided into two broad parts: the luminescence spectrometer and a modification of the IBM-PC data acquisition board. The first part describes the general principles of the new instrument, computer interfacing, excitation source modulation and detection for time-resolved spectrometry, the use of the instrument for RTP and RTP lifetime measurements, and the software developed for instrument control and data acquisition are also discussed. The second part describes a modification of the IBM-PC data acquisition board used later in this research with the Perkin-Elmer LS-5B spectrometer.

The RTP lifetime measurements and the study of decay kinetics in micellar-stabilized RTP are presented in chapter VI. Various methods for determining RTP lifetimes are discussed first. And then, a new decay kinetics model in micellar stabilized RTP is proposed. In the last part, RTP lifetime data for polyaromatic hydrocarbon molecules, pyrene, naphthalene, biphenyl, and 2-bromonaphthalene are presented. Also various factors affecting the observed RTP lifetime are discussed with the experimental data.

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Chapter VII presents RTP data of organic compounds in mixed organized media: the azaparacyclophane-surfactant system. The strength of molecular association of azaparacyclophane with anionic and neutral compounds in solution is compared to cyclodextrin. The factors affecting RTP in this system as well as an analytical figure of merit are discussed along with the experimental data.

Conclusions and future prospects on instrumentation, application of synthetic macrocyclic compounds, and RTP decay lifetimes in chemical analysis are described in the final chapter. Source codes for the software developed during this research are presented in the appendix.

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CHAPTER II

MOLECULAR LUMINESCENCE

The emission of light by electronically excited molecules is the basis of molecular luminescence spectroscopy. Photoluminescence originates from atoms and molecules that are excited by an external light source, as opposed to bioluminescence, chemiluminescence, and electroluminescence, which are excited by a biological process, by a chemical reaction, and by electrical energy, respectively. Fluorescence and phosphorescence are the two major types of luminescence which occur in molecules as a result of photophysical process after absorption of light. Fluorescence is a radiative transition from the lowest excited singlet state to the ground state. Phosphorescence is the result of a radiative transition from the lowest excited triplet state to the ground state.

This chapter describes the nature of the photophysical processes of photoluminescence. The theoretical aspect of photoluminescence is discussed first. The next two parts deal with the dependence of photoluminescence upon molecular structure and the molecular environments. The last part briefly discusses quenching effects for the two major types of photoluminescence, fluorescence and phosphorescence.

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A. Absorption and Emission of Light by Molecules.

A light wave may be thought of as electromagnetic radiation traveling with a speed of 3.0×10^{10} cm/s. When a molecule is exposed to electromagnetic radiation, it can absorb a photon of that radiation. The absorption or emission process entails the interaction of the electric field associated with the exciting light with the loosely bound p or nonbonded electrons of the absorbing molecule. This interaction distorts the electronic distribution of the absorbing molecule and causes energy to be absorbed from the electric field of the exciting light wave.

Electronic absorption entails the promotion of an electron, by the absorption of energy, from an originally occupied bonding or nonbonding orbital to an originally unoccupied molecular orbital. Because σ electrons are usually bound very tightly by the molecule, a great deal of energy (which is beyond the conventional luminescence spectroscopic region) is required to promote these electrons to vacant molecular orbitals. On the other hand, delocalized π electrons are not as tightly bound as σ electrons. Hence, their electronic promotion do not require higher energy than do the σ electrons. The nonbonding electrons are only slightly lower in energy than atomic valance shell electrons. As a result, the energy gap between the n -orbitals and vacant π orbitals is very small (1). In most organic molecules of spectroscopic interest it is only the σ

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electrons and n electrons which are involved in electronic transition to vacant π molecular orbitals.

The amount of light absorbed by the molecules is given by the Beer-Lambert law:

$$A = \log \left(\frac{P_0}{P} \right) = \epsilon bc \quad (2.1)$$

where A is the absorbance, P_0 and P are the incident and transmitted light intensity, respectively, ϵ is the molar absorptivity of the molecule, and c is the molar concentration of the absorber in light path length b . To utilize the above equation properly there are several requirements: the solution must be sufficiently dilute so that changes in the refractive index are negligible, the radiation must be monochromatic, and stray light must be negligible. Quantum mechanically the electronic excitation of particular molecular species occurs only if the exciting radiation energy corresponds to the difference in energy between the electronically excited state and the ground state of the absorber.

There are several vibrational levels within each electronic state in molecules. In the ground electronic state, almost all molecules occupy the lowest vibrational level at room temperature. When a molecule absorbs a particular frequency of radiation it can be excited to one of several vibrationally, as well as electronically excited energy levels (Figure 2.1). If the absorbed radiant power by

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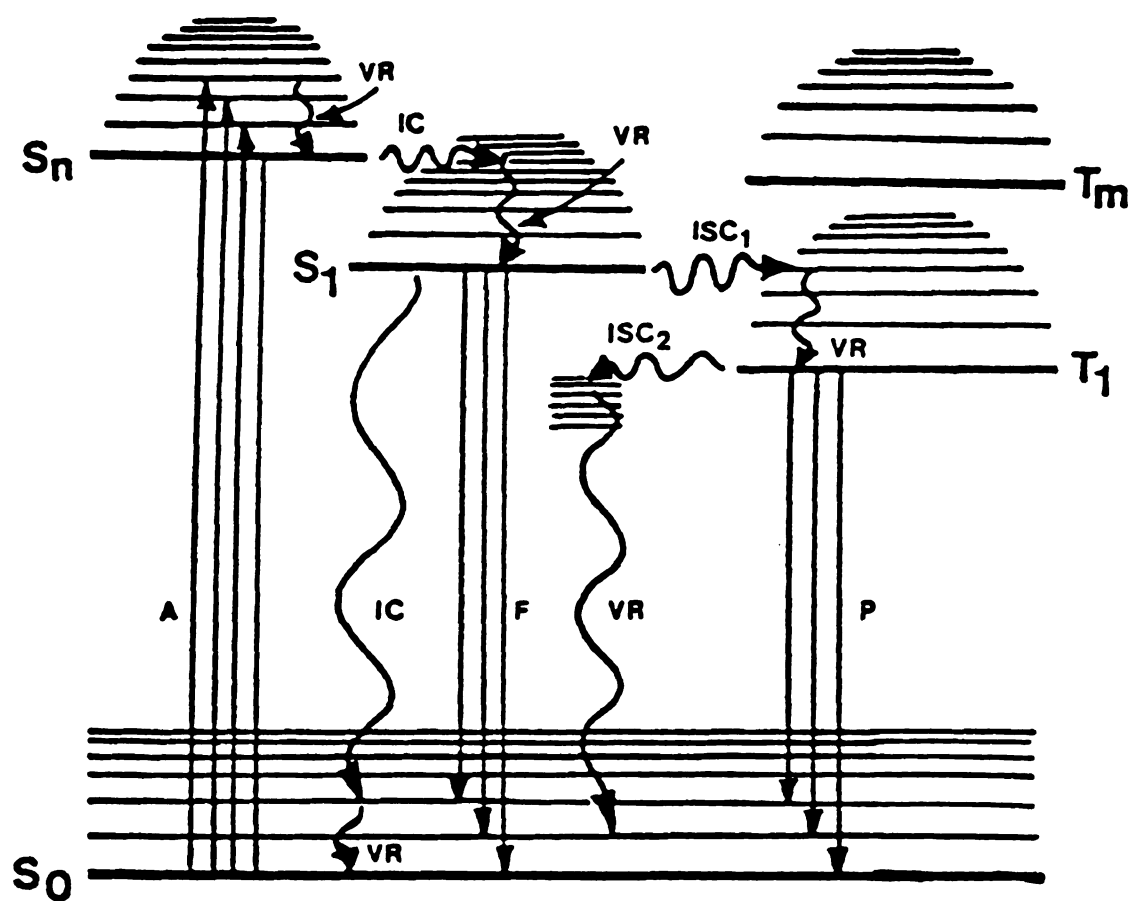


Figure 2.1. Jablonskii diagram of a molecule upon excitation. (A, absorption; F, fluorescence; IC, internal conversion; ISC, inter-system crossing; P, phosphorescence; VR, vibrational relaxation).

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the molecules is measured as a function of the wavelength of the radiation, a molecular absorption spectrum can be obtained (2).

The excess vibrational and electronic energy is then dissipated by rapid (10^{-13} - 10^{-12} s) radiationless processes, vibrational relaxation and internal conversion. Vibrational relaxation occurs when the excited molecule loses vibrational energy within a given electronic state, and internal conversion occurs when the molecule undergoes a radiationless transition from various excited energy levels to the lowest vibrational level of the lowest excited electronic singlet state. The excited molecules may return to the ground electronic state with emission of radiant energy whose frequency is governed by the gap between the lowest excited singlet state and the ground electronic state. This radiative transition between excited and ground states of the same spin multiplicity occurs in a time frame of 10^{-11} - 10^{-7} s after excitation, and is called fluorescence.

Some portion of the molecules in the lowest excited singlet state may deactivate by crossover to the lowest excited triplet state, a process called intersystem crossing. This crossover entails a change in spin angular momentum. This is, of course, forbidden in absorption quantum mechanically. In the excited singlet state, the spins of the promoted electrons are still paired with the ground state electrons; however, in the triplet state the

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spins of the electrons have become unpaired and are thus parallel with the ground state electron. Molecules in the lowest triplet state can return to the ground state without radiation being emitted by triplet - singlet intersystem crossing, or with radiation being emitted, which is called phosphorescence. Because phosphorescence is spin forbidden process, the lifetime of the triplet state is very long (10^{-4} - 10 s) as compared with an average lifetime of 10^{-8} - 10^{-8} s for an excited singlet state (3).

Molecules in the excited states which do not deactivate with radiation through either fluorescence or phosphorescence usually deactivate without radiation. Deactivation of an excited electronic state may involve interaction and energy transfer between the excited molecules and the solvent or other solutes (quenchers). This process is called external conversion. The details of external conversion processes are not well understood. External conversion competes so successfully with phosphorescence that phosphorescence could not be observed without reducing the effectiveness of the external conversion.

B. The Effect of Molecular Structure on Photoluminescence.

Molecular structure can have a profound effect on the photoluminescence. Both fluorescence and phosphorescence are most often observed in highly conjugated organic molecules

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with rigid molecular skeletons. Those molecules whose molecular structure have less vibrational and rotational freedom may have high probability that the energy gap between the ground state and the lowest excited singlet or triplet state will be large and require deactivation by luminescence. Typically, aromatic hydrocarbon molecules show intense fluorescence and can give high phosphorescence quantum yield in certain situations.

Aromatic hydrocarbon molecules containing freely rotating substituents, or lengthy aliphatic side chains, usually tend to luminescence less intensely than those without those substituents (4). This results from the introduction of a large number of rotational and vibrational degrees of freedom by the exocyclic substituents.

The energy difference between the ground state and the lowest excited singlet or triplet states becomes smaller in those molecules which have a more extended conjugated system. Therefore, benzene, naphthalene, and anthracene fluoresce maximally at 262 nm, 320 nm, and 379 nm, respectively.

Certain substituents strongly affect fluorescence (5). A substituent that delocalizes the π electrons, such as $-\text{NH}_2$, $-\text{OH}$, $-\text{F}$, $-\text{OCH}_3$, and $-\text{NHCH}_3$ groups, often enhances fluorescence. These electron-donating substituents tend to increase the transition probability between the lowest excited singlet state and the ground state.

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Electron-withdrawing groups containing -Cl, -Br, -I, -NHCOCH₃, -NO₂, and -COOH decrease or quench the fluorescence. The influence of halogen substituents is strong. As the atomic number of the halogen substituent increases, the fluorescence of the molecule decreases, and the probability for intersystem crossing to the triplet state increases at the expense of the excited singlet state. This heavy atom effect will be discussed more in detail in chapter III.

Molecular rigidity lessens the probability of competing nonradiative transitions by restricting the vibrational and rotational degrees of freedom of the molecule. The influence of the molecular rigidity results in a decreased probability of collisional deactivation and intersystem crossing. The formation of chelates of certain organic molecules with metal ions also promotes fluorescence by promoting rigidity and minimizing internal vibrations.

C. Medium Effects.

The chemical environment affects the photophysical processes of the molecules to a large extent. The effects of some of these environmental variables are considered briefly in this section.

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1. Effect of Temperature and Solvent.

The quantum efficiency of photoluminescence by most molecules decreases with increasing temperature because the increased frequency of collisions at elevated temperatures improves the probability for deactivation by external conversion.

Solvent interactions with solute molecules are largely electrostatic. It is usually the differences between the electrostatic stabilization energies of the ground and excited states that contribute to the relative intensities, and spectral positions of fluorescence and phosphorescence in different solvents.

Solvents containing heavy atoms, or other solvents with such atoms in their structure, also have a substantial effect on the photoluminescence of solute molecules. Atoms of high atomic number in the solvent cage of the solute molecule enhance spin-orbital coupling in the lowest excited singlet state of the solute (6). This increases the population of the lowest triplet state at the expense of the lowest excited singlet state. Thus, the intensity of fluorescence becomes less intense while that of phosphorescence becomes more intense in heavy atom solvents.

2. Effect of pH and Dissolved Oxygen.

Many aromatic compounds containing acidic or basic ring substituents show a dependence on the pH of the medium (7). Both ionized and nonionized forms of the compound are likely

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to show different wavelength and intensity due to a change in the nature and rates of the photoluminescence. Protonation and dissociation can alter the relative separation of the ground and excited states of the reacting molecules. The protonation of electron-withdrawing groups, such as carbonyl and nitrogen, results in a shift of the luminescence spectra to lower wavelengths, while the protonation of electron-donating groups, such as the amino groups, produces spectral shift to shorter wavelengths.

The presence of dissolved oxygen reduces the emission intensity of the photoluminescent molecules. This effect is more severe in phosphorescence than in fluorescence spectra. Triplet state oxygen and other paramagnetic species are highly effective in deactivating excited triplet states (8). Micromolar amount of oxygen can completely quench the phosphorescence of most aromatic compounds in solution. Due to the long intrinsic lifetime of the triplet state engendered by the spin-forbidden nature of phosphorescence, there is a comparatively long period for oxygen to interact with excited triplet molecules.

3. Effect of Solute Concentration.

At lower solute concentrations, a plot of the photoluminescence of the solution versus concentration of the emitting species is normally linear. But at higher solute concentrations, there is a tendency for molecules to form aggregates in both ground and excited states. Molecular

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aggregation can substantially affect the photoluminescence of molecules (9).

Self-quenching and self-absorption are the two well known quenching effects due to the high solute concentration. The former is the result of collisions between excited state solute molecules. Radiationless transfer of energy occurs from the excited state of the solute molecules to the solvent molecules. Self-quenching increases with solute concentration.

Self-absorption occurs when the wavelength of emission overlaps an absorption peak. This is due to the absorption of emitted radiation (secondary absorption) by analyte. But, there is also absorption of the incident radiation (primary absorption) which can cause nonlinearity. These two effects are often called the inner filter effect (3).

4. Effect of Coordination by Metal Ions.

Photoluminescence of aromatic ligands can be affected by coordination with metal ions. Nontransition metals will shift the wavelength of fluorescence and phosphorescence of luminescing ligands to which they are coordinated. This results from the positive polarization, caused by the metal ion, at the sites of coordination on the ligand (10). The luminescence of the ligand may be somewhat enhanced or quenched by coordination, depending on the influence of the metal ion has on the nonradiative processes competing with luminescence.

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The coordination of aromatic ligands with transition metal ions usually produces electronic spectral shifts which are much greater than the shifts produced by complexation of the same ligands with nontransition metal ions.

The fluorescence and phosphorescence of luminescing aromatic ligands are usually quenched by complexation with transition metal ions. The reasons for this quenching of transition metal complexes are not completely understood. One of the proposed theories is that the paramagnetic and heavy atom effects of the transition metal ion cause spin-orbital coupling which populates low lying states which are then deactivated by internal conversion (11).

D. Quenching of Photoluminescence.

Quenching of luminescence is the result of the interaction of the chromophore, either in ground or excited state, with the various other species present in the system. Quenching processes may be divided into two broad categories depending on the state of the chromophore when it actually interacts to give radiationless deactivation.

Static quenching occurs when an interaction takes place between the chromophore and the quencher in the ground state forming a nonluminescent complex. The efficiency of quenching is governed by the formation constant of the complex as well as the concentration of the quencher.

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In dynamic quenching, the quenching species and the potentially luminescent molecules interact during the lifetime of the excited state of the chromophore with no resulting radiation. As a result, the efficiency of dynamic quenching depends on the viscosity of the solution, the lifetime of the excited state of the luminescent species, and the concentration of the quencher. The Stern-Volmer equation describes this dynamic quenching process as follows:

$$P/P_0 = \frac{1}{1 + k_q \tau [Q]} \quad (2.2)$$

where k_q is the bimolecular quenching constant, P_0 and P are the quantum yields of the luminescence in the absence and presence of the quencher $[Q]$, respectively, and τ is the lifetime of the luminescent molecule in the absence of the quencher.

It should be noted that in dynamic quenching, the quantum yields of fluorescence and phosphorescence are governed by the kinetics of the photoreaction. However, in static quenching, they are generally governed exclusively by the strength of ground state complexation.

Static and dynamic quenching can be distinguished by various methods. In static quenching, the observed lifetime of the luminescence is unaffected by the quencher, while it decreases in dynamic quenching due to a quenching process which occurred during the lifetime of the luminescent species. Also, absorption spectra of the possible

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luminescent species are different in static quenching from that in the absence of the quencher due to a complex formation between lumiphore and quencher in the ground state.

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CHAPTER III

ROOM TEMPERATURE PHOSPHORESCENCE

Phosphorescence was identified first by Lewis and Kasha (1) in 1944 as the emission of light as a result of a transition from the lowest excited triplet state of a molecule to the ground state. A number of features of phosphorescence distinguish it clearly from fluorescence. Most importantly, phosphorescence is distinguished from fluorescence by its much longer lifetime. Fluorescence lifetimes are typically in the range of 10^{-8} - 10^{-6} s, whereas phosphorescence lifetimes normally lie between 10^{-4} s and 10 s. These long lifetimes are potentially valuable in analysis, as they enable phosphorescence to be distinguished from fluorescence and scattered light. Also phosphorescence with different lifetimes may be distinguished using time-resolved methodology. Unfortunately, long lifetimes are also the principle disadvantage of phosphorimetry: in these lengthy periods, the excited molecules are normally deactivated by collisions with solvent molecules and other quenchers such as oxygen molecules. As a result, traditional phosphorimetry is performed in rigid media at liquid nitrogen temperature (77 K) to minimize the radiationless deactivation by various quenching effects.

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Sample preparation in low temperature phosphorimetry involves lowering a long capillary cell into a quartz Dewar flask filled with liquid nitrogen. The rate of cooling of the sample cell, the chemical nature, and the composition of the solvent system will determine whether the cooled matrix will be a clean glass, a cracked glass, or a snow. Thus, the selection of the proper solvent is critical. It should form a clear glass at 77 K and have a low phosphorescence background. Also, the analyte should be readily soluble in the solvent at 77 K. Experimental difficulties and limitations associated with traditional methods in phosphorimetry, have been the primary factors in preventing broader application of low temperature phosphorimetry. However, recent developments have shown that a wide variety of organic compounds exhibit strong phosphorescence at room temperature when certain conditions are applied to the sample system (2).

Although the studies by Roth (3) and by Schulman and Walling (4) generalized room temperature phosphorescence (RTP) first in an analytical application, perhaps the first RTP phenomenon was observed by Wiedemann and Schmidt (5) in 1896. Phosphorescence spectra obtained at room temperature are generally similar to those observed at 77 K, but they show less vibrational fine structure due to increased vibrational freedom of the molecule at the higher temperature. RTP emission peaks are also shifted to slightly longer wavelengths at room temperature compared with low

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temperature phosphorescence spectra, although shifts are typically less than 10 nm (6). RTP lifetimes are almost invariably shorter than phosphorescence lifetimes at 77 K and rarely exceed a few hundred ms (7). RTP intensities are also generally lower than those obtained at 77 K; however, RTP is still capable of low detection limits for certain molecules.

This chapter describes several features of room temperature phosphorescence. The conditions necessary for RTP are discussed first, and then various RTP techniques are described. The last two parts cover the most important and necessary conditions in RTP: deoxygenation methods and the heavy atom effect.

A. Conditions for the Observation of RTP.

Most organic compounds which emit strong fluorescence in fluids, commonly do not give any phosphorescence at room temperature. Because of the long lifetime of the triplet state, collisional deactivation is highly effective in bringing about radiationless decay of triplet states in fluid media. Quenching of the triplet state by a small amount of dissolved oxygen is very efficient in preventing phosphorescence in a liquid solution. As a result, some technique must be used to reduce or to prevent such quenching in order to observe phosphorescence at room temperature. The essential requirements for RTP are;

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deoxygenation of the sample solution, a rigid molecular microenvironment, and a heavy atom source.

Deoxygenation can be done physically or chemically during sample preparation and emission measurement. By bubbling the sample solution with an inert gas such as nitrogen or argon, the concentration of dissolved oxygen can be reduced below micromolar amounts. Chemical deoxygenation is another way to reduce molecular oxygen in a sample solution by converting it into other species. Sodium sulfite (8), chromium(II) (9), and zinc (10) have been used successfully to convert dissolved molecular oxygen to other inactive species in solution.

It has been observed that, a rigid microenvironment generally increases the phosphorescence intensity by reducing collisional quenching of the triplet state. Because collisional quenching is very efficient at room temperature and is often considered as the main pathway for loss of triplet state energy, a rigid molecular environment for the triplet state is very important for the observation of the phosphorescence at room temperature. In solid state RTP, the rigid matrix is formed by the hydrogen bonding of ionic organic molecules to hydroxyl groups of the filter paper. In micellar stabilized RTP, sample molecules are confined inside micelles which are semi-rigid organized media. In cyclodextrin RTP, molecules form trimolecular inclusion complex with cyclodextrin and a heavy atom. Because sample molecules are included inside the cyclodextrin cavity, there

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is less probability for collisional quenching of the triplet state.

Although RTP can be observed in deoxygenated rigid media at room temperature, the observed intensity of RTP is generally too low to be useful in analytical work. However, by adding a heavy atom such as Tl^+ , I^- , or Ag^+ , into the sample solution, the intensity of RTP can be increased significantly. This is due to an increase in the intersystem crossing rate from excited singlet state to triplet state through spin-orbital coupling induced by the heavy atom. These effects are discussed in more detail later in this chapter.

B. Methods for RTP Measurement.

Since the introduction of analytical RTP by Roth (3) in 1967, numerous methods for RTP measurement have been developed. These are solid surface RTP, micellar-stabilized RTP, sensitized RTP, cyclodextrin RTP, and collidal or microcrystalline RTP. Though all these different methods of RTP measurement look different from each other, they have many common factors. Each different RTP technique tries to minimize collisional quenching which is believed to be the main pathway for radiationless deactivation of the triplet state, by employing different methods to fulfill the three requirements discussed in the above section. This section discusses the major RTP methods developed thus far.

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1. Solid Surface RTP.

In 1967, Roth (3) reported a new analytical method based on the RTP emission from a variety of organic compounds adsorbed on the filter paper. Schulman and Walling (11) observed strong RTP from salts of a wide variety of polynuclear carboxylic or sulfonic acids, phenols, and amines adsorbed on paper, silica, alumina, and other supports. The observed phosphorescence lifetimes of several molecules were in the range of 100-700 ms, which were somewhat shorter than those observed at liquid nitrogen temperature. In 1974, Paynter et al (12) reported the range of linearity and detection limits for several compounds adsorbed on filter paper. Calibration curves had a wide linear range, detection limits were in the nanogram region, and precision ranges from about 3% to 10% depending on the solid surface and experimental conditions.

In most cases of solid surface RTP, drying of the samples was essential to enhance the RTP signal. It was noted that moisture on the solid substrate caused radiational quenching. It seemed that moisture acts to disrupt any binding of analyte on the solid surface so that the rigid molecular environment becomes loose and aids in the transport of oxygen into the sample matrix.

Although no general model has been developed to explain the interactions involved in the production of RTP from compounds adsorbed on filter paper, it seems that hydrogen

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bonding of ionic organic molecules to hydroxyl groups of the solid surface is the primary mechanism for providing the rigid matrix for RTP.

The ionic nature of the analyte molecules plays an important role in producing RTP. While ionic molecules show intense RTP signals when spotted on solid surfaces from solvents containing a large excess of a strong acid or base, nonionic compounds exhibit extremely weak or no RTP even from acidic or basic solutions (11). It is believed that the ionic state of the molecule results in great molecular rigidity via adsorption to the substrate, which reduces radiationless decay due to collisional deactivation (12).

The major disadvantage of solid surface RTP using filter paper, is the strong background from the solid substrate. Most grades of paper exhibit substantial background phosphorescence, which is difficult to remove in the wavelength range 400-600 nm. Attempts to reduce the background emission from paper have been described by Ward et al (13). Experiments designed to wash the contaminants from the paper have met with little success (14). Beteh and Winefordner (15) tried to photobleach the lignins or hemicelluloses believed to be a constituent of the background. They concluded that such a pretreatment improved the absorption characteristics of the filter paper, but did not significantly reduce the phosphorescence background.

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Other substrates used for solid surface RTP include sodium acetate, starch, inorganic substances, polymer-salt mixtures, and silica gel. Wandruszka and Hurtubise (16) observed RTP from a number of compounds adsorbed on sodium acetate. The main interaction proposed with this system was the formation of hydrogen bonds of the analyte with the carbonyl group of sodium acetate.

2. Sensitized RTP.

Sensitized phosphorescence refers to the process whereby an acceptor (or emitter) having no appreciable absorption in a given region of the spectrum, is made to emit radiation upon excitation as a result of triplet energy transfer from a donor (or sensitizer) molecule. The overall process of sensitized phosphorescence is shown in Figure 3.1. The analyte is excited by means of light absorption. Subsequently, these non-fluorescent compounds with a high efficiency of intersystem crossing, deactivate without radiation through intersystem crossing. However, in the presence of an acceptor, the triplet energy of the donor molecule can be transferred to the triplet state of the acceptor molecule, and the excited acceptor molecule deactivates with radiation.

There are several requirements for successful sensitized phosphorescence. In general, a suitable acceptor should have a triplet energy lower than that of the donor, a low molar

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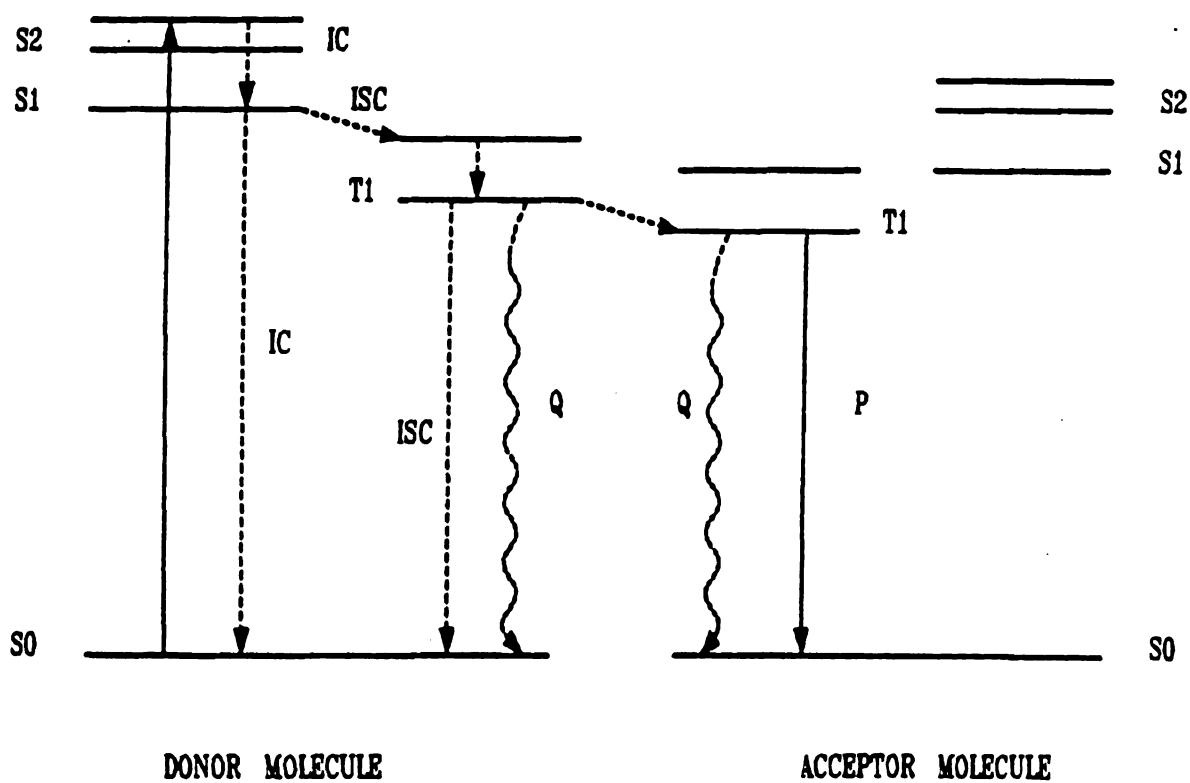


Figure 3.1. Schematic representation of sensitized phosphorescence. The analyte (acting as the energy donor) absorbs light, and the acceptor molecule emits phosphorescence. (IC, internal conversion; IS inter-system crossing; S_n , singlet state; T_n , triplet state; Q, quenching).

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absorption in the excitation region of the donor, and a strong phosphorescence yield at room temperature. The energy gap between the donor and acceptor triplet state should be sufficiently high so that it prevents the reverse transfer mechanism (2). Biacetyl has favorable properties in this context: its molar absorptivity is exceedingly small over a wide wavelength range (17). For a given analyte concentration, the maximum sensitized RTP signal is reached if the excitation wavelength chosen corresponds to the maximum in the excitation spectrum of the analyte.

Donkerbroek et al (18) introduced sensitized and quenched RTP detection for flow injection analysis and liquid chromatography. In sensitized RTP, the analyte, after absorption of light, induces biacetyl to emit phosphorescence. The quenched RTP detection method is based on a dynamic quenching process in which the analyte acting as a quencher, reacts with excited biacetyl, thus prohibiting phosphorescence emission. The limits of detection for several substituted aromatic compounds in acetonitrile:water (1:1) were in the range of 10^{-8} M (19).

DeLuccia and Cline Love (20) demonstrated that sensitized biacetyl RTP can be enhanced via molecular organization for many aromatic compounds. Micelles composed of sodium dodecyl sulfate (SDS) and β -cyclodextrin were used to enhance the energy transfer reaction by organizing the reactants in close proximity to one another. Both organized media provided more favorable environments for induction of

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sensitized RTP than homogeneous solutions of the reactants in acetonitrile.

3. Micellar Stabilized RTP.

Surfactants (Surface Active Agents) are amphiphilic molecules composed of a hydrophobic portion and a charged or polar portion. The hydrophobic backbone of the surfactant can vary in length from eight to twenty carbons and the hydrophilic portion can be a partially dissociable carboxylate (soap), a fully ionized moiety (such as anionic sulfate or cationic trimethyl ammonium), or an uncharged polar species (such as alcohol).

A fascinating feature inherent in aqueous surfactant solutions is the phenomenon of self-organization. At low concentrations in solution, the surfactants exist mostly as monomers. Above a certain concentration, the critical micelle concentration (CMC), surfactant molecules associate spontaneously to build up structural entities of colloidal dimensions called micelles. The architecture of these agglomerates is such that the interior contains the hydrophobic alkyl chain of the amphiphile, while the hydrophilic head groups are located at the surface and are in contact with bulk water.

Micelle formation is believed to be the result of three primary forces: hydrophobic repulsion between the hydrocarbon chains and the aqueous environment; charge

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repulsion of ionic head groups; and, the van der Waals attraction between the alkyl chains (21). Micelles are roughly spherical in shape and they consist of typically 60-100 monomers.

Most micelles are optically clear and they do not cause measurable light scattering in conventional spectrometry. The concentration of micelle at various bulk surfactant solution can be calculated from the aggregation number and the CMC

$$[\text{micelle}] = \frac{[\text{surfactant}] - \text{CMC}}{\text{aggregation number}} \quad (3.1)$$

where the aggregation number is the number of monomers incorporated into a micelle (22,23).

The shape of micelles depends upon the solvent and the packing parameters of the surfactant molecule in the micellar assembly. When surfactants are dissolved in polar solvents, the polar head groups and counterions orient together in an outward fashion in contact with the water phase. However, in apolar solvents the orientation of the head groups and the aliphatic tail is reversed (reversed micelle) such that the hydrophobic tails contact the apolar solvent.

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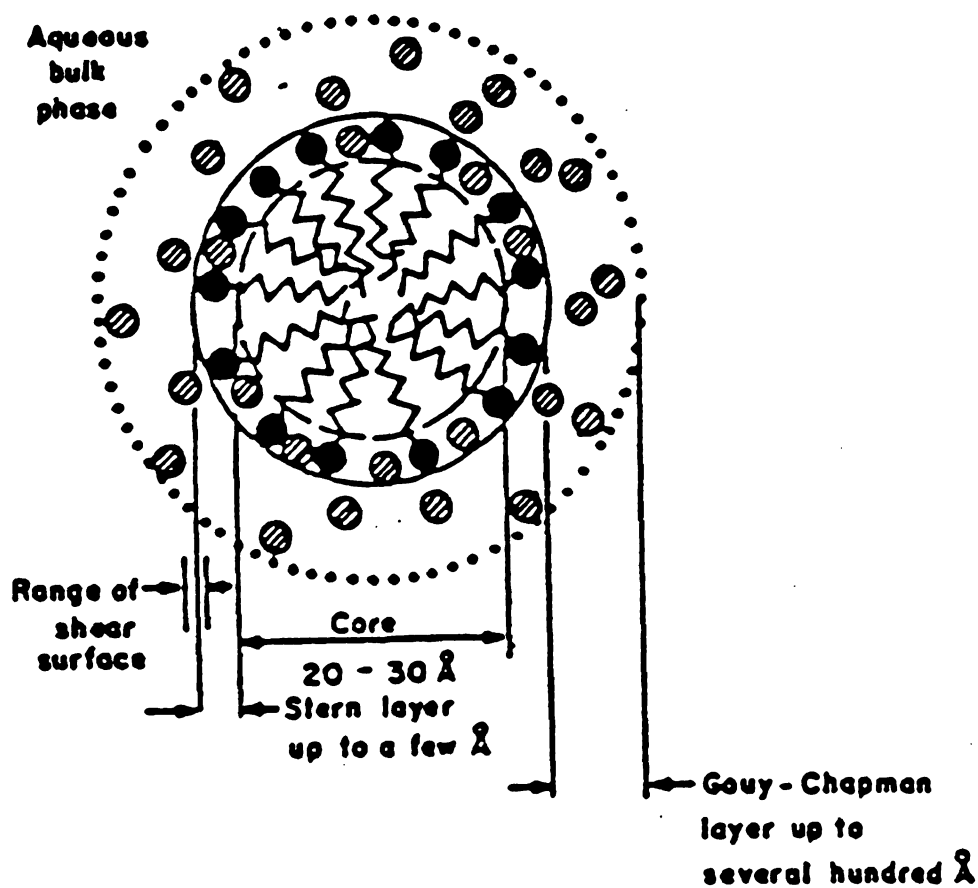


Figure 3.2. Simplified model representation of the ionic micelle. The electric double layer is composed of a Gouy-Chapman layer outside, and a Stern layer inside the shear surface.

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The most advanced theoretical model of an ionic micelle discussed so far is a combination of a Stern layer with a rough rather than a smooth sphere and a Gouy-Chapman diffuse double layer (24) as shown in Figure 3.2. The aqueous Stern layer is between the smooth surface of the core and the shear surface, which contains ionic heads of the micellized surfactant ions. The Gouy-Chapman region is immediately outside of the Stern layer and is a diffuse double layer containing unbound counterions.

Micelles are responsible for many of the practical applications of detergents, such as enhancement of the solubilities of organic compounds in water, and catalysis of organic reactions. Furthermore, micelles have been proposed as simple model systems for a variety of important "two-phase" systems, such as monolayers, colloids, proteins, enzymes, and membranes. Among the primary reasons for the usefulness of micelles as models are: (a) the thermodynamic stability and reproducibility of many micellar systems; (b) the simplicity of the structure of the micelle; (c) the two-phase nature of the micelle; and (d) the ionic composition of many micellar surfaces (25).

RTP of aromatic hydrocarbons was first observed in micelles by Kalyansundaram et al (26). The solutions were deoxygenated by bubbling them with nitrogen gas for 30 min. The ready observation of RTP in micellar solution was attributed to the protective screening of the triplet probe from external quenchers by the micelle assembly. Cline Love

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and coworkers (27) further developed micellar-stabilized RTP (MS-RTP) for analytical applications. In addition to deoxygenation by an inert gas, heavy atoms were required to enhance the sensitivity. Thallium counterions were found to give higher signals than silver counterions in 0.15 M surfactant solutions, and about 90 % of the fluorescence was quenched by heavy atoms. The limits of detection were typically in the nanomolar range, and the calibration curves had a wide linear dynamic range for several hydrocarbons.

The incorporation of the analyte molecule into the core of a micelle imparts certain advantages (28):

- (i) The structural conformation of the micelles protects the triplet state of the analytes from external quenchers.
- (ii) The orientational constraint decreases vibrational deactivation.
- (iii) The altered microenvironment can provide favorable polarity and acid/base equilibrium for enhanced phosphorescence quantum efficiency.
- (iv) The micellar solutions can improve the detection limits for hydrophobic species in aqueous solution by increasing their solubility.
- (v) The proximity of interacting species (phosphors and heavy atoms) is increased, and can result in a more effective spin-orbital coupling.

4. Cyclodextrins

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4. Cyclodextrin Enhanced RTP

Cyclodextrins (CDs) are a series of macrocyclic oligosaccharides produced by the bacterial degradation of starch. The most widely used CDs consist of 6, 7, and 8 glucose monomers arranged in torus shapes and denoted as α , β , and γ CD, respectively (29). The coupling of the glucose moieties gives a rigid, torus molecular structure with a hollow interior that contains one or more water molecules. This water can easily be displaced by other species which can enter the CD cage. The interior of the cavities are composed of two rings of C-H groups with a ring of glycosidic oxygen in between, allowing them to be hydrophobic in nature. The internal diameters of these cavities are approximately 5.7, 7.8, and 9.5 Å, respectively, and the depths are roughly 7.8 Å as shown in Figure 3.3.

The hydrophobic nature of the cavities enables the CDs to trap compounds, such as aromatic and alkyl halides as guest molecules in their interior, resulting in the formation of inclusion complexes (30). The stability of an inclusion complex depends on the size of the CD cavity and on intermolecular forces such as hydrogen bonding, van der Waals attraction, and hydrophobic interactions. These macrocyclic carbohydrate molecules can be discriminating in their inclusion complexing tendencies toward different structural, positional, or stereomeric molecules. A more

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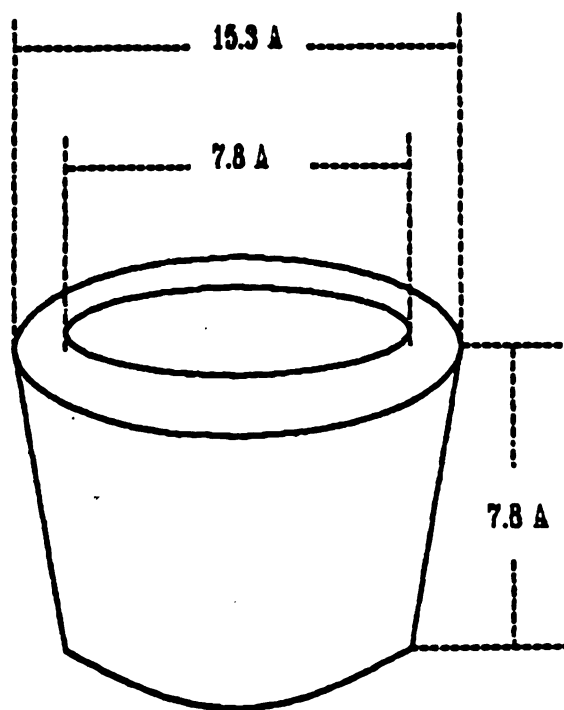


Figure 3.3. Simplified representation of β -cyclodextrin structure.

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detailed discussion of macrocyclic compounds is given in the next chapter.

The CD molecule provides a favorable microenvironment for luminescent probes. Turro et al (31) reported cyclodextrin-enhanced RTP in 1982. They investigated the emission of several 1,3-bichromophoric systems in aqueous solutions which contained various cyclodextrins. In all cases, CDs revealed structural (size and shape) selectivities toward inclusion complex guest molecules. The tight CD-lumiphor complex also showed partial immunity to oxygen quenching. They could observe RTP of 4-bromo-1-naphthoyl groups in aqueous solution even under 1 atm of oxygen (32). Oxygen completely quenched the fast decay, but only partially quenched the slow decay.

Following the work of Turro's group, Cline Love and coworkers further developed cyclodextrin enhanced RTP for analytical applications. Scypinski and Cline Love (33) demonstrated cyclodextrin enhanced RTP for the analysis of polynuclear aromatic hydrocarbons (PAHs). They found that a heavy atom source, 1,2-dibromoethane, was necessary, and deoxygenation with nitrogen gas was required to enhance the sensitivity. The proposed inclusion complex is a trimolecular complex of CD-lumiphor-heavy atom. Only molecules that can physically enter the CD cavity are phosphorescent, which provides considerable selectivity based on lumiphor size. Shapes of analytical calibration curves are similar to those obtained for micellar-stabilized

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The unique inclusion capability of cyclodextrins was utilized in other areas of RTP. Vo-Dinh et al (34) reported RTP of anthracene on cyclodextrin treated filter paper. The binding constant of anthracene with β -CD was 32. Bello and Hurtbuisse (35) observed RTP of several aromatic hydrocarbons on a solid surface of CD-sodium chloride. By utilizing the size requirements of the CD molecule, they could identify between seven to nine PAHs in the mixtures at nanogram levels.

5. Other Methods of RTP.

As mentioned earlier, in order to observe RTP, certain conditions are required: (i) a rigid molecular environment; (ii) deoxygenation; and (iii) a heavy atom source. Other methods of RTP try to fulfill these requirements with different methods. These methods include RTP of colloidal or microcrystalline suspensions and silicalite RTP.

Weinberger and Cline Love (36) reported RTP observation of PAHs in colloidal suspensions in water. In this method, the PAH molecule, which is insoluble in the aqueous media, is injected rapidly into the aqueous solution. This results in the formation of a microcrystalline or colloidal suspension of the sample in the aqueous media. The observed spectrum was very similar in resolution, shape and symmetry to

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spectra observed at 77 K. Also, this system is insensitive to quenching by dissolved oxygen. The microcrystals act in a manner analogous to solid-surface RTP, in that the quenching pathways that normally operate at room temperature are minimized. The microcrystals serve as their own substrate and provide stability to the triplet state. Since the solute is present as a solid in suspension, diffusion of molecular oxygen through the solute is not possible, thus rendering it immune to oxygen quenching (37).

Casal and Scaiano (38) observed RTP of several aromatic ketones included in the channels of silicalite, a hydrophobic zeolite. They found that the lifetime of β -phenylpropiophenone at room temperature is enhanced by over five orders of magnitude by inclusion in silicalite cavities. This enhancement was attributed to the steric restrictions imposed on the included guest molecules in the channels of the silicalite. Silicalite (99 % SiO_2) forms part of the class of zeolite molecular sieves with a new topologic type of tetrahedral framework. The channel system of this zeolite consists of near circular zig-zag channels which are cross-linked by elliptical straight channels (38). The diameter of the circular channels is 5.4 Å and the free cross-section of the elliptical ones is 6 Å. Therefore, the limiting size for adsorption is around 6 Å at room temperature. One of the remarkable properties of this zeolite is its hydrophobicity, which ensures no deactivation effect due to water.

C. Deoxygenation

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C. Deoxygenation Methods.

Oxygen quenches RTP so efficiently that every method of RTP requires protection of the triplet state from oxygen. In solution RTP, deoxygenation is a necessary condition. In this section, the deoxygenation methods developed to date for solution RTP are discussed.

1. Physical Methods of Deoxygenation.

The most common method for deoxygenation in solution RTP, is bubbling of the sample solution with an inert gas, such as, nitrogen and argon. If the solution is bubbled for 30 min with oxygen-free inert gas, the concentration of dissolved oxygen can be reduced to less than micromolar amounts. The disadvantages of this method are the time consuming process and the fact that it generates excess foam in a micellar solution.

Reim (40) used semipermeable membranes to remove dissolved oxygen. Silicon rubber was used as membrane because of its high oxygen permeability and chemical inertness. Since oxygen transport is directly proportional to the pressure difference across the membrane, evacuation around the membrane was necessary. With this method about 95 % of the dissolved oxygen could be removed in 25 min.

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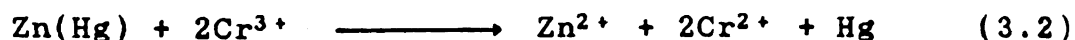
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Other methods are vacuum degassing and repeated freeze-thaw cycles. However, these methods are not popular due to the difficult and time consuming process involved for degassing of sample solution.

2. Chemical Methods of Deoxygenation.

In most chemical methods the dissolved oxygen is converted into inactive other species by a chemical reaction.

Rollie and coworkers (41) used chromium(II) to convert dissolved molecular oxygen into water. Chromium(II) is formed by the reduction of chromium(III) with Zn(Hg), and is in contact with the sample solution through a semipermeable membrane. The chemical reaction is as follows:



Although this method is effective, the long equilibration time required for the reaction prohibits further application in RTP.

MacCrehan and May (42) used a zinc column to remove dissolved oxygen. A sample solution is passed through a column packed with zinc particles and allowed to react with oxygen as follows:

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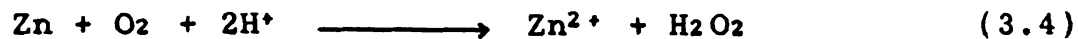
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Since zinc is oxydized by the reaction, the zinc column has a finite lifetime.

Recently, Diaz Garcia and Sanz-Medel (43) reported a deoxygenation method based on the following chemical reaction of sulfite with oxygen:



The reaction time for oxygen consumption showed a dependence on the surfactant concentration in MS-RTP. As the concentration of surfactant increases, it took more time for a complete reaction due to the restriction of ionic movement by the micelles. This method has been shown to be an easy and effective deoxygenation method.

D. The Heavy Atom Effect.

It has been observed that the presence of heavy atoms affects luminescence significantly. The use of external heavy atoms to increase phosphorescence yields has become a common practice in RTP. By mixing two chemical species, one of which contains a heavy atom, the probability of singlet-triplet crossover increases. This phenomenon is referred to as the external heavy atom effect (44), in contrast to the

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internal heavy atom effect, observed when the heavy atom is chemically affixed to the molecular skeleton whose intercombinational transition it perturbs (45). These interactions between the heavy atom and the sample molecule may frequently result in an increase in the phosphorescence quantum yield, and a corresponding decrease in the fluorescence yield. Also, the observed phosphorescence lifetimes with the heavy atom are usually shorter than without the heavy atom.

The heavy atom effect has been ascribed to increased spin-orbital coupling induced by the heavy atom perturber (46). The external heavy atom effect may take place in two ways: one, by complex formation with the heavy atom, and another, by a long range interaction through a statistical distribution of the heavy atom molecules around the phosphorescing molecule (47). It has been shown that the rate constants of both $S_1 \rightarrow T_1$ and $T_1 \rightarrow S_0$ processes are increased (48). This is evident from the observed RTP lifetimes of probe molecules in micelles with heavy atoms which decrease substantially as the heavy atom concentration increases. Furthermore, it has been also demonstrated that the $T_1 \rightarrow S_0$ radiationless transition is not much enhanced, and the observed reduction of triplet lifetimes is mainly due to an increase in the radiative transition probability (49).

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aromatic hydrocarbon molecules have been proposed, it is still not well understood. The various schemes which have been previously proposed share a common factor in that the singlet states are ultimately responsible for the reduced phosphorescence lifetime (50). That is, the triplet state of the emitting molecule is mixed with the singlet state of the other. The sources of the singlet state can be the same molecule (51), the perturber (52), or charge transfer state of the molecule-perturber complex (53).

Bower and Winefordner (54) investigated the effects of several heavy metal ions on the RTP of PAHs adsorbed on paper, and found the following enhancement trend: $Tl^+ > Ag^+ > Pb^{2+} > Hg^{2+}$. It has been suggested that π -complex formation between the heavy metal ions and the PAH might be providing an internal heavy atom effect in some of these cases.

White and Seybold (55) have investigated in detail the effect of the addition of various sodium halide salts on the RTP of sodium 2-naphthalenesulfonate adsorbed on paper. The enhancement in RTP follows the trend of $I^- > Br^- > Cl^- > F^-$. Hence, the heavier ions induce the greater RTP enhancement. In this study (55), the heavy atom perturber was found to affect the radiative RTP emission rate more than the nonradiative rate.

The external heavy atom effect is extensively used in MS-RTP (56). The RTP of many PAHs is not detectable in a 0.1 M solution of NaLS. However, when thallium(I) is used to

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replace 30 % of the sodium counterions in NaLS, an intense RTP signal can be detected.

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CHAPTER IV

APPLICATION OF MACROCYCLIC COMPOUNDS IN CHEMICAL ANALYSIS

There has been an increasing amount of research interest in the general properties and applications of macrocyclic compounds. Macrocyclic compounds typically contain central cavities ringed with long frameworks. Many of these macrocyclic compounds have been shown to possess very interesting and unusual binding properties. Various metal ions and molecules can be encapsulated inside the cavity of the macrocyclic compounds. The complexes thus formed are of great analytical interest. But relatively few studies have been done on the analytical applications of these compounds compared to the fundamental studies performed.

Although metal complexes of naturally occurring macrocyclic compounds, such as, porphyrins and certain antibiotics have been known for over 50 years, it is only during the past two decades that a large number of synthetic macrocyclic compounds capable of binding metal ions or molecules have been prepared and investigated (1). These synthetic macrocyclic compounds include cyclic polyethers, polythioethers, polyamines, and cyclophanes. There are numerous publications and patents on applications of naturally occurring macrocyclic compounds, cyclodextrins and porphyrins (2). The important properties and usefulness of

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these compounds have become well understood during the past decade (3).

Inclusion complexes are formed with macrocyclic compounds having a variety of selectivities, depending on the physical and chemical property of the host macrocyclic compounds. These unique properties of macrocyclic compounds in inclusion complex formation stimulated chemists to devise organic compounds which will perform functions similar to those performed by naturally occurring macrocyclic compounds. Since these macrocyclic compounds are synthetic in nature a high degree of selectivity in binding of guest molecules or ions can be obtained by designing host molecules for specific purposes (4). A wide variation is possible in designing host structures which will have the most appropriate size, shape, and functionality for specific molecular recognition.

Water soluble heterocyclophanes are synthetic macrocyclic compounds which have been proposed as enzyme models (5). The macrocyclic ring is composed of a wall of benzene rings and quaternary nitrogens. Several variations of the structure were made to provide host cyclophanes with different functionality and substrate specificity. Heterocyclophanes have been shown to be excellent inclusion hosts toward certain organic substrates. A unique substrate specificity was observed due to their cavity size and functionality.

This chapter discusses analytical applications of macrocyclic compounds. In the first part, a brief

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introduction to host-guest chemistry and its usage in chemical analysis is discussed. Next, potential applications of the synthetic enzyme model compounds, cyclophanes, in molecular luminescence are addressed. The last part reviews the current status of applications of other macrocyclic compounds in chemical analysis.

A. Host-Guest Molecular Inclusion.

A basic understanding of the specific binding and catalytic behavior of enzymes is one of the significant driving forces for studying host-guest chemistry. In a host-guest complex, the host molecule is the larger, and the guest molecule is the smaller of the two. The host molecule usually contains one or several binding sites for complex formation (inclusion complex) with guest molecules. The host molecule must recognize and complex best those guest molecules that contain binding sites and steric features that complement those of the host. The self-evident postulate that two objects cannot occupy the same space at the same time indicates that host and guest must be compatible with respect to shape if they are to complex. The simple electrostatic attraction of opposite charges accounts for much of the binding forces between host and guest molecules (4).

Various investigations have been carried out in recent years on some new synthetic catalysts which show enzyme-like

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behavior (6). Micellar surfactants and water soluble polymers bearing various functional groups have shown phenomenological similarities with enzyme systems (7). Micellar surfactants have been known to form a hydrophobic site through micelle formation. The hydrophobic interaction between substrates and micelles results in the formation of intra-complexes. Additionally, micelles provide proximity effect by raising local concentrations of substrates effectively. The formation of hydrophobic binding sites in these cases is in dynamic equilibrium with the bulk phase, and subject to the external medium effects, consequently. Thus, these mobile structures may provide a limited substrate specificity. On the other hand, macrocyclic compounds may exhibit several novel and unique characters due to the following effects (8).

(i) The macrocyclic cavity provides a stable binding site due to a characteristic ring conformation of hydrophobic nature.

(ii) A high substrate specificity can be brought about by the geometric requirements for binding substrates into these macrocyclic cavities, as well as by spatial geometries of substrate molecules incorporated into these cavities.

(iii) A high possibility of introducing a charge relay or an electrostatic antenna system, which is provided by spatial arrangements of appropriate functional groups. Consequently, a well-designed macrocyclic compound may

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Substrate specificity is understood to indicate that a specific substrate has a "best fit" to a unique array of binding-site residues and that the spatial arrangement of atoms relevant to the catalysis is particularly favorable for the stabilization of the transition state (9). Although the specific mechanism and requirements for a stable host-guest inclusion complex formation may be different for each complex, it is known that the main binding forces are hydrogen bonding, van der Waals forces, hydrophobic interaction, and steric and electrostatic interactions (10).

The stability of host-guest inclusion complexes can be affected by various forces which are at the core of the discriminative nature of substrate binding. The factors affecting the formation and stability of host-guest complexes include: (i) the type or types of binding sites in the ring; (ii) the number of binding sites in the ring; (iii) the relative sizes of the guest and the macrocyclic cavity; (iv) the physical placement of the binding sites; (v) steric hindrance in the ring; and (vi) the external charge of the guest (11).

In room-temperature phosphorescence, quenching of the triplet state of a molecule is the most annoying problem especially in solution. Judging from the above discussion, the inclusion behavior of macrocyclic enzyme model compounds could provide an ideal environment for observing room-

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temperature phosphorescence. Triplet quenching should be reduced by inclusion of the triplet molecule inside the cavity of the host molecule. At the same time, it might be possible to observe a high degree of selectivity due to the requirements for the host-guest inclusion complex formation. Also, since the host molecule is synthetic in nature, specific host molecules could be tailored for a specific guest in chemical analysis. These are the underlying ideas of this research.

B. Synthetic Enzyme Model Compounds: Cyclophanes.

Since the discovery of crown ethers by Pedersen twenty years ago, the chemistry of synthetic hosts for the selective complexation of organic and inorganic guests has developed rapidly. Soon after the fundamental studies on molecular complexation by cyclodextrins, Cramer investigated the enzyme-like catalytic properties of these systems (12). Following this, a large variety of stimulating and innovative studies on the catalytic properties of the supramolecules have been conducted by many research groups (13,14,15). Of the fascinating synthetic enzyme model compounds, water-soluble cyclophanes seem quite interesting for the examining possible RTP applications

In aqueous solution, cyclophanes form stoichiometric inclusion complexes with aromatic guest molecules; these can approach enzyme-substrate complexes in their stabilities

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(16). Diederich and his coworkers (17) introduced water soluble cyclophanes. For the construction of water-soluble hosts with apolar cavity binding sites shaped by hydrocarbon residues, ionic groups remote from the cavity were introduced as shown in Figure 4.1. Complexation studies showed stable 1:1 complexes with various hydrocarbon molecules in solutions of a wide polarity range (18).

Urushigawa et al (19) reported the synthesis of various azaparacyclophanes (APCs) containing four benzene rings. Tabushi and his coworkers (20) studied substrate specificity of the water-soluble heterocyclophane, N,N,N',N',N'',N'',N''',N'''-octamethyl-2,11,20,29-tetraaza[3,3,3,3]paracyclophane-tetraammonium tetraborate (methyl-APC). Strong inclusion complexes were formed through hydrophobic, electrostatic, and steric interactions between the host and guest molecules. Solubilization of the host methyl-APC molecules in water was achieved by the introduction of the four quarternary nitrogens around the macrocyclic ring.

The methyl-APC molecule in Figure 4.2-A has a hydrophobic cavity resembling a square box surrounded by a wall of benzene rings that form angles of 60° with respect to a pseudo molecular plane defined by the four N atoms (21). The size of macrocyclic cavity is 5.5-7 Å wide and 6 Å deep. An NMR study showed that the benzene rings of the methyl-APC molecule favor a "face" conformation, in which benzene rings are perpendicular to the hypothetical molecular plane (22).

H₃C

H₃C

N[•]

N[•]

Figure

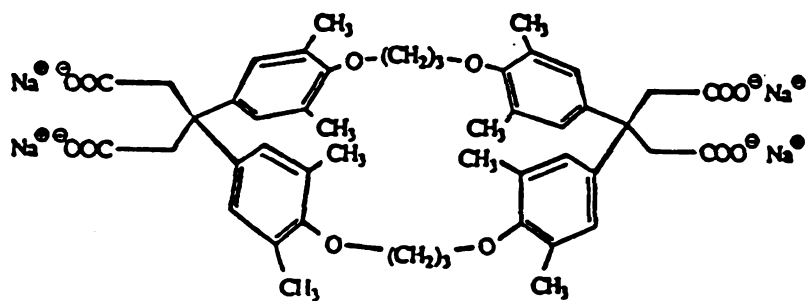
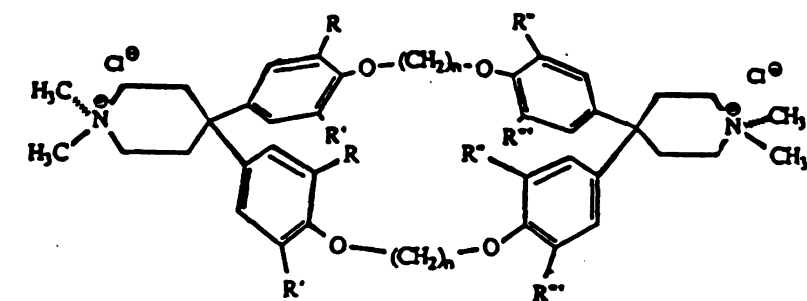
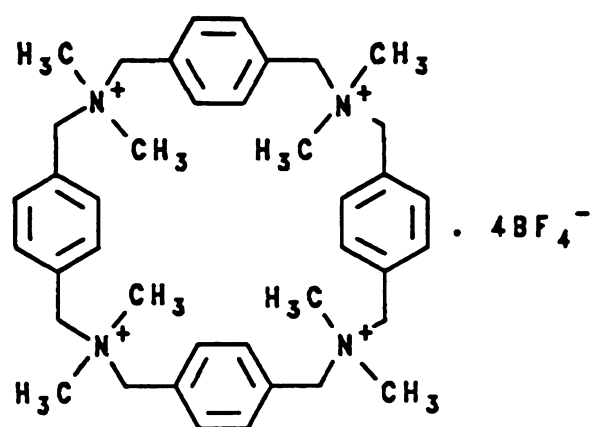
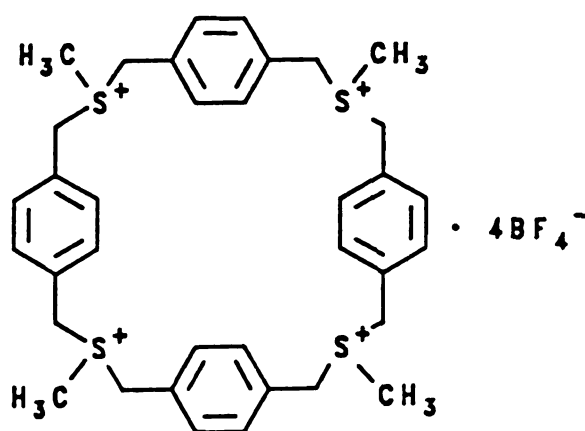


Figure 4.1. Diederich's water-soluble cyclophanes.

Figure



A



B

Figure 4.2. Water-soluble heterocyclophanes.

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Variations of the basic structure of methyl-APC were made to improve the enzyme-like action of the host molecule. Nitrogen atoms in the original structure of methyl-APC were replaced with sulfur atoms as shown in Figure 4.2-B. The original methyl-APC was more stable in an aqueous media of a wide pH range, while sulfonium heterocyclophane was unstable in aqueous solution above pH 9, where a remarkable change in its electronic spectrum was observed (20).

The basic methyl-APC molecule without modifications with hydrophobic substituents, containing a relatively small and shallow cavity for host-guest interactions. APC molecules with long alkyl chains of different functional groups have been introduced (Figure 4.3) to improve substrate specificity further. Anionic APC (Figure 4.3-A) favors cationic guests (23), while cationic APC (Figure 4.3-B) favors anionic guests (24). Octopus-like APC (Figure 4.3-C) having eight long alkyl chains gives extra hydrophobic binding efficiency (25). By using two rigid macrocyclic skeletons, a capped APC (Figure 4.3-D) with four flexible hydrocarbon chains connecting the macrocycles was synthesized (26).

These synthetic enzyme model compounds (APCs) are highly promising macrocyclic compounds for various applications in chemical analysis. Their most prominent characteristics are:

- (i) The macrocyclic skeleton may provide a stable binding site through hydrophobic interaction.
- (ii) The stable binding site may show a high substrate

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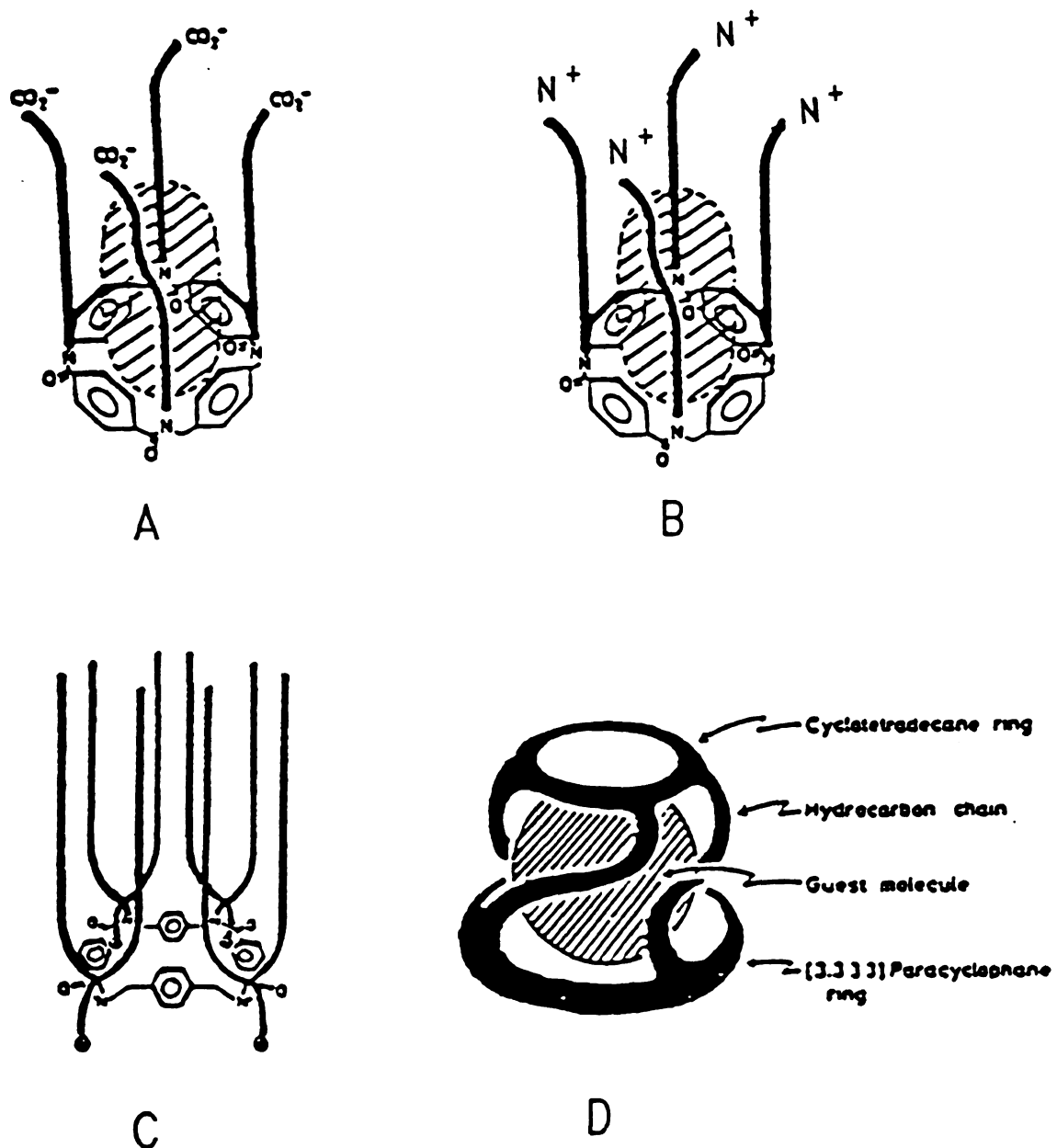


Figure 4.3. Schematic representations of modified water-soluble heterocyclophanes.

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specificity due to electrostatic and geometrical requirements for host-guest interaction.

(iii) A rigid microenvironment may be obtained due to the wall of benzene rings.

Thus, the APCs may provide a favorable microenvironment for triplet state molecules. Triplet molecules included inside the macrocyclic cavity may be protected from quenching by dissolved oxygen or impurities in the solution. Also, a high selectivity in RTP is expected due to the size and charges of the APC. In this research, the possibility of using APC molecules in RTP was examined.

C. Other Macrocyclic Compounds in Chemical Analysis.

In 1967, Charles Perdesen reported the synthesis of a new class of macrocyclic compounds called crowns (27). Crown ethers are cyclic polyethers having various sizes and structures. These compounds have the ability to selectively complex metal ions, and in particular, the alkalies. They can also solubilize inorganic and organic solutes in polar and nonpolar solvents. Over 60 cyclic polyethers have been synthesized with ring structures containing from 9 to 60 total atoms, from 3 to 20 oxygen atoms, and from 1 to 4 attached hydrocarbon rings (28).

The nomenclature recommended by Perdesen for his cyclic polyether compounds does not follow complicated IUPAC rules. Generally, the groups attached to the crown are mentioned

Figure

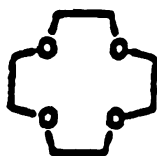
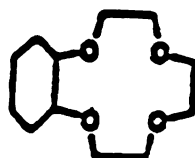
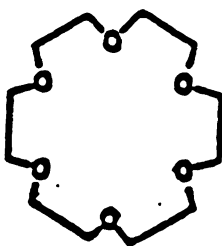
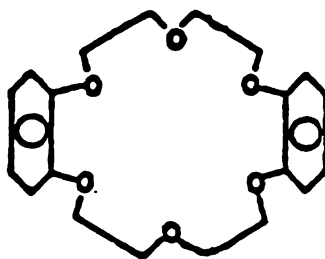
**12-Crown-4****Cyclohexyl-12-crown-4****18-Crown-6****Dibenzo-18-crown-6**

Figure 4.4. Some representative crown ethers.

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first, then the number of atoms in the ring, followed by "crown" and then by the number of oxygen atoms in the crown. Examples are given in Figure 4.4. The cyclic polyethers have been found to form primarily 1:1 polyether:metal complexes with a large array of metal ions. However, there are some indications that, depending on the ratio of the cavity to the metal ion diameter, 2:1 and 3:2 complexes are also formed. The 1:1 complexes are generally assumed to consist of the metal ion bound in the cavity of the polyether ring. On the other hand, 2:1 complexes are "sandwich" type structures, in which the metal ion is located between two cyclic polyether molecules. The 3:2 complexes are "club sandwich" types, in which two metal ions are located between polyethers.

Crown ethers have been used in extraction studies (29,30), membrane transport (31,32), ion-selective electrodes and related electrochemical procedures (33), ion-exchange resin preparation (34), and liquid chromatographic separation of ionic and neutral species (35,36).

In 1967, Lehn and his coworkers introduced the macrocyclic ligands called cryptands (37). Cryptands form complexes with metal ions, in which the metal ion is located within the central cavity (crypt). In the cryptates, the complexed cation is completely enclosed in three dimensions by ligands containing O or N which bind to the cation. Thus, the ion becomes isolated from external species if it fits, or is smaller than the diameter of the cavity. The cavity is

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hydrophillic, and hydrophobic hydrocarbon groups form the outside envelope to make cryptands quite soluble in organic solvents. Thus, the cryptand is a three-dimensional ether, which has a hydrophillic cavity and hydrophobic exterior hydrocarbon groups (38,39).

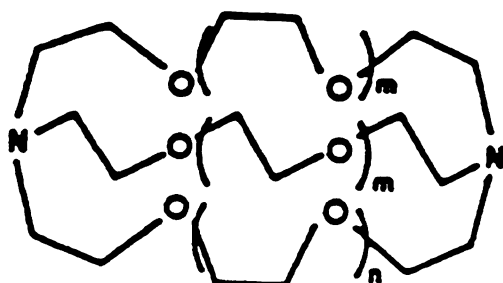
The cryptands promise to become of great analytical importance, as their complexes with alkali and alkaline earth ions are much more stable than those with other macrocyclic compounds, such as crown ethers. The cryptands, 2.1.1., 2.2.1, and 2.2.2. (Figure 4.5), are analytically important and readily available.

The usage of cryptands in chemical analysis is similar to crown ethers. Kirch and Lehn (40) used cryptands in alkali metal ion extraction. Czerwenka and Scheubeck (41) determined sodium and potassium by potentiometric titration using cryptands. Cryptands were used for the preparation of ion-exchange resins (42).

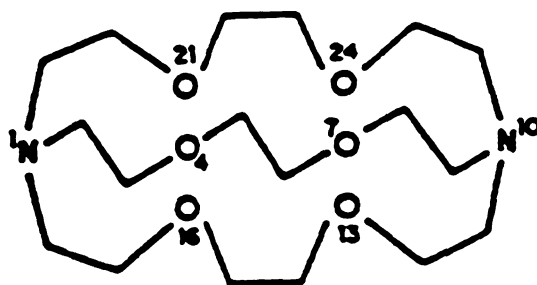
Recently, cyclodextrins have been used extensively in chemical analysis. The structure, properties, and their application in luminescence are discussed in the previous chapter. Mueller and Rodin (43) studied inclusion complex formation of cyclodextrins with metal ions. Cramer et al (44) studied cyclodextrins as enzyme model system.

Cyclodextrin polymers are used as filling materials for column chromatography (45). When compared with customary Sephadex, it is apparent that cyclodextrin polymers have the advantage in that they strongly retard molecules of an

Figur



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| <u>3</u> | [2.2.1] | $m=1, n=0$ |
| <u>4</u> | [2.2.2] | $m=n=1$ |
| <u>5</u> | [3.2.2] | $m=1, n=2$ |
| <u>6</u> | [3.3.2] | $m=2, n=1$ |
| <u>7</u> | [3.3.3] | $m=n=2$ |



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Figure 4.5. Lehn's cryptands.

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appropriate size. Molecules with similar molecular weight or even isomers can be separated. Armstrong and his coworkers (46,47) used cyclodextrins extensively in liquid chromatographic separations. They demonstrated that many difficult separations, such as enantiomers, diastereomers, and positional, geometric, and structural isomers can be accomplished (48).

Recently, an attempt was made to increase the solubility of cyclodextrins in the aqueous phase. Using urea, the solubility of cyclodextrins in aqueous solution was increased up to 10 times (49). Tabushi and his coworkers (50,51) modified cyclodextrins to provide more functionality and structural rigidity. Considerable enhancement of a certain elementary recognition interaction was observed with modified cyclodextrins.

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CHAPTER V

INSTRUMENTATION

Although RTP has grown as an important analytical method over the last two decades, there are few commercial instruments available for RTP. Modifications to commercial instruments for RTP studies have been made to aid individual needs depending on the RTP technique employed. Vo-Dinh et al (1) designed an automatic phosphorimeter for solid-surface RTP with a continuous filter paper device. Cline Love et al (2) constructed a phosphorimeter from commercially available components and used it in micellar-stabilized RTP studies. Nithipatikom and his coworkers (3) designed a spectrometer for determining single and multiple RTP lifetimes.

The use of microcomputers for instrument control and data acquisition has become a common tool in scientific laboratories. In many cases, experimental control and data acquisition require complex timing and synchronization with other experimental conditions. Instrument control, data acquisition, complex timing and synchronization, time consuming event monitoring, tedious calculations, and data analysis can be done with a personal computer. This ability of the personal computer has enabled scientists to do experiments easier with more sophisticated instrumentation than previously possible.

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In this chapter, the construction of a versatile computer controlled luminescence spectrometer for measuring fluorescence, RTP, and RTP lifetimes with comprehensive data acquisition and analysis software is described. Also, the modification of an IBM-PC data acquisition and control adaptor board is presented. This board was used for the Perkin-Elmer LS-5B spectrometer later in this research.

A. Luminescence Spectrometer.

In this work, an Aminco-Bowman spectrofluorometer (SPF-500) was modified and interfaced to the IBM-XT compatible personal computer for keyboard control of the instrument, and data acquisition. This instrument allows semiautomatic luminescence data acquisition and analysis on a personal computer.

The Aminco-Bowman spectrofluorometer (SPF-500) contains a Xenon lamp which produces an intense 250 W Xenon arc. The monochromators are single-pass Czerny-Turner type with a 250 mm focal length and $f/4$ aperture. Both excitation and emission monochromators use 600 line/mm gratings blazed for optimal wavelength response. These monochromators are controlled by precision 500 step/rev stepping motors. This allows precise control of position and scanning rate of both monochromators. The interface connector at the back of the spectrometer contains all the necessary control lines for the monochromator control.

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1. Principle of Operation of the Instrument.

In this work, the optical unit, excitation and emission monochromators, PMT, xenon lamp with power supply, and sample compartment unit of the spectrometer, were used without modification. A block diagram of the instrument is shown in Figure 5.1. A continuous Xe-arc lamp (250W) was used as an excitation source. The exciting radiation was modulated by a rotating single-disk type chopper. This allows phosphorescence to be measured after any fluorescence has decayed.

For data acquisition and monochromator control, a data acquisition board (Lab Master, Scientific Solution, Solon, Ohio) was used with an IBM-XT compatible computer. Digital outputs from the Lab Master board controlled both monochromators for scanning. Menu driven software written in Turbo Pascal and Macro Assembler was used for instrument control, data acquisition, and data manipulation.

A photodiode was placed at the opposite side of the excitation beam to sense the modulation of the excitation beam. The pulsed signal from the optointerrupter was adjusted to be in-phase with photodiode signal. This adjusted chopper signal was used to trigger the AM9513 counter/timer for time-resolved data collection.

The current signal from the PMT (Hamamatsu R928) was converted into a voltage signal, and a variable gain

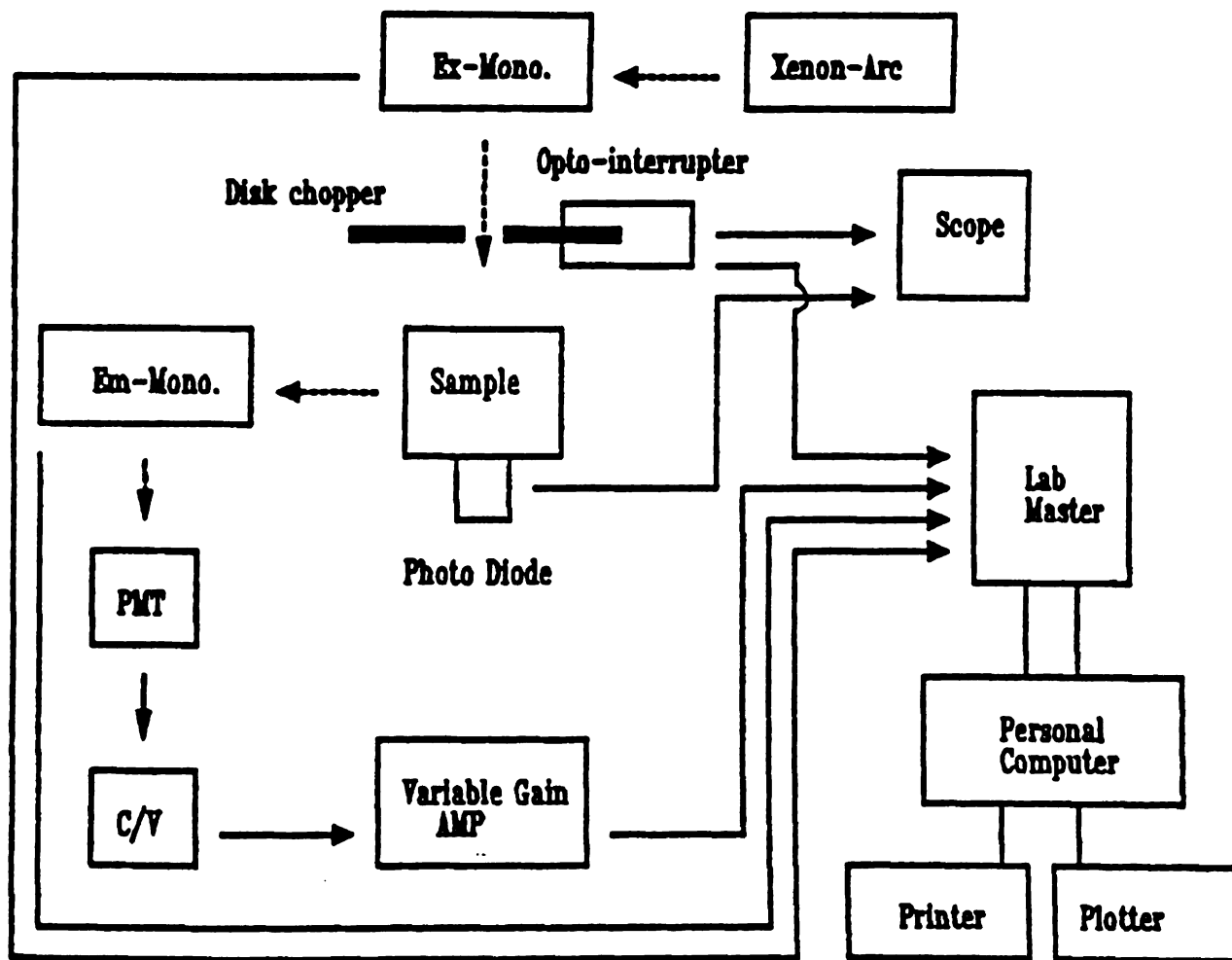


Figure 5.1. Block diagram of the luminescence spectrometer.

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amplifier (AD507, Analog Device) was used to get the desired input voltage signal (0-10 V) for the analog-to-digital converter (ADC). To reduce high frequency noise, a low-pass filter was used for fluorescence and RTP measurements placed between the current-to-voltage converter and the main amplifier. It was turned off by a manual switch when RTP decay curves were to be measured.

2. Hardware Construction.

(a) Computer Interfacing to Spectrometer.

The Lab Master board (4) contains a 12 bit analog-to-digital converter (ADC) with a built-in 16 channel multiplexer (MP 6812), a 24 bit parallel port (Intel 8255), two 12 bit digital-to-analog converters (DACs), and a five channel 16 bit counter/timer, AM9513, as shown in Figure 5.2. The AM9513 LSI chip is one of the most powerful counter/timers (5). The Lab Master data acquisition board installed on the IBM-XT compatible computer controlled the scanning module of the spectrometer, and digitized analog signals from the spectrometer.

The Lab Master board address was set to 1808 (decimal) and I/O mapped. The last channel of the multiplexer was set to channel 15. Input mode was set to pseudo-differential 0-10 V range, and no interrupts were used. Channel 15 of the ADC was used for luminescence data read, and channel 13 and 14

Figure
board

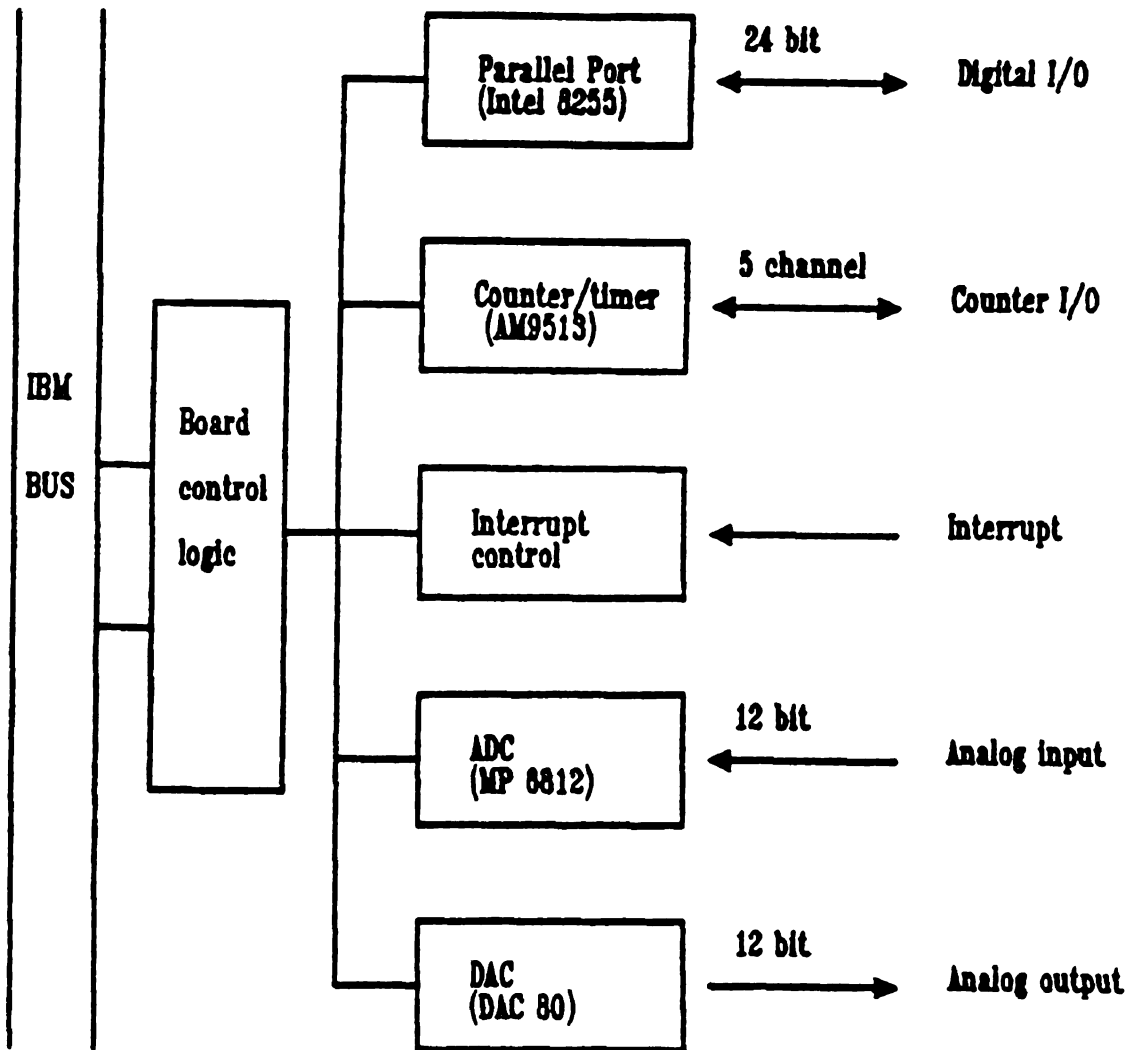


Figure 5.2. Schematic representation of the Lab Master board.

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were used for reading positions of the both monochromators. Pin 24 (analog output of excitation wavelength) of the interface connector of the spectrometer must be connected to ADC channel 13, and pin 23 (analog output of emission wavelength) must be connected to ADC channel 14.

For control of both excitation and emission monochromators, a parallel output was used along with a latch (74LS75). The direction of rotation and the number of turns of the monochromator to scan wavelength were controlled by the software. This was done by sending the appropriate digital outputs to the latch inputs, whose outputs were connected to the control lines of both monochromators. This allowed total computer control of the scanning modes (emission, excitation, synchronous, excitation-emission) and automatic initial wavelength selection of both monochromators.

An Intel 8255-5 parallel I/O port on Lab Master board was programmed to work in mode 0, and digital data bits 0-5 of port A were used to send control pulses for both monochromators. Data bits 1-4 were latched by a 74LS75 quad latch in order to keep each control signal of the scanning module until next operation. Each digital signal and latch connection is shown in Figure 5.3.

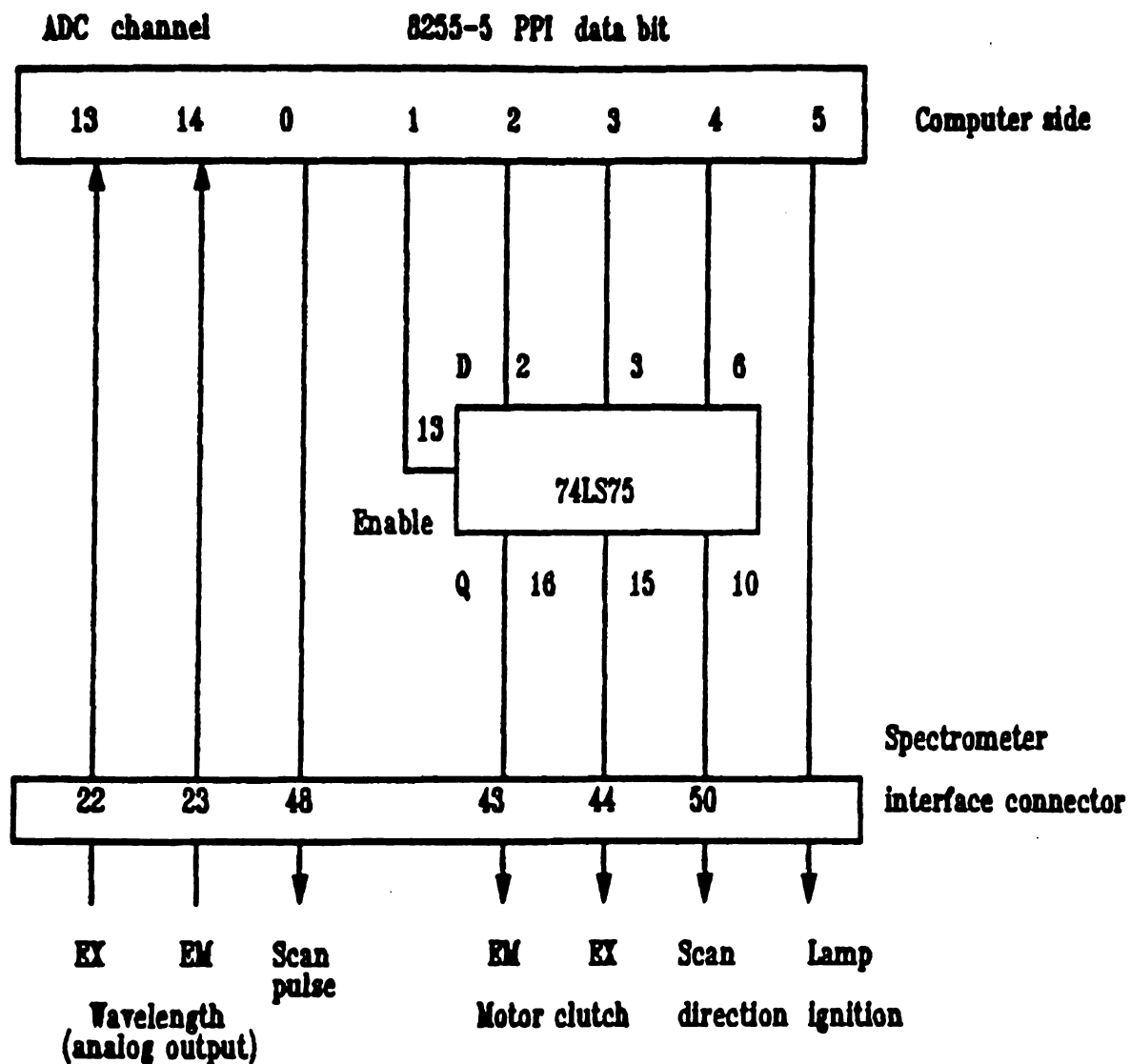
In the phosphorescence mode, counter 5 of the AM9513 counter/timer was used to trigger the ADC start conversion after a given delay time. In the RTP lifetime mode, counter 4 was used to control the delay time after lamp excitation,

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- To control scanning module directly by computer pin 33 (motor master hold) and pin 49 (auto direction enable) must be connected to pin 6 (5V return).

Figure 5.3. Interfacing of the control signals from Lab Master to the Aminco-Bowman spectrometer (SPF-500).

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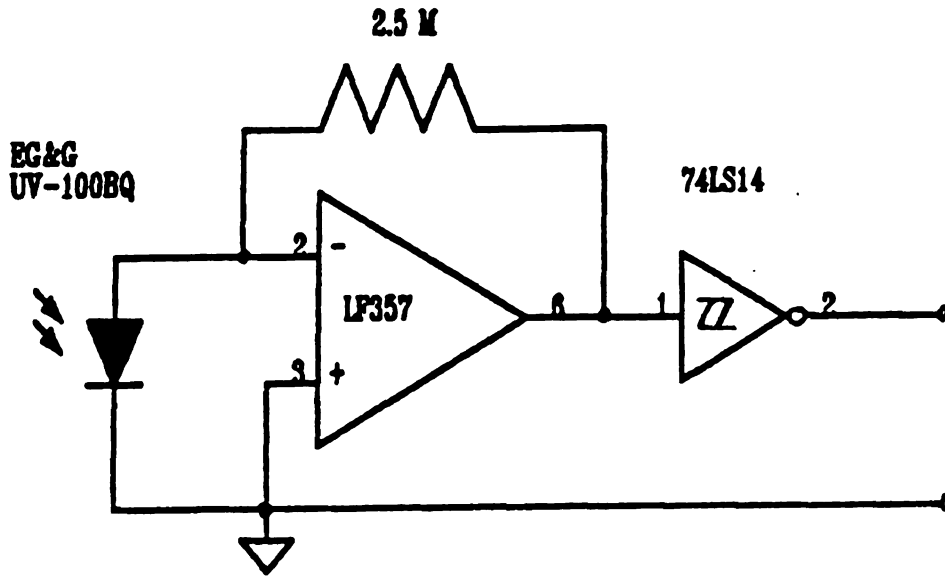
and counter 3 was used to control the sampling rate of the ADC. OUT4 of the counter/timer must be connected to GATE3. GATE4 and GATE5 must be connected to the chopper signal in order to count the delay time in microseconds. OUT3 and OUT5 of the counter were connected to the control pin of the external start conversion of ADC through a rotary switch in order to select external start conversion signals of ADC between OUT3 and OUT5. More detailed explanations about the operation of the counter/timer will be shown later in this section.

(b). Light source Modulation and Detection.

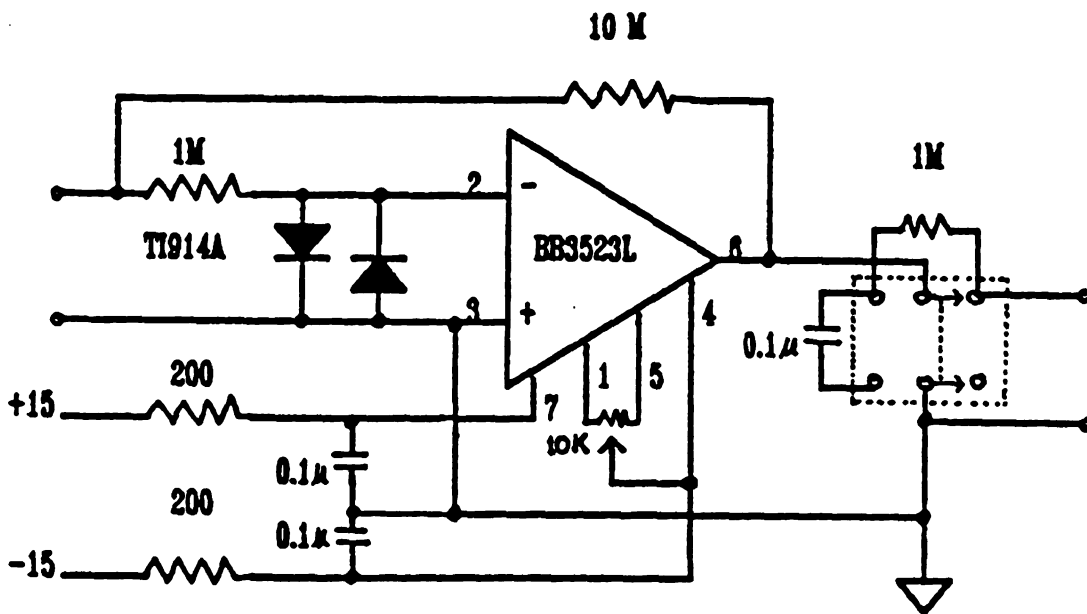
Several commercial spectrophosphorimeters, including the Aminco-Bowman (SPF-500), use a rotating can to modulate the exciting light source. The major disadvantage of the rotating-can type phosphoroscope is that the modulation frequency of the excitation source, and the luminescence detection time window are limited. But the RTP lifetimes of most organic phosphors are very short, especially in solution. As a result, the rotating-can type phosphoroscope gives very low or negligible luminescence signals for short-lived phosphors. The short delay time will increase the RTP sensitivity of the short lived phosphors. A pulsed source, or a single-disk type chopper, gives better performance in terms of flexibility for selecting appropriate delay times and gate times for the experimental requirements.

Figur

(B)



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Figure 5.4. Circuits built for (A) photodiode light sensor, (B) current-to-voltage convertor.

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In this design, the Xe-arc lamp was modulated by a replaceable rotating single-disk type chopper. The chopper disk was constructed with a stiff, thin plastic blade (10 cm diameter) and coated with non-glare black paint. The chopper was driven by a synchronous motor, and its speed was controlled by a variable, precision DC power supply. Two types of disks, which have a different number of open slots, were used depending on the type of experiment (RTP or RTP lifetime). When fluorescence was measured, the disk was stationary with its open slot facing the incoming excitation beam, so that all the exciting light could pass through the chopper disk.

The phase detection of the rotating chopper was accomplished through the use of a photodiode and an opto-interrupter. A photodiode (EG&G UV-100BQ) with a Schmitt trigger (74LS14) was placed on the opposite side of the chopper across the sample cell, and an opto-interrupter (GEH21B1) with monostable multivibrator (74LS121N) was placed under the chopper blade. The phase detection signal was adjusted in the visible range as described below.

At the beginning of the experiment, signals from photodiode circuit and opto-interrupter circuit were displayed concurrently on a dual-trace oscilloscope. The output of the photodiode circuit is the true chopped signal of the excitation source, whereas the output from the opto-interrupter circuit is not the true source chopping signal, since its signal depends on the mounting position of the

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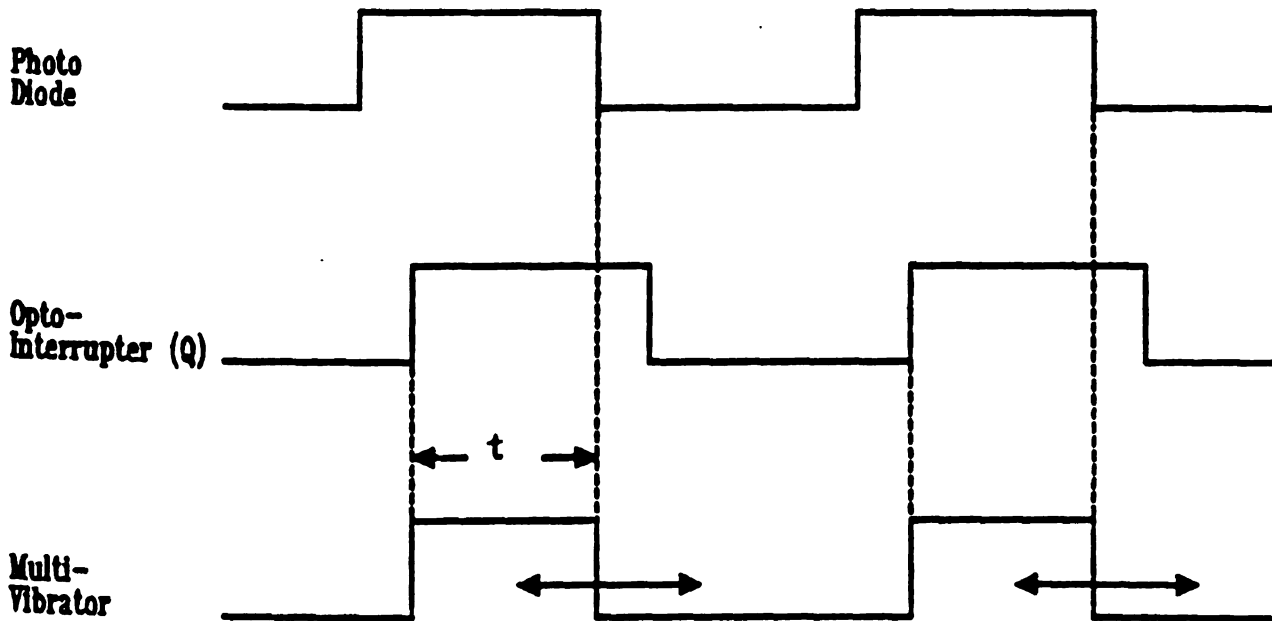
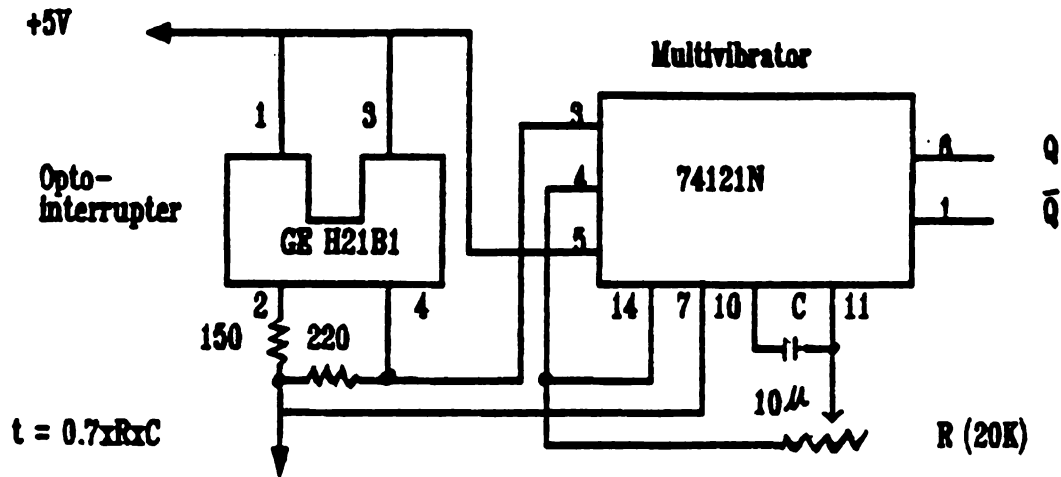


Figure 5.5. Timing diagram of the chopper phase detector. The opto-interrupter output is connected to the multivibrator and the pulse width of the multivibrator is adjustable with a variable register.

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opto-interrupter under the chopper blade. The low-going pulse output of the opto-interrupter was adjusted manually by a variable register of the multivibrator, until the falling edges of both signals from the photodiode and the opto-interrupter were exactly matched (Figure 5.5).

(c). RTP and RTP Lifetime Measurement.

For RTP and RTP lifetime measurements, a timer/counter, AM9513, on the Lab Master board was used. A single-disk type chopper with four opening slots was rotated to obtain a 200-500 Hz modulated source for RTP measurements. The chopper signal from the one-shot of the phase detector circuit was connected to gate 1 of the counter 1, and counter 1 was programmed to generate a pulse at the terminal count. The on board 1.000 MHz clock was used as the clock source. The output of counter 1 was connected to the ADC external start conversion pin. By varying the count number of the counter 1, data can be acquired at different time delays after excitation for time-resolved phosphorimetry (Figure 5.6). For RTP lifetime measurement, two counters were used simultaneously. A chopper with one or two open slots was rotated to obtain a 5-10 Hz modulated source. Gate 1 of the counter 1 was connected to the chopper signal, and the output of the counter 1 was connected to gate 2 of counter 2. The analog-to-digital conversion was triggered by the output of counter 2. At the falling edge of the chopper

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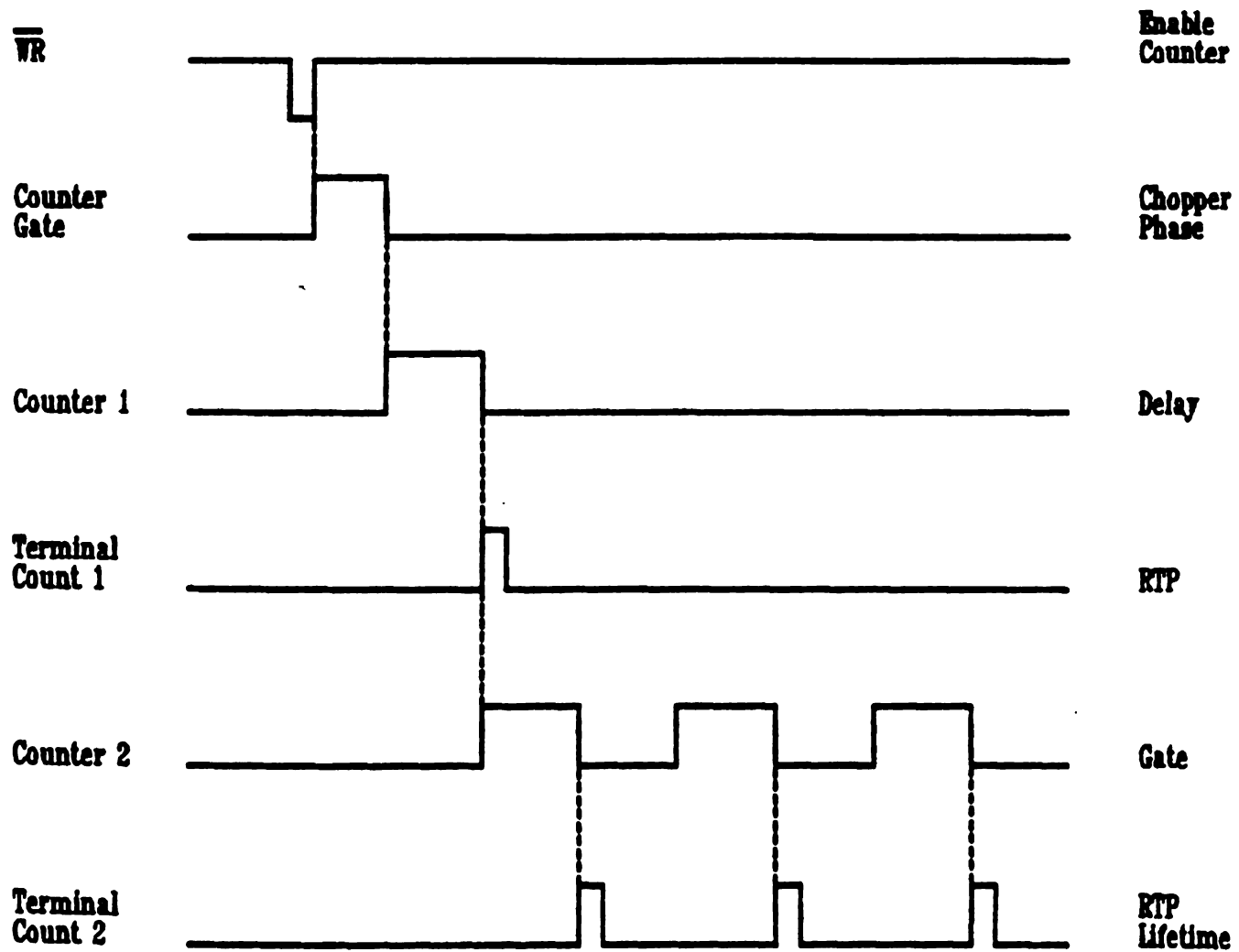


Figure 5.6. Timing diagram of the counters for RTP and RTP-lifetime measurement.

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signal, counter 1 starts the count down for a given delay time and generates a pulse at the terminal count. The rising edge of the terminal count signal of counter 1 then initiates counter 2 to count down for a given gate time, repetitively, to generate pulses at each gate time interval. At each terminal count pulse of counter 2, an RTP signal was read to obtain a decay signal. The delay time is the time delay from the falling edge of the chopper signal to the starting point of the data reading. The gate time ($33 \mu\text{s}$ minimum) is the time interval between the successive data read points.

At the beginning of each experiment, the user can specify the number of data points to read at each emission wavelength for RTP measurement, or the number of decay cycles to repeat per experiment, and the number of data points per each decay cycle for RTP lifetime measurement. The final data are obtained by signal averaging of the collected raw data in real time.

3. Software Development.

A menu driven program, EMISPEC, was developed using TURBO PASCAL (Version 4.0, Borland International) and MACRO ASSEMBLER (Version 4.0, MicroSoft) for instrument control, luminescence data acquisition, and manipulation on the IBM-XT compatible computer. A block diagram of the program, EMISPEC, is shown in Figure 5.7. There are six items in the

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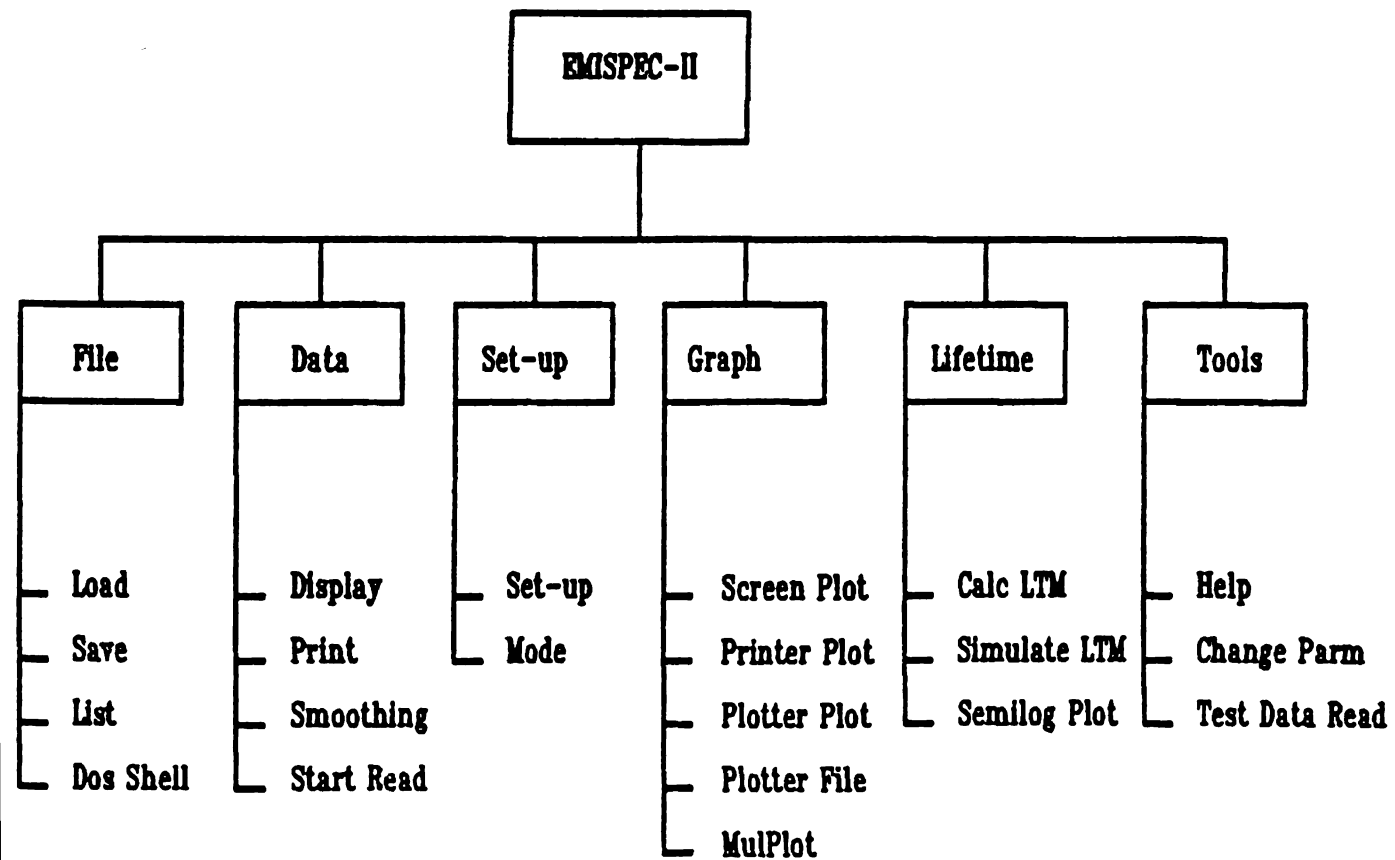


Figure 5.7. Block diagram of the EMISPEC program.

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main menu: File, Data, Set-up, Plot, Tools, and Lifetime. For noisy data, smoothing can be employed using a Fourier transformation technique, according to the unique algorithm developed by Eric E. Aubanel (6). Set-up parameters include mode (Fluorescence, RTP, RTP Lifetime), initial setting and scan ranges for both monochromators, number of data per wavelength or number of decay cycles for signal averaging, scan mode (emission, excitation, synchronous, excitation-emission), delay time, and gate time. Fluorescence and RTP spectrum data are plotted on the screen in real time as the monochromator scans. The plot routine plots data in five ways: screen plot, printer plot, plotter plot, plotter file. The spectrum drawn on the screen can be printed with either an EPSON printer or an HP plotter. Data plot can also be routed into a file in HPGL format for use with other software.

4. Results and Discussion.

The primary output of any experiment in which chemical information is to be extracted, is the signal which measures the phenomenon under observation. In most cases the signal measured has some superimposed noise which arises from various sources. Noise can be distinguished from the signal by its frequency characteristics and by the time of occurrence or phase coherence of their frequency component (7). The removal of noise can be performed by hardware and

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The three most common methods for smoothing data are moving-average, least-squares, and Fourier transformation. In the moving-average method (8), each data point is replaced by the average of itself and n neighboring points on either side of it. The advantage of this method is that it is very easy to program. The disadvantage is that the first and the last data points are not smoothed to the same degree as the rest of the data set, because they don't have n neighbors on each side of them. This method flattens the signal more than the other methods.

The least-squares method identifies the line of the order you specify which minimizes the sum of the squares of the distances between the data points and the calculated line. The advantages of this method are that it generates statistical information on the goodness of the fit, and it does not require that the data be collected at regular time intervals. The disadvantage of this method is that it generates disproportionately biased data by one or two very bad data points, because it will twist the line of fit to spread the error over the entire data set.

Fourier transformation and inversion (9) is probably the best method, since it lends itself naturally to identifying and eliminating noise. The reason for this is that noise is usually present at high frequencies, whereas, the signal components are usually present at low frequencies. Fourier transformation produces the frequency spectrum. By

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eliminating the high frequency portion of the spectrum and performing an inverse Fourier transformation, one can obtain the original data without much of the noise.

In this system, we employed both hardware and software smoothing methods. A simple passive low-pass filter (cut-off frequency 10 Hz) was placed between the current-to-voltage converter and the variable gain amplifier (10) for RTP and fluorescence measurements. Because the low-pass filter has a long time constant which can affect the phosphorescence decay signal, it was turned off by a manual switch when RTP lifetime was being measured. Two other methods, signal averaging and Fourier smoothing, were used to further enhance the signal to noise ratio (S/N) of the measurement. If the noise is random and the number of measurements is N , the noise is proportional to the square root of N , while the signal is proportional to N . As a result, the S/N will be improved by the square root of N with signal averaging (11). Also, by performing Fourier smoothing, one can further reduce noise components from the measured data. The method of Fourier smoothing used is a modified Discrete Fourier Transformation (DFT), which runs much faster than regular DFT. In this method, the user can control the degree of smoothing, and the data size is not limited to a multiple of 2, which is the limiting factor of the Fast Fourier Transformation (FFT).

The effect of the degree of smoothing of the data is well demonstrated in Figure 5.8. A typical luminescence data set

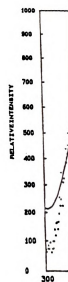
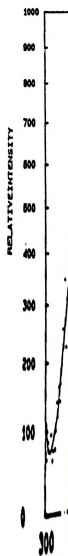
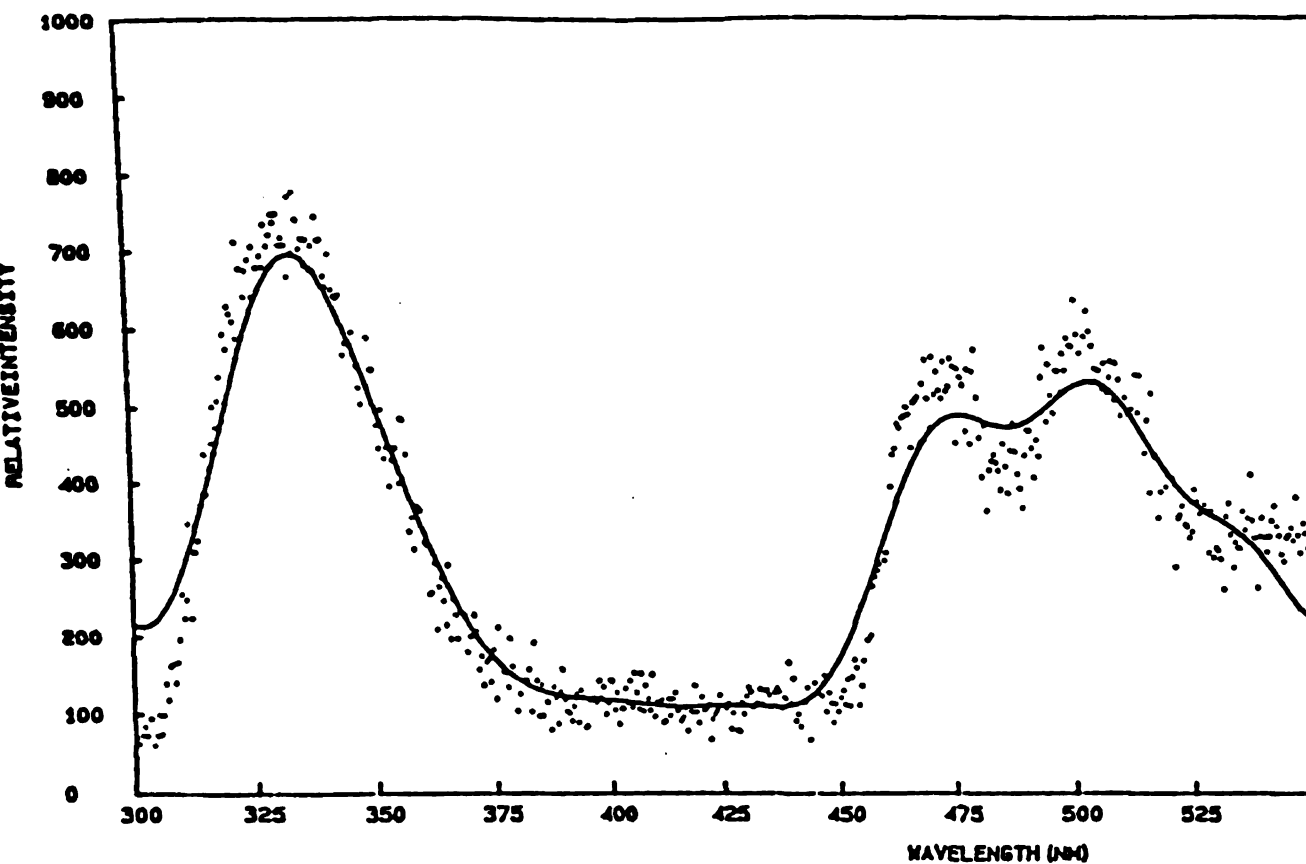
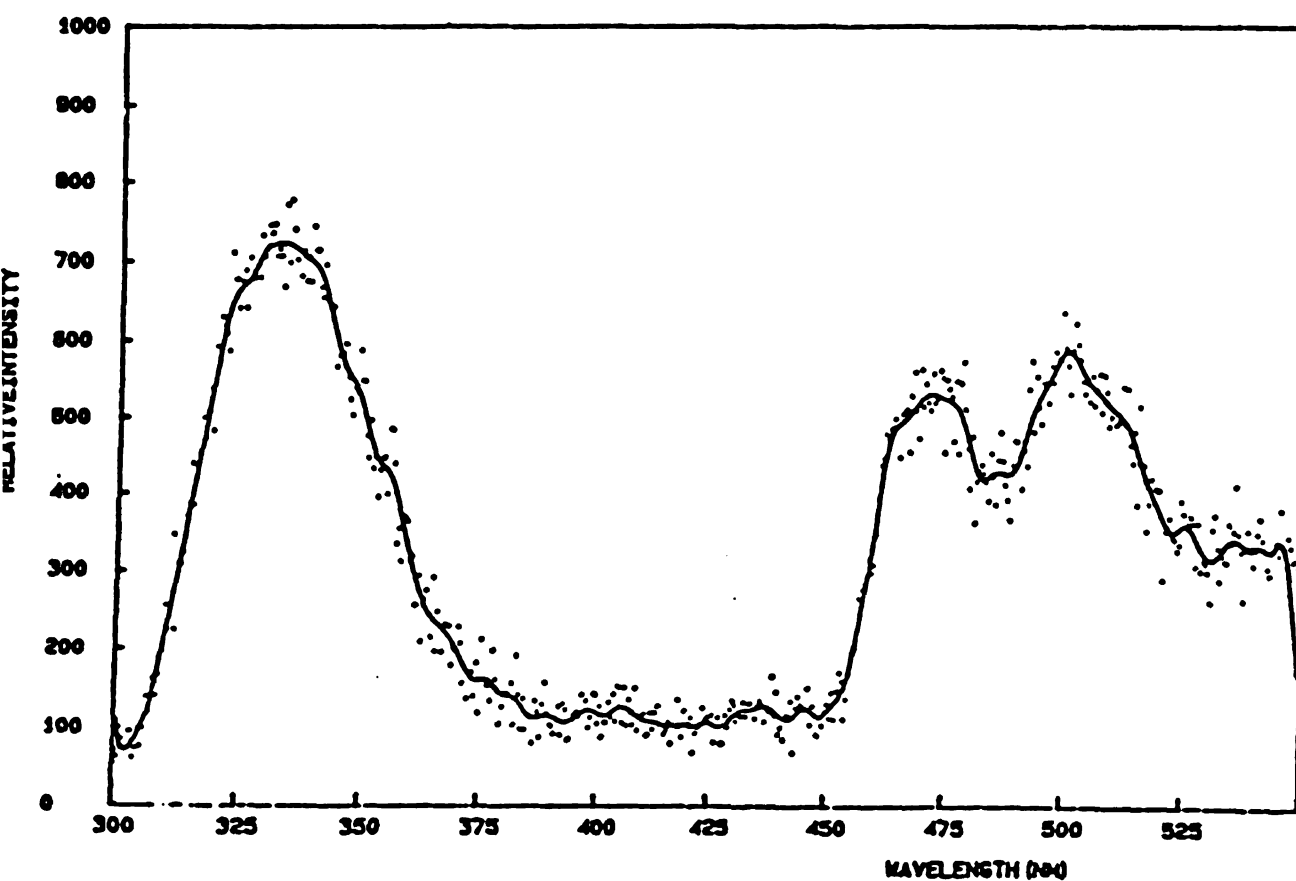


Figure 5.8.A. - Four
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5.8.A. Fourier smoothing of the luminescence spectrum of Naphthalene in micelle(SDS). Degree of smoothing: 9, raw data: dot, smoothed data: line.



5.8.B. Fourier smoothing of the luminescence spectrum of Naphthalene in micelle(SDS). Degree of smoothing: 25, raw data: dot, smoothed data: line.

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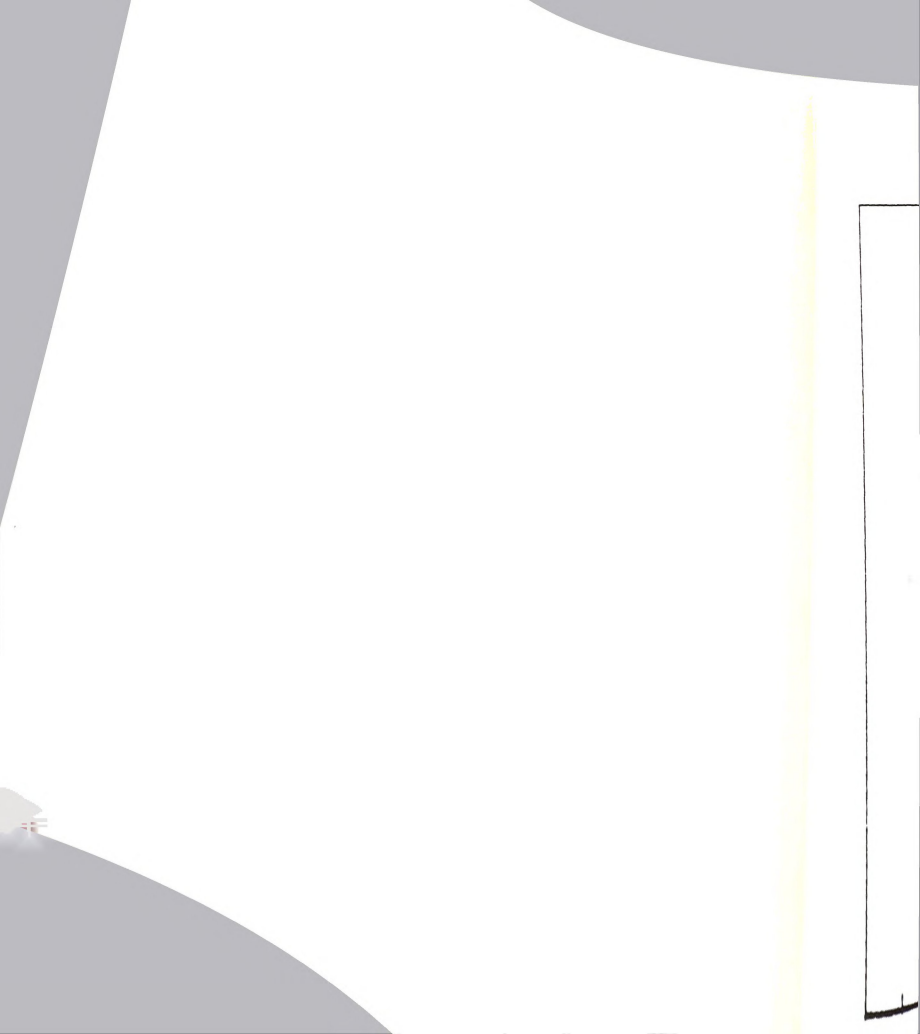
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in Figure 5.8 was obtained with an aqueous solution of naphthalene in a sodium dodecyl sulfate (SDS) micellar solution. Peaks below 400 nm are fluorescence, and the peaks above 400 nm are phosphorescence at room temperature. The dots in Figure 5.8 are the raw data without signal smoothing, and the solid line is the smoothed data after Fourier smoothing.

Figure 5.8.A shows oversmoothed data with a degree of smoothing of 9, while with a degree of smoothing of 25, undersmoothed data were obtained as shown in Figure 5.8.B. Choosing the right degree of smoothing is a matter of trial and error. With a degree of a smoothing of 15, properly smoothed data were obtained as shown in Figure 5.9. In Figure 5.8, both smoothed data sets show "end effects" in which the first and last part of the data set are distorted. This end effect is due to the discontinuity between the beginning and the end of the data set. The DFT treats the data as periodic; that is, it assumes that the last data points are followed by replicas of the initial points. In Figure 5.9, this "end effect" was corrected by the straight line method (6).

The general principles and applications of time-resolved phosphorimetry were well demonstrated by Winefordner (12). Fisher and Winefordner (13) showed a detailed comparison of the pulsed source and continuous mechanical phosphoroscope system in phosphorimetric instrumentation. The major advantage of pulsed source phosphorimetry over the rotating



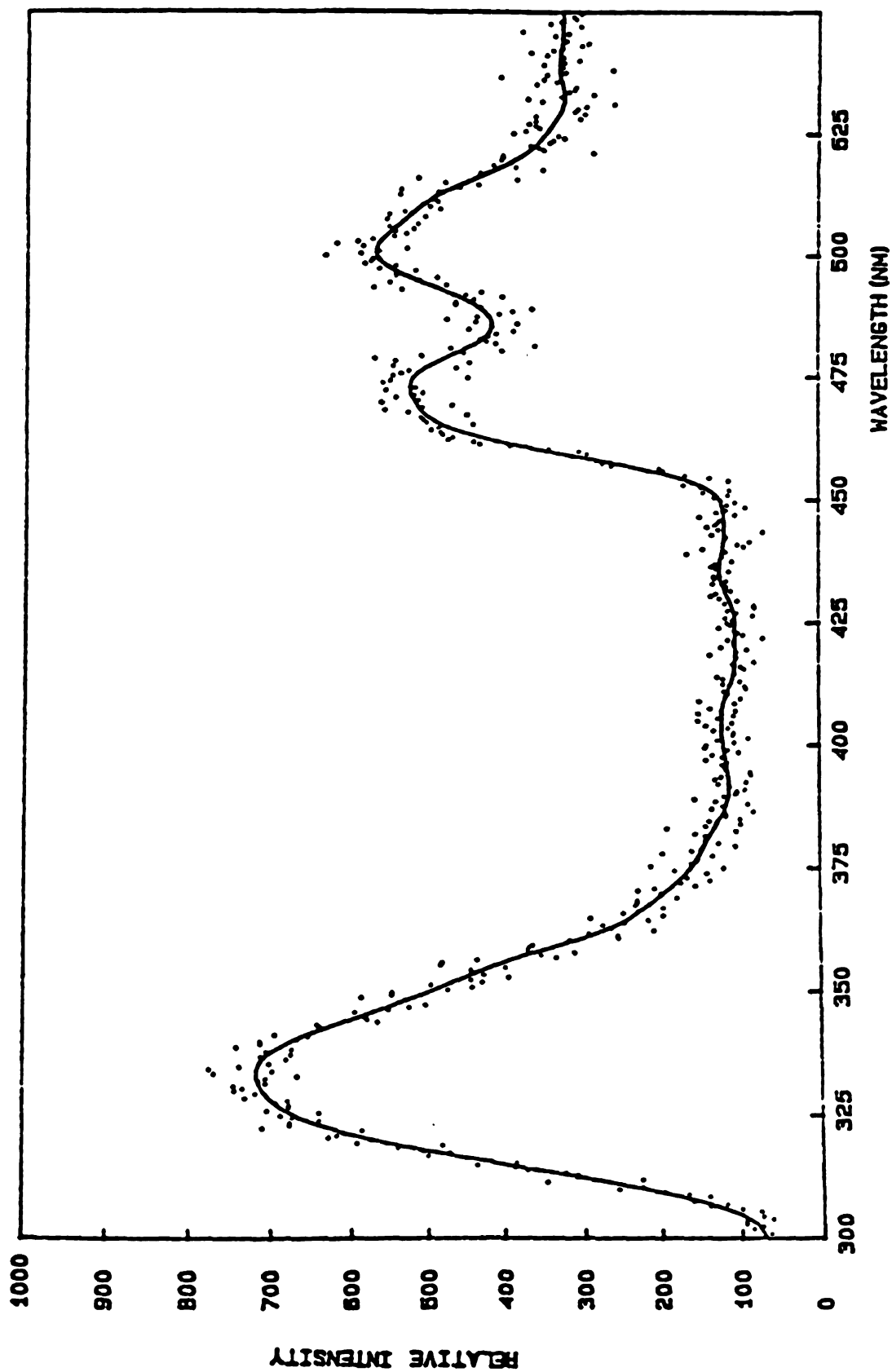


Figure 5.9. Fourier smoothed luminescence spectrum of Naphthalene (1.5×10^{-5} M). Degree of smoothing: 15. The 'End-effect' is corrected at both ends.

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can type mechanical phosphoroscope, is the possibility of selecting experimental conditions, such as, delay time, and gate time, so that the species of interest is measured under optimum conditions. For measurement of short-lived RTP, the rotating can phosphoroscope was found to be inadequate since it takes a relatively long time (1.5 ms minimum) to open the emission window by rotating 90 degrees after excitation. As a result, the intensity of a short-lived RTP signal has decreased significantly before the detection of actual RTP signal is started. On the other hand, if the exciting light source is modulated by a disk type blade or pulse, the detector is always open to the phosphorescence signal. The measurement of the phosphorescence signal can be initiated at any time by choosing the proper delay time after excitation for time-resolved spectrometry. This system gives more versatility in the measurement of short-lived RTP over the rotating can type phosphoroscope.

Accurate detection of the end of the modulated excitation source pulse is very important in RTP and RTP lifetime measurements. Typically, an opto-interrupter or photodiode is used to detect the chopper phase. However, manual adjustment of the position of the opto-interrupter so that its output matches exactly with the true source chopping, is very difficult. Also, most photodiodes (even UV sensitive) are not very sensitive to light below 300 nm; however, many organic compounds require excitation under 300 nm. In

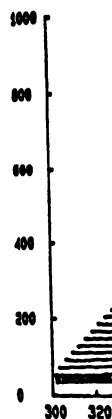


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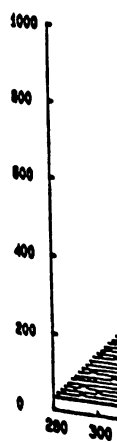


Figure 5.10.B. E
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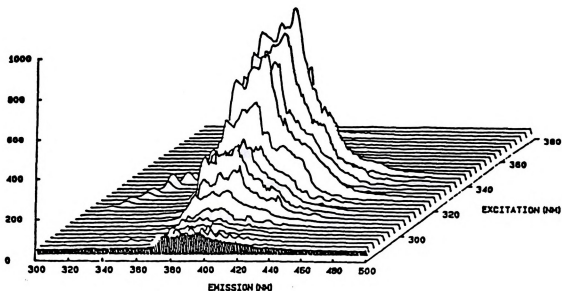


Figure 5.10.A. Fluorescence spectrum of Pyrene (1.5×10^{-6} M) in micelle (SDS 0.1 M).
Excitation scan range: 280–380, emission scan range: 300–500 nm.

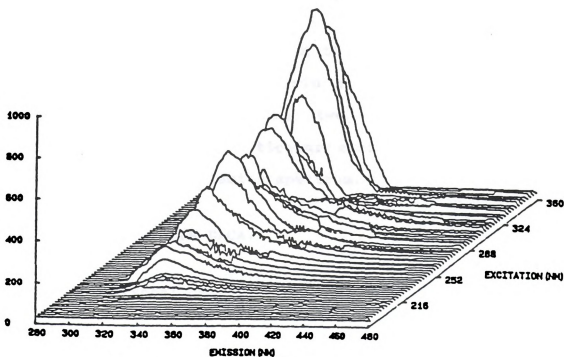


Figure 5.10.B. Excitation-Emission spectrum of Naphthalene at room temperature.
Excitation scan range: 180–360, emission scan range: 280–480.

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addition, typical RTP sample solutions are turbid due to the high concentration of heavy atoms. These conditions make it difficult to obtain a reliable and accurate phase detection signal from the rotating chopper. To overcome these difficulties, we used a combined system using a photodiode and an opto-interrupter for chopper phase detection. This system gives a very reliable and accurate chopper signal, which is independent of any variation of the motor speed of the chopper, excitation source wavelength, and condition of the sample solution.

Figure 5.10 shows the excitation-emission spectrum of pyrene and naphthalene in SDS micellar solution. The entire emission spectrum was obtained by changing excitation wavelength at certain intervals. The excitation-emission spectrum is useful for examining the spectral response of the sample molecules over certain excitation and emission wavelength ranges. Also, any two similar spectra of different samples at a particular excitation will show a different excitation-emission spectrum.

B. Modification of IBM-DACA.

Recently, personal computers and data acquisition adapters have become commercially available at much lower prices. One of the big bargains at the time of this writing is the IBM-DACA (IBM Personal Computer Data Acquisition and Control Adaptor). The IBM-DACA provides both analog and digital I/O

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1. Hardware

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capabilities as well as an on-board counter/timer (14). For general purpose data acquisition and system control which does not require complex timing, process control, or high throughput, it is satisfactory. But for more complex data acquisition and system control, it lacks two very important functions: a hardware trigger capability for the analog-to-digital conversion; and total control of the three on board timer/counters.

This section describes a modification of the IBM-DACA board for general purpose laboratory data acquisition and system control, along with a software package developed for this modified IBM-DACA. By this modification, data collection can be initiated by a software trigger as in the unmodified original IBM-DACA, as well as by a hardware trigger, by simply switching the jumpers installed on a small jumper board. Also all three channels of the on-board timer/counter can be programmed and used for complex timing and counting operations. This board was used later in this research with Perkin-Elmer LS5 spectrometer.

1. Hardware Modification.

(a) Hardware Triggered Analog-to-digital Conversion.

The analog input device of the IBM-DACA has four channels which are multiplexed into a single analog-to-digital converter (ADC), AD574K (15). The ADC converts the analog

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signal in one of three ranges to digital values in the range of 0 to 4095 (12 bit). The AD574K is designed to interface to most popular microprocessors with peripheral interface controllers. The control signals CE, $\overline{CS}$ and $R/\overline{C}$ of AD574K, control the operation of ADC. The state of $R/\overline{C}$, when CE and $\overline{CS}$ are both asserted, determines whether a data read ($R/\overline{C}=1$) or a convert ($R/\overline{C}=0$) is in progress. Either chip enable (CE) or chip select ($\overline{CS}$) signal may be used to initiate analog-to-digital conversion if $R/\overline{C}$ is low. In the IBM-DACA, both CE and $\overline{CS}$ are already enabled in the original design, which means only the $R/\overline{C}$ signal controls the analog-to-digital conversion. To trigger the analog-to-digital conversion, the start convert signal is ANDed with the ADC CE signal from the external distribution panel board, and its inverted signal is fed to the $R/\overline{C}$ pin of AD574K. The ADC CE signal from the distribution panel can only delay the analog-to-digital start conversion when it is low, but it can not guarantee a synchronized hardware trigger of the analog-to-digital conversion. Thus, a true hardware triggered analog-to-digital conversion is not possible in the original design.

In this modification, we have enabled a true hardware trigger of the analog-to-digital conversion by disconnecting the line between the $R/\overline{C}$ (pin 5 of AD574K) and the start conversion signal line (pin 3 of U28) on the printed circuit board. Lines were extended from both sides of the disconnected point to a jumper daughter board which was

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GATE 1 (14)

GATE 2 (16)

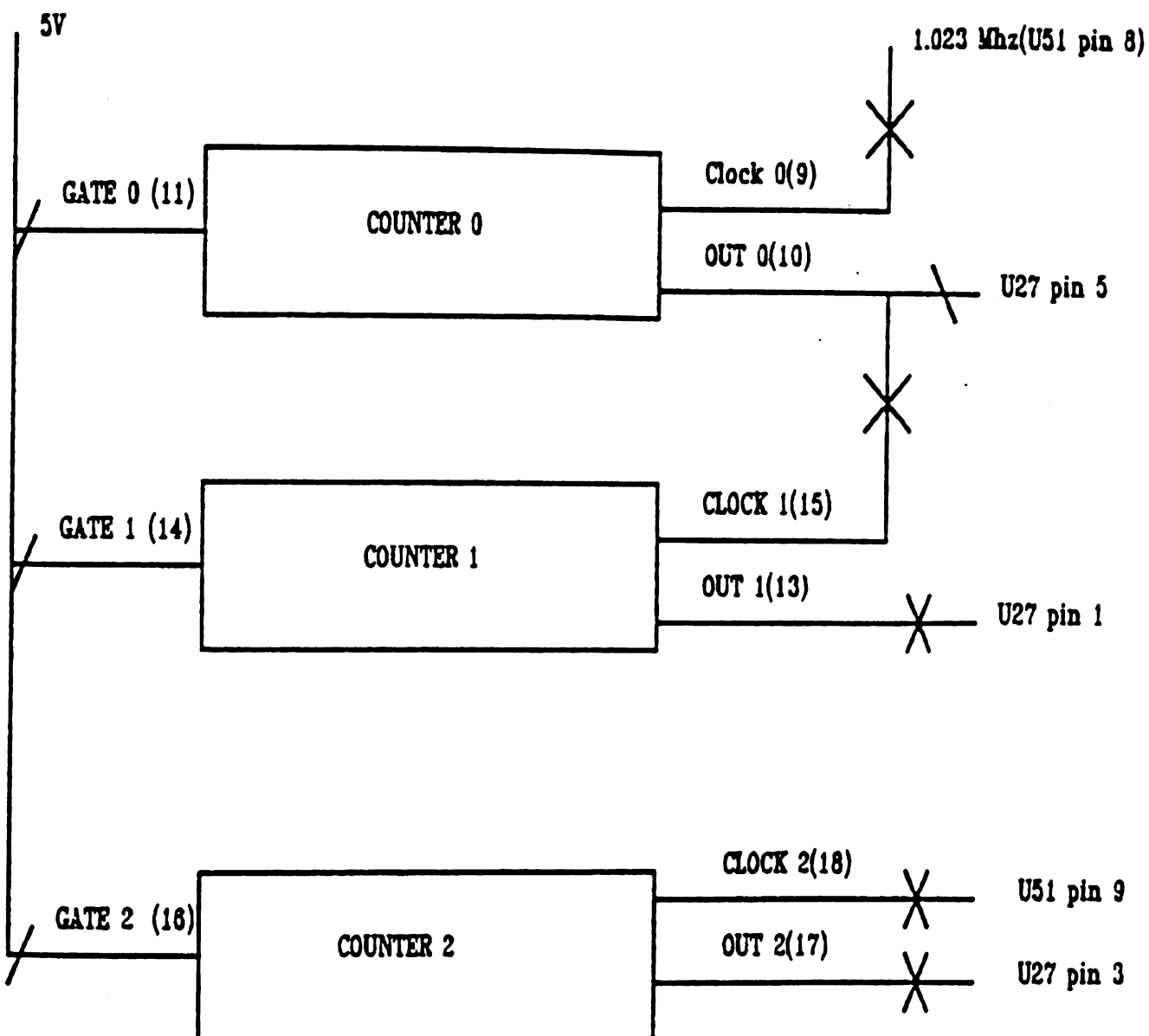
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 X : Disconnect line and extend lines from both ends to the jumper board

Figure 5.11. Modification of the timer/counter circuit of the IBM-DACA.

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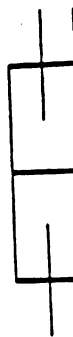
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installed on the IBM-DACA to implement the switching options (a hardware or software trigger of the ADC). If a jumper is placed between these two pins, the IBM-DACA will work with a software trigger as it does normally with an unmodified IBM-DACA board. If the $R/\bar{C}$ pin is connected to another triggering pulse source, then the ADC start conversion will be controlled by that pulse. The AD574K requires 25 μs to complete a 12-bit analog-to-digital conversion. Therefore, the external trigger source must not exceed a pulse width of 25 μs . If a low-going pulse longer than 25 μs is used, multiple conversions will be made on a single trigger pulse. To prevent this, we used a monostable multivibrator (74LS121 or 74LS123) which generates a single pulse with a 5 μs pulse width on every low-going trigger source, regardless of its pulse width. This low-going pulse of the multivibrator is used to trigger the ADC start conversion.

(b) Modification of 8253-5 Timer/counter Circuit.

The Intel 8253-5 timer/counter on the IBM-DACA has three 16-bit counters (16). However, counter 0 and counter 1 are cascaded to provide a single 32-bit counter in the original design of the board. All three gate inputs are disabled and the clock input of the counter 0 is connected to a 1.023 MHz system clock. The second clock input is connected to the output of counter 0 for cascaded operation of the two

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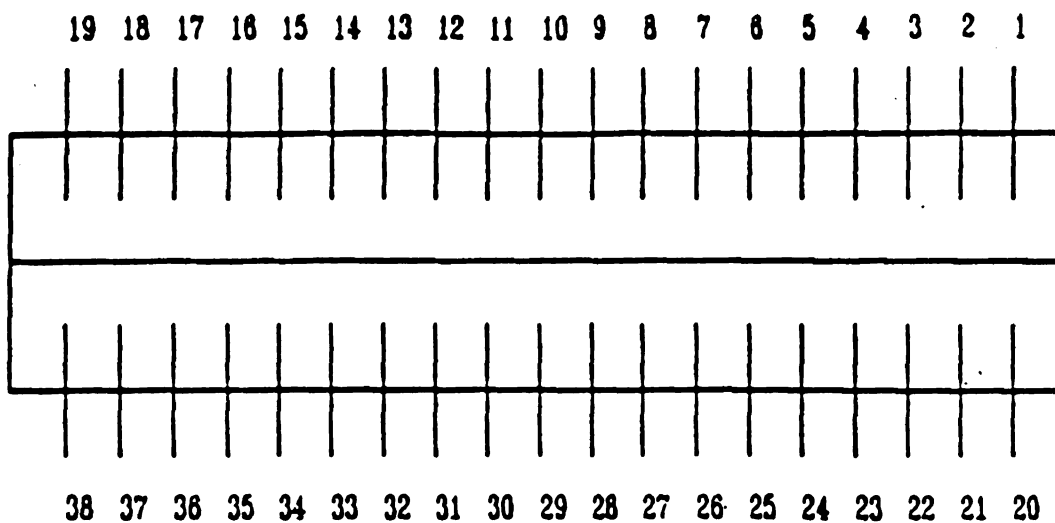
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Figure 5.



Pin connections

1- B1(74LS123 pin 1)	2- B2(74LS123 pin 9)	3- 74LS14 pin 1(optional)
4- 74LS14 pin 3(optional)	5- 74LS14 pin 5(optional)	6- 74LS04 pin 1(optional)
7- 74LS04 pin 3(optional)	8- 74LS04 pin 5(optional)	9,10- +5V
11-G0(8253-5 pin 11)	12-G1(8253-5 pin 14)	13-G2(8253-5 pin 16)
14-Delay(U27 pin 1)	15-Rate(U27 pin 5)	16-Count in(U51 pin 9)
17-Count out(U27 pin 3)	18-Soft trigger(U28 pin 3)	19-Ground
20-Q1(74LS123 pin 4)	21-Q1(74LS123 pin 13)	22-Q2(74LS123 pin 12)
23-Q2(74LS123 pin 5)	24-74LS14 pin 2(optional)	25-74LS14 pin 4(optional)
26-74LS14 pin 6(optional)	27-74LS04 pin 2(optional)	28-74LS04 pin 4(optional)
29-74LS04 pin 6(optional)	30-Out 0(8253-5 pin 10)	31-Out 1(8253-5 pin 13)
32-Out 2(8253-5 pin 17)	33-1.023 Mhz(U46 pin 9)	34- Clk 0(8253-5 pin 9)
35-Clk 1(8253-5 pin 15)	36-Clk 2(8253-5 pin 18)	37-R/C(AD574K pin 5)
38-Ground		

Figure 5.12. Jumper board pin layout.

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counters. Thus, only one clock input is available to the users for their own configurations as shown in Figure 5.11. In our modification, all three clock inputs, three gate inputs, and three counter outputs were enabled to make each counter work independently. Also, the lines were extended from these timer input/output ports to the daughter jumper board for user configuration (Figure 5.12).

By enabling all three clock inputs, three gate inputs, and the three outputs of the timer/counter, each timer/counter channel can be configured according to the user's needs. Also, the Delay-out, Rate-out, Count-in, and Count-out lines from the distribution panel were extended to the jumper board for easy access to timer/counter control lines from outside of the IBM-DACA board. Optional installation of a hex inverter and a Schmitt trigger for control and signal conditioning gives even more flexibility to the IBM-DACA board.

2. Software Development.

To manipulate the extended functions of the modified IBM-DACA board, menu-driven software has been developed. The main program was written in TURBO PASCAL (Version 4.0, Borland International) and MACRO ASSEMBLER (Version 4.0, Microsoft) to optimize the ADC throughput. This program was written for general purpose use so as to be utilized in various scientific applications. It is a complete package

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to acquire data and manipulate data on an IBM-PC or compatible personal computer. Data can be displayed in real time, either in numerical format or in a graphics plot, on the screen. If desired, source code can be modified by the user for specific applications. This program can also be used with an unmodified IBM-DACA. However, the modifications will allow extended modes of operation of the IBM-DACA for data acquisition.

Set-up information for data acquisition is displayed in the lower half of the main menu screen to show the operation mode of the IBM-DACA. In set up, the user can implement the data-read mode, the number of data points to read, the input channel, the input signal voltage range, the polarity, and the rate of data read. Mode 0 is reserved for the software trigger mode, which is the default data acquisition mode of the unmodified IBM-DACA. All other modes are for the modified IBM-DACA and require a hardware trigger source. If the user wants to use the unmodified IBM-DACA, mode 0 must be selected. Mode 9 is reserved for fast data acquisition with a hardware trigger of the ADC start conversion and uses an assembly language subroutine. This mode gives the fastest data read speed, which is dependent on the trigger source. If mode 0 is selected, a jumper must be placed between $R/\bar{C}$ and the software enable pin. If another mode (mode 2-9) is selected, a jumper must be placed between the $R/\bar{C}$ line and the Q output of the monostable multivibrator, and the trigger pulse source must be fed to the B input of

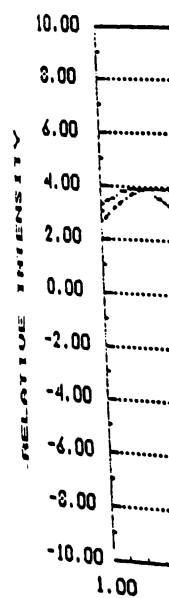
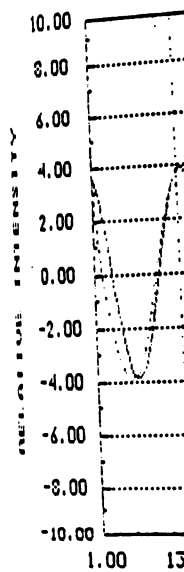


Figure 5.13.
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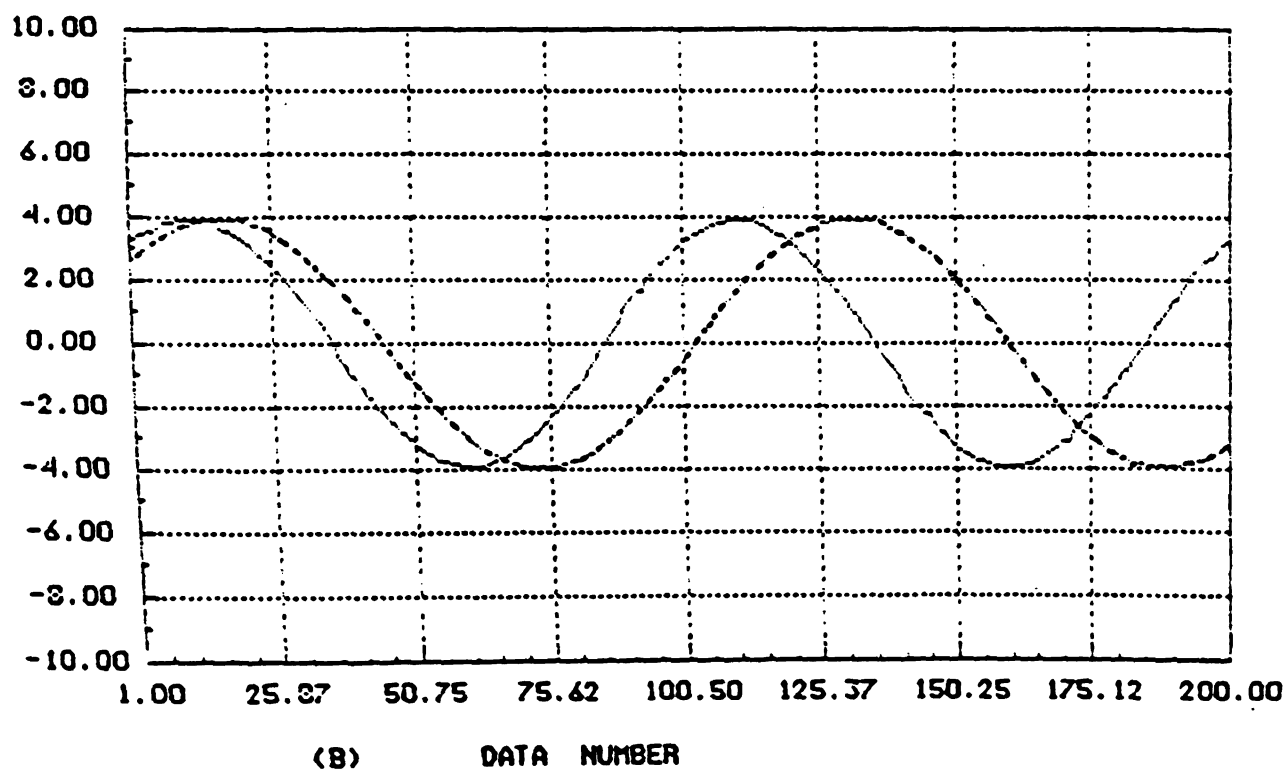
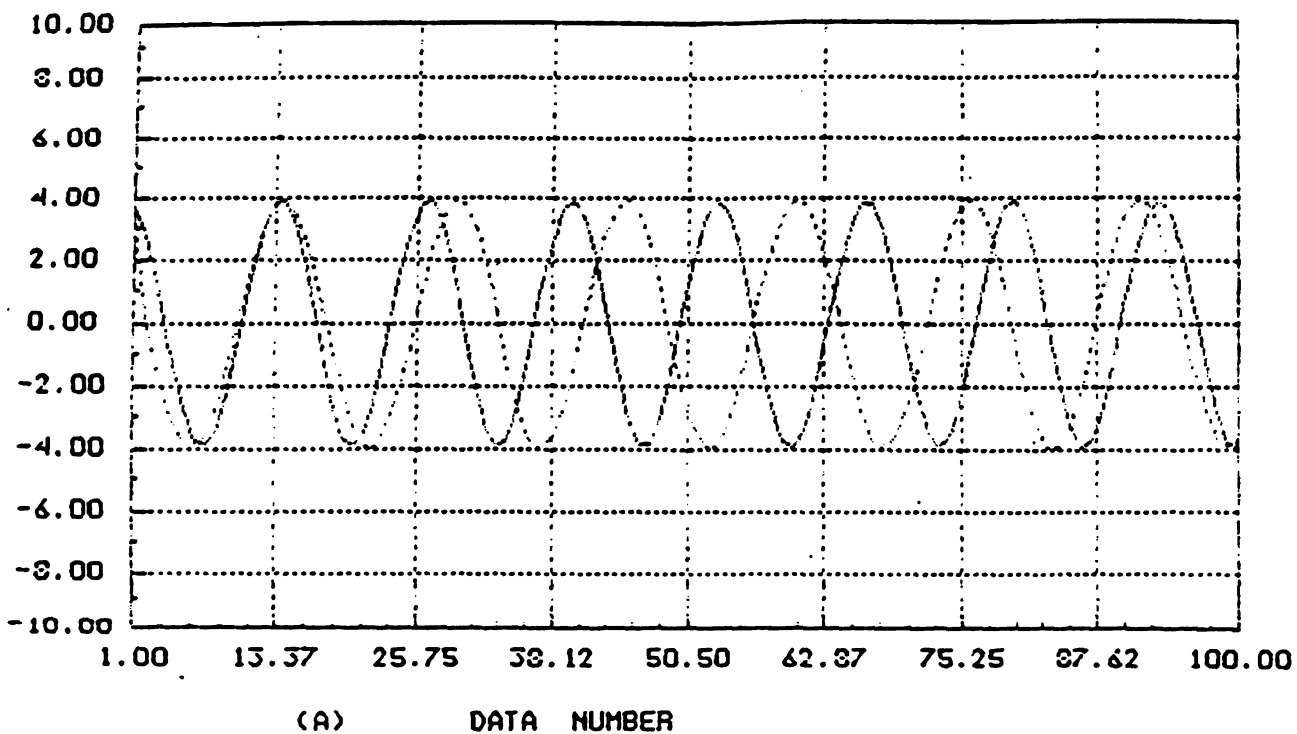


Figure 5.13. Sine wave (6250 $\mu\text{s}/\text{cycle}$) captured in mode 0 (A, line), in mode 3 (A, dotted line), in mode 9 with trigger pulse interval of 27 μs (B, dotted line) and 62 μs (B, line).

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The user can also program the three timer/counter channels. A timer/counter set up window will pop up if modes other than mode 0 are selected so that the users can program each timer/counter channel for their desired mode of operation. Each counter/timer of the Intel 8253-5 is individually programmed by writing a desired mode of operation and a count number. There are six modes of counter operation for each counter/timer. But if more than two counters are cascaded, then very complex and sophisticated modes can be obtained. The plotting routine employs device independent graphics to plot data on various types of graphics monitor. The hard copy of the plot can be obtained with Epson printer.

3. Results and Discussion.

To test the performance of the modified IBM-DACA we used a sine wave of 160 Hz ($6250 \mu\text{s}/\text{cycle}$) as the input source. In mode 0 (software trigger of the ADC start conversion) a maximum speed of 2130 samples/s was obtained (Figure 5.13.A line). In the hardware trigger modes (modes 2-8) which still use high level language for data read, a throughput of 2500 samples/s was obtained (Figure 5.13.A dotted line). The on-board timer/counter output, which was programmed to operate in counter mode 2, was used as a hardware trigger source for the ADC start conversion. A $400 \mu\text{s}$ pulse interval gave the fastest and most satisfactory performance.

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In mode 9, which uses an assembly language subroutine to read data and a hardware trigger source for the ADC conversion, the throughput was increased dramatically. A maximum speed of 18520 samples/s was obtained when a timer output with a 27 μ s pulse interval was used as the trigger source (Figure 5.13.B dotted line). But this data has no accurate timing relationship with the trigger source, because if each pulse is used for ADC trigger then 37037 samples/s throughput must be obtained. An optimum speed, with true timing relationship with trigger source, was obtained with a 62 μ s pulse interval, which gave a throughput of 16130 samples/sec (Figure 5.13.B line).

C. Conclusions.

In conclusion, the instrument designed in our laboratory for luminescence measurements shares most of the advantages of the pulsed source phosphorimeter, but does not share the disadvantages of the conventional rotating-can type mechanical phosphoriscopes. The major advantages of this instrument over the conventional phosphorimeters are:

- (1) Fluorescence, RTP, and RTP lifetimes can be measured on the same instrument without additional instrumental modification.
- (2) Unlike the rotating-can type mechanical phosphoriscopes, there is no limitation on the time discrimination procedure.

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Delay time and gate time can be easily changed by software without adjustment of the rotation speed of the chopper.

(3) It is less dependent on the precision of the modulation frequency of the excitation source. The chopper phase detector detects every closing edge of the exciting source accurately.

(4) It gives equally good performance in RTP and RTP lifetime measurements for short-lived phosphors and long-lived phosphors. By changing the delay time, gate time or rotation speed of the chopper, an optimum detection time window can be obtained.

(5) It gives higher reproducibility and less RF interference than a pulsed source, due to a chopped continuous source.

(6) Finally, this instrument is controlled by a low cost IBM-XT compatible personal computer. Luminescence data are acquired and manipulated easily on site.

The modified IBM-DACA allows initiation of analog-to-digital conversion by a hardware trigger as well as by software. A dramatic increase in throughput was observed by this modification using the hardware trigger. Also, all three channels of the on-board timer/counter can be fully utilized by the user for complex modern data acquisition.

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CHAPTER VI

ROOM-TEMPERATURE PHOSPHORESCENCE LIFETIMES

The phosphorescence decay lifetime of the excited triplet state is important in studies of kinetic processes associated with the triplet state of compounds in a given chemical system. The lifetime of the excited triplet state is a characteristic property of a molecule and its molecular environment. Phosphorescence lifetimes frequently provide a diagnostic tool since compounds having very similar spectral characteristics can have greatly different lifetimes.

Since the phosphorescence decay of any single component is known to be first order, it can be represented by the following equation:

$$dI/dt = -kI \quad (6.1)$$

where I is the phosphorescence intensity and k is the decay rate constant. Integration of equation 6.1 yields

$$I = I_0 \exp(-kt) \quad (6.2)$$

where I_0 represents the phosphorescence intensity at $t=0$. The phosphorescence lifetime, τ , is defined as the time required for the intensity of phosphorescence emission to

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decrease to $1/e$ of its initial intensity following excitation (1). As a result, the phosphorescence lifetime is given by

$$\tau = 1/k \quad (6.3)$$

and the phosphorescence intensity at this time equals to $1/e$ of its initial intensity.

However, experimentally observed phosphorescence intensity contains a finite background or blank intensity as in equation 6.4.

$$I(t) = I_0 \exp(-kt) + B \quad (6.4)$$

The background signal is a convolution of the detector dark current, instrumental offset, ground loops, light scatter signal, etc., with solvent or substrate and sample impurity signals.

The phosphorescence lifetimes of compounds at low temperature (77 K) have been well studied compared to RTP lifetimes. In the frozen state at liquid nitrogen temperature, collisional quenching processes of the triplet state by molecular oxygen and other impurities is greatly minimized, and the temperature of the sample is constant. These conditions give reliable and consistent phosphorescence lifetimes which can be used as a qualitative descriptor of the luminescent species. However, RTP

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lifetimes of compounds are extremely sensitive to environmental conditions, especially for solution RTP. Because the phosphorescence decay lifetime is strongly dependent on the molecular environment (temperature, solvent type, dissolved oxygen, other concomitant species, etc.), the RTP lifetimes of compounds can vary from system to system.

This chapter describes general methods of RTP lifetime determination, a new proposed decay kinetics model, and finally, experimental data of micellar-stabilized RTP (MS-RTP) spectra and the decay kinetics experiment of PAHs.

A. Methods for Determining Decay Lifetimes.

The accurate measurement of the phosphorescence decay constant and the subsequent calculation of the corresponding lifetime of the triplet state is very important. The most common methods for measuring excited state lifetimes involve monitoring the time dependence of the sample emission while exciting it with a pulsed or modulated source.

After source excitation, the phosphorescence intensity starts to decay exponentially according to equation 6.2. Usually, the source excitation function is not an instantaneous impulse as shown in Figure 6.1. Instead, it entails a rather smooth lagged tail. A delay time is required to discriminate phosphorescence signal against this source tail, stray light, and fluorescence. Actual sampling

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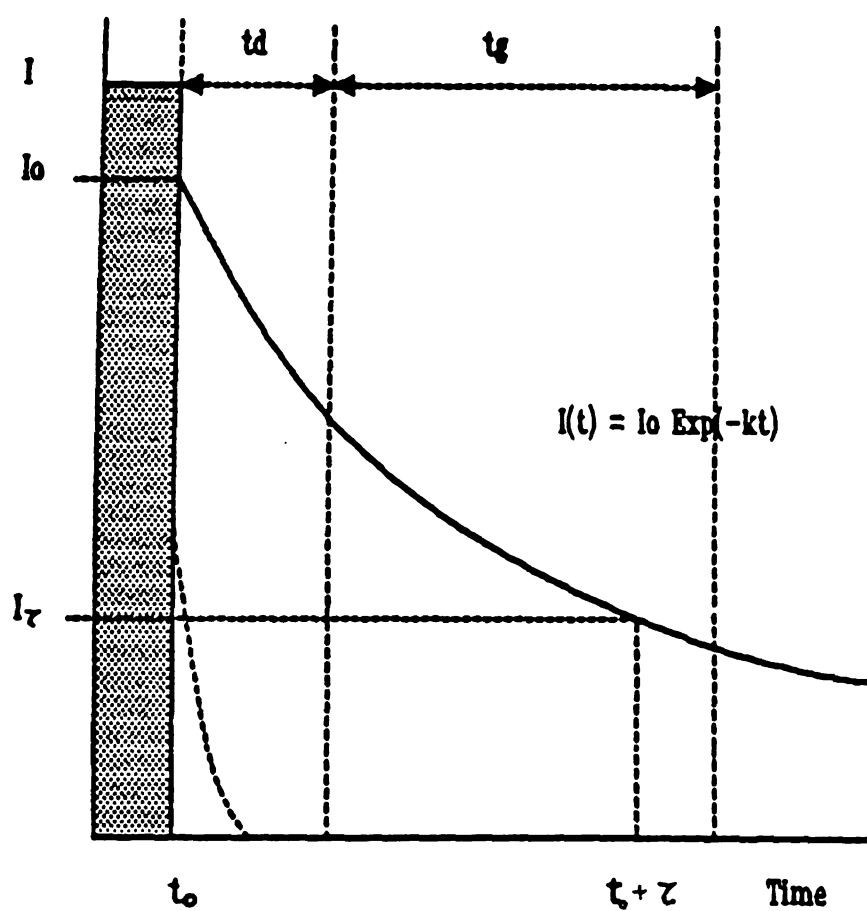


Figure 6.1. Schematic representation of typical RTP decay curve and data acquisition process.

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of the phosphorescence decay signal is done during the gate time, t_g , after delay time t_d .

There are many different methods for measuring luminescence decay with variations in excitation source, detection device, and display. Generally, photomultipliers (PMTs) are used to measure the luminescence signal, but photodiodes may be used if an intense laser source is used.

For recording the luminescence decay signal, an oscilloscope coupled with a camera or photosensitive paper, was the standard choice until recently. But nowadays, single photon counting instruments and transient digitizers along with microcomputers are more common.

The detection-display system may distort the observed luminescence decay (2). Distortions are introduced by the detector, cabling, amplifiers, etc. These distortions may affect observed lifetimes seriously when a short-lived luminescence signal is to be measured. Resistors and capacitors used in PMTs and amplifiers must be chosen carefully. The RC time constants of these systems must be fast enough to trace the fast decaying luminescence signal. Amplifiers must have a high slew rate and wide bandwidth response characteristics.

Phosphorescence lifetimes also depend on the method of data reduction and deconvolution for the calculation of decay lifetimes. Many of the simple techniques such as improper background correction and signal sampling can lead to erroneous interpretation of the data. Popular methods for

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the determination of phosphorescence lifetimes from single exponential decays involve the linear least-squares method, the Guggenheim method, the phase-plane method, and more recently, the rapid lifetime determination (RLD) method.

The linear least-squares method (3) is probably the most popular one. By taking the logarithm of equation 6.2, the following equation is obtained.

$$\ln I(t) = -kt + \ln I_0 \quad (6.5)$$

The decay constant, k , can be obtained from the slope of the $\ln I(t)$ vs time plot, and the decay lifetime is calculated from the negative reciprocal of the slope.

Ideally, a plot of $\ln I(t)$ vs time should yield a straight line for single component decay. The straight line indicating a single exponential decay can be used as a criterion to judge the validity of the obtained data. The linear least-squares method assumes that as time approaches infinity, the observed signal level decreases to zero. But experimentally, the observed signal level is not only a function of phosphorescence signal intensity, but also a function of dark current, amplifier offsets, emission from impurities, etc. As a result, the experimentally observed decay signal always contain a finite value of background. Therefore, low noise, a stable base line, and background subtraction are required for good results.

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For simple exponential decays with a constant but unknown background, the Guggenheim method (4) is popular. By this method, measurements of decay signal are performed in pairs such that each pair is separated by a constant time interval dt .

$$I_t = I_0 \exp(-kt) + B \quad (6.6)$$

$$I_{t+dt} = I_0 \exp\{-k(t+dt)\} + B \quad (6.7)$$

If background is constant in both intensity values then by taking the difference in intensity values one can remove the background term.

$$I_t - I_{t+dt} = I_0 \exp(-kt) \{1 - \exp(-kdt)\} \quad (6.8)$$

$$\ln(I_t - I_{t+dt}) = -kt + \ln\{I_0(1 - e^{-kdt})\} \quad (6.9)$$

A plot of $\ln(I_t - I_{t+dt})$ versus time should be linear. From the slope and intercept values one can obtain lifetime and initial intensity values.

The Guggenheim method is popular because it eliminates the need for acquiring base line, and is simple and fast. However, the Guggenheim method has several disadvantages. The fitting region and dt must be properly selected for accurate results. Margerison (5) suggested that the decay should be followed over 3-4 lifetimes, depending on the accuracy of the individual measurements. If the data acquisition period is shorter than 3-4 lifetimes, smaller dt

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values must be used (6). Also the linear fit $I(t)$ and the reconstruction of $I(t)$ using the best fit parameters and equation 6.4 is complicated since the base line B is not available.

Bacon and Demas (7) modified the phase plane method for the treatment of luminescence lifetimes. The modified phase-plane method permits direct calculation of initial intensity, base line, and decay lifetimes. Furthermore, the phase-plane method is more forgiving in the fitting ranges and the noise levels than the Guggenheim method. Additionally, the phase plane method requires no operator selection of optimum fitting parameters, and no initial guess of the parameters is required.

The more recent rapid lifetime determination (RLD) method (8) is a significantly faster method allowing for real time analysis. In the RLD method the data are collected at equal time intervals, and all data points from the experimental start-to-stop time must be used. The decay data are divided into three equal parts, and the sums; S_0 , S_1 , and S_2 are obtained from each portion. Decay lifetime can be calculated from the following equations:

$$Y = (S_1 - S_2) / (S_0 - S_1) \quad (6.10)$$

$$\text{Lifetime} = -N \, dt / \ln(Y) \quad (6.11)$$

where N is the number of data in each divided part of the decay data, and dt is the fixed time interval between data

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$$A = (S_0 - S_1)^2 / (S_0 - 2S_1 + S_2) \quad (6.12)$$

$$I_0 = A (1 - Y^{1/N}) / (1 - Y) \quad (6.13)$$

$$B = (S_0 - A) / N \quad (6.14)$$

Ballow and Demas (9) performed an error analysis of the RLD method for the evaluation of single exponential decays. The RLD method was comparable to the weighed linear least-squares method in terms of precision and accuracy, and was found to be 10-800 times faster. Also, the RLD method calculates the base line and initial intensity for ready reconstruction of a decay curve, and calculation of fit errors. The disadvantage of the RLD method is that it gives no warning of more complex decays or no statistical parameters for the evaluation of the goodness of the fit.

In this research, both linear least-squares method and RLD method were used for RTP lifetime determination. The RLD method was primarily used with home-built luminescence spectrometer, and linear least-squares method was used with the less noisy and stable Perkin-Elmer LS-5B spectrometer.

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B. RTP Decay Lifetimes of Organic Compounds in Micellar Solutions.

1. Decay Kinetics Model for Micellar Stabilized RTP.

Studies of micelle dynamics were performed to understand the quenching and decay kinetics of luminescence in micellar solution. In this section, a new decay kinetic model of MS-RTP is proposed. To successfully apply this proposed model, several assumptions are made as follows.

- (i) The detectable RTP comes mainly from triplet molecules located inside the micelle. This assumption is valid because RTP cannot be observed without the micelle.
- (ii) Quenching of the triplet state of the molecule occurs mainly at the micellar surface and in the aqueous phase. This assumption is based on our experimental results in which as the concentration of quencher increases the observed triplet lifetime approaches a steady-state value. If quenching occurs mainly inside or outside the micelle, the triplet lifetime must decrease continuously as the quencher concentration, increases. But with a fixed micelle concentration the micellar surface area is also fixed to a limiting value for triplet quenching. This supports the assumption of micellar surface quenching.
- (iii) The probe molecules must be far more soluble in the micellar phase than in the aqueous phase. This condition is

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(iv) The concentration of the phosphorescent probe is so low that self-quenching of the excited state is unimportant.

The proposed decay model is shown as follows. The partitioning of a phosphorescent probe P^* between an aqueous phase and micelle (M) is given by



where P^* and MP^* denote the triplet probe located inside and outside the micelle, respectively. The triplet emission mainly comes from the phosphorescent probe located inside the micelle.



Triplet quenching by quencher Q occurs at the micellar surface and in the aqueous phase.



As a result, the rate of disappearance of MP^* is given by

$$-d[MP^*]/dt = k_-[MP^*] - k_+[M][P^*] + k_{mp}[MP^*] + k_{mq}[MP^*][Q] \quad (6.19)$$

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$$-d[P^*]/dt = k_+ [M][P^*] + k_q [P^*][Q] - k_- [MP^*] \quad (6.20)$$

If the phosphorescent probe P^* is far more soluble in the micellar solution than in aqueous phase, such that $[MP^*] \gg [P^*]$, the steady-state approximation can be applied.

$$-d[P^*]/dt = 0 \quad (6.21)$$

From equation 6.20 and 6.21 we obtain

$$[P^*] = k_- [MP^*] / (k_+ [M] + k_q [Q]) \quad (6.22)$$

Substitution of equation 6.22 into 6.19 yields

$$-d[MP^*]/dt = [MP^*] [k_- + k_{mp} + k_{mq} [Q] - \{k_+ k_- [M] / (k_+ [M] + k_q [Q])\}] \quad (6.23)$$

Thus, the disappearance of MP^* follows a first-order kinetic process. Therefore, the observed disappearance rate constant of MP^* , k_o , which is reciprocal of lifetime τ is given by

$$k_o = 1/\tau = k_- + k_{mp} + k_{mq} [Q] - \{k_+ k_- [M] / (k_+ [M] + k_q [Q])\} \quad (6.24)$$

This equation shows that at a fixed concentration of micelle, the observed lifetime decreases as the quencher concentration increases until $[Q]$ reaches the saturated

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value $[Q]_s$ at the micellar surface. At a very low quencher concentration, the observed rate constant k_o will be equal to the decay rate constant of the triplet probe located inside the micelle, k_{sp} .

2. Experimental Section.

Reagents:

Sodium sulfite (J.T. Baker) was recrystallized from warm water (0.5 ml/g) by cooling to 0 C. Thallium(I) nitrate (99.99%, Aldrich), sodium bromide (99.9%, J.T. Baker), sodium dodecyl sulfate, SDS (99%, Sigma), and methanol (GR grade, EM Science) were used as received. The analytes used in this research, naphthalene (Eastman), pyrene (J.T. Baker), 2-bromonaphthalene (97%, Aldrich), and biphenyl (J.T. Baker), were all recrystallized once from absolute ethanol. Distilled and deionized water was used for RTP sample preparation throughout the experiment.

Apparatus:

RTP and RTP lifetimes were measured by both the home-built instrument and the Perkin Elmer LS-5B spectrometer. The operation of the home-built instrument is described in detail in chapter V. For the Perkin Elmer LS-5B data acquisition, the chart recorder output (1V max) of the spectrometer was amplified 10 times and digitized by the IBM-PC data acquisition board (see chapter V) which was

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installed on an IBM-XT compatible computer. Software written for this purpose was used for luminescence data acquisition and analysis. The range of delay time and gate time for RTP measurements were 0.03 - 0.05 ms and 1-3 ms, respectively.

Sample Preparation and General Procedure:

Stock solutions of naphthalene, pyrene, biphenyl, and 2-bromonaphthalene were made by dissolving appropriate amounts of analytes into 10 ml methanol and diluted to 100 ml final volume with water. For the external heavy atom source, thallium nitrate stock solution (0.25 M) was used. A sodium dodecyl sulfate stock solution (0.5 M) was prepared for the micellar solution, and sodium sulfite stock solution (0.4 M) was used for deoxygenation of the sample solution.

An aliquot of methanolic analyte solution was pipetted into a 10 ml volumetric flask and solvent was evaporated to a minimum volume using nitrogen gas. Sample solutions were made by adding the appropriate amounts of stock solutions of thallium nitrate, SDS, and sodium sulfite into previous 10 ml volumetric flask. These solutions were diluted to 10 ml final volume with the distilled and deionized water.

After thorough mixing, the solution was transferred to a cuvette with a Teflon stopper. The progress of chemical oxygen scavenging was monitored by measuring the RTP intensity development at the emission maximum with the appropriate excitation wavelength on the spectrometer.

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Spectra were obtained after the RTP intensity reached steady state and remained the same for at least 5 minutes.

3. Results and Discussion.

Micellar stabilized RTP (MS-RTP) is the most popular method for solution RTP. Deoxygenation of the surfactant solution was the most troublesome aspect of the MS-RTP, because of a lot of foam formed during the deoxygenation process by inert gas. This can be eliminated by chemical deoxygenation using sodium sulfite.

Air Tight Capping of the Cuvette.

A tight seal of the sample cuvette to prevent any leakage of air was necessary throughout RTP measurements. Figure 6.2 shows RTP development of 2-bromonaphthalene after addition of a chemical scavenger, sodium sulfite, into a micellar sample solution. The concentrations of surfactant (SDS) and heavy atom (TlNO_3) were 0.05 M and 1.5×10^{-2} M, respectively. After the addition of 0.01 M sodium sulfite into the solution, the development of RTP intensity was monitored at the emission maximum for 20 min. RTP began to show up after 8 minutes and reached a steady-state maximum after 15 min.

The dotted line of Figure 6.2 shows the leakage of air into the sample solution with no capping the sample cuvette. As fresh oxygen enters into the solution, RTP fluctuates

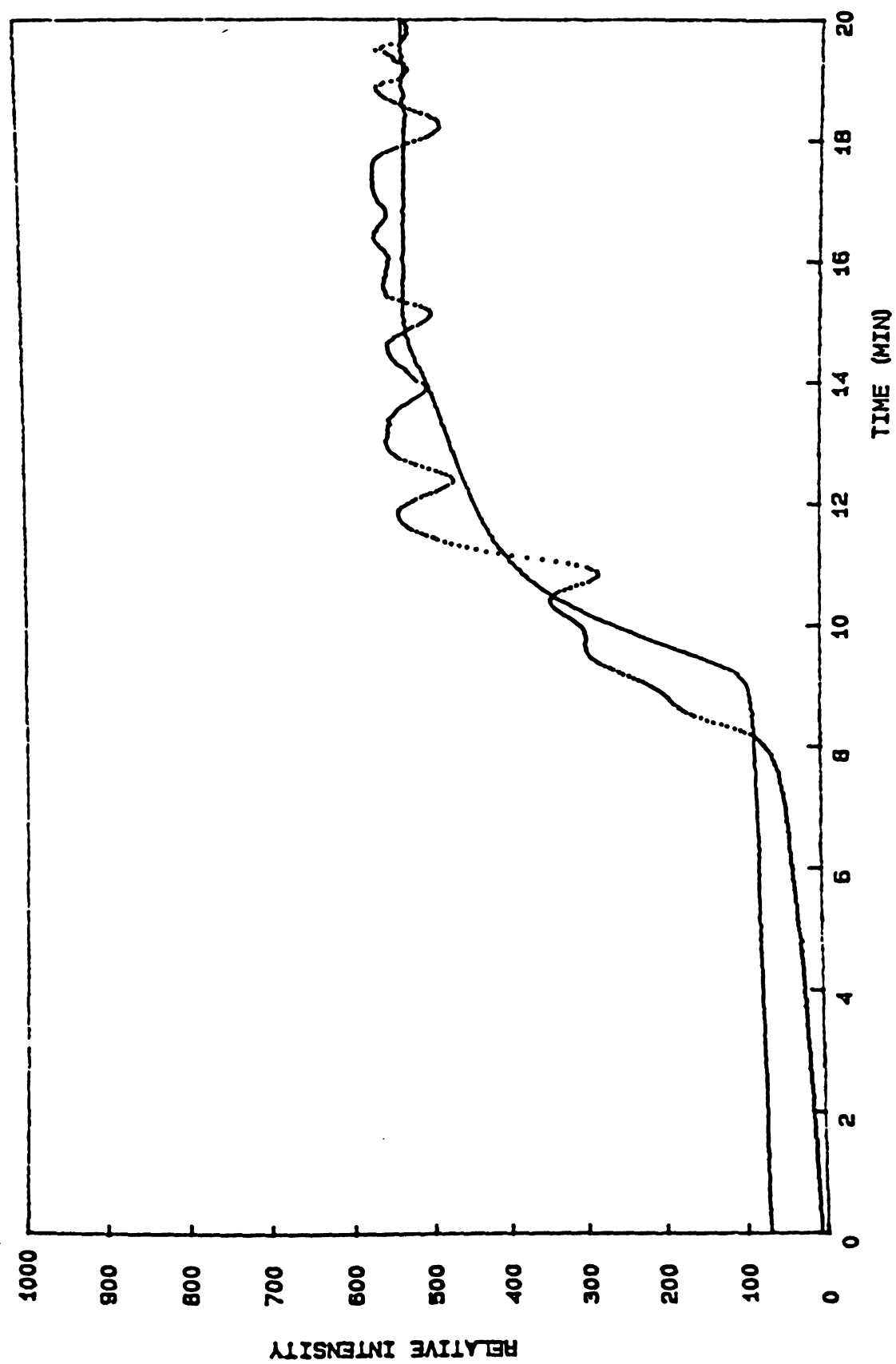


Figure 6.2. RTP development of 2-bromonaphthalene with time.

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sinusoidally, indicating a new chemical equilibrium by oxygen and sodium sulfite. With an air tight seal of the sample cuvette with a Teflon top, the RTP intensity remained very stable with time.

Optimum Concentration of Sodium sulfite.

Various amounts of sodium sulfite were added into a solution of a fixed amount of naphthalene, SDS, and heavy atom (Tl^+), to find an optimum concentration of sodium sulfite for chemical deoxygenation. The concentrations of SDS and Tl^+ were 3.5×10^{-2} M and 1.5×10^{-2} M, respectively. RTP intensities were measured 15 min after solution mixing. The optimum concentration range of sodium sulfite was 0.8×10^{-2} - 1.2×10^{-2} M, as shown in the upper graph of Figure 6.3. Below this optimum concentration, the RTP intensity decreased due to the incomplete and slow deoxygenation reaction. Above this optimum concentration range, the RTP intensity decreased due to high sodium ion concentrations, which at such levels could displace thallium ions from the micellar surface.

The Effect of SDS Concentration on RTP Intensity.

The reported value of the critical micelle concentration (CMC) of SDS is 8.2×10^{-3} M (10). Thus, the effective screening of the triplet sample molecules by the micelle is expected at surfactant concentrations above the CMC. Into a solution of a fixed amount of Tl^+ , sodium sulfite,

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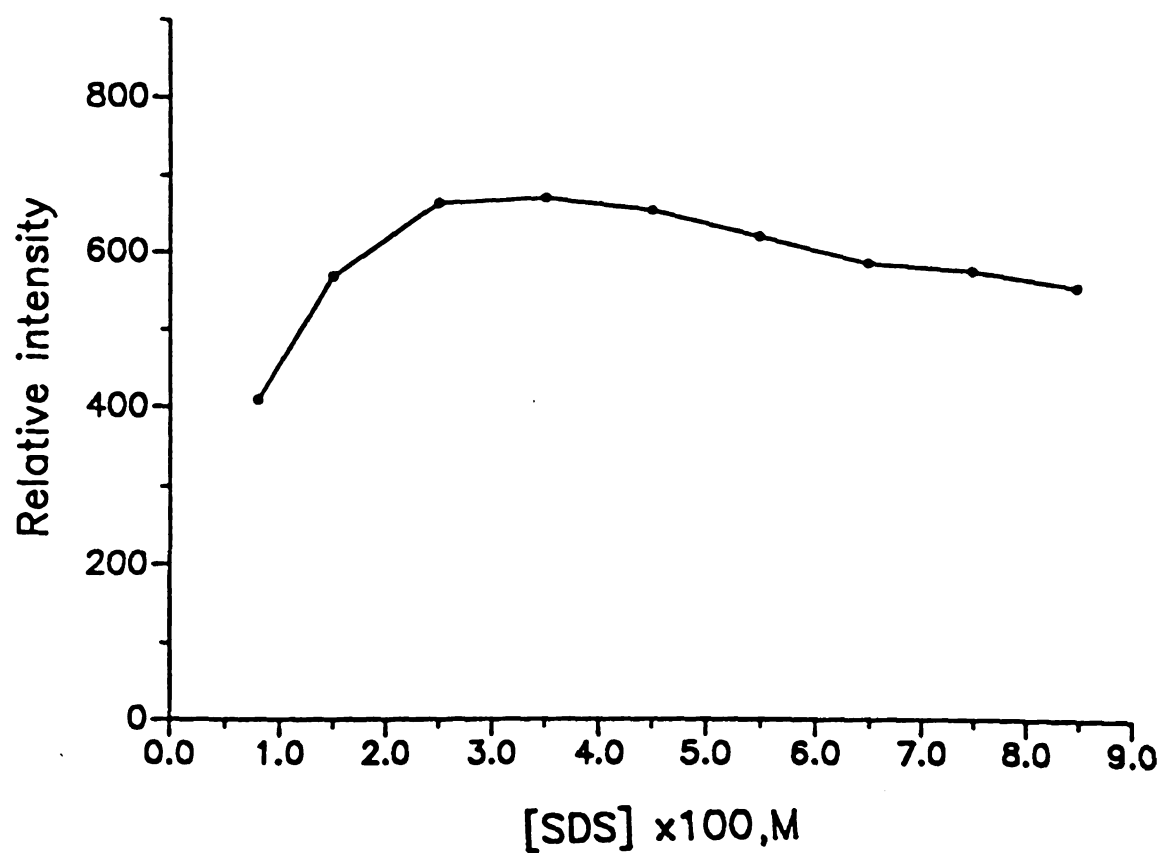
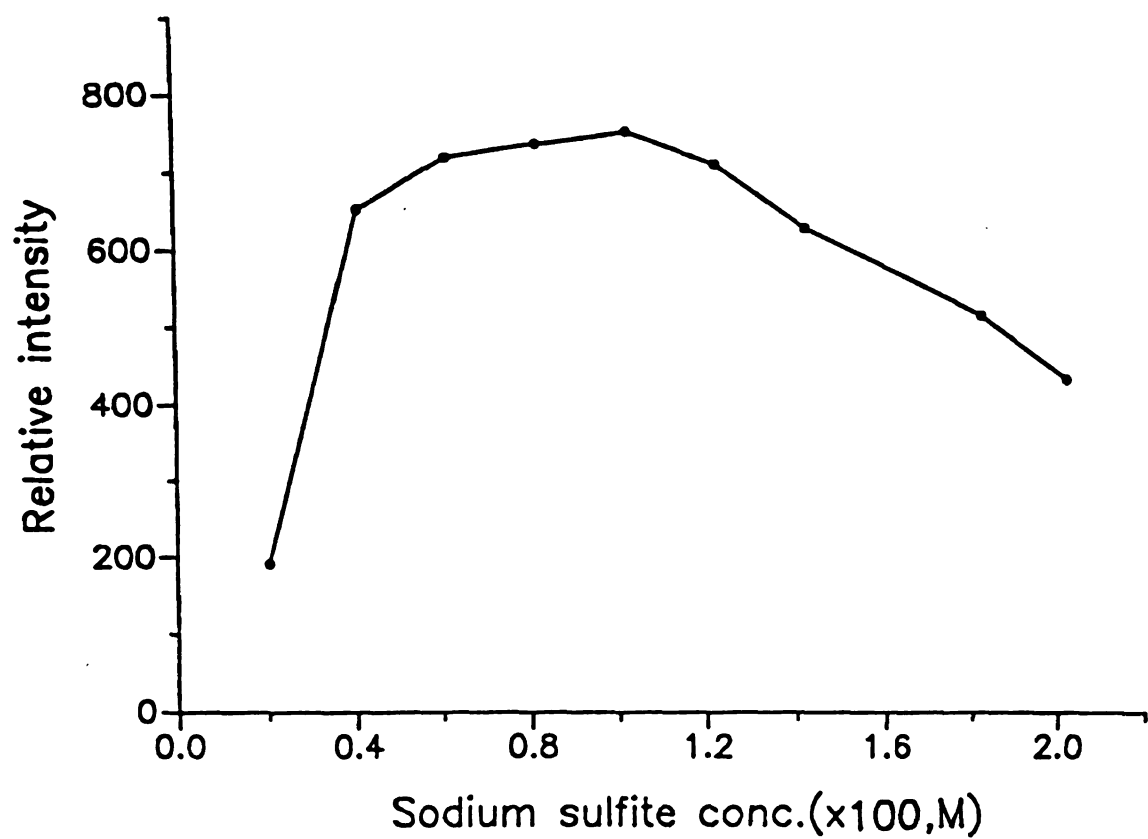


Figure 6.3. The effect of sodium sulfite and SDS on the RTP intensity of naphthalene.

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naphthalene, various amounts of SDS were added to find the optimum concentration of SDS. The maximum RTP was observed in the 1.5×10^{-2} - 4.5×10^{-2} M concentration range. RTP intensity began to decrease at SDS concentrations above 4.5×10^{-2} M, as shown in the lower graph of Figure 6.3.

The reduced RTP intensity at a high SDS concentration can be explained in several ways. It is expected that the number of heavy atoms per micelle will decrease as the concentration of SDS increases. As a result, the effective concentration of the heavy atom will be decreased, which will result in reduced spin-orbital interaction between the singlet molecules and heavy atoms. Also the size, shape, and dynamic motion of the micelle will change, which could affect the stability of triplet molecules. It was reported that the removal of dissolved oxygen by sodium sulfite is more difficult at a higher surfactant concentration than at less concentrated SDS concentration (11). Also, there are more possibilities of RTP quenching by impurities of SDS at higher SDS concentration.

MS-RTP Spectra of Polyaromatic Hydrocarbon Molecules.

Excitation and emission spectra of naphthalene, pyrene, biphenyl, and 2-bromonaphthalene are shown in Figure 6.4 and 6.5. The concentrations of lumiphors are kept at 5×10^{-5} M to avoid any undesired quenching processes due to high sample concentrations. MS-RTP spectra were obtained in 0.05 M SDS and 0.01 M sodium sulfite with 15 mM TlNO_3 .

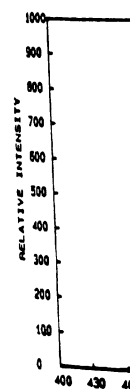
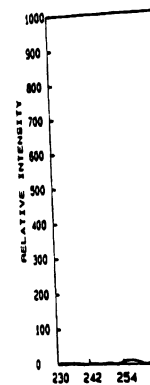


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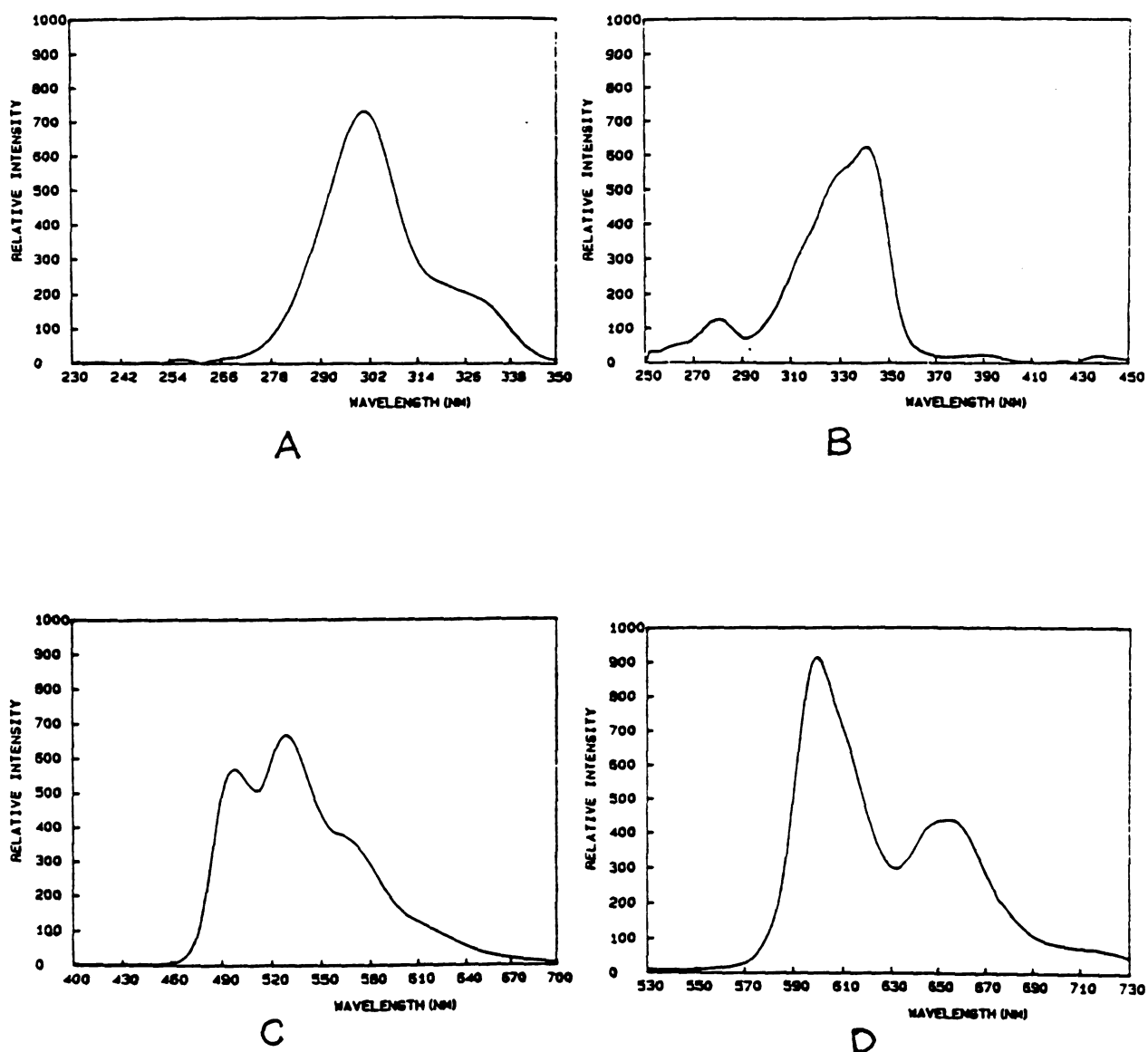


Figure 6.4. Excitation and emission spectra of 2-bromonaphthalene (A,C) and pyrene (B,D). A and B are excitation spectra and C and D are emission spectra.

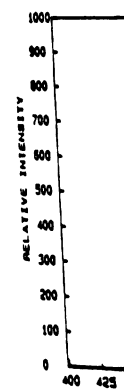
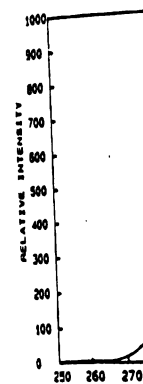


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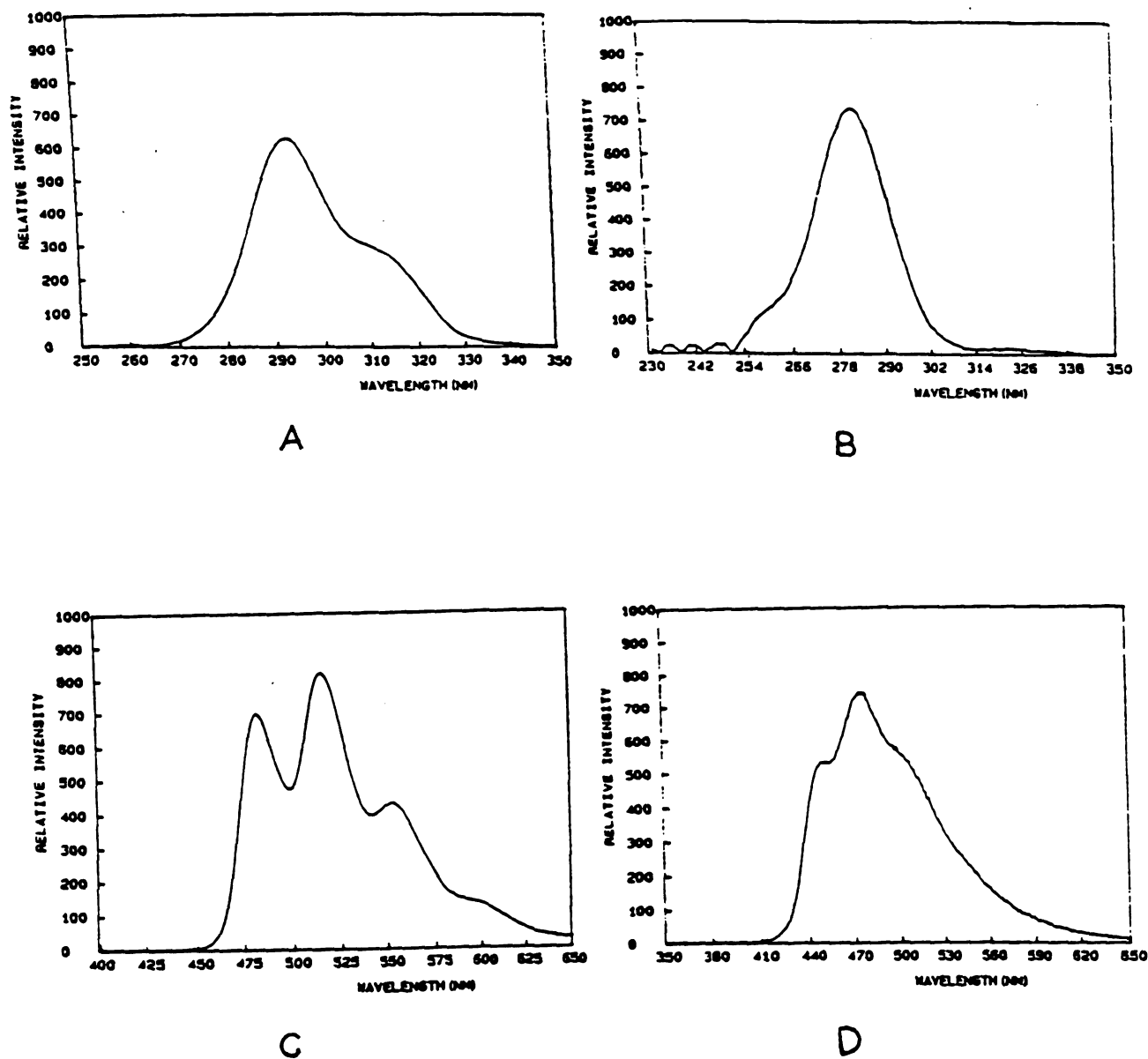


Figure 6.5. Excitation and emission spectra of naphthalene (A,C) and biphenyl (B,D). A and B are excitation spectra and C and D are emission spectra.

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2-Bromonaphthalene showed three broad RTP peaks at 497, 527, and 562 nm. Pyrene strongly absorbs light at 337 nm and has two distinct RTP peaks at 595 and 653 nm. Naphthalene showed a very similar spectral response as 2-bromonaphthalene. RTP peaks of naphthalene are more sharp and blue shifted than that of 2-bromonaphthalene. Biphenyl showed featureless broad peaks at 450, 474, and 504 nm. Excitation and emission spectral characteristics of polyaromatic hydrocarbon compounds are summarized in Table 6.1.

Table 6.1. MS-RTP Spectral Characteristics of Selected PAHs.

Compound Name	Excitation (nm)	Emission peaks (nm)
Naphthalene	293	481, 513, 552
Pyrene	337	595, 655
2-Bromonaphthalene	298	497, 527, 567
Biphenyl	281	450, 474

MS-RTP Lifetimes of Polyaromatic Hydrocarbon Molecules.

Although triplet lifetimes of organic compounds at low temperature are well established, RTP lifetimes in micellar solution may vary from system to system. This is attributed

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to the fact that triplet molecules in micellar solution are in dynamic equilibrium with various sources of triplet quenchers. This is a serious problem if RTP lifetimes are to be used for analytical purposes. To understand this problem better, it was necessary to study the microenvironment and decay kinetics associated with the triplet state.

Almgren et al (12) proposed a decay kinetic model in the micellar system using SDS and nitrate as triplet quencher. It was found that the phosphorescence lifetime depends strongly on the exit rate of the probe molecule from the core of the micelle. The exit rate of 1-bromonaphthalene from SDS micelle was determined as $2.5 \times 10^{-4} \text{ s}^{-1}$. They assumed that the main quenching of the excited state occurs in the bulk aqueous phase.

Bolt and Turro (13) studied the exit rates of phosphorescent detergent probes with varying hydrocarbon length from host micelles using Co(III) hexamine as an aqueous soluble triplet quencher. The log of the exit rate was a linear function of the number of methylene in the probe alkyl chain.

Cline Love et al (14) extended a kinetic model of micelle-analyte interactions to several limiting cases. But they failed to present experimental data to support their model due to experimental difficulties involved in the limiting cases. The limiting cases of their model involved quenching in the bulk aqueous phase as well as inside the micelle. But none of the previous models accounts for the quenching

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process at the micellar surface and the effect of the external heavy atom source.

MS-RTP lifetimes of PAHs in micellar solution are very sensitive to environmental conditions. MS-RTP lifetimes of PAHs are found to increase as increasing surfactant concentration up to 0.05 M. At higher surfactant concentrations, they decreased indicating the possible increase in impurities in surfactant solution.

Table 6.2. MS-RTP Lifetimes of Selected Arenes.

Compound	Excitation(nm)	Emission(nm)	Lifetime(ms)*
Naphthalene	290	511	1.25±0.07
2-Bromonaphthalene	293	527	0.82±0.08
Biphenyl	280	475	1.12±0.07
Pyrene	337	595	8.31±0.05

* All sample concentrations were 5×10^{-5} M, [SDS] = 0.05 M [Na₂SO₃] = 0.02 M, [TlNO₃] = 0.025 M. Above lifetimes were calculated with linear least-squares method and correlation coefficients were above 0.998 in all cases.

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MS-RTP lifetimes of PAHs were measured at room temperature. MS-RTP lifetimes of selected PAHs under optimum conditions are shown in Table 6.2.

These MS-RTP lifetimes are considerably longer than other reported lifetime values. Cline Love et al (15) reported MS-RTP lifetimes of selected PAHs. Their reported values for several compounds rarely exceed 1.2 ms. On the other hand, Kalyanasundaram et al (16) observed much longer MS-RTP lifetimes of several PAHs in various surfactant solutions. Their reported MS-RTP lifetime values lie between 1.2 - 20 ms range, which are, in some cases, much longer than our values.

The differences in MS-RTP lifetimes among different systems indicates that RTP lifetimes are extremely sensitive to environmental conditions. The concentrations of dissolved oxygen, heavy atom, temperature, and other impurities in the sample solution directly affect the observed RTP lifetime.

Figure 6.6 shows triplet decay data of naphthalene (upper) and pyrene (lower). Dotted lines are raw data and solid lines are simulated data. The RLD method generates base line, decay lifetime, and initial intensity which can be used to simulate the decay data. The RLD method works well, as can be judged visually in the overlapped data plotted over the noisy raw data. The calculated RTP lifetime of naphthalene in 0.02 M SDS and 0.04 M TiNO_3 was 0.486 ms. But with decreased concentrations of heavy atoms, much longer RTP lifetimes were observed (Table 6.2). The RTP lifetime of

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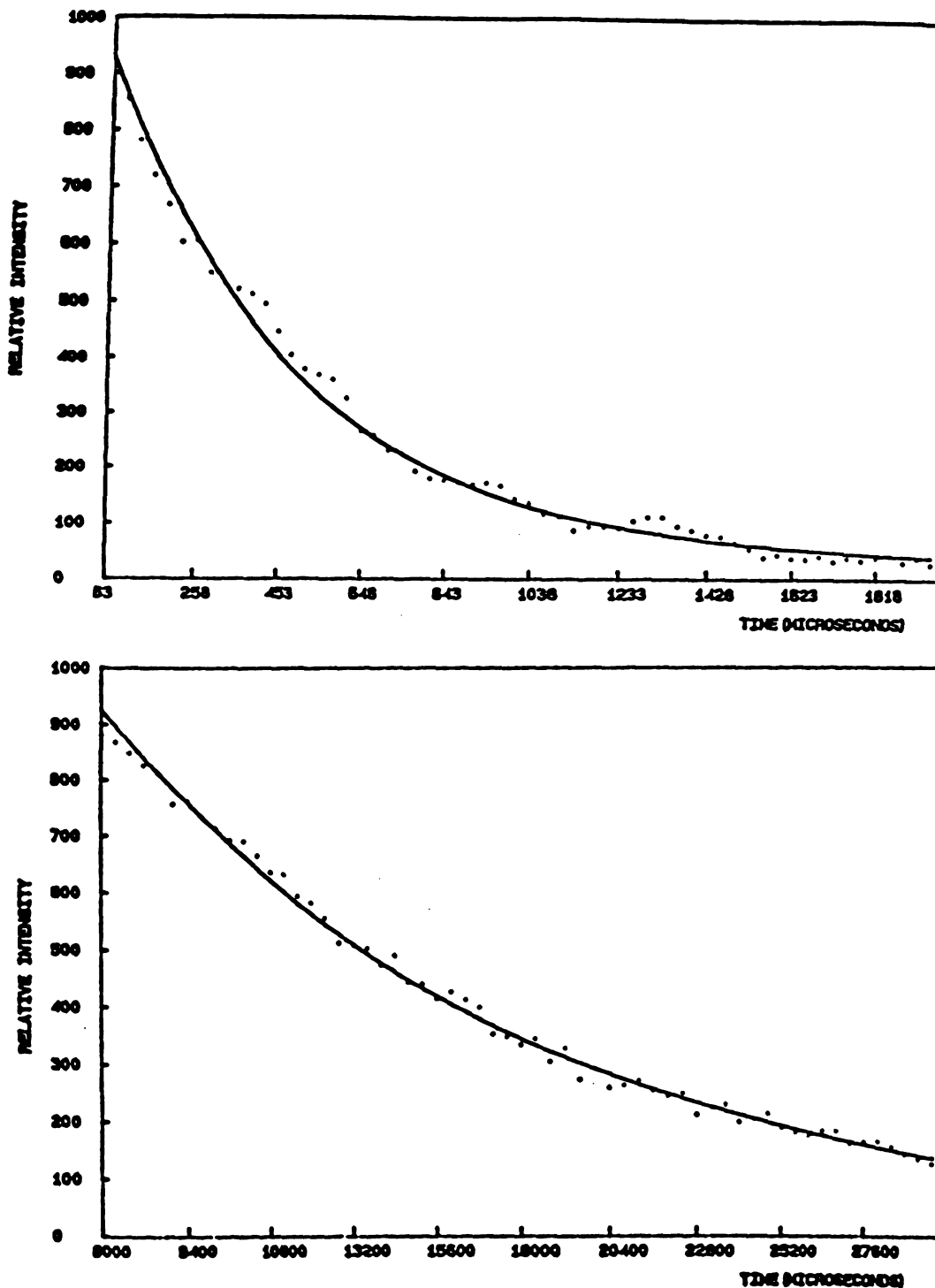


Figure 6.6. MS-RTP decay of 5×10^{-5} M naphthalene (upper) and pyrene (lower). Solutions were deoxygenated with 0.01 M sodium sulfite, and the concentrations of SDS were 0.05 M for both, the concentrations of Tl^+ were 0.04 M for naphthalene and 7.5×10^{-3} M for pyrene.

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pyrene in 0.03 M SDS and 7.5×10^{-3} M Tl^+ solution was 11.2 ms, which is 10 times longer than Cline Love's value, but five times shorter than Kalyanasundaram's value.

The effect of temperature on the MS-RTP lifetime was investigated. The changes in RTP intensity and triplet lifetime were monitored at different solution temperatures. The RTP intensity of pyrene decreased from 656 to 468, and the RTP lifetime also decreased by 19% from 6.02 to 4.89 ms as temperature increased from 15 °C to 35 °C as shown in Table 6.3.

Table 6.3. The effect of temperature on RTP of pyrene.

Temp. (C)	RTP intensity	RTP lifetime (ms)*
15	656	6.02±0.03
20	590	5.36±0.04
25	532	5.02±0.04
30	502	4.93±0.06
35	468	4.89±0.06

* Sample solutions were deoxygenated with 0.02 M sodium sulfite and concentrations of SDS and Tl^+ were 0.05 M and 0.035 M, respectively. Excitation=337 nm, emission=595 nm.

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These data shows that temperature affects both the RTP intensity and the lifetime significantly. For accurate and consistant RTP lifetime data, it seems that temperature control of the sample solution is a necessary condition.

The effect of the external heavy atom on the RTP lifetime was also investigated using thallium ions as the heavy atom source. The use of an external heavy atom is very common in micellar stabilized RTP. Usually, heavy atoms increase the sensitivity of RTP but decrease the observed triplet lifetimes. The RTP intensity and lifetime of pyrene were measured as increasing the thallium ion concentration.

Table 6.4. The Effect of Heavy Atom on RTP Lifetime.

[Tl ⁺] $\times 10^2$ (M) RTP Intensity Lifetime(ms) k_0 (=1/lifetime, s ⁻¹)			
0.75	345	11.70 $\pm$ 0.04	86
1.50	488	9.35 $\pm$ 0.05	107
2.25	635	8.30 $\pm$ 0.05	121
3.00	717	7.01 $\pm$ 0.07	143
3.75	763	5.85 $\pm$ 0.07	171
4.50	783	5.65 $\pm$ 0.08	177
* [Pyrene] = 5 $\times 10^{-5}$ M, [SDS] = 0.05 M, [Na ₂ SO ₃] = 0.025 M.			

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The RTP intensity increased up to 0.06 M thallium ion concentration, while the fluorescence intensity decreased rapidly (Table 6.4). At higher concentrations above 0.06 M thallium, precipitation took place in the solution. On the other hand, the RTP lifetimes of pyrene decreased continuously up to 0.05 M thallium concentration. The same trend was observed with naphthalene even though the RTP lifetimes were rather unstable at lower heavy atom concentrations. The use of silver ion instead of thallium did not work due to a very low solubility of Ag_2SO_3 , which was formed by the reaction of dissolved silver ions and sulfite ions.

It was found that the decrease in RTP lifetime is a function of heavy atom concentration. The plot of reciprocal lifetime, k_0 , versus thallium ion concentration, is linear (Figure 6.7) according to the equation 6.24, where the heavy atom is treated as a triplet quencher. From the slope and intercept of the plot, k_{sq} and k_{sp} were calculated to be 2560 and 65, respectively. This result is very similar to other triplet quenchers, such as nitrite (12).

It has been known that heavy atoms reduce phosphorescence lifetimes through spin-orbital interactions (17). The spin and orbital motions of the electrons are not independent, even in atoms with very small nuclear charge. The orbital motion of the electron induces a magnetic field which interacts with its spin magnetic momentum. This spin-orbital interaction leads to a change in the direction of the spin-

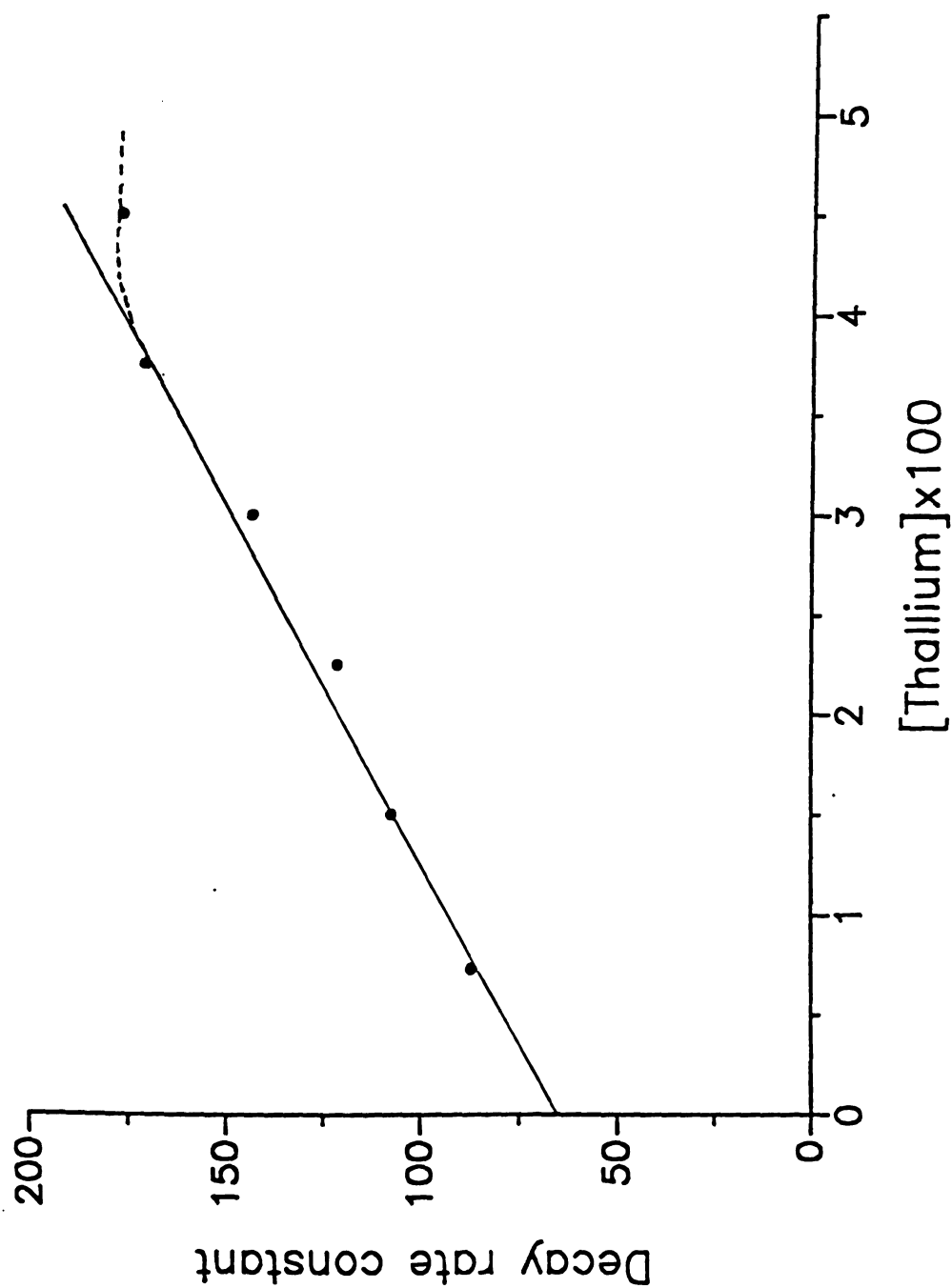


Figure 6.7. Plot of observed decay rate constant of pyrene versus heavy atom (Tl⁺) concentration in SDS micelle.

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angular momentum of one electron, thereby changing a singlet state into triplet state, and vice versa.

The external heavy atom effect may take place in two ways: one, by a complex formation with heavy atom, and another, by a long range interaction through a statistical distribution of the latter molecules around the phosphorescing molecule (18). It has been shown that the rate constants of both $S_1 \rightarrow T_1$ and $T_1 \rightarrow S_0$ processes are increased (19). This is evident from the reduced RTP lifetimes of the probe molecules at high heavy atom concentrations. Furthermore, it has also been demonstrated that $T_1 \rightarrow S_0$ radiationless transition is not enhanced much and the observed reduction in the triplet lifetimes is mainly due to an increase in the radiative transition probability (20).

While various mechanisms to explain the enhanced spin-orbital interaction and the reduced triplet lifetime have been proposed, they are still not well understood (21). The various schemes which have been previously proposed share a common factor in that the singlet state is ultimately responsible for introducing reduced phosphorescence lifetimes. That is, the triplet state of the emitting molecule is mixed with the singlet state of the other. The source of the singlet state can be the same molecule (22), the perturber (23), or a charge transfer state of the molecule-perturber complex (24).

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4. Conclusions.

RTP lifetimes of PAHs in micellar solution were a function of various environmental factors. A major contributor to the magnitude of the observed RTP lifetimes was the external heavy atom. Also, the method of deoxygenation and solution temperature affect observed RTP lifetime significantly. This results in different RTP lifetimes under different experimental conditions, which is undesirable from an analytical point of view.

The similar behavior of heavy atoms to other triplet quenchers was striking since heavy atoms are not usually treated as triplet quenchers. It is not clear whether heavy atoms actually quench triplet molecules at the micellar surface, but it seems quite probable that a main contact reaction between the probe molecule and the heavy atom takes place at the micellar surface. We tried to test the proposed decay kinetics model using other heavy atoms such as silver and other triplet quenchers without heavy atoms. But it was very difficult to obtain consistent RTP data under the above conditions.

It is recommended that the use of k_{ap} instead of RTP lifetime will give a more valid criterion in identifying different compounds in MS-RTP. The MS-RTP lifetimes may vary drastically depending on the experimental conditions employed, but k_{ap} , which is the triplet decay rate constant in the micelle will give rather consistent values.

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CHAPTER VII

ROOM-TEMPERATURE PHOSPHORESCENCE OF COMPOUNDS IN MIXED
ORGANIZED MEDIA: SYNTHETIC ENZYME MODEL-SURFACTANT SYSTEM

Room-temperature phosphorimetry (RTP) is quite different from the classic low temperature phosphorescence technique which is typically performed in glass matrices at liquid nitrogen temperature (1). Since the discovery of RTP, numerous techniques have been developed to induce RTP from various molecules. These include solid-state RTP (2), micellar-stabilized RTP (3), sensitized RTP (4), cyclodextrin-enhanced RTP (5), and colloidal or microcrystalline RTP (6). The probability of observing RTP (especially in solutions) is enhanced in a rigid molecular environment due to a reduced quenching effect by oxygen or other impurities, and in the presence of a heavy atom, due to an increase in the rate of intersystem crossing.

Photophysical and photochemical properties of organic molecules included in the cavity of cyclodextrins (CDs) have been of considerable interest in the past decade (7). Cyclodextrins form complexes with hydrophobic organic and organometallic molecules in aqueous solution. Although there is no direct proof for a fixation of the guest molecules within the void space of the cyclodextrin, the complexes are usually regarded as inclusion compounds, host-guest

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compounds, in which hydrogen bonding, van der Waals forces, and hydrophobic interactions are the main binding forces (8). When lumiphores are included inside the cyclodextrin molecules, the resulting RTP shows enhanced intensity and lifetime because the cyclodextrin protects the lumiphores from quenchers (9).

The synthetic macrocyclic enzyme model compounds, azaparacyclophanes (APCs), can also act as inclusion hosts capable of molecular organization by forming complexes with a variety of hydrophobic molecules (10). Water-soluble azaparacyclophane (APC), N,N,N',N',N'',N'',N''',N'''-octamethyl-2,11,20,29-tetraaza[3.3.3.3]paracyclophanetetraammonium tetrafluoroborate (methyl-APC), is an excellent inclusion host toward certain organic substrates. A unique substrate specificity was observed due to its cavity size and functionality (11). The macrocyclic cavity is surrounded by the wall, which is formed by four benzene rings and four quarternary ammonium residues around the macrocyclic ring.

In this chapter, RTP results of anionic, 6-(p-toluidinyl)-naphthalene-2-sulfonate (TNS) and the neutral compound, 2-bromonaphthalene in premicellar surfactant solutions using methyl-APC and cyclodextrin as host, are described. The binding ability and selectivity of methyl-APC in complex formation with anionic and neutral polyaromatic hydrocarbon molecules in a mixed organized media, methyl-APC - surfactant, are also discussed.

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A. EXPERIMENTAL SECTION

1. Materials and Methods.

Reagents:

Sodium sulfite (J.T. Baker) was recrystallized from warm water. Thallium(I) nitrate (99.999%, Aldrich), sodium bromide (99.9%, J.T. Baker), sodium dodecyl sulfate, SDS (99%, Sigma), 2-bromonaphthalene (97%, Aldrich), 8-anilino-1-naphthalene sulfonic acid, ANS, ammonium salt (Aldrich), 6-toluidino-2-naphthalene sulfonic acid, TNS, potassium salt (Aldrich), and β -cyclodextrin(Sigma) were used as received. Methanol (GR grade, EM Science) was used without further purification. Distilled and deionized water was used for RTP sample preparation.

The compounds, α, α' -dibromo-p-xylene(98%, Aldrich), terephthaloyl chloride (97%, Aldrich), trimethyloxonium tetrafluoroborate (Aldrich), LiAlH_4 (Aldrich), and methylamine (40% in water, Matheson Coleman & Bell) were used to synthesize the methyl-APC as described below. Distilled benzene was used as a solvent throughout the synthesis experiment.

Apparatus:

All spectra were obtained with a Perkin-Elmer LS-5B spectrometer. The chart recorder output (1 V maximum) of the spectrometer was amplified 10 times and digitized by an IBM-

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PC data acquisition board installed on the IBM-XT compatible computer. Laboratory written software was used for the luminescence data acquisition and data manipulation (12). The range of delay and gate times for RTP measurements was 0.03-0.05 ms and 1-3 ms, respectively.

Sample Preparation and General Procedure.

The macrocyclic compound, methyl-APC, was synthesized in highly dilute conditions according to the reported procedure by Tabushi et al (11). The sample solutions for RTP were deoxygenated by adding sodium sulfite solutions (0.01 - 0.04 M) to the sample solution as reported by Garcia et al (13). To a 10 mL volumetric flask, appropriate aliquots of lumiphor, sodium sulfite, heavy atom, and surfactant solutions were added and diluted to 10 mL final volume with distilled and deionized water. After thorough mixing, the solution was transferred to a cuvette with a Teflon stopper, and the RTP intensity was monitored at the emission maximum with the appropriate excitation wavelength on the spectrometer. Uncorrected spectra were obtained after the RTP intensity reached the steady state and remained the same for at least 5 minutes.

2. Synthesis of Azaparacyclophane.

In this research a simple APC , N,N,N',N',N'',N'',N''',N'''-octamethyl-2,11,20,29-tetraaza[3,3,3,3]paracyclophanetetra-

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ammonium tetrafluoroborate (methyl-APC), was chosen as a model host compound. For the synthesis of methyl-APC, N,N'-dimethyl-p-xylenediamine was synthesized first (Figure 7.1). Macrocyclic ring was obtained by the condensation of N,N'-dimethyl-p-xylene with terephthaloyl chloride in a highly dilute condition. For the analysis of the compounds, Varian EM360 nmr spectrometer and Nermag R10-10 mass spectrometer were used.

N,N'-Dimethyl-p-xylenediamine (1).

Gaseous methylamine generated by dropping aqueous methylamine solution (40 %) into the solid KOH, was passed through a drying tube (KOH) and trapped as a liquid in the 2 L three-neck flask which was cooled by dry ice in acetone. Thus trapped methylamine solution was collected up to 200 mL (150 g). To this methylamine solution, 66 g (0.25 mole) of p-xylene bromide in 600 mL of THF was slowly added under gentle stirring for 6 h at -15 - -20 °C. After an additional 2 h stirring, THF and excess amounts of methylamine were evaporated, and the residue was made strongly alkaline with 30% aqueous NaOH solution. The product was extracted three times with 200 mL ether. The HCl gas, which was generated by dropping conc. H₂SO₄ into conc. HCl, was introduced into the combined ether solution to obtain the precipitate (2HCl salt) of N,N'-p-xylenediamine. The final product was obtained by recrystallization from the 3:1 (v/v) ethanol-water solution (yield 62 %). The N,N'-p-xylenediamine was

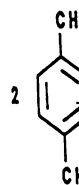


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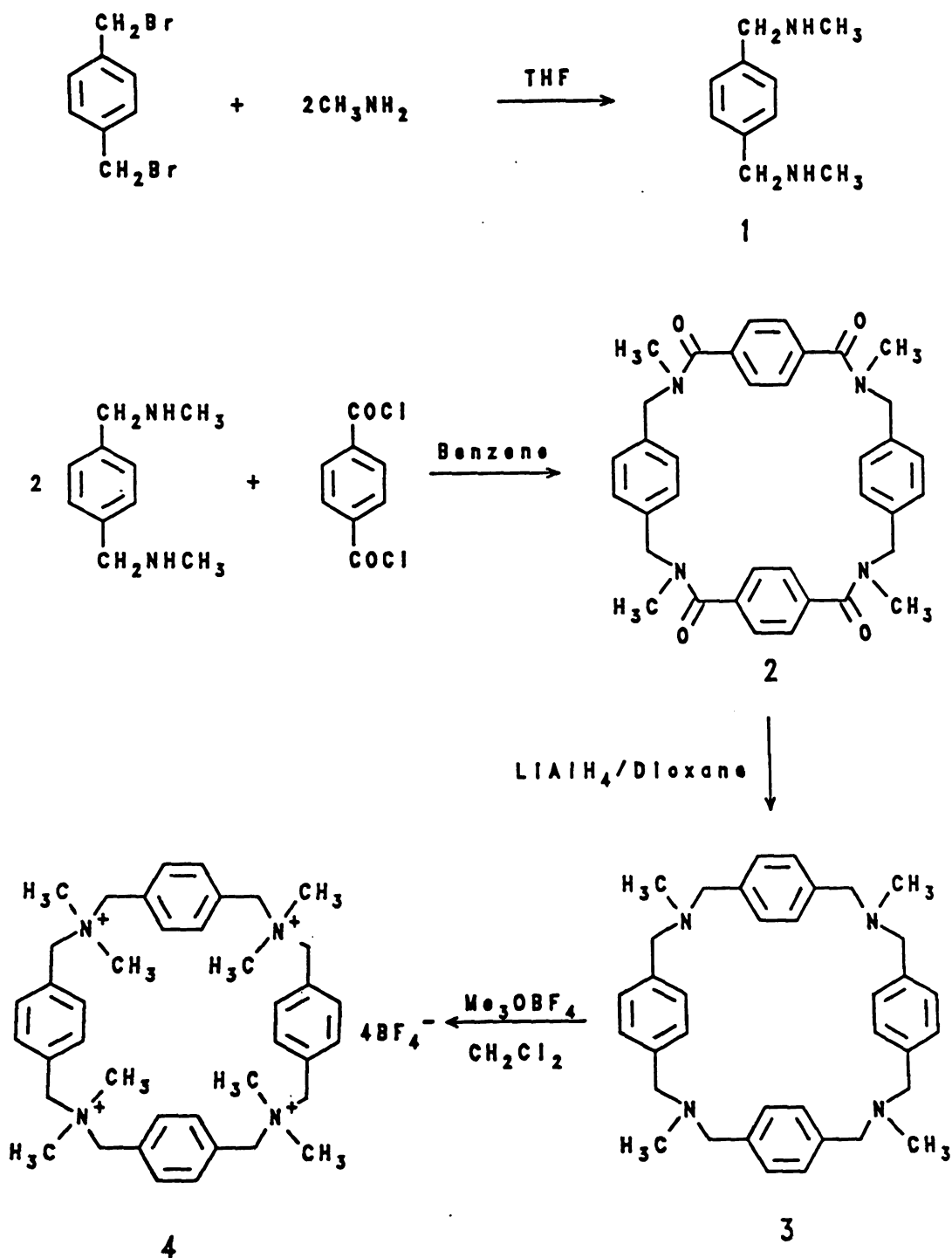


Figure 7.1. Synthesis of N,N,N',N',N'',N'',N''',N'''-octamethyl-2,11,20,29-tetraaza[3,3,3,3]paracyclophanetetraammonium tetrafluoroborate.

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recovered when needed by dissolving it into water under strong alkaline (pH 12) and by extraction with anhydrous ether. Evaporation of the solvent gives a yellow oily product 1.

N,N',N'',N'''-Tetramethyl-1,12,19,30,tetraoxo-2,11,20,29-tetraaza[3,3,3,3]paracyclophane (2).

Ring closing was done under highly dilute conditions to prevent unwanted polymer byproducts. Distilled benzene (2 L) was placed in a three-neck flask and refluxed under stirring. Reactant solutions of terephthaloyl chloride (1.5 g, 0.007 mole) and N,N'-p-xylenediamine (2.5 g, 0.015 mole) in each 250 mL dropping funnel were added dropwise to the hot benzene solution for 8 h, whose dropping rates were maintained as equally as possible. After this addition was complete, the mixture was refluxed for an additional 2 h. The resulting hot solution was filtered to remove the salt of diamine and evaporated under vacuum. Residues were collected using chloroform. The final product 2 (white solid) was obtained by recrystallization in dioxane (yield 11 %).

N,N',N'',N'''-Tetramethyl-2,11,20,29-tetraaza[3,3,3,3]paracyclophane (3).

A mixture of 200 mL dioxane and 2.2 g LiAlH₄ was placed in a 250 mL flask and refluxed under stirring. To this mixture 0.7821 g of 2 was added in small portions for 30 min and refluxed for 2 days. To the reaction mixture, 3 mL NaOH

(15%) and 6 mL water were added slowly to convert the excess LiAlH_4 into $\text{Li}(\text{OH})_2$, which is a white solid in dioxane. After an additional 1 h refluxing, solution was filtered to remove the white solid. The final product 3 (white crystals) was obtained by recrystallization in methylene chloride (yield 20 %). The nmr and mass spectrum of 3 are shown in Figure 7.2 and Figure 7.3, respectively.

**N,N,N',N',N'',N'',N''',N'''-Octamethyl-2,11,20,29-tetraaza
[3,3,3,3]paracyclophanetetraammonium tetrafluoroborate (4).**

0.32 g of 3 and 0.4 g of trimethyloxonium tetrafluoroborate were dissolved in a 200 mL dioxane and stirred for 6 h at room temperature. The product precipitated from the solution as a white powder and was filtered and combined with additional crop, which was obtained by the concentration of the filtrate solution one fifth by volume. Recrystallization from 1:1 (v/v) acetonitrile-water gave a colorless crystal of 4 (yield 55%).

Figure 7.2 NMR spectrum N,N',N'',N'''-Tetramethyl-2,11,20,29-tetraaza[3,3,3,3]paracyclophane.

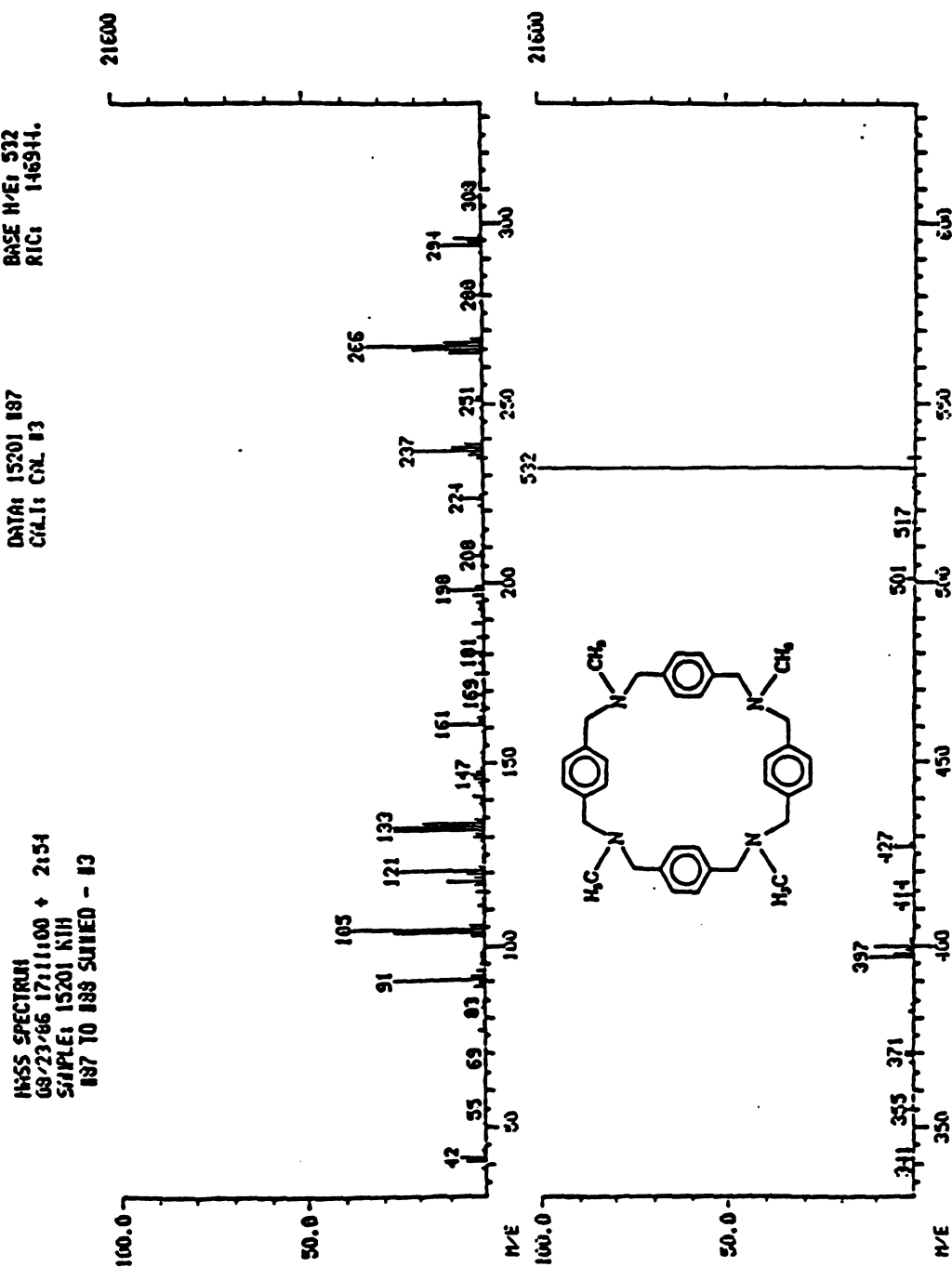


Figure 7.3 Mass spectrum N,N',N'',N'''-Tetramethyl-2,11,20,29-tetraaza[3,3,3,3]paracyclophane.

B. RESULTS AND DISCUSSION

The interesting characteristics of enzymes have stimulated chemists to design and synthesize various kinds of enzyme models which can show enzyme-like behavior (14). Substances capable of functioning as enzyme models or receptors should have binding sites or molecular cavities and functionalities so as to be able to form inclusion complexes. The substrate specificity of enzymes is controlled largely by the specific reaction pathways, the geometry of the substrate, and the shape and size of the substrate.

The methyl-APC molecules used in this study have a cavity surrounded by four positively charged active sites. The unique molecular shape of methyl-APC resembles a "square box" (Figure 7.4), and the cavity size is 5.5-7 Å wide and 6 Å deep (15). The box-like opening of the methyl-APC can vary in size, due to the single-bond character of the four quarternary nitrogen atoms and adjacent hydrocarbon chains, to include guest molecules of various size by "induced-fit binding" (16).

Association constant or complex formation constant between host and guest molecules can be measured from steady-state fluorescence intensities. The underlying theory for employing steady-state fluorescence intensities to calculate equilibrium constants have been reported by Benesi (17) and Bright (18). For a simple 1:1 host-guest complex, an equilibrium will exist between the fluorescent substrate, S,

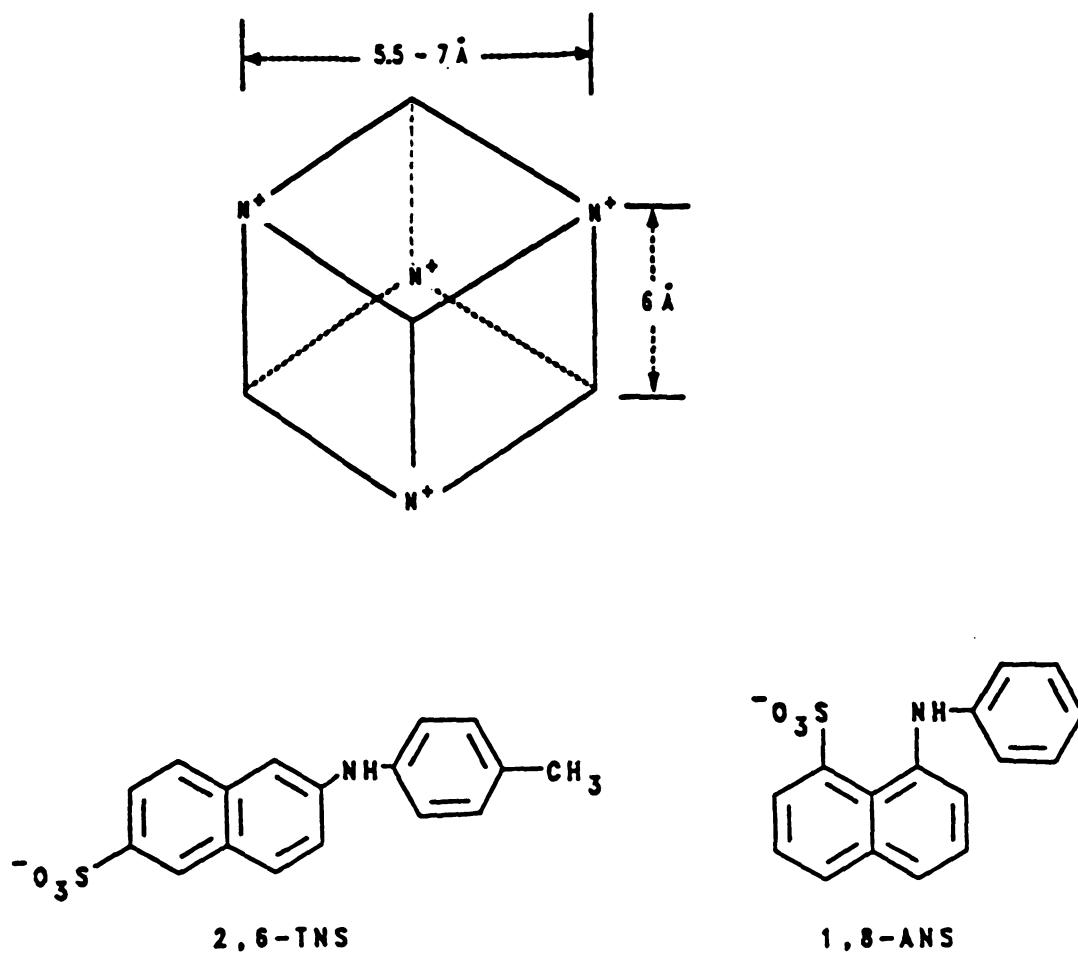


Figure 7.4. Simplified structural representation of host molecule, methyl-APC (upper), and anionic guest molecules.

and the host macromolecule M.



The equilibrium constant, K, is expressed as

$$K = [MS] / [M][S] \quad (7.2)$$

Substituting mass balance expressions for S and M, one obtains

$$K = [MS] / \{([M]_i - [MS])([S]_i - [MS])\} \quad (7.3)$$

where $[M]_i$ and $[S]_i$ are the initial concentrations of the host and substrate, respectively. If the condition is made so that $[M]_i \gg [MS]$ then equation 7.3 can be written as

$$K = [MS] / [M]_i([S]_i - [MS]) \quad (7.4)$$

We assume that the fluorescence enhancement of the probe in the observed spectrum, after addition of host, is due only to the presence of the MS complex.

The quantum yield expression for the complex is given by

$$Q = F / k[MS] \quad (7.5)$$

where Q is the quantum yield for complex MS, F is the fluorescence intensity of MS, and k is an instrumental constant. By substitution of equation 7.5 into equation 7.4 and rearrangement, one obtains following final relation.

$$[S]_i / F = (kQ)^{-1} + (KkQ)^{-1}([M]_i)^{-1} \quad (7.6)$$

This equation is now in the form, $y=ax+b$. Thus, a reasonable estimation of K can be obtained from a plot of $[S]_i/F$ versus $1/[M]_i$, by simply dividing the intercept by slope.

Fluorescence Enhancement of Lumiphors by APC.

The fluorescence probe molecules, ANS and TNS, are substituted naphthalene family that have found extensive use as fluorescence probes due to their highly sensitive nature towards hydrophobic binding (Figure 7.4). They normally show very weak fluorescence in aqueous solutions, but are highly fluorescent when bound to bovine serum albumin or to several proteins (19). The binding ability of the methyl-APC molecule was tested with ANS and TNS in aqueous solutions.

Figure 7.5 shows the change in fluorescence spectrum of guest molecules on addition of the host molecule, methyl-APC. The fluorescence intensity of TNS (2.5×10^{-5} M) in water was negligible (bottom), but it increased by a factor of twenty upon the addition of β -cyclodextrin (1×10^{-4} M) with an emission maximum at 470 nm. Upon addition of methyl-APC (1×10^{-4} M), the fluorescence intensity of TNS was enhanced

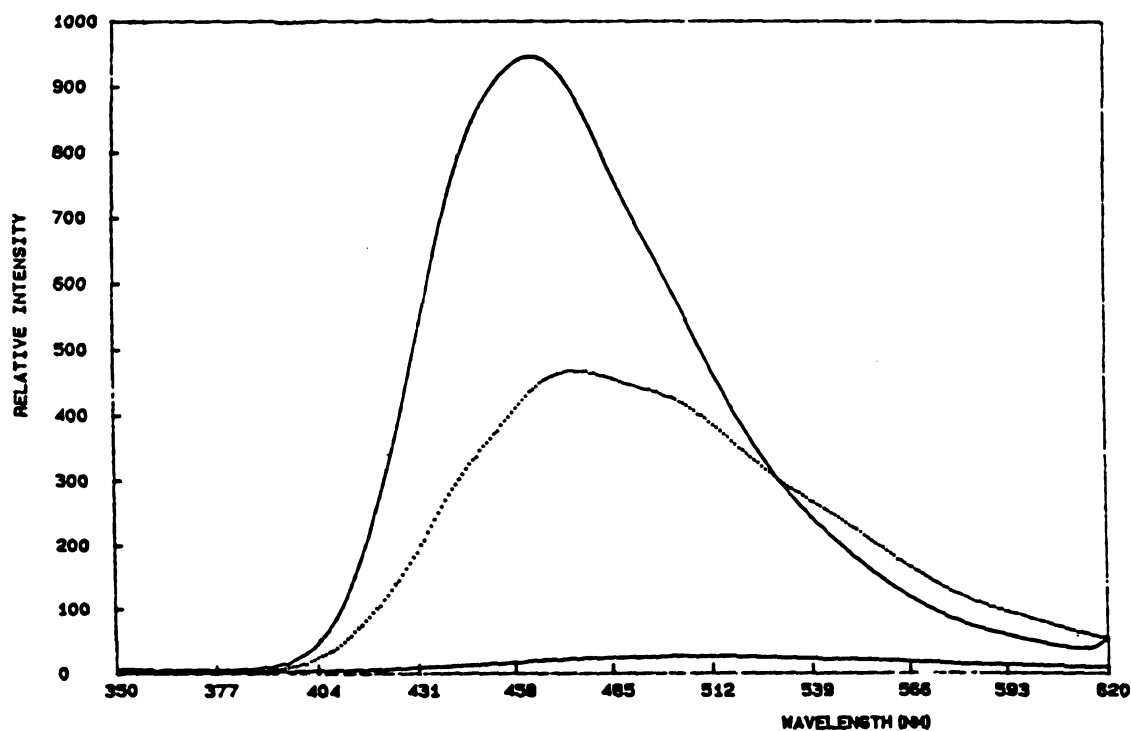
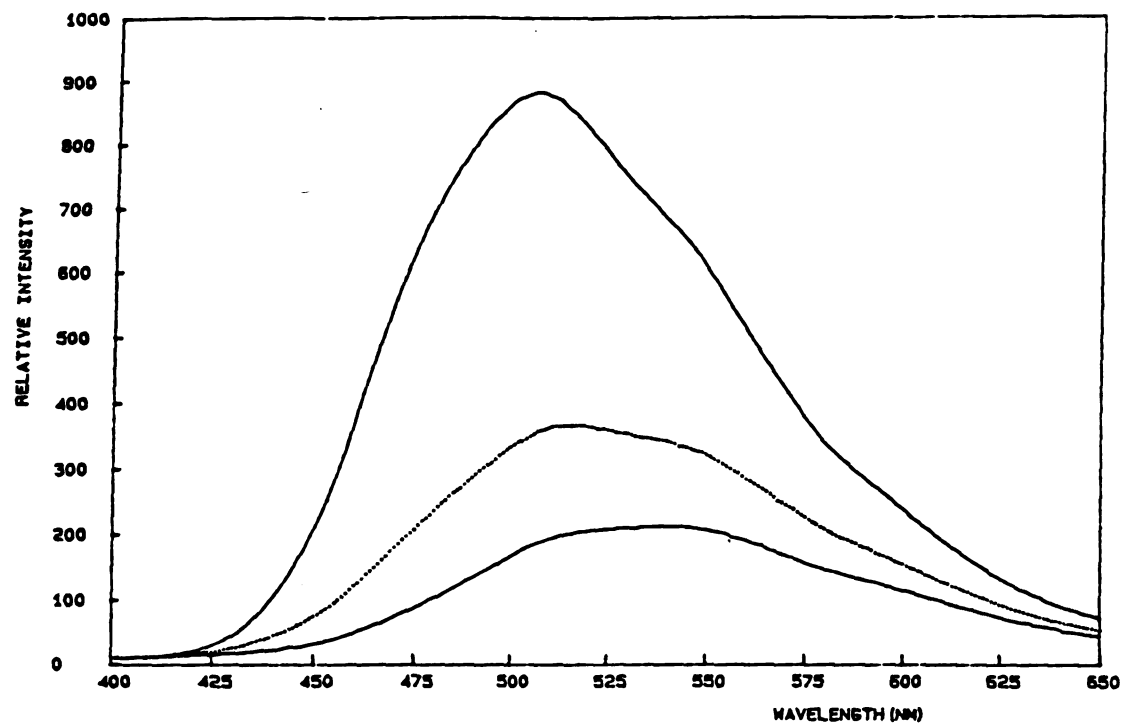


Figure 7.5. Fluorescence enhancement of ANS (2.5×10^{-5} M, excitation=375nm, upper spectrum) and TNS (2.5×10^{-5} M, excitation=337nm, lower spectrum) on addition of 1×10^{-4} M of b-cyclodextrin (dotted line) and methyl-APC (upper solid line).

more than twice that with β -cyclodextrin (β -CD), and the peak maximum blue shifted to 460 nm. Also with the methyl-APC, the fluorescence peak became narrower than that with β -CD. The same trend was observed when ANS was used as a guest molecule instead of TNS. Upon addition of β -CD (1×10^{-4} M) to the ANS (2.5×10^{-5} M) solution the fluorescence intensity of ANS increased by a factor of two, but when the same concentration of methyl-APC was added, the fluorescence intensity increased by a factor of five. The fluorescence peak blue shifted 8 nm with β -CD, and 25 nm with methyl-APC when compared to the emission maximum of ANS alone.

Plotting the fluorescence intensity of guest molecules against the reciprocal of the concentration of APC according to the equation 7.6, produces a straight line as shown in Figure 7.6. The association constant of ANS was calculated from the slope and intercept value to give 1.6×10^4 /mole. This value was much greater than the association constant of ANS with β -CD. The association constant of ANS with β -CD was reported recently by Catena et al (20) to be 110/mole at 25 °C.

The spectral changes are due to the changes in the microenvironment of the ANS or TNS molecules. In both cases, methyl-APC showed a larger blue shift and a greater fluorescence enhancement than β -CD. These data suggest that methyl-APC molecules provide stronger and more hydrophobic binding sites for TNS and ANS molecules than β -CD.

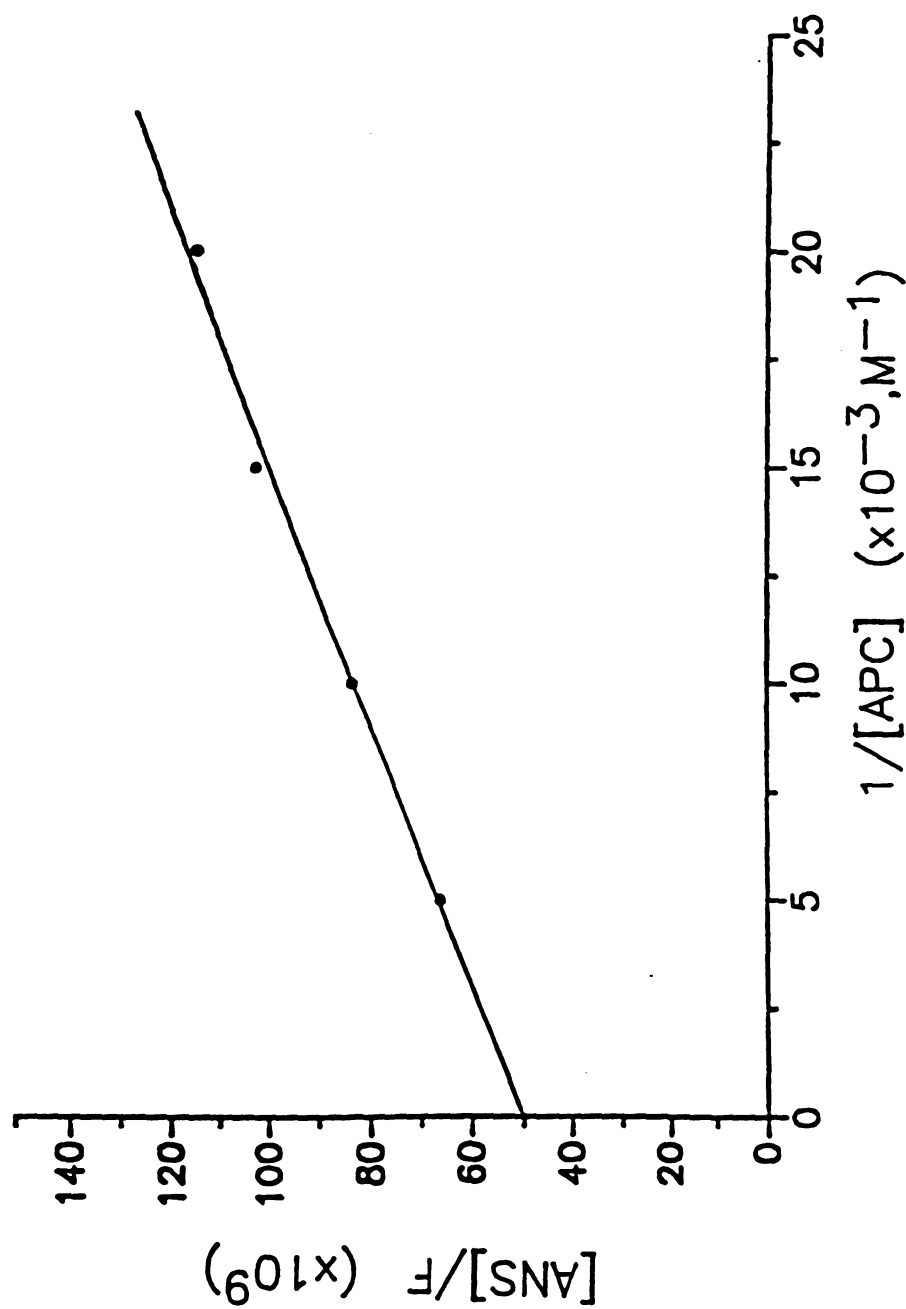


Figure 7.6. Double reciprocal plot for ANS - APC complex.

RTP Enhancement of Lumiphors by Host.

If the host molecules can provide an environment to protect excited triplet state molecules from quenching, it is expected that the resulting phosphorescence will increase.

Sample solutions were made for the observation of RTP with various amounts of methyl-APC or β -CD. The TNS solutions for RTP were deoxygenated with sodium sulfite (0.04 M) and, thallium nitrate (0.02 M) was used as an external heavy atom source. With the above conditions we could not observe any RTP of TNS. However, RTP of TNS began to show up when premicellar concentrations of SDS were added to the solution mixture. RTP enhancement of TNS (2.5×10^{-5} M) are shown in the upper spectrum of Figure 7.7. The RTP intensity of TNS increased by a factor of two when methyl-APC (0.1 mM) was added to the sample solution containing the premicellar concentration of SDS (5 mM), but it decreased slightly when the same concentration of β -CD was added to the solution. There were no noticeable spectral changes of the resulting phosphorescence when either host was added.

In the case of 2-bromonaphthalene under the similar experimental conditions, except for the heavy atom source (NaBr was used instead of TlNO_3), the RTP intensity of 2-bromonaphthalene also increased on addition of the host molecules. But again, the methyl-APC showed better RTP enhancement as shown in the lower spectrum of Figure 7.7.

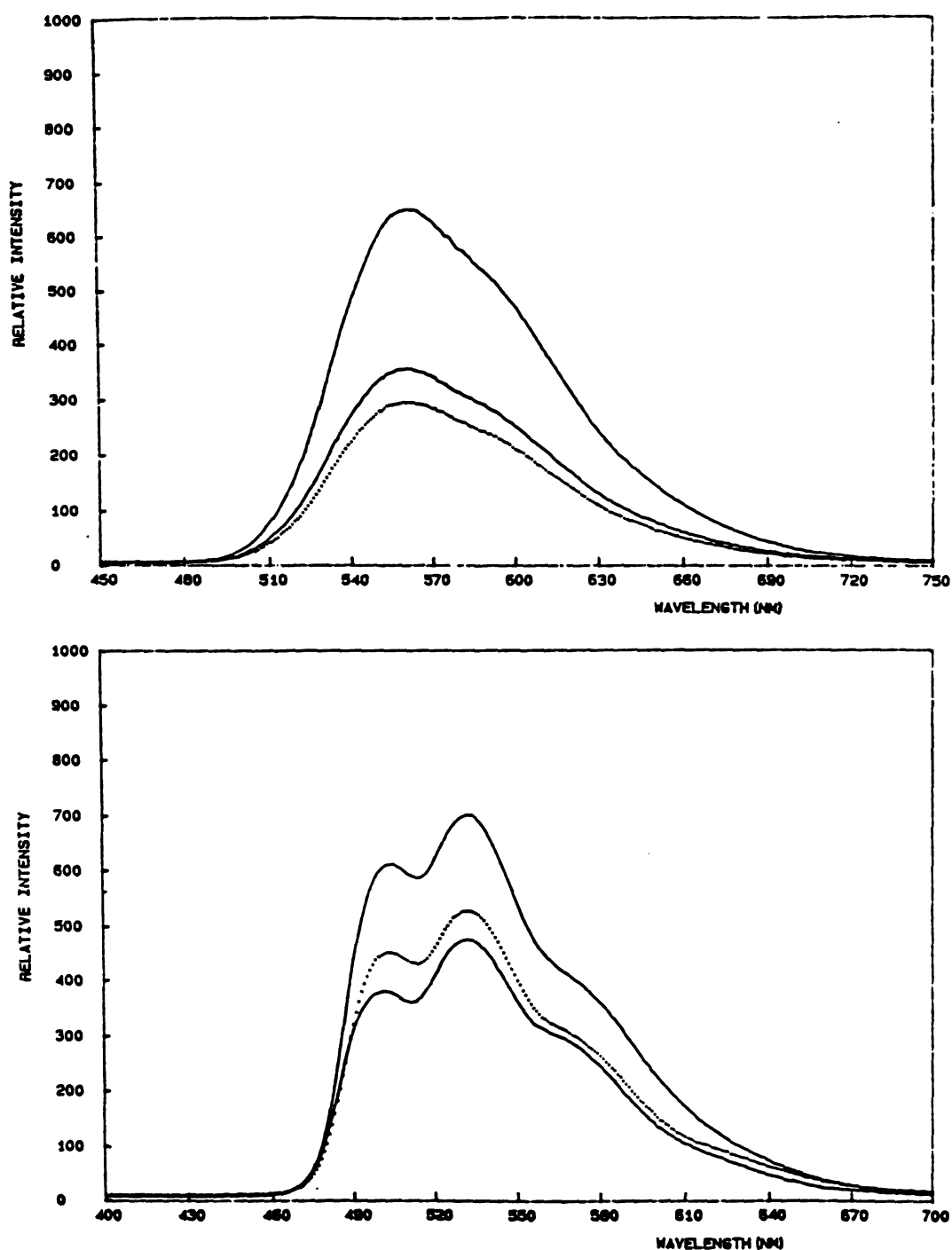


Figure 7.7. RTP spectrum of TNS (2.5×10^{-5} M, upper spectrum) and 2-bromonaphthalene (2.5×10^{-5} M, lower spectrum) upon addition of 0.1 mM solutions of β -CD (dotted line) and APC (upper solid line). The bottom solid lines in both spectra were obtained with SDS only. Excitation: TNS=337; ANS=293nm.

The RTP of 2-bromonaphthalene could be observed without an external heavy atom by careful sample purification and solution deoxygenation, but the RTP intensity in that case was much weaker than that with additional external heavy atom. Although the fluorescence of ANS was enhanced by the addition of methyl-APC, we could not observe any RTP from ANS under similar experimental conditions.

The critical micelle concentration (CMC) for SDS is reported to be 8×10^{-3} M (21). Also, the CMC of SDS was found to increase on addition of β -CD in aqueous solution due to the association of the SDS molecules with β -CD. This results in a decrease in the amount of available surfactant monomers for micelle formation (20). The concentration of SDS employed was 5 mM, so we expect some premicellar aggregates of SDS in a solution without any host. However, we should not expect any micelles in the above solutions containing host molecules. Although methyl-APC was not the only substance responsible for the observation of RTP, it appears that methyl-APC enhances RTP by efficient organization of lumiphors and SDS. The RTP of TNS at SDS concentrations above the CMC still showed enhanced intensity with methyl-APC.

The enhancement of RTP intensity on the addition of methyl-APC, strongly indicates that SDS molecules aggregate around the TNS-APC complex through electrostatic and hydrophobic interaction. This provides a favorable microenvironment that stabilizes the triplet state of TNS

molecules. It is quite surprising that the RTP of TNS decreased even though fluorescence of TNS increased in the aqueous solution on addition of β -CD. It is believed that the decrease in phosphorescence intensity of TNS on addition of β -CD is due to a preference for the aqueous bulk phase over the neutral cyclodextrin by TNS molecules. Also, the number of available surfactant monomers for TNS in aqueous bulk phase is expected to decrease due to the aggregation of surfactant molecules around the β -CD.

The Effect of Host, Heavy Atom, and SDS on RTP.

The effect of host concentration on RTP was studied at fixed concentrations of SDS, lumiphor, heavy atom, and sodium sulfite. The concentration of host was varied from 0 to 0.6 mM and the concentration of SDS was maintained at 5 mM. As increasing the concentration of methyl-APC, the RTP intensity of TNS steadily increased up to 0.3 mM and decreased thereafter. The same trend was observed with 2-bromonaphthalene. On the other hand, the RTP intensity of TNS continuously decreased when increasing the β -CD concentration as shown in the upper graph of Figure 7.8. The decrease in RTP intensity of TNS above 0.3 mM may be due to an impurity of methyl-APC and decrease in the number of SDS molecules available for methyl-APC molecules.

The increase in the SDS concentration with the fixed amount of heavy atom and sodium sulfite enhanced the RTP intensity of lumiphors up to an SDS concentration of 0.1 M.

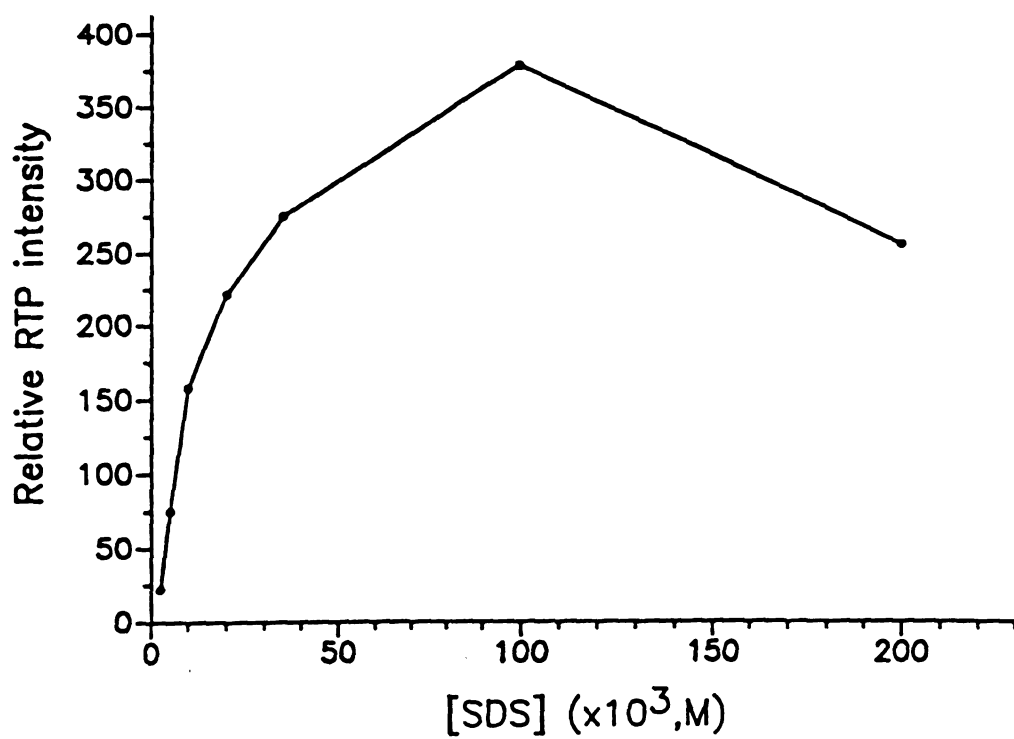
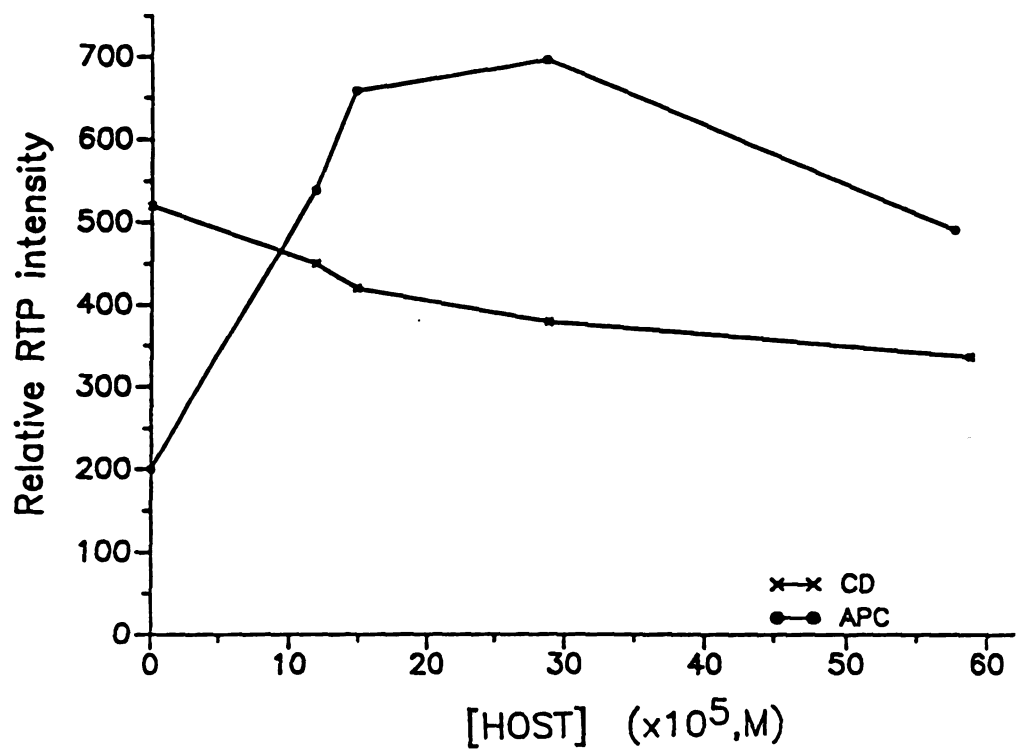


Figure 7.8. The effect of host (upper graph) and SDS (lower graph) on the RTP intensity of TNS ($2.5 \times 10^{-5} M$).

Beyond this the RTP intensity began to decrease. This type of change in RTP intensity was also observed in micellar stabilized RTP of many PAHs. But, the inflection point of SDS concentration at which RTP intensity starts to decrease normally lies in the 0.04-0.05 M range, which are much lower than that of methyl-APC - SDS mixed organized system.

The exact mechanism of these RTP changes is not clear at this time, but it seems that the aggregation of SDS around the methyl-APC plays a major role. Adsorption of surfactant monomers on a hydrophilic solid surface such as silica has been observed by studying the fluorescence decay of pyrene dissolved in surfactant-silica media (23). The proposed models on the basis of the adsorption data admit the existence of condensed (or organized) molecular assemblies on the surface, either in the form of micellar-like aggregates (hemimicelles) or in the form of a more extended lamellar phase (24).

The effects of heavy atoms on the RTP were quite interesting. No RTP of TNS was observed from anionic TNS with bromide ions as an external heavy atom source. In the case of nonionic 2-bromonaphthalene, both Br^- and Tl^+ enhanced RTP, but the bromide ions enhanced the RTP intensity more than twice that of thallium ions as shown in Figure 7.9. These data indicate that the effective enhancement of RTP by heavy atoms through intersystem crossing requires a closer approach of heavy atoms to the lumiphors. It is expected that thallium ions cannot approach

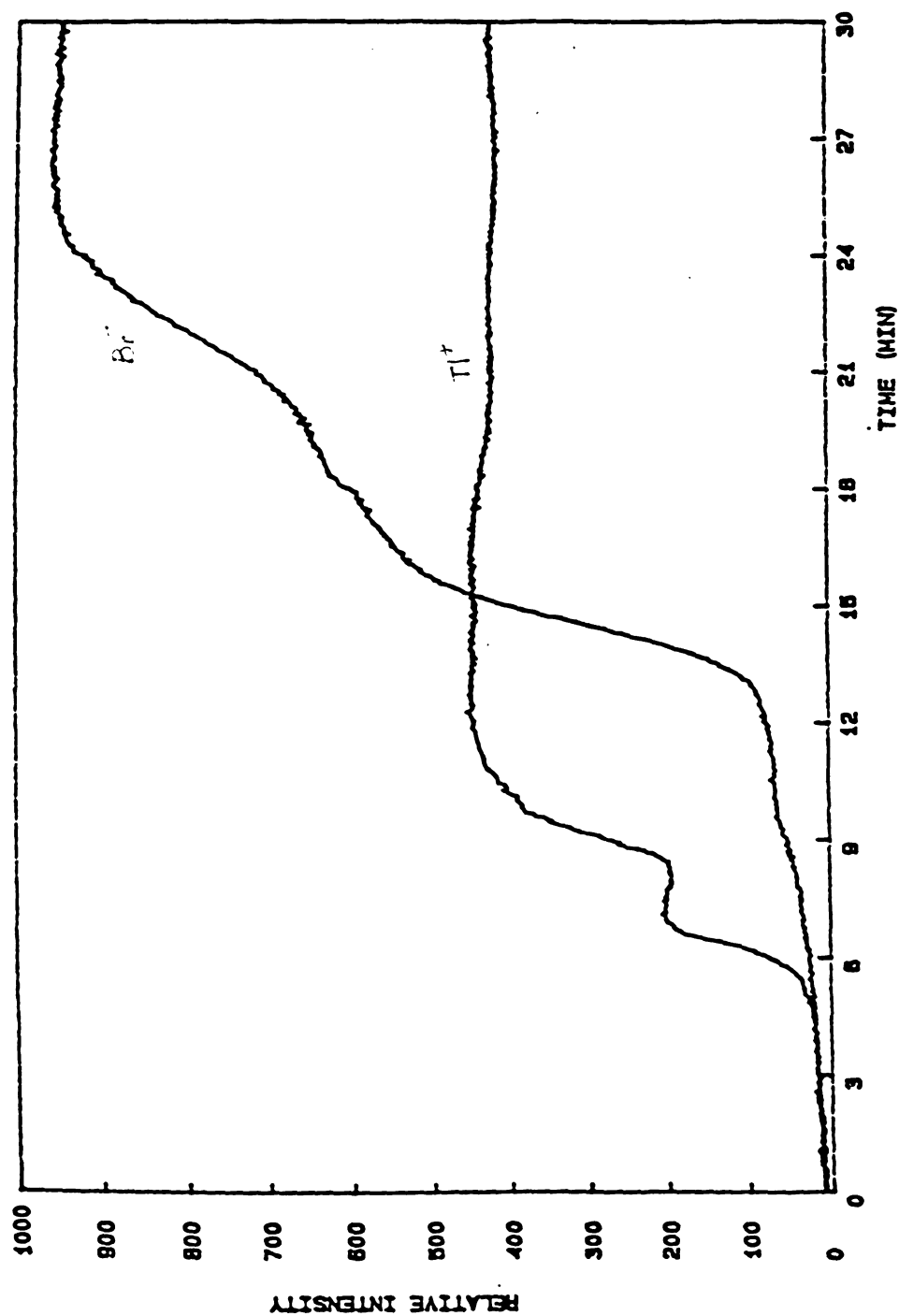


Figure 7.9. RTP development of 2-bromonaphthalene (5×10^{-5} M) with 0.02 M sodium bromide (lower line) and 0.02 M thallium nitrate (upper line). $[\text{Na}_2\text{SO}_3] = 0.04$ M; $[\text{SDS}] = 5$ mM; $[\text{APC}] = 0.1$ mM. Excitation = 293 nm; emission = 525 nm.

close to the methyl-APC due to the electrostatic repulsions between positively charged sites of the methyl-APC and thallium ions. However, anionic bromide ions can approach closely to the methyl-APC due to attractive forces between them. It is worth noting in Figure 7.9 that the RTP development of 2-bromonaphthalene by bromide ions took more time than with the thallium ions due to the electrostatic interactions of bromide ions with anionic SDS molecules around the methyl-APC.

Sodium sulfite has proven to be an excellent oxygen scavenger in solution (13) even though the reaction of sulfite ions with oxygen is a slow process in this system. The RTP development with time directly indicates the oxygen consumption process in the sample solution. It appears to be a two-step process in both the bromide and the thallium system. These can be also observed in a highly concentrated micellar solution. The oxygen consumption by sulfite ions should be a diffusion controlled process. Also the diffusion process of oxygen in the bulk phase and inside the SDS-APC-lumiphor complex will be different. These data suggest that the solution is composed of two different microenvironments for lumiphors.

RTP Decay of Lumiphors.

Since the RTP lifetime is extremely sensitive to a molecules's microenvironment, information about the

molecular interaction of the lumiphors in the APC-surfactant mixed media can be obtained from the RTP decay lifetimes.

RTP lifetimes of TNS and 2-bromonaphthalene were measured in aqueous solutions containing a heavy atom and 5 mM SDS. RTP lifetimes were calculated with the linear least-squares method. Figure 7.10 shows the change in RTP decay of TNS on addition of the host. The RTP lifetime of TNS in SDS solution without any host was 124 ± 22 μ s with a correlation coefficient of 0.999 (dotted curve). In the 0.2 mM β -CD solution, the RTP lifetime of TNS was decreased slightly (lower curve) to 117 ± 22 μ s, as expected from the decreased RTP intensity upon addition of β -CD. But when 0.2 mM methyl-APC was added to the TNS solution, the RTP lifetime of TNS increased (upper curve) to 406 ± 20 μ s with a correlation coefficient of 0.999. The RTP decay of 2-bromonaphthalene under similar experimental conditions except for the heavy atom (Br^- instead of Tl^+), showed increased lifetime on addition of host. The RTP lifetime of 2-bromonaphthalene was increased from 412 ± 11 μ s to 455 ± 12 μ s upon addition of 0.1 mM β -CD and increased further to 529 ± 36 μ s with 0.1 mM methyl-APC.

Differences in decay lifetimes can generally be attributed to changes in the deactivation pathways of the excited state or changes in the interaction of the excited state with the surroundings (25). The increase in RTP lifetime of lumiphors on the addition of methyl-APC indicates that the microenvironment of lumiphors in the methyl-APC - surfactant

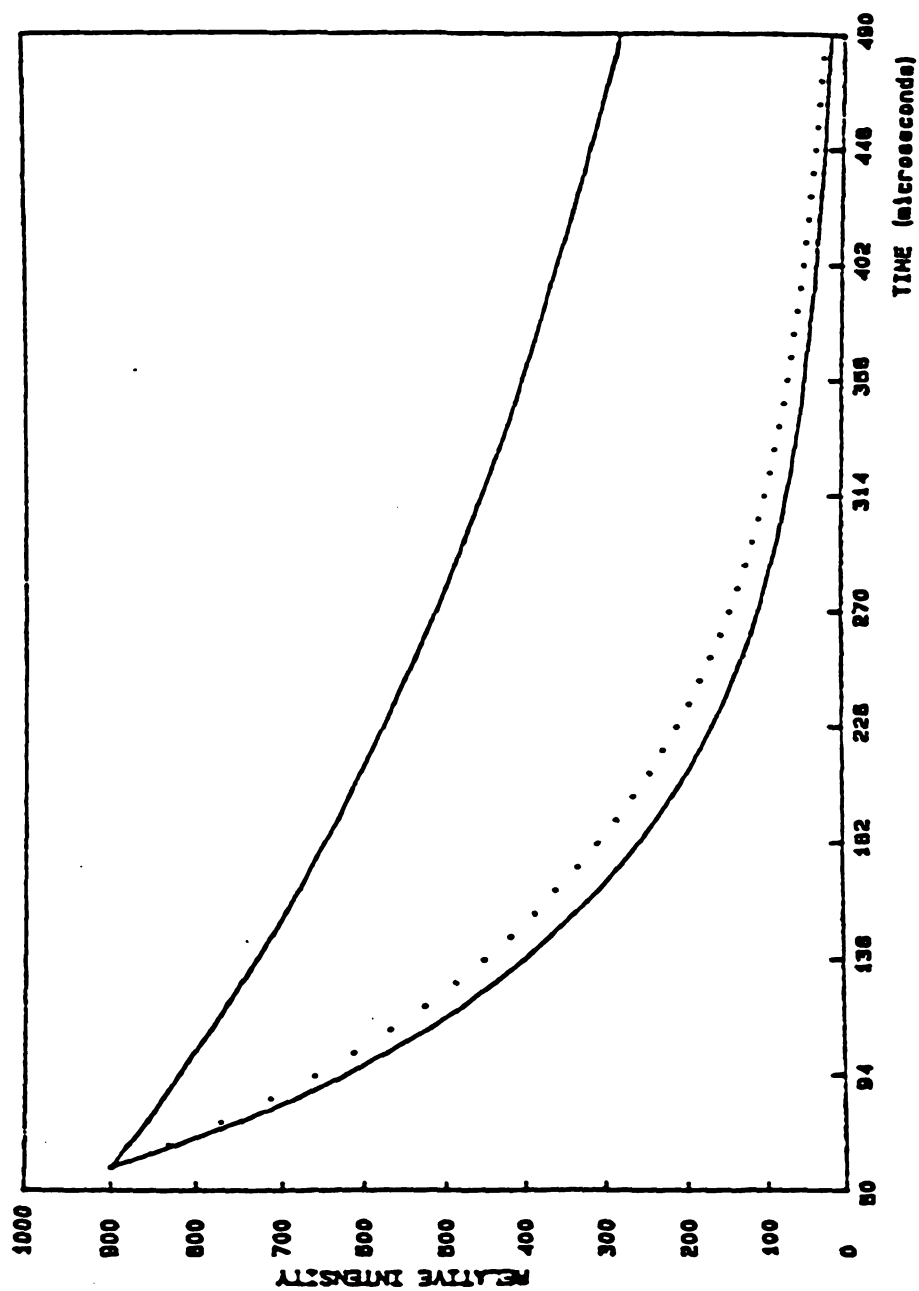


Figure 7.10. RTP decay of TNS (5×10^{-5} M) in 5 mM SDS and 0.02 M TlNO₃; dotted line, no host; bottom line, on addition of 0.2 mM β -CD; upper line, on addition of 0.2 mM APC.

mixed media stabilizes the excited triplet state of molecules.

D. CONCLUSIONS

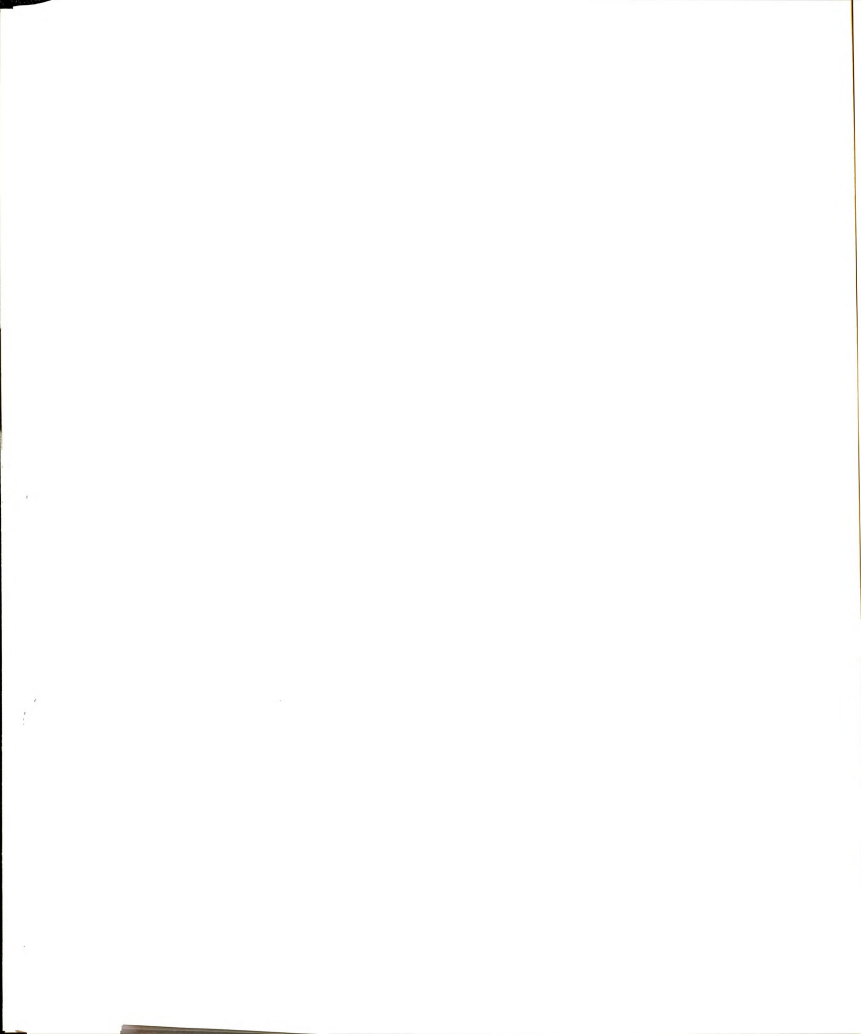
The comparative RTP study of anionic and neutral lumiphors in a mixed organized media, surfactant-host, has demonstrated that synthetic enzyme model molecules can provide a favorable microenvironment for lumiphors through electrostatic and hydrophobic interactions. Since the host enzyme model molecules are synthetic in nature, a highly selective microenvironment can be obtained by designing host molecules for specific purposes. Consequently, the synthetic enzyme model molecules could open a new avenue for chemical analysis due to their inherent potential for controlled specific host-guest interaction.

In the future, such synthetic enzyme model molecules may find applications in luminescence, chromatography, biological immunoassay, flow injection analysis, and many other research areas.

CHAPTER VII REFERENCES

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CHAPTER VIII

CONCLUSIONS AND FUTURE PROSPECTS

The ultimate goal of this research was to develop a new RTP methodology using the synthetic enzyme model compounds, the azaparacyclophanes. To carry out this goal two additional areas had to be studied. These were luminescence instrumentation for RTP and RTP lifetime measurements, and the decay kinetics study of triplet state molecules.

In this final chapter, the three major research projects are reviewed to draw conclusions. Luminescence instrumentation is discussed first, then the RTP lifetime study of PAHs in micellar solution follows. Finally, the RTP study of PAHs in mixed organized media, APC-SDS system, is described. Also in each section, future prospects for that particular research area is discussed.

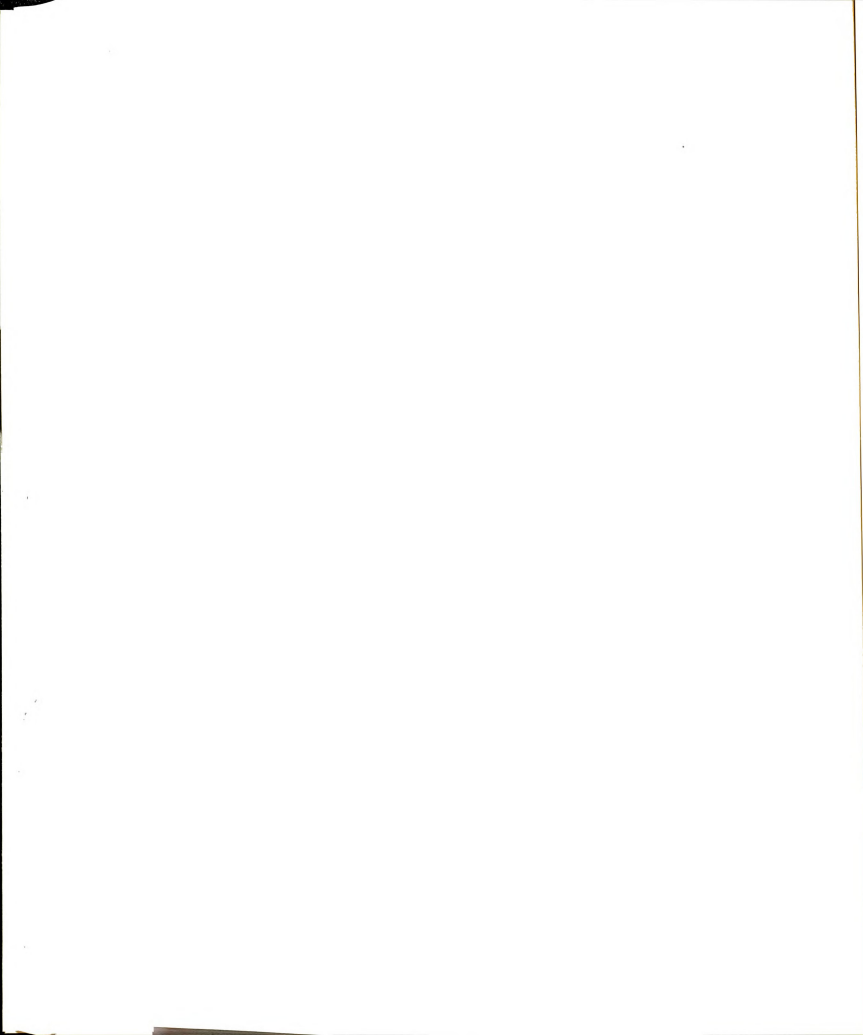
A. Luminescence Instrumentation.

The intended goal of this instrumentation, was to modify a commercial spectrofluorometer, and to develop a computer-controlled, versatile, and flexible luminescence data acquisition and analysis system. This would allow semiautomatic luminescence data (fluorescence, RTP, RTP lifetime) acquisition and analysis with minimal cost.

These goals were carried out successfully (1,2), and the details are documented in chapter V. This luminescence instrument shares most of the advantages of the pulsed source phosphorimeter, but does not share the disadvantages of the conventional rotating-can type mechanical phosphoroscope. The major advantages of this instrument over the conventional phosphorimeters are:

- (i) Fluorescence, RTP, and RTP lifetimes can be measured on the same instrument without additional instrumental modifications.
- (ii) Unlike the rotating-can type mechanical phosphoroscope, there is no limitation on the time discrimination procedure. Delay time and gate time can be easily changed by software, without adjustment of the rotating speed of the chopper.
- (iii) This instrument gives equally good performance in RTP and RTP lifetime measurements for short-lived and long-lived phosphors. By changing the delay time, gate time, or rotational speed of the chopper, one can obtain an optimum detection time window.
- (iv) This instrument gives a more reliable reproducibility and less RF (radio-frequency) interference than a pulsed source, due to a chopped continuous source.
- (v) Finally, this instrument is controlled by a low-cost IBM-XT compatible personal computer. Luminescence data are acquired and manipulated easily on site.

The biggest and most persistent problem of this instrument was an unstable Xenon-arc lamp. In the worst case, the



source intensity fluctuated up to 20% of its maximum intensity. We could not replace the source because that spectrofluorometer model (SPF-500) had been discontinued by the manufacturer. Also, the use of the slow ADC board and a computer did not allow the measurement of fluorescence lifetimes.

The instrument designed for this research can be improved in several ways with extra investment. Replacement of the unstable high pressure lamp with a high power pulsed laser will give much more power. Also, a fast ADC board and computer will enable the measurement of fluorescence lifetimes. The PMT can be replaced by photodiode array for a high speed multidimensional luminescence data acquisition.

In the software part, a spectral library routine could be added for spectral data base applications, such as, compound identification from the obtained spectrum by spectral peak matching. An ideal intelligent instrument could be build by the incorporation of artificial intelligence (3).

B. RTP Lifetimes of PAHs in Organized Media.

MS-RTP lifetimes of PAHs measured in this system were found to be significantly longer than those of other literature values. Similar results were obtained by other researchers in this field (4). The reason for these longer RTP lifetimes may be explained in several ways. It was found that the RTP lifetimes of PAHs change from system to system

depending on the experimental conditions employed. Any comparisons based on the numerical values of the RTP lifetimes could lead to a serious misinterpretation of the obtained lifetime data.

Heavy atoms were found to affect observed RTP lifetimes significantly. Today, the use of heavy atoms in RTP is very common. Heavy atoms usually increase the RTP sensitivity while reducing triplet lifetimes. Since oxygen is such an efficient triplet quencher, the deoxygenation method also affects the observed RTP lifetimes significantly. Sodium sulfite has proven to be a very efficient oxygen scavenger for RTP solution.

Unlike conventional frozen phosphorescence samples, every component in the RTP solution is in dynamic equilibrium. As a result, temperatures showed profound effects on the observed RTP. For accurate and reproducible results, precise temperature control of the sample was necessary.

The rapid lifetime determination (RLD) method for the calculation of lifetimes gave good results with an unknown background and noisy data. Even though statistical parameters which can judge goodness of the data fit are not available, regeneration of decay data by simulation with the obtained background, initial intensity, and lifetime value, enables direct comparison of the regenerated data with raw data. This RLD method is very fast, and may be adapted for real-time lifetime analysis.

Since RTP lifetimes are highly dependent on the experimental conditions, there should be some kind of normalized conditions for the RTP lifetime experiment. Unless some normalization of the RTP lifetime data which were obtained in different systems is made, the analytical usefulness of RTP lifetimes is questionable.

C. Application of Synthetic Enzyme Model Compounds in Chemical Analysis.

The importance of organized media has been well understood during the last two decades. The applications of macrocyclic compounds and various types of organized media are now emerging rapidly in chemical analysis (5,6).

Initial attempts were made to synthesize octopus-like azaparacyclophanes which carry negative or positive charges at their long tails. But their synthesis was not easy to accomplish. As a result, basic azaparacyclophane without long tails (methyl-APC) was chosen for testing and evaluating for RTP applications.

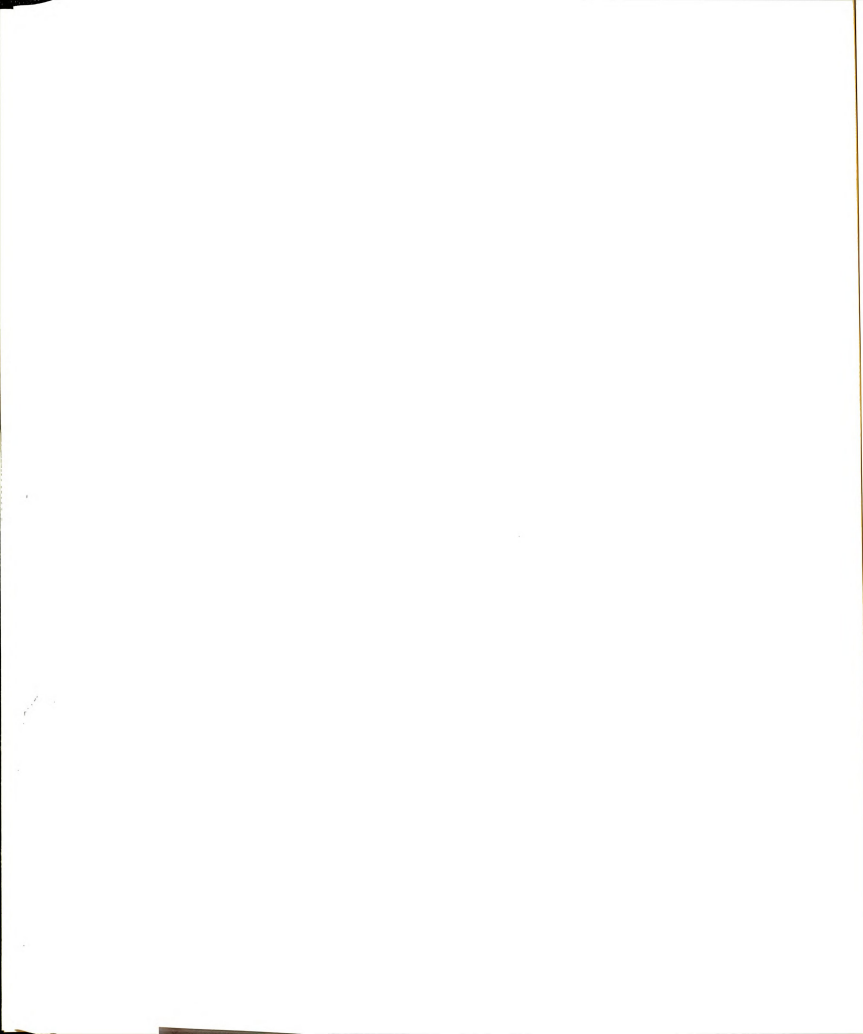
The basic model compound was found to be an excellent host for anionic as well as neutral compounds. The binding constants of several anionic compounds are several orders higher than that of well known cyclophanes. These were attributed to the strong electrostatic and hydrophobic interactions between APC and PAH. Although methyl-APC strongly binds many PAHs, the open ends at the top and

bottom of the methyl-APC made included lumiphors to be exposed to outside quenchers. This prevented any observation of RTP in the solution containing only methyl-APC. The addition of premicellar concentrations of surfactant seemed to cover up both open ends of the APC-PAH complexes, thus inducing RTP. This result suggests that octopus-like compounds might be better for RTP in this sense.

It was found that close approach or complex formation of heavy atoms to lumiphors is necessary to induce an analytically useful RTP signal. Also, electrostatic interaction between the heavy atoms and the organized assembly plays a very important role to induce a so-called external heavy atom effect (7).

Immediate use of APCs in chemical analysis is prohibited by tedious synthetic process required, because they are not available commercially at this time. The necessity of highly dilute conditions and low yield of the product in the synthesis of azaparacyclophanes required labor intensive devotion. But as more scientists recognize their importance in chemical analysis they will be available commercially sometime in near future.

The potential application of water-soluble APCs in analytical chemistry may be wide ranging, including applications in luminescence and chromatography. One particularly interesting application of charged APCs would be as a separation medium in capillary electrokinetic chromatography.



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APPENDIX A

**SOURCE CODE OF
TPLIB**

(Library routines for Turbo Pascal)


```

    fgrnd:Byte);
Procedure OpenWindow(Window: Integer);
Procedure HeaderOn(Window: Integer);
Function CloseWindow;
Function PullDownMenu(Window, NumMenu: Integer;
    Sub1, Sub2, Sub3, Sub4, Sub5, Sub6, Sub7,
    Sub8, Sub9, Sub10: String20): Byte;
Function BarMenu(Line, NumMenu, Bkgrnd, Fgrnd: Byte;
    TxtMenu: String80): Byte;
Procedure ExitProcess;
Procedure GrInitLize;
Procedure GetDriverAndMode(var DriveStr, ModeStr: String);
Procedure PrintScreen(Inverse: Boolean; Mode: Byte);
Procedure FindMenuIn(var DataSet: DataArray; NumData: Integer;
    var MaxVal, MinVal: real);
Function IntToStr(L: Integer): String;
Function RealToStr(M, Sign: Real): String;
Procedure DefaultColors;
Procedure FullPort;
Procedure DrawBorder(Color: Integer);
Procedure DrawBox(x1, y1, x2, y2, Bgrnd, Fgrnd: Integer);
Procedure DrawPlotAxis(x1, y1, x2, y2,
    Xmin, Xmax, Ymin, Ymax: Integer; Grid: Boolean);
Procedure DrawArtText(x, y, Direction, Font, Size, Color: Integer;
    GLabel: String255);
Procedure PlotData(D: DataArray; First, Last,
    PlotStyle, Color: Integer; Bipolar: Boolean);
Implementation
(-----)
(----- CursorOn sets cursor on or off -----)
(-----)
Procedure CursorOn(Option: Boolean);
Var
    I, J: Byte;
    GraphicsCard: Integer;
    Regs: Registers;
Begin
    GraphicsCard := Detect;
    If GraphicsCard = CGA Then
        Begin
            I := 6; J := 7;
        End
    Else
        Begin
            I := 12; J := 13;
        End
    With Regs Do
        If Option = True
            Then Begin
                AL := 0; AH := 1;
                CL := J; CH := I;
            End
            Else Begin
                AL := 0; AH := 1;
                CL := 15; CH := 32;
            End
        End;
    Intr(16, Regs);
End;
(-----)

```

```

(----- Write text at col, row -----)
(-----)
Procedure Msg(Col, Row, Color: Integer; Text: String80);
Begin
    ( Print a message at location Col, Row )
    GotoXY(Col, Row); TextColor(Color);
    Write(Text);
End;
(----- Centers a text of L length at col, row -----)
(-----)
Procedure Center(Col, Row, L: Integer; S: String255);
( Center a string on a line of L length at Col, Row )
Var L: Integer;
Begin
    GotoXY(Col, Row);
    For I := 1 To L Do Write(' ');
    GotoXY(Col*(L-Length(S)) Div 2, Row); Write(S);
End;
(----- Prints a text in Inverse Video -----)
(-----)
Procedure InVideo(InVStr: String255);
Begin
    TextBackground(15); TextColor(0); Write(InVStr);
    TextBackground(0); TextColor(7);
End;
(----- Change color of the screen -----)
(-----)
Procedure Color(Bgrnd, Fgrnd: Integer);
Begin
    ( Change the text background & foreground color )
    TextBackground(Bgrnd); TextColor(Fgrnd);
End;
(----- Beeps for duration of frequency tone -----)
(-----)
Procedure Beep(Tone, Duration: Integer);
Begin
    Sound(Tone); Delay(Duration); NoSound;
End;
(----- Deletes a selected portion of the line -----)
(-----)
Procedure DelLine(Bgrnd, R, C1, C2: Integer);
Var I, J, K: Integer;
Begin
    K := C2 - C1 - 1;

```

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<pre> GotoXY(C1,R1);TextBackground(Bgrnd); For I:=1 to K do Write(' '); End; (-----) (-----) Format a real number (-----) Function FormatReal(num : real; len,dec: Integer): String20; Var s1,s2,Temp : string20; C,I,J,K,Cd : Integer; Begin Str(num,s1); S1 := copy(s1,pos(1,s1)+1,20); Val(s1,C,cd); Str(num;dec,s1); S2 := copy(s1,pos(1,s1)+1,dec); S1 := copy(s1,pos(1,s1)-1); J:=s1; K:=0; For I := length(s1) downto 1 do Begin If ((J mod 3) = 0) and (I <> 1) then If (I=2) and (copy(s1,I)= '-') then S1:=S1 else S1:=copy(s1,length(s1)-J-K)+','+copy(s1,I, length(s1)-J-K+1); K:=K+1; End; J:=J+1; End; Temp := S1+'.'+S2; If length(Temp) > len then Temp:=Temp; If length(Temp) < len then Begin Repeat Temp:=' '+Temp; until length(Temp)=len; End; FormatReal := Temp; End; (-----) (-----) Draw a divided box (-----) (-----) Procedure DoubleBox(Border,M,C1,R1,C2,R2: Integer); Var I,J,K: Integer; ul,ur,ll,lr,hb,vb: Char; Begin If Border = 1 Then Begin ul := #218; ur := #191; ll := #192; lr := #188; hb := #196; vb := #179; End Else Begin ul := #201; ur := #187; ll := #200; lr := #186; hb := #205; vb := #186; End; K:= C2-C1-1; GotoXY(C1,R1); write(ul); For I:=1 to K do write(hb);Write(ur); For I:=R1+1 to R2-1 do </pre>	<pre> Begin GotoXY(C1,I); write(vb); If I = M then Begin For J:=1 to K do write(hb); End; GotoXY(C2,I); write(vb); End; GotoXY(C1,R2); write(ll); For I:=1 to K do write(hb); Write(lr); End; (-----) (-----) Clear a selected portion of the screen (-----) (-----) Procedure ClrWd(C1,R1,C2,R2: Integer); Var I,J,K: Integer; Begin K:=C2-C1-1; For I:=R1 to R2 do Begin GotoXY(C1,I); for J:= 1 to K do write(' '); End; End; (-----) (-----) Save whole screen in memory (-----) (-----) Procedure SaveTextScreen; Var mono: boolean; Begin If (mem[0000:1040] and \$30) = \$30 then Mono:=true If mono then move(memobuffer[1],memory_scr[1],4000) Else move(memory_scr[1],memory_scr[1],4000); End; (-----) (-----) Restore saved screen image (-----) (-----) Procedure RestoreScreen; Var mono: boolean; Begin If (mem[0000:1040] and \$30) = \$30 then Mono:=true else Mono:=false; If mono then move(memory_scr[1],monobuffer[1],4000) Else move(monobuffer[1],memory_scr[1],4000); End; (-----) (-----) Display Caps Num,Insert states at col,row (-----) (-----) Procedure Say_Cap_Num(Col,Row: Integer); Var Value : Integer; Begin Value := Mem[0000:1047]; (test for caps,numbers,cursor cntrl) </pre>	6

```

GotoXY(Col,Row);
Case Value of
0 : begin LowVideo; write(' ');
32 : begin LowVideo; write(' '); InvVideo('NUM');
64 : begin LowVideo; write(' '); InvVideo('CAPS');
96 : begin LowVideo; write(' '); InvVideo('NUM');
128 : begin LowVideo; write(' '); InvVideo('Insert');
160 : begin LowVideo; write(' '); InvVideo('NUM');
192 : begin LowVideo; write(' '); InvVideo('CAPS');
224 : begin LowVideo; write(' '); InvVideo('NUM');
End; ( Case )
End;

(----- Sets Caps,Num,Insert keys -----)
(-----)
(-----)
Procedure Set_Cap_Num(Caps,Num,Insert : Char);
Var J : Integer;
Begin
If Insert='I' then J:=128 else J:=0;
Case Caps of
'C': begin If Num='M' then Memd[0000:1047]:= 96+J
else
: : begin If Num='M' then Memd[0000:1047]:= 32+J
else
: : begin If Num='M' then Memd[0000:1047]:= 0+J;
End; ( Case )
End;

(----- Read keyboard to check function keys -----)
(-----)
(-----)
Procedure Ck_edit_key(var Ch: Char);
Begin
Ch:=ReadKey;
Begin Case of IBM specific key pressed)
Case Ch of
'N': Ch:='E' ; (Up-arrow)
'P': Ch:='X' ; (dn-arrow)
'M': Ch:='D' ; (rt-arrow)
'K': Ch:='S' ; (left-arr)
'S': Ch:=#127 ; (Del)
'G': Ch:='V' ; (Insert)
'O': Ch:='Q' ; (Home)
'I': Ch:='O' ; (End)
'R': Ch:='R' ; (Pg-Up)
'D': Ch:='F' ; (Pg-Dn)
'U': Ch:='A' ; (F1)
'V': Ch:='B' ; (F2)
'W': Ch:='C' ; (F3)
'X': Ch:='D' ; (F4)
'Y': Ch:='E' ; (F5)

```



```

Begin
  Answer:= ''; X:=1; GotoX;
  Write(template); GotoX; PosX;
End;

(----- End local procedures -----)

Begin
  If Datatype = 'A' then OChars:=('...'); (Alphabet)
  Else OChars:=('0...9...!...~'); (Numbers)
  Temp := OChars; DF := false; NoChange:=true;
  Case Datatype Of
    'A','M','S': Begin
      FillChar(template,80,995);
      template:=Copy(template,1,length);
      If Datatype='S' Then
        Begin
          X := 0; GotoX; HighVideo; Write('S');
        End;
      If
        : Begin
          Get template(length,template);
          length := length(template);
          If Copy(template,1,1) <> 995 then DF := true;
        End;
      End;
      Answer:= ''; Enter:=false;
    End;
  Color(8grnd,fgnd); Ch:=BackKey;
  If Keypressed Then Ck_edit_key(ch);
  DelOK := true;
  If (Ch = #13) or (Ch = #80) Then DelOK := false;
  If (Datatype='F') and (X=1) and (Default<>'') and (Ch<>'')
  And (DelOK) Then Del_Ans;
  Case Ch of
    ^ : Begin
      If #10 then clear ;
      If (X>length(Answer)+1) or (X>length) then X:=X-1;
      GotoX;
    End;
    ^S : Begin
      ( Move cursor left : left-arr )
      If Datatype='F' then Del_Ans else
        Begin
          X:=X-1; If X<1 then X:=1;
          GotoX;
        End;
    End;
    ^O : Begin
      ( Move cursor to end of line )
      X:=length(Answer)+1; If X>length then X:=length;
      GotoX;
    End;
    ^G : Begin
      ( Move cursor to beginning of line )
      X:=1; GotoX;
    End;
    ^W : Begin
      ( Delete left char: Back Space )
      X:=X-1;
      If (length(Answer)>0) and (X>0) then
        Begin

```

```

Delete(Answer,X,1); GotoX;
WriteCopy(Answer,X,length(Answer)-X+1),995);
GotoX;
End;
Else X:=1;
End;
#12:Begin
  Delete(Answer,X,1);
  WriteCopy(Answer,X,length(Answer)-X+1),995); GotoX;
End;
'E : Begin
  If NoChange Then Answer := Default;
  ExitOK := true; ( If Up arrow then exit)
  GotoX := true; ( and go back to previous field)
End;
'M : ExitOK := true; (If Enter key then exit)
'X : Begin
  If NoChange Then Answer := Default;
  ExitOK := true; (If Down arrow then exit)
End;
'[' : Begin
  (If Escape then get answer and exit)
  ExitOK := true;
  Enter := true; Answer := Default;
End;
Else
  If (length(Answer)+1 <= length) or (Not InsertOn) then
  Begin
    If Ch in OChars then
      Begin
        InsertOn then
          If length(Answer) < length then
            Insert(Ch,Answer,X);
          Case Datatype Of
            'A','M','S': WriteCopy(Answer,X,
              length(Answer)-X+1);
            'F': Write(Ch);
          End;
        End;
        ( OK to Insert )
        End else
        If X <= length then
          Begin
            Write(Ch);
            If X>length(Answer) then Answer:=Answer+Ch
            Else Answer[X]:=Ch;
          End;
        If X+1 <= length then X:=X+1;
        If (X > length(Answer)) and (template[X]<>995) then PosX;
        End
      Else If (Ch <> 'V') then Beep(400,150);
      GotoX;NoChange:=false;
      If (Datatype<'F') and (length(Answer) > length) and (Ch <> 'V')
      Then Beep(400,100);
      End;
      ( Non IBM function key )
      If (Datatype<'F') and (length(Answer) > length) and (Ch <> 'V')
      Then Beep(400,100);
      Until ExitOK = true;
      X:=1; GotoX; Write(Answer);
      X:=length(Answer)+1; GotoX;
      For J:= 1 to length(X-1) do Write(' '); (remove trailing template)
      ExitOK := false; Color(0,7);
      If (DF) and (length(Answer)=1) then
        Begin
          GotoXY(col,row); Write(' '); Answer:= '';
        End;
      End;

```

```
(-----
(      Inputs data from single or multiple fields
(-----)
```

```
Procedure FieldInput(InputCode: String5; var Escape: boolean);
```

```
Label 100;
```

```
Var
  Enter_Valid : Boolean;
  Datatype, InputType : Char;
  Default_Template : String80;
  Row, Col, Length : Integer;
  Count, First_Var_Num,
  Last_Var_Num, FilVar_Num, Bgnd, Fgnd,
  DefaultNum : Integer;
```

```
Procedure Get_Var;
```

```
Begin
  With Fdofine[Count] Do
    Begin
      Bgnd := fBgnd;
      Fgnd := fFgnd;
      Val(Copy(fStr, 1, 2), FilVar_Num, Code);
      Val(Copy(fStr, 3, 2), Col, Code);
      Val(Copy(fStr, 5, 2), Row, Code);
      Val(Copy(fStr, 7, 3), Length, Code);
      Datatype := fStr(10);
    End;
  End;
```

```
Procedure Display_Rec;
```

```
Var I : Integer;
```

```
Begin
  For I := First_Var_Num to Last_Var_Num do
    Begin
      If Datatype <> 'M' Then
        Begin
          Count:=1; Get_Var;
          Gotoxy(Col, Row);
          Write(FilVar(FilVar_Num));
        End;
      End;
    End;
  End;
```

```
Begin
  InputType := InputCode(1);
  Val(Copy(InputCode, 2, 2), First_Var_Num, Code);
  Val(Copy(InputCode, 4, 2), Last_Var_Num, Code);
  If InputType = 'M' Then
    For I := 1 To MaxFilNum Do FilVar(I) := fDefault;
  Count := First_Var_Num; Enter := False;
  Else
    Begin
      Repeat
        Get_Back := False;
        Get_Var; template:='';
      Until Get_Back = True;
    End;
  100: Default := FilVar[Count];
  Input(Datatype, Default, Col, Row, Length, Bgnd, Fgnd, Enter);
  If Get_Back = True Then
    Begin
      FilVar[Count] := Answer;
```

```
Count := Count-1;
If Count < First_Var_Num Then Count := Last_Var_Num;
Get_Var; Get_Back := False;
Goto 100;
```

```
End;
```

```
If Enter Then
```

```
Begin
```

```
ExitOK := True; Enter := False;
```

```
End
```

```
Else
```

```
Begin
```

```
FilVar[Count] := Answer;
```

```
Count:=Count+1;
```

```
If Count>Last_Var_Num then
```

```
Begin
```

```
Escape:=False; ExitOK := True;
```

```
End Else ExitOK := False;
```

```
End;
```

```
Until ExitOK = True;
```

```
If Count > first_var_num then Escape:= false;
```

```
End; ( typ <> '0' )
```

```
ExitOK := False;
```

```
End;
```

```
(-----
```

```
(      Lotus Style Menu
```

```
(-----)
```

```
Procedure LMenu(Col, Row: Integer; Title: String255; var Ch: Char);
```

```
Var
```

```
  c: String;
```

```
  MenuLen
```

```
  MenuStr
```

```
  MenuStrs
```

```
  Counter
```

```
  Integer;
```

```
Begin
```

```
Counter := 0; Set_Cap_Mum(' ', ' ', ' ');
```

```
Repeat
```

```
Counter := Counter + 1;
```

```
I[Counter] := Copy(FilVar[Counter], 1, Pos(' ', FilVar[Counter])-1);
```

```
I[Counter] := Copy(I[Counter], 1, Pos(' ', I[Counter])-1);
```

```
Delete(FilVar[Counter], 1, Pos(' ', FilVar[Counter]));
```

```
Until (FilVar[Counter] = ' ') or (FilVar[Counter] = ' ');
```

```
MenuLen := Counter-1;
```

```
MenuStr := ' ';
```

```
MenuStrs := ' ';
```

```
For Counter := 1 to MenuLen do
```

```
Begin
```

```
MenuStr := MenuStr + I[Counter] + ' ';
```

```
MenuStrs := MenuStrs + Copy(I[Counter], 1, 1);
```

```
End;
```

```
Counter := 1; Write Title in top right corner **
```

```
Gotoxy(1, 1); clrcol; Gotoxy(79-length(title), 1);
```

```
Textbackground(15); textcolor( 0); write(title);
```

```
Lowvideo;
```

```
Repeat
```

```
Gotoxy(Col, Row);
```

```
Lowvideo;
```

```
WriteLn (MenuStr); (** Write menu choices string in low video )
```

```
Clrcol;
```

```
(** find location for and write highlighted menu choice **)
```

```
Gotoxy((Col-1) + pos(I[Counter], MenuStr), Row);
```

05/31/1989 00:06	Filename: IPLIB.PAS	Page
<pre> Textbackground(15);textcolor(0);write(1[Counter]); Textbackground(0);textcolor(15); (* write Prompt for highlighted item *) GotoXY(Col Row-1); clrscr; writeln (F1[Var[Counter]]); GotoXY(80,1); Ch := Readkey; If Keypressed then ck_edit_key(Ch); Ch := UpCase(Ch); #13Case Ch:och 'D','E','#32 : Counter := Menu[tr][Counter]; 'S','X : Counter := Counter + 1; 'G : Counter := Counter - 1; '0 : Counter := MenuLen; End; (case Ch) If Pos(Ch,Menu[tr]) <> 0 then Counter := Pos(Ch,Menu[tr]) Else If not (Ch in ('13','D','E','#32','S','X','G','0')) then Beep(350,200); If Counter < 1 then Counter := MenuLen; If Counter > MenuLen then Counter := 1; Until Pos(Ch,Menu[tr]) <> 0; LowVideo; End; </pre>	<pre> 185/31/1989 00:06 Filename: IPLIB.PAS Page 14 Var HeaderDef : HeaderType; Header: String(40); Begin With HeaderDef Do Begin d8grnd := Bgrnd; d1grnd := Fgrnd; d1header := Header; End; HeaderSet(Window) := HeaderDef; End; (----- Get Absolute position of Cursor into parameters x,y -----) (-----) (-----) Procedure Get_Abs_Cursor (var x,y :Integer); Var Active_Page: byte absolute \$0040:\$0062; (Current Video Page Index) Crt_Pages : array[0..7] of Integer absolute \$0040:\$0050; Begin x := Crt.Pages[active_page]; (Get Cursor Position) y := Hi(x)+1; (y Get Row) x := Lo(x)+1; (x Gets Col position) End; (----- Turn the Video On/Off to avoid Read/Write snow -----) (-----) (-----) Procedure Video (Switch:boolean); Begin If (Switch = Off) Then Port(CrtAdapter+4) := (VideoMode - VideoEnable) Else Port(CrtAdapter+4) := (VideoMode or VideoEnable); End; (----- InitWin Saves the Current (whole) Screen -----) (-----) (-----) Procedure InitWin; Begin If CrtMode = 7 Then Video_Buffer := \$8000 (Set Ptr to Monobuffer) Else Video_Buffer := \$8800; (or Color Buffer) With Win.Dim Do Begin x1:=1; y1:=1; x2:=Crtwidth; y2:=25; End; Win.Depth:=0; InitDone := true; (Show Initialization Done) End; (----- DrawWindow Draws Window Border -----) (-----) (-----) </pre>	
<pre> (----- Open whole screen as a working window -----) (-----) (-----) (True option clears screen and changes window color -----) (-----) Procedure FullScreen(Bgrnd,Fgrnd: Integer; Option: Boolean); Begin Window(1,1,60,25); If Option = True then Begin Textcolor(Fgrnd); Textbackground(Bgrnd); Clrscr; End; End; (----- DefWindow defines windows used -----) (-----) (-----) Procedure DefWindow(Window,x1,y1,x2,y2,Border,Bgrnd:Integer; Fgrnd:byte); Var WindowDim : WindowDef; Begin With WindowDim Do Begin dx1 := x1; dy1 := y1; dx2 := x2; dy2 := y2; dBorder := Border; d8grnd := Bgrnd; d1grnd := Fgrnd; End; WindowSet(Window) := WindowDim; End; (----- Defines Headers for Windows -----) (-----) (-----) Procedure DefHeader(Window, Bgrnd, Fgrnd: Integer; </pre>		


```

(-----)
Get graphics Driver and Mode
)

Procedure GetDriverAndMode(var DriveStr, ModeStr: String);
Begin
  DriveStr := DriverNames[GraphDriver];
  GraphMode := GetGraphMode;
  Case GraphDriver of
    CGA : Case GraphMode of
      CGAC0: ModeStr := 'CGAC0';
      CGAC1: ModeStr := 'CGAC1';
      CGAC2: ModeStr := 'CGAC2';
      CGAC3: ModeStr := 'CGAC3';
      CGAHI: ModeStr := 'CGAHI';
    End;
    MCGA : Case GraphMode of
      MCGAC0: ModeStr := 'MCGAC0';
      MCGAC1: ModeStr := 'MCGAC1';
      MCGAC2: ModeStr := 'MCGAC2';
      MCGAC3: ModeStr := 'MCGAC3';
      MCGAHI: ModeStr := 'MCGAHI';
    End;
    EGA : Case GraphMode of
      EGALo: ModeStr := 'EGALo';
      EGANi: ModeStr := 'EGANi';
    End;
    EGA64 : Case GraphMode of
      EGA64Lo: ModeStr := 'EGA64Lo';
      EGA64Hi: ModeStr := 'EGA64Hi';
    End;
    HerkHono: ModeStr := 'HerkHonoHi';
    EGAHono: ModeStr := 'EGAHonoHi';
    VGA : Case GraphMode of
      VGALo: ModeStr := 'VGALo';
      VGANi: ModeStr := 'VGANi';
    End;
  End;
End;

(-----)
Hard copy of graphics screen
)
(-----)
Hard copy of graphics screen with Epson printers(NK,RX,FX).
)

Procedure PrintScreen(Inverse: boolean; Mode: byte);
( Graphics screen dump routine for EPSON compatible
( printers. Pre-FX series of EPSON printers should
( only use Mode 1.
(
( Mode: 1 = Double-Density 120 dots per inch
( 2 = High-Speed D-0 120 dots per inch
( 3 = Quadriple-Density 240 dots per inch
( 0, 4, 5 = 80 dots per inch
( 6 = 90 dots per inch
Const
  Esc = 27;
  XScreenMaxLib = 639;
  YMaxLib = 349;
  GrafBase: Word = $8000;
Var
  ScanLine: Integer; ( The current scan line )
  n1, n2: byte; ( 2 byte printer control code )

```

```

Procedure SendByte(B: byte);
( Send one byte to the printer )
const
  LPTPortNum = 1; ( Defaults to LPT1. 2 = LPT2 )
var
  Regs: Registers;
begin
  Regs.AL := 0;
  Regs.AH := 0;
  Regs.DX := Pred(LPTPortNum);
  Intr($17, Regs);
end; ( SendByte )

(SB+) ( Turn off short circuit boolean evaluation )

Function BaseAddress(Y: word): word;
( Calculate the address of scanline Y )
begin
  BaseAddress := (Y and 3) shl 13 + 90 * (Y shr 2);
end; ( BaseAddress )

Function ConstructByte(X, Y: Integer): byte;
( Construct a print byte by reading bits from the graphics screen buffer )
const
  Bits: array[0..7] of byte = (128,64,32,16,8,4,2,1);
var
  CByte, Bit: byte;
begin
  Y := Y shl 3; ( Y := Y * 8 )
  CByte := 0;
  for Bit := 0 to 7 do
    if (Mem[BaseAddress(Y*8) + X shr 3] and Bits[Bit])
    then CByte := CByte + Bits[Bit];
  ConstructByte := CByte;
end; ( ConstructByte )

(SB-) ( Turn on short circuit boolean evaluation )

Procedure Doline;
( Dumps one print line to the printer )
var
  XPixel: Integer;
  PrintByte: byte;
begin
  if Mode = 1 then
    begin
      SendByte(Esc);
      SendByte(Ord('L'));
    end
  else
    begin
      SendByte(Esc);
      SendByte(Ord('i'));
      SendByte(Mode);
    end;
    SendByte(n1);
    SendByte(n2);
    for XPixel := 0 to XScreenMaxLib do
      begin
        PrintByte := ConstructByte(XPixel, ScanLine);
        if Inverse then
          PrintByte := not PrintByte;
        SendByte(PrintByte); ( Send print byte )
      end;
      SendByte(10); ( Send line feed )
    end;

```



```
(-----)
Procedure DrawPlotAxis(x1,y1,x2,y2,
  Xmin,Xmax,Ymin,Ymax:Integer; Grid:Boolean);
Var
  SigF,SigFY,Labval,dx,dy:Real;
  i,tx,ty,Xinc,Yinc:Integer;
  LabelStr:String20;
Begin
  With AxisData Do
    Begin
      ax1:=x1; ay1:=y1; ax2:=x2; ay2:=y2;
      axmin:=Xmin; axmax:=Xmax; aymin:=Ymin; amax:=Ymax;
    End;
    DefaultColors;
    SigF := (Xmax-Xmin)/40; SigFY := (Ymax-Ymin)/20;
    SetLineStyle(SolidN,0,NormalWidth);
    Rectangle(x1,y1,x2,y2);
    dx := (x2-x1)/40; dy := (y2-y1)/20;
    SetTextJustify(CenterText,CenterText);
    For i := 0 To 20 Do
      Begin
        Yinc := Trunc(y1+i*dy);
        If (i Mod 2)=0 Then ty := 6 Else ty := 2;
        Line(x1,Yinc,x1+ty,Yinc);
        If (Grid) And ((i Mod 2)=0) Then
          Begin
            SetLineStyle(DottedN,0,NormalWidth);
            Line(x1,Yinc,x2,Yinc);
            SetLineStyle(SolidN,0,NormalWidth);
          End;
        If (i Mod 2)=0 Then
          Begin
            Label := (Ymax-i*SigFY);
            Labval := RealToStr(Labval,SigFY);
            OutTextXY(x1-TextWidth('M')/4,Yinc,LabelStr);
          End;
        End;
        SetTextJustify(CenterText,BottomText);
        For i := 0 To 40 Do
          Begin
            Xinc := Trunc(x1+i*dx);
            If (i Mod 5)=0 Then tx := 6 Else tx := 2;
            Line(Xinc,y2,Xinc,y2-tx);
            If (Grid) And ((i Mod 5)=0) Then
              Begin
                SetLineStyle(DottedN,0,NormalWidth);
                Line(Xinc,y1,Xinc,y2);
                SetLineStyle(SolidN,0,NormalWidth);
              End;
            If (i Mod 5)=0 Then
              Begin
                Labval := Xmin+i*SigF;
                LabStr := RealToStr(Labval,SigF);
                OutTextXY(Xinc,(y2-TextHeight('M')/2),LabelStr);
              End;
            End;
          End;
        End;
      End;
    End;
  End;
  Draw any Text on Screen
End;
(-----)
Procedure DrawDrText(x,y,Direction,Font,Size,Color: Integer;
```

```
GrLabel: String255);
Begin
  SetTextJustify(CenterText,CenterText);
  SetTextStyle(Font,Direction,Size);
  SetColor(Color);
  OutTextXY(x,y,GrLabel);
  SetTextStyle(0,0,1);
End;
(-----)
(-----)
Plot Date on Screen
(-----)
(-----)
Procedure PlotData(D:DataArray; First,Last,
  PlotStyle,Color:Integer; Bipolar:Boolean);
(PlotStyle: 0=solid line,1=dotted line,2=centered line)
Var
  PlotNumData,i,dx,dy,Xp,Yp,FirstPoint,YaxisMax: Integer;
  ScaleX,ScaleY,Xinc: Real;
Begin
  SetLineStyle(PlotStyle,0,NormalWidth);
  SetColor(Color);
  PlotNumData := (Last-First)+1;
  With AxisData Do
    Begin
      ScaleX := (x2-ax1)/(amax-axmin);
      ScaleY := (y2-ay1)/(amay-aymin);
      Xinc := (x2-ax1)/(PlotNumData-1);
      Yp := ay1; YaxisMax := ay1;
      If Bipolar
        Then Yp := ay2-Round((ay2-ay1)/2)
        Else Yp := ay2;
      (x interval per data)
      (y max value)
    End;
    FirstPoint := Yp-Round(0(First)*ScaleY);
    If FirstPoint < YaxisMax Then FirstPoint := YaxisMax;
    MoveTo(Xp,FirstPoint);
    For i := 0 To PlotNumData-1 Do
      Begin
        dy := Yp-Round(0(First+i)*ScaleY);
        dx := Xp-Round(i*Xinc);
        If Not Bipolar And (dy > Yp) Then dy := Yp;
        If dy < YaxisMax Then dy := YaxisMax;
        LineTo(dx,dy);
      End;
    End;
  End;
(-----)
(-----)
End of TPLIB Unit
(-----)
(-----)
```

APPENDIX B

**SOURCE CODE OF
EMISPEC-II**

```

*****
*
*      EMISPEC-II LUMINESCENCE DATA STATION
*
*      Version 3.0 Main Program
*
*      Written by Maldong Kim
*
*      Department of Chemistry, Michigan State University
*
*****
* This program must be compiled with unit EMISP.PAS and IPLIB.PAS *
*****
($M 55360,0,655360)

```

Program EMISPEC;

Uses Crt,Dos,Printer,Graph,IplIb,Emisp;

Procedure MultiPlot; Forward;

Function Pow(a,b: Real): Real;
(Returns a value of b power of a)

```

Begin
  Pow := Exp(b*Ln(a));
End;

```

Procedure HelpGraph;

```

Begin
End;

```

Procedure Help;
(Print Help message on the screen. This routine
can be called on MainMenu,Spectrum graph routines.)

Var Ch : Char;

```

Begin
  OpenWindow(8);HeaderOn(8);
  GotoXY(2,2);Write(' F1 : Help');
  GotoXY(2,3);Write(' F2 : Test Data Read');
  GotoXY(2,4);Write(' F3 : Save Screen');
  GotoXY(2,5);Write(' F4 : Print Graph');
  GotoXY(2,6);Write(' F5 : Plot Multiple Data');
  GotoXY(2,7);Write(' F6 : Change Plot Range');
  GotoXY(2,8);Write(' Esc : Return to Main Menu');
  Ch := ReadKey;
  CloseWindow;
End;

```

Procedure ResetADC(ADCNode: Integer);
(This procedure resets LabMaster data acquisition board with
the ADCNode and Channel specified.)

```

Var
  Reset: Integer;
Begin

```

```

  Port(ADCControl) := ADCNode;
  Port(ADCChannel) := Channel;
  Delay(1);
  Port(ADCStart) := 0;
  Repeat
    Done := Port(ADCControl);
  Until Done > 128;
  Reset := Port(ADCStart);
  Delay(1);
End;

```

Function ReadWavelength(Channel: Integer):Real;
(This function reads current wavelength(nm) specified by the
channel number(13: Excitation,14: Emission) and returns current
wavelength(nm).)

```

Var
  Datum: Integer;
  PWavelength,SumpWave: Real;
Begin
  ADCNode := 128;
  ResetADC(ADCNode,Channel);
  SumpWave := 0;
  For i := 1 To 50 Do
    Begin
      Port(ADCStart) := 0;
      Repeat
        Done := Port(1812);
      Until Done > 128;
      LowByte := Port(1813);
      HighByte := Port(1814);
      Datum := 256 * HighByte + LowByte;
      PWavelength := 200 + (Datum/406)*1000; (12 bit, 0 V=200 nm)
      SumpWave := SumpWave + PWavelength;
    End;
    ReadWavelength := SumpWave/50;
  End;

```

Procedure Scan(ScanNH: Real);
(This procedure scans monochromator(s) selected by InitScan
procedure ScanNH at a time)

```

Var
  ScanStep: Integer;
Begin
  ScanStep := Round(4*ScanNH);
  For i := 1 To Abs(ScanStep) Do
    Begin
      For j := 1 To 5 Do
        Begin
          Port(ParlPortA) := 0;
          Delay(1);
          Port(ParlPortA) := 1;
          Delay(1);
        End
      End
    End;
  End;

```

Procedure InitScan;
(InitScan initializes both monochromators specified by
ScanNode, and scans both monochromators to the starting wavelength.)

```

Var
  ScanNH: Integer;
Begin
  DialogueA;

```

```

Msg(15,3,Black,'Initializing Monochromators .....');
Port[ParCtrl] := $80;      (Set 8255A to mode A for monochromator)
Channel := 13;             (Excitation wavelength port)
ScanM := Ekm - Round(ReadLavelength(Channel));
If ScanM > 0
  Then Port[ParPortA] := 10 (forward direction)
  Else Port[ParPortA] := 26; (Reverse direction)
Scan(ScanM);
Channel := 14;             (Emission wavelength port)
ScanM := Ekm - Round(ReadLavelength(Channel));
If ScanM > 0
  Then Port[ParPortA] := 6 (forward direction)
  Else Port[ParPortA] := 22; (Reverse direction)
Scan(ScanM);
Port[ParPortA] := 14;      (Disengage both monochromators)
Msg(15,3,Black,'Press ENTER to start data reading. ');
ReadLn; CloseWindow;
Case ScanMode Of
  1 : Port[ParPortA] := 6;      (Emission mode)
  2 : Port[ParPortA] := 10;     (Excitation mode)
  3 : Port[ParPortA] := 2;      (Synchronous mode)
End;
(End InitScan)

Procedure WaitKey;
Begin
  Repeat
    Ch := ReadKey;
    If Ch = #0
    Then Begin
      Ch := ReadKey;
      If (Ch = #62) Then PrintScreen(False,6);
    End;
  Until (Ch = #27);
  ExitProcess;
  HaltMenuScreen;
End;

Procedure PhsTimer;
(AMD 9513 timer is set to proper mode for phosphorescence
data collection. For phosphorescence measurement Mode C is
used to trigger the A/D start conversion.)
Begin
  Port[TimerCtrl] := 23;      (Set up Data Pointer register to point
to Master mode register(10111))
  Port[TimerData] := 0;      (Set up Master Mode(BCD,disable increment)
comparators and 100(1100000100000000).)
  Port[TimerData] := 193;    (8 bit,FOUT on,divide by 1,F1 source,disable
comparators and 100(1100000100000000).)
  Port[TimerCtrl] := 5;      (Set CounterMode of counter 5(Active low edge)
of Gate5,count on rising edge of F1,disable)
  Port[TimerData] := 1;      (of Gate5,count on rising edge of F1,disable)
  Port[TimerData] := 235;    (special gate,reload from load,count once,binary
count,count down,active high TC(11101011000000001))
  Port[TimerCtrl] := 13;     (Select Load register 5)
  Port[TimerData] := Lo(Gate); (Load gate time(minimum 28 usec))
  Port[TimerData] := Hi(Gate); (Load gate time(maximum 255 usec))
  Port[TimerCtrl] := 80;     (Get higher byte)
  Port[TimerCtrl] := 80;     (Load counter 5)
End;

Procedure LtmTimer;
(Setup timer for phosphorescence lifetime measurement.
OUT3 must be connected to external start conversion of A/D
and photodiode signal must be connected to Gate4 and OUT4
must be connected to GATE3.)
Begin
  Port[TimerCtrl] := 23;      (Set data pointer to master mode reg.)
  Port[TimerData] := 0;      (Master mode(BCD,disable increment),
FOUT on,divide by 1,F1 source,disable
both comparators and 100.)
  Port[TimerCtrl] := 4;      (Counter mode of counter4(Mode C)
of F1,disable special gate,reload from
load,count once,binary count,count down
TC toggled: Counter4 controls delay time)
  Port[TimerData] := 235;    (Counter mode of counter3(Mode E)
of F1,disable special gate,
rising edge of F1,disable special gate,
reloaded from load,repitive binary count,
down,active high TC: Counter3 controls sampling rate.)
  Port[TimerCtrl] := 12;     (Load counter4 with delay time(IDelay))
  Port[TimerData] := Lo(IDelay);
  Port[TimerCtrl] := Hi(IDelay);
  Port[TimerCtrl] := 11;     (Load counter3 with gate time(usec))
  Port[TimerData] := Lo(Gate);
  Port[TimerData] := Hi(Gate);
End;

Procedure GetAverage;
(This procedure averages data which is in buffer. The maximum number
of data to be collected at a position is 1000. After get average value
it is stored into data array specified by Index.)
Var
  SumData,Average: Real;
Begin
  SumData := 0;
  For I := 1 To NumData Do
    SumData := SumData + Buffer(I);
  Average := (SumData/NumData)/4.096;
  If (ScanMode = 4) And (ExScanStep Mod 2 = 0)
  Then Data[ScanRange+1-Index] := Average
  Else Data[Index] := Average;
End;

(SL ADCR)
Procedure ReadData(Var Buffer:Integer; Var Count,DataType:Integer);
External;

(This routine is a machine language code for speedy
data collection. Each data type must be specified by
DataType(1=fluorescence,2=phosphorescence,3=Lifetime).)

Procedure ReadFLS;
(This procedure reads fluorescence data at a given position.
The NumData is the number of data to be collected at a position.)
Begin
  ResetADC(ADCMode,Channel);
  Count := NumData;
  DataType := 1;
  ReadData(Buffer,Count,DataType);
End;

Procedure ReadPHS;
(Chopper signal is used to trigger the ADC start conversion.
FOUT 5 must be connected to ADC external start gate.)
Begin
  ResetADC(ADCMode,Channel);

```

```

Count := NumData;
DataType := 2;
ReadData(Buffer, Count, DataType)
End;

```

Procedure ReadData;
(Phosphorescence decay data are collected and plotted on the screen.)

```

Var
  X : Integer;
  SumBuffer : Array[1..1000] Of Real;

Begin
  ConvertParam;
  Dialogues;
  Count := NumData; DataType := 3;
  For j := 1 To NumData Do
    SumBuffer[j] := 0; (Initialize SumBuffer)
  For i := 1 To NumDecay Do
    Begin
      GotoXY(12,3); Write('Current decay : ', i);
      ResetADC(ADCCode, Channel);
      ReadData(Buffer, Count, DataType);
      For j := 1 To NumData Do
        SumBuffer[j] := SumBuffer[j] + (Buffer[j] / 4.096)
      End;
    End;
  For i := 1 To NumData Do
    Data[i] := SumBuffer[i] / NumDecay;
  DataLoaded := True; Grinitalize;
  DrawPlotAxis(90, 20, 600, 250, First, Last, 0, 1000, True);
  PlotData(Data, 1, TotalData, 0, White, False);
  WaitKey;
End;

```

Procedure FPSPlot;
(This routine plots fluorescence and phosphorescence data on the screen in real time. It first reads emission data and at each 1 nm increment data are plotted on the screen.)

```

Var
  Ch : Char;
  ScaleX, ScaleY, Xinc: Real;
  dx, dy, Ap, Yp, PlotStep, StartPoint: Integer;

Begin
  ConvertParam;
  Grinitalize;
  DrawPlotAxis(90, 20, 600, 250, First, Last, 0, 1000, True);
  DrawGrText(15, 140, 1, 0, 1, White, 'Relative Intensity');
  DrawGrText(250, 265, 0, 1, White, 'Wavelength(nm)');
  ScaleX := (600-90) / (Last-First);
  ScaleY := (250-20) / 1000;
  Xinc := (600-90) / TotalData;
  Yp := 90; Yp := 250;
  For Index := 1 To TotalData Do
    Begin
      Case Mode of
        1 : ReadFLS;
        2 : ReadPLS;
      End; (Case)
    End;
    GetAverage;
    dx := Yp * Round((Data[Index] * ScaleY)); (Y-coordinate)
    dx := Xp * Round((Index * Xinc)); (X-coordinate)
    PutPixel(dx, Yp, White);
    If KeyPressed then

```

```

      Begin
        Ch := ReadKey;
        If (Ch = Chr(27)) Then
          Begin
            ExitProcess;
            MainMenuScreen;
            Exit;
          End;
        End;
        Scan(0.5);
        End;
        WaitKey;
      End;
      (Scan 0.5 nm forward)
      (Scanning and plotting)

      Procedure InitEEM;
      (Draws 3-D graph window for EEM spectrum.)

      Var
        X, dx1, dy1, dx2, dy2 : Integer;
        Y : String[6];

      Begin
        ConvertParam; Grinitalize;
        Line(49, 116, 49, 315);
        Line(49, 315, 438, 315);
        Line(438, 315, 598, 211);
        dy1 := 315;
        For i := 0 To 10 Do ( y ticks)
          Begin
            dx1 := 49; x := Round((EScan/10));
            For j := 0 To 10 Do ( x ticks)
              Begin
                dx2 := Trunc(dx1 + 39 * i);
                Line(dx2, 315, dx2, 320);
                Str(Emm + i * x, Y);
                OutTextXY(dx2 - TextWidth('Y'), 315 + TextWidth('Y'), Y);
              End;
            OutTextXY(46 - TextWidth('Y') * 4, dy2 - TextWidth('Y'), Y);
            dx1 := 49; x := Round((EScan/10));
            For j := 0 To 10 Do ( x ticks)
              Begin
                dx2 := Trunc(dx1 + 39 * i);
                Line(dx2, 315, dx2, 320);
                Str(Emm + i * x, Y);
                OutTextXY(dx2 - TextWidth('Y'), 315 + TextWidth('Y'), Y);
              End;
            x := Round((EScan/10));
            For i := 0 To 10 Do
              Begin
                Str(Emm + i * x, Y);
                dx1 := Round(438 + i * 16); dy1 := Round(315 - i * 10.4);
                dx2 := Round(445 + i * 16);
                Line(dx1, dy1, dx2, dy1);
                OutTextXY(445 + i * 16 + TextWidth('Y'), 310 - i * 10, Y);
              End;
            DrawGrText(280, 340, 0, 0, 1, White, 'EMISSION(nm)');
            DrawGrText(580, 295, 0, 0, 1, White, 'EXCITATION(nm)');
            End;
          End;
        Procedure TotalScan;
        (This routine collects data over the emission ScanRange at each
        excitation wavelength and continues over the EScan Excitation
        wavelength range to plot 3-D spectrum.)

        Var
          Ch : Char;
          i, x1, y1, x2, y2, db, n, d, e, f, g, h, Plot : Integer;
          Zmax: Array[1..600] of Integer;
          x, y, da, dx, dy, dz, day: Real;

```

05/31/1989 00:32	Filename: EMISPEC.PAS	Page	
<pre> InitEEM; ConvertParam:OutputDate; For d := 1 to 600 Do Zmax(d) := 310; dx := 389/ScanRange; dy := (106/(ExScan/ExScanStep)); dz := 0.199; dxy := (160/(ExScan/ExScanStep)); dda := (day/dx); ddb := Round(0.199*dxy); For i := 1 to trunc(ExScan/ExScanStep)*1 Do Begin Delay(100); If i Mod 2 = 0 Then Port[ParPortA] := 22 Else Port[ParPortA] := 6; For Index := 1 to ScanRange Do Begin ReadKey; GetAverage; If KeyPressed Then Begin Ch := ReadKey; If (Ch=80) Then Begin ExitProcess; HaltMenuScreen; Exit; End; End; End; Scan(1.0); End; If (i = 1) Then For e := 1 to ScanRange Do Begin Zmax(e) := Round(310-dz*Data(e)); x1 := Round(50+e*dx); y1 := Round(Zmax(e)); (Convert into) x2 := Round(50+(e-1)*dx); y2 := Round(Zmax(e-1)); Line(x1,y1,x2,y2); End Else Begin x := 50+(i-1)*dx; y := 310-(i-1)*dy; h := Round(1*da); For g := 1 to ScanRange-1 Do Begin x1 := Round(x+(g-1)*dx); y1 := Round(y-dz*Data(g)); x2 := Round(x+g*dx); y2 := Round(y-dz*Data(g+1)); If g >= (ScanRange-da) Then Begin If g = ScanRange-1 Then Plot := 4 Else Plot := 1; End Else Begin If (y1 < Zmax(g+h)) And (y2 < Zmax(g+h+1)) Then Plot := 1 Else (Plot full) If (y1 < Zmax(g+h)) And (y2 > Zmax(g+h+1)) Then Plot := 2 Else (Plot first half) If (y1 > Zmax(g+h)) And (y2 < Zmax(g+h+1)) Then Plot := 3 Else Plot := 0; (Plot second half) End; Case Plot Of 1 : Begin Zmax(g+h) := Round(y1); Line(x1,y1,x2,y2); End; End; End; End; End; End; End; End; End; Zmax(g+h) := Round(y2); Line(Round((x1+x2)/2),Round((y1+y2)/2),x2,y2) End; Zmax(g+h) := Round(y2); Line(Round((x1+x2)/2),Round((y1+y2)/2),x2,y2) End; 4 : Line(x1,y1,x2,Round(y)) End; (End Case) End; (End for g) End; (End Else) Append(OutFile); (Append data to disk file) For n := 1 to ScanRange Do WriteLn(OutFile,Data(n)); Close(OutFile); Port[ParPortA] := 10; Delay(100); If i < trunc(ExScan/ExScanStep)+1 Then Scan(ExScanStep); (Increment EXnm by ExScanStep) End; WaitKey; End; </pre>	<pre> 2 : Begin Zmax(g+h) := Round(y1); Line(x1,y1,Round((x1+x2)/2),Round((y1+y2)/2)) End; 3 : Begin Zmax(g+h) := Round(y2); Line(Round((x1+x2)/2),Round((y1+y2)/2),x2,y2) End; 4 : Line(x1,y1,x2,Round(y)) End; (End Case) End; (End for g) End; (End Else) Append(OutFile); (Append data to disk file) For n := 1 to ScanRange Do WriteLn(OutFile,Data(n)); Close(OutFile); Port[ParPortA] := 10; Delay(100); If i < trunc(ExScan/ExScanStep)+1 Then Scan(ExScanStep); (Increment EXnm by ExScanStep) End; WaitKey; End; </pre>	<pre> Procedure Fluorescence; (Fluorescence routine initializes various variables for fluorescence data collection.) Begin DataLoaded := False; InitScan; ADCHode := 128; Channel := 15; If ScanMode = 4 Then TotalScan Else FPSPlot; DataLoaded := True; End; </pre>	<pre> Procedure Phosphorescence; (Counter 5 waits Gate usec until it receive active low chopper signal and then start countdown to generate ADC external start conversion signal.) Begin DataLoaded := False; InitScan; Phatimer; ADCHode := 132; (ADC mode:disable autoIncrement and all) Channel := 15; (Interrupts,enable external start conv.) FPSPlot; DataLoaded := True; End; </pre>
<pre> 05/31/1989 00:32 Filename: EMISPEC.PAS Page </pre>	<pre> 05/31/1989 00:32 Filename: EMISPEC.PAS Page </pre>	<pre> 05/31/1989 00:32 Filename: EMISPEC.PAS Page </pre>	<pre> 05/31/1989 00:32 Filename: EMISPEC.PAS Page </pre>


```

Ch : Char;
Code,X: Integer;
Begin
  If Not DataLoaded Then
    Begin
      ShowError;
      Exit;
    End;
  ConvertPam;
  If ScanMode = 4
    Then Begin
      InputData;
      EmPlot;
    End
  Else
    Begin
      DialogueA;
      Msg(10,3,Black,'Enter the plot style(0: Line, 1: Dot): ');
      ReadIn(Code);ClosedInow;
      GrInitialize;
      DrawPlotAxis(90,20,600,250,First,Last,0,1000,True);
      PlotData(Data,1,TotalData,Code,White,False);
      DrawGrText(15,140,1,0,1,White,RELATIVE INTENSITY');
      If Mode = 3
        Then DrawGrText(350,285,0,0,1,White,'TIME(ussec)')
        Else DrawGrText(350,285,0,0,1,White,'WAVELENGTH(NM)');
      End;
      WaitKey;
    End;
End;

Procedure PrinterPlot;
Var Code: Integer;
Begin
  If Not DataLoaded Then
    Begin
      ShowError;
      Exit;
    End;
  If ScanMode = 4
    Then Begin
      InputData;
      EmPlot;
    End
  Else Begin
    DialogueA;GotoXY(10,3);
    Write('Enter plot style(0: Line, 1: Dot): ');
    ReadIn(Code);ClosedInow;
    GrInitialize;
    DrawPlotAxis(90,20,600,250,First,Last,0,1000,True);
    PlotData(Data,1,TotalData,Code,White,False);
    DrawGrText(15,140,1,0,1,White,RELATIVE INTENSITY');
    If Mode = 3
      Then DrawGrText(350,285,0,0,1,White,'TIME(ussec)')
      Else DrawGrText(350,285,0,0,1,White,'WAVELENGTH(NM)');
    End;
    PrintScreen(False,6);
    ExitProcess;
  End;
End;

Procedure DrawFrame(Start,Stop: Integer;PlotItA,PlotItB: StrL);
(Draw graph frame with plotter for data plot)
Var

```

```

Pld,P1x,X,Y: Integer;
Begin
  If Mode = 3
    Then Begin
      Pld := Round((Stop-Start)/10);
      Unitx := 'TIME(ussec)';
    End
  Else Unitx := 'WAVELENGTH(NM)';
  P1x := Round((LastPoint-FirstPoint)/10);
  Writeln(Com1,'IW:SPI:IP1250,2100,9250,7350:');
  Writeln(Com1,'SCI:FirstPoint,',LastPoint,',0,1000:');
  Writeln(Com1,'PU:FirstPoint,',0P0',LastPoint,',0,',LastPoint,
    ',1000,',FirstPoint,',1000,',FirstPoint,',0PU:');
  Writeln(Com1,'SI:15,22;TL1.5,0:');
  X := Start;
  If GoPlotted Then Repeat Until FreeSpace;
  For I := 0 To 10 Do
    Begin
      Writeln(Com1,'PA: X-Start,',0,XT:');
      Writeln(Com1,'CP:1,-1:');
      If Mode = 3
        Then Writeln(Com1,'LB',Start+I*Pld,Chr(3))
        Else Writeln(Com1,'LB',Start+Round((X-Start)/2),Chr(3));
      X := X+P1x
    End;
    Writeln(Com1,'PA6.5,0:CP:57,-2.5:LB',Unitx,Chr(3));
    Writeln(Com1,'SI:15,22;TL',7,0:');
    If GoPlotted Then Repeat Until FreeSpace;
    Y := 0;
    For I := 0 To 10 Do
      Begin
        Writeln(Com1,'PA: FirstPoint,',400:CP:0,0:D10,1;LBRELATIVE',
          ' INTENSITY',Chr(3),011,0:');
        If GoPlotted Then Repeat Until FreeSpace;
        Writeln(Com1,'PA: FirstPoint,',-200:SI:LB',PlotItA,Chr(3));
        Writeln(Com1,'PA: FirstPoint,',-250:LB',PlotItB,Chr(3));
      End;
    End;
  End;
  Procedure MultiPlotter(PlotStyle: Integer);
  (Plots multiple data on the same graph frame)
  Var
    X,Y: Integer;
  Begin
    If GoPlotted Then Repeat Until FreeSpace;
    For X := FirstPoint To LastPoint-1 Do
      Begin
        I := Round(Data(X+1));
        If PlotStyle = 0
          Then Writeln(Com1,'PA',X,',',Y,'PD')
          Else Writeln(Com1,'PA',X,',',Y,'PD:PU:');
        If GoPlotted Then Repeat Until FreeSpace;
      End;
      Writeln(Com1,'PU:');
      Writeln(Com1,'SP0:');
    End;
  End;
End;

```

Procedure StartPlot;
 (This routine plots data with HP7475A. Computer com1 used to
 communicate with plotter. Com1 DB25 connector must be configured
 as dnn-terminal by connecting pin 20 to pin 6 and pin 4 to pin
 5,6. Only three lines(2,3,7) is used for communication. Data flow

control is checked and controlled by software. HP7475A must be configured as 4800 baud Direct connection mode.)

```

Var
  StartStop,PlotStyle,PlotChoice: Integer;
  PlotItA,PlotItB: String[80];

Begin
  If PlotNum = 0
  Then Begin
    DialogueC:GotoXY(2,2);
    WriteLn('The range of this data is ',First,'-',Last);
    GotoXY(2,3);WriteLn('Enter the starting data point to plot: ');
    ReadLn(Start);GotoXY(2,4);
    WriteLn('Enter the last data point to plot : ');
    ReadLn(Stop);GotoXY(2,5);
    WriteLn('Enter the 1st line label(X-axis): ');
    GotoXY(2,6);ReadLn(PlotItA);GotoXY(2,7);
    WriteLn('Enter the 2nd line label(X-axis): ');
    GotoXY(2,8);ReadLn(PlotItB);
    GotoXY(2,9);WriteLn('Enter plot style(0: Line, 1: Dot) :');
    ReadLn(PlotStyle);
    Then Begin
      FirstPoint,LastPoint are the data array
      (number-1 to plot)
      FirstPoint := Round((Start-First)/Gate);
      LastPoint := FirstPoint+Frunc((Stop-Start)/Gate);
    End
  Else
    Begin
      FirstPoint := 2*(Start-First);
      LastPoint := 2*(Stop-Start)+FirstPoint;
    End;
    GotoXY(22,11);WriteLn('*** Working ***** ');
    InitPlotter;
    DrawFrame(Start,Stop,PlotItA,PlotItB);
    MultiPlotter(PlotStyle);
    PlotNum := PlotNum+1;
  End
  Else (If PlotNum > 0)
  Begin
    DialogueC;
    Msg(7,3,Black,'Enter plot style(0: Line, 1: Dot): ');
    ReadLn(PlotStyle);
    Msg(14,5,Black,'*** Working *****');
    WriteLn(Concat('SPI:'));
    MultiPlotter(PlotStyle);
  End;
  ClosedWindow;

  Procedure PlotterPlot;
  Label PlotAgain;
  Var
    Yes : Char;
    ExitPlot: Boolean;
  Begin
    If Not DataLoaded Then
      Begin
        ShowError;
        Exit;
      End;
      PlotNum := 0; GoPlotter := False;
      DialogueA;
      PlotAgain;
      If PlotNum = 0 Then
        Begin
          Msg(2,2,Black,'Enter plot file name: ');
          ReadLn(PlotFileName);
          If PlotFileName = '' Then
            Begin
              ClosedWindow;
              Exit;
            End;
          End;
          If ScanMode = 4 Then
            Begin
              InputData;
              TotalPlot;
              ClosedWindow;
              Exit;
            End
          Else StartPlot;
          Msg(1,3,Black,'Append another data to previous file? (y/n): ');
          ReadLn(Yes); Yes := UpCase(Yes);
          If Yes = 'Y' Then

```

```

Begin
  Mag(1,4,Black,'Enter data file name: ');ReadLn(Filename);
  InputData;
  Goto PlotAgain;
End
Else CloseWindow;
End;

Procedure LogPlot;
(Plots decay data in log unit)

Var
  X,Y : Integer;
  TempData : DataArray;
Begin
  If Not DataLoaded Then
    Begin
      ShowError;
      Exit;
    End;
  For I := 1 To TotalData Do
    TempData(I) := Ln(Data(I));
  Grinitalize;
  DrawPlotAxis(90,20,600,250,First,Last,0,10,True);
  DrawGrText(15,140,0,1,White,'RELATIVE INTENSITY(LN)');
  DrawGrText(350,285,0,1,White,'TIME(USEC)');
  PlotData(TempData,1,NumData,0,White,False);
  WaitKey;
End;

Procedure MulPlot;
(MulPlot plots multiple data on the same graphics screen.)

Label GotoExt;
Var
  Filespec : Array [1..5] of String[15];
  I,Code,NumFiles,OutputPort : Integer;
Begin
  Dialogues; NumFiles := 0;
  Write(' Output to (0: Screen, 1: Printer, 2: Plotter): ');
  ReadLn(OutputPort);
  For I := 1 To 5 Do Begin
    Write(' Data file name(' ,I, '): ');ReadLn(Filespec(I));
    If Filespec(I) = '' Then Goto GotoExt
  Else NumFiles := NumFiles + 1;
  End;
GotoExt:
  If NumFiles = 0 Then Exit;
  Case OutputPort of
    0,1: Begin
      CloseWindow;
      Grinitalize;
      FileName := Filespec(1);
      InputData;ConvertPar;
      DrawPlotAxis(90,20,600,250,First,Last,0,1000,True);
      DrawGrText(15,140,0,1,White,'RELATIVE INTENSITY');
      If Mode = 3
      Then DrawGrText(350,285,0,1,White,'TIME(USEC)');
      Else DrawGrText(350,285,0,1,White,'WAVELENGTH(NM)');
      If Not (NumFiles = 0) Then
        For I := 1 To NumFiles Do
          Begin
            If (I Mod 2 = 0)
            Then Code := 0 Else Code := 1;

```

```

      FileName := Filespec(I);
      InputData;ConvertPar;
      PlotData(Data,1,TotalData,Code,White,False);
    End;
  If OutputPort = 1 Then PrintScreen(false,6);
  WaitKey;
End;
3:Begin
  PlotNum := 0;
  WriteLn;WriteLn;WriteLn;
  Write(' .....');
  For I := 1 To NumFiles Do
    Begin
      FileName := Filespec(I);
      InputData;ConvertPar;
      PlotterPlot;
    End;
  CloseWindow;
  End; (End case)
End;

Procedure CalcLifetime;
(Calculate lifetime,initial decay signal, and background.
See Cline Love et al,1964,56,1984, Anal., Chem.)

Var
  Dt : Integer;
  Sum1,Sum2,Sum3,Y,R : Real;
Begin
  If Not DataLoaded Then
    Begin
      ShowError;
      Exit;
    End;
  If Mode <> 3 Then Exit;
  Dialogues;
  Dt := Trunc(TotalData/3);
  Sum1 := 0; Sum2 := 0; Sum3 := 0;
  For I := 1 To Dt Do
    Begin
      Sum1 := Sum1 + Data(I);
      Sum2 := Sum2 + Data(I+Dt);
      Sum3 := Sum3 + Data(I+2*Dt);
    End;
  Y := (Sum2 - Sum3)/(Sum1 - Sum2);
  Lifetime := -(Dt*Gate)/Ln(Y);
  R := Sqr(Sum1-Sum2) / (Sum1-2*Sum2+Sum3);
  Background := (Sum1 - R)/Dt;
  DConst := R*(1-Pow(Y,1/Dt)) / (1-Y);
  GotoXY(10,2);Write(Lifetime(USEC));
  GotoXY(10,3);Write(Background);
  GotoXY(10,4);Write(Decay Amplitude);
  GotoXY(10,5);Write('Press ENTER key to continue. ');
  ReadLn;CloseWindow;
End;

Procedure SimLifetime;
(Simulate decay data with given parameters.)

Var
  Lifetime,Background,Idconst: Real;
Begin
  Dialogues;GotoXY(16,1);
  Write('Enter 0 to use default value!');GotoXY(2,2);

```

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Write('Enter Lifetime(uscc) : '); ReadLn(TLifetime);
If TLifetime < 0 Then TLifetime := 1; ReadLn(TLifetime);
Write('Enter Background signal : '); ReadLn(TBackground);
If TBackground < 0 Then TBackground := TBackground; GotoXY(2,4);
Write('Enter Decay Amplitude : '); ReadLn(TDconst);
If TDconst < 0 Then TDconst := TDconst;
GotoXY(22,6); Write('Working .....');
For I := 0 To TotalData-1 Do
  Data[I] := Round(TDconst*Exp(-(I*Gate)/LTifetime)
    +Background);
GotoXY(15,6); Write('Done! Press any key to continue. ');
Ch := ReadKey; CloseWindow;
End;

Procedure MainMenu;
Begin
  MainMenuScreen;
  Repeat
    Action1 := BarMenu(1,7,White,Black,
      File Data Setup Graph Life_Time Tools Exit);
    Case Action1 of
      1: Begin
        Action2 := PullDownMenu(1,4,
          'Load Data', 'Save Data', 'List File', 'Dos Shell', '',
          '', '', '', ' ');
        Case Action2 of
          1: LoadData;
          2: SaveData;
          3: ListDir;
          4: Exec('command.com', '');
        End;
      End;
    2: Begin
      Action2 := PullDownMenu(2,4, 'Display', 'Print', 'Smoothing',
        'Start Read', '', '', '', '', ' ');
      Case Action2 of
        1: DisplayData;
        2: PrintData;
        3: FilterData;
        4: AcquireData;
      End;
    3: InputSetup;
    4: Begin
      Action2 := PullDownMenu(3,5, 'Screen Plot', 'Printer Plot',
        'Plotter Plot', 'Plotter File', 'Multiple Plot', '', '', ' ');
      Case Action2 of
        1: ScreenPlot;
        2: PrinterPlot;
        3: PlotterPlot;
        4: PlotterFile;
        5: MulPlot;
      End;
    End;
    5: Begin
      Action2 := PullDownMenu(4,3, 'Calculate Life Time',
        'Simulate Life Time', 'Seallog Plot', '', '', ' ');
      Case Action2 of
        1: CalcLifetime;
        2: SimLifetime;
        3: LogPlot;
      End;
    End;
  6: Begin
    Action2 := PullDownMenu(5,3, 'Help',
      'Change Parameter', 'Test Data Read', '', '', ' ');
    Case Action2 of
      1: Help;
      2: InputSetup;
      3: Tester;
    End;
  7: Begin
    OpenWindow(6);
    Write('Are you sure? (y/n) ');
    Ch := ReadKey; Ch := UpCase(Ch);
    If Ch = 'Y'
      Then Begin
        FullScreen(Black,White,True);
        ClrScr; Halt;
      End
    Else CloseWindow;
  End;
  (End Case Action1)
Until Action1 = 10;
End;

Begin
  (Main)
  FirstSetup := True;
  DataLoaded := False;
  DefinedIndows;
  MainMenu;
End.

```



```

DefineField(14,White,Black,ID2,'141422063A');
End;

Procedure ConvertParam;
Begin
  If Mode = 3 Then
    Begin
      First := TDelay + Gate;      (Start reading from 1st gate)
      Last := TDelay + (NumData)*Gate;
      TotalData := NumData;
    End
  Else
    Begin
      Case ScanMode of
        1,3,4:Begin
          ScanRange := EmScan;
          TotalData := 2*EmScan;
          First := EmNm;
          Last := EmNm + EmScan;
        End;
        2 : Begin
          ScanRange := ExScan;
          TotalData := 2*ExScan;
          First := ExNm;
          Last := ExNm + ExScan;
        End;
      End;
    End;
  End;
End;

Procedure ExtractSetup;
(Extract integer setup values)
Begin
  FileName := DF.FileName;
  Val(DF.Mode,Mode,ErrorCode);
  Val(DF.ExNm,ExNm,ErrorCode);
  Val(DF.Exm,Exm,ErrorCode);
  Val(DF.NumData,NumData,ErrorCode);
  Val(DF.ScanMode,ScanMode,ErrorCode);
  Val(DF.ExScan,ExScan,ErrorCode);
  Val(DF.ExScanstep,ExScanstep,ErrorCode);
  Val(DF.TDelay,TDelay,ErrorCode);
  Val(DF.Gate,Gate,ErrorCode);
  Val(DF.NumDecay,NumDecay,ErrorCode);
End;

Procedure FillSetup;
(Extract string setup values and fill DF)
Begin
  DF.FileName := FileName;
  Str(Mode,DF.Mode);
  Str(ExNm,DF.ExNm);
  Str(Exm,DF.Exm);
  Str(NumData,DF.NumData);
  Str(ScanMode,DF.ScanMode);
  Str(EmScan,DF.ExScan);
  Str(ExScanstep,DF.ExScanstep);
  Str(TDelay,DF.TDelay);
  Str(Gate,DF.Gate);
  Str(NumDecay,DF.NumDecay);

```

```

End;

Procedure SetupDefault;
Var I: Integer;
Begin
  With DF Do
    Begin
      FileName := ''; Mode := '1';
      ExNm := '300'; Exm := '300';
      ScanMode := '1'; EmScan := '100';
      ExScan := '0'; ExScanstep := '0'; NumData := '100';
      TDelay := '0'; Gate := '0'; NumDecay := '0';
      ID1 := '1'; ID2 := '1';
    End;
  End;
End;

Procedure WriteSetup;
Begin
  DrawFindBox(2,14,70,24,2,Black,White);
  Window(1,80,25); GotoY(25,13);
  InvVideo('Data Acquisition Mode Set-up ');
  Msg(3,15,White,Mode(1:FS,2:PS,3:LT)); Write(FileName);
  Msg(3,16,White,ExNm(1:FS,2:PS,3:LT)); Write(Mode);
  Msg(3,17,White,Exm(1:FS,2:PS,3:LT)); Write(ExNm);
  Msg(3,18,White,EmScan(1:FS,2:PS,3:LT)); Write(Exm);
  Msg(3,19,White,ExScan(1:FS,2:PS,3:LT)); Write(NumData);
  Msg(3,20,White,ExScanstep(1:FS,2:PS,3:LT)); Write(ID1);
  Write(ScanMode);
  Msg(3,21,White,Comment 1: ''); Write(DF.ID1);
  Msg(3,22,White,Comment 2: ''); Write(DF.ID2);
  Msg(3,23,White,EM Scan Range(nm)); Write(EmScan);
  Msg(3,24,White,EM Scan Range(m)); Write(ExScan);
  Msg(3,25,White,EM Scan Range(mm)); Write(ExScanstep);
  Msg(3,26,White,EM Scan Range(um)); Write(TDelay);
  Msg(3,27,White,EM Scan Range(nm)); Write(Gate);
  Msg(3,28,White,EM Scan Range(mm)); Write(NumDecay);
  Msg(3,29,White,EM Scan Range(um)); Write(NumDecay);
End;

Procedure Ask_Exit;
Begin
  DelLine(Black,24,10,70);
  Msg(26,24,White,'Do you want to save? (y/n): ');
  Ch := ReadKey; Ch := UpCase(Ch);
  If (Ch = 'Y') Then Change := False Else Change := True;
  If Change Then
    Begin
      DelLine(Black,24,10,70);
      GotoXY(10,24); InvVideo('F10'); Write(' Delete Entry');
      GotoXY(35,24); InvVideo('Esc'); Write(' Exit Set-up');
    End;
  End;

Procedure InputSetup;
(Set up data acquisition mode)
Label SetupAgain;
Begin
  GotoXY(10,24); InvVideo('F10'); Write(' Delete Entry');
  GotoXY(35,24); InvVideo('Esc'); Write(' Exit Set-up');
  DefineSetupFields;

```



```

Begin
  For i := 0 To TotalData-1 Do
    AnyPlot(i,1) := (Delay + (i+1)*Gate);
  End
Else
  Begin
    For i := 0 To TotalData-1 Do
      AnyPlot(i,1) := (First + i*0.5);
    End;
  End;
  For i := 0 To TotalData-1 Do
    AnyPlot(i,2) := Data[i*(1)]; (Make Y coordinate array)
  End;
  Procedure ListDir;
  Var
    Count,NextCol,NextRow: Integer;
    Filespec: String[12];
    DirInfo: SearchRec;
  Begin
    DialogBoxA;
    Msg(2,2,8,Black,'Enter the filespec. to list: ');
    Readln(Filespec);
    CloseWindow;
    DialogBoxB;
    If Filespec = '' Then Filespec := '.*';
    FindFirst(Filespec,Archive,DirInfo);
    Count := 0; NextCol := 4; NextRow := 1;
    While DosError = 0 Do
      Begin
        If NextRow = 14 Then
          Begin
            WriteLn;WriteLn;
            Write('Press any key for next page.....');
            Ch := ReadKey;
            NextCol := 4;NextRow := 1;
            Count := 0;ClrScr;
          End;
          Count := Count+1;
          GotoXY(NextCol,NextRow);Write(DirInfo.Name);
          If Count = 5 Then
            Begin
              Count := 0;
              NextCol := 4;NextRow := NextRow+1;
            End
            Else
              NextCol := NextCol+14;
              FindNext(DirInfo);
            End;
          WriteLn;WriteLn;
          Msg(20,20,Black,'Press ENTER to continue.....');
          Readln;
          CloseWindow;
        End;
      End;
    Procedure InputData;
    ($I-)
    Begin
      If FileName = '' Then Exit;
      Assign(InFile,FileName);
      Reset(InFile); ErrorCode := IOResult;
      If ErrorCode <> 0 Then Exit;
      Readln(InFile,FileName);Readln(InFile,Mode);
      Readln(InFile,ExNm);Readln(InFile,ExNm);
      Readln(InFile,NumData);Readln(InFile,ScanMode);
    End;
    ($I-)
    Begin
      If FileName = '' Then Exit;
      Assign(OutFile,FileName);
      Rewrite(OutFile); ErrorCode := IOResult;
      If ErrorCode <> 0 Then Exit;
      WriteLn(OutFile,FileName);WriteLn(OutFile,Mode);
      WriteLn(OutFile,ExNm);WriteLn(OutFile,ExNm);
      WriteLn(OutFile,NumData);WriteLn(OutFile,ScanMode);
      WriteLn(OutFile,ExScan);
      WriteLn(OutFile,ExScanStep);
      WriteLn(OutFile,TDelay);WriteLn(OutFile,Gate);
      WriteLn(OutFile,NumDecay);WriteLn(OutFile,DF.ID1);WriteLn(OutFile,DF.ID2);
      WriteLn(OutFile,First);WriteLn(OutFile,Last);
      WriteLn(OutFile,TotalData);
      WriteLn(OutFile);
      For i := 1 To TotalData Do
        WriteLn(OutFile,Data[i]);
      Close(OutFile)
    End;
    ($I+)
    Procedure SaveData;
    (Save data on disk as a file)
    Label Done;
    Begin
      DialogBoxA;
      If ScanMode = 4 Then
        Begin
          Msg(2,5,Black,'*** Data already saved! Press any key. ');
          Repeat Until KeyPressed;
          Exit;
        End;
        Msg(2,2,Black,'Enter the file name to be saved: ');
        Readln(FileName);
        If FileName = '' Then Goto Done;
        Msg(2,5,Black,'Saving data to disk .....');
        OutputData;
        Done;
        CloseWindow;
      End;
    End;
    Procedure LoadData;
    ($I-)
    Begin
      If FileName = '' Then Exit;
      Assign(InFile,FileName);
      Reset(InFile); ErrorCode := IOResult;
      If ErrorCode <> 0 Then Exit;
      Readln(InFile,FileName);Readln(InFile,Mode);
      Readln(InFile,ExNm);Readln(InFile,ExNm);
      Readln(InFile,NumData);Readln(InFile,ScanMode);
    End;
  End;
End;

```

(This procedure can load either screen data or text data)
(81-)

Label Done;

Begin

Msg(2,2,Black,'Enter the file name to be loaded: ');
Readln(fileName);

If fileName = '' Then Goto Done;

Msg(2,5,Black,' Loading data from disk');

FileSetUp;

ConvertPars;

FirstSetup := False;

DataLoaded := True;

Done;

CloseWindow;

WriteSetup;

(81+)

End;

Procedure DisplayData;
(Output data to screen.)

Var

Ys : Char;

More : Boolean;

RawDataX,RawDataY: String(6);

Start,Stop: Integer;

Begin

If Not DataLoaded Then

Begin

ShowError;

Exit;

End;

If ScanMode = 4 Then Exit;

More := True;

Repeat

Dialogues;GotoXY(2,2);

WriteLn('The range of this data is ',First,' - ',Last);

GotoXY(2,3);

Write('Enter the starting data point: ');Readln(Start);

GotoXY(2,4);

Write('Enter the last data point : ');Readln(Stop);

CloseWindow;ClrScr;

WriteLn;WriteLn;

For i := 1 To 80 Do

Write(Chr(205));WriteLn;

WriteLn('File Name: ',FileName);

WriteLn('Comment: ',pf.ID1);WriteLn(pf.ID2);

WriteLn('First data point: ',First,' ',Last data point: ',Last);

If Mode = 3 Then

WriteLn('Delay Time(usec): ',Tdelay,' ',Gate Time(usec): ',Gate);

For i := 1 To 80 Do

Write(Chr(205));

WriteLn('Data Pts. Data Pts. Data Pts. Data');

For i := 1 To 80 Do

Write(Chr(205));

WriteLn('Data Pts. Data Pts. Data Pts. Data');

WriteLn;WriteLn;

FirstPoint := Round((Start-First)/Gate);

LastPoint := FirstPoint + Trunc((Stop-Start)/Gate);

End
Else
Begin
FirstPoint := 2*(Start-First);
LastPoint := 2*(Stop-Start) + FirstPoint;

End;

For i := FirstPoint To LastPoint Do

Begin

Str(ArYPlot(1,1):6:1,RawDataX);

Str(ArYPlot(1,2):6:1,RawDataY);

Write(RawDataX,RawDataY, '');

If i = LastPoint Then WriteLn

End;

For i := 1 To 80 Do

Write(Chr(205));

Write('More? (y/n): ');Readln(Ys);

Ys := UpCase(Ys);

If (Ys = 'N') Then More := False;

Until Not More;

MainMenuScreen;

End;

Procedure PrintData;

(Print data with printer)

Var

Ys : Char;

RawDataX,RawDataY: String(6);

Start,Stop,ErrorCode: Integer;

Begin

If Not DataLoaded Then

Begin

ShowError;

Exit;

End;

If ScanMode = 4 Then Exit;

Dialogues;GotoXY(2,2);

WriteLn('The range of this data is ',First,' - ',Last);

GotoXY(2,3);

Write('Enter the starting data point: ');Readln(Start);

GotoXY(2,4);

Write('Enter the last data point : ');Readln(Stop);

GotoXY(16,6);

Write('Printing data');

WriteLn(Last); ErrorCode := IResult;

If ErrorCode <> 0 Then

Begin

ShowError;

CloseWindow;

Exit;

End;

WriteLn(Last);

For i := 1 To 78 Do

Write(Last,' ');WriteLn(Last);

WriteLn(Last,'File Name: ',FileName);

WriteLn(Last,'Comment: ',pf.ID1);WriteLn(Last,PF.ID2);

WriteLn(Last,'First data point: ',First,' ',Last data point: ',Last);

If Mode = 3 Then

WriteLn(Last,'Delay Time(usec): ',Tdelay,' ',Gate Time(usec): ',Gate);

For i := 1 To 78 Do

Write(Last,' ');

WriteLn(Last);

WriteLn(Last,'Data Pts. Data Pts. Data Pts. Data');

For i := 1 To 78 Do

Write(Last,'Data Pts. Data Pts. Data Pts. Data');

WriteLn;WriteLn;


```

Write(Lst,'');
WriteLn(Lst);
MakeArray;
If Mode = 3 Then
  Begin
    FirstPoint := Round((Start-First)/Gate);
    LastPoint := FirstPoint + Round((Stop-Start)/Gate);
  End
Else
  (FLS,PHS data)
Begin
  FirstPoint := 2*(Start-First);
  LastPoint := 2*(Stop-Start) + FirstPoint;
End;
For I := FirstPoint To LastPoint Do
  Begin
    Str(ArYPlot(I,1),6:1,RawDataX);
    Str(ArYPlot(I,2),6:1,RawDataY);
    Write(Lst,RawDataX,' ',RawDataY,' ');
    If (I-1) Mod 4 = 0 Then WriteLn(Lst);
    If I = LastPoint Then WriteLn(Lst)
  End;
For I := 1 To 78 Do
  Write(Lst,' ');
  WriteLn(Lst);WriteLn(Lst);
CloseWindow;
End;

Procedure FilterData;
(Modified Discrete Fourier Transform routine is used for
high frequency noise reduction. )
Const
  PI = 3.141592;
Type
  CalcArY = Array(0..1000) Of Real;
Var
  Xf: FilterArray;
  U,V,R,I: CalcArY;
  D,O,E,J,K,L,I,J1,J2,M,M2,P,P1: Integer;
  B,F,F1,F2,T,T2,G,M,S,S1,S2,X1,X2,Ult Reel;
Begin
  If Not DataLoaded Then
    Begin
      ShowError;
      Exit;
    End;
  If ScanMode = 4 Then Exit;
  DialogBoxA;GotoXY(2,2);
  Write(' Enter the degree of smoothing(7-25): ');
  ReadLn(E);GotoXY(2,3);
  Write(' Enter the # of data to correct the end effect: ');
  ReadLn(D);GotoXY(8,5);
  Write(' Working on Fourier Transform ..... ');
  For J := 0 To D-1 Do
    Begin
      S1 := 0; S2 := 0;
      S := S1*Data[I+1];
      S2 := S2*Data[M-J];
    End;
    X1 := S1/D; X2 := S2/D;
    M := (X2-X1)/(M-D);
    B := (X1+X2)/2 - M*M/2;
    G := 0;
    For J := 0 To M-1 Do

```

```

    Begin
      Data[J+1] := Trunc(Data[J+1]-M*J-B);
      G := G + Data[J+1];
    End;
    R(0) := G/M; Q := 1;
    J2 := Trunc((M-1)/2);
    P1 := Trunc(Ln(2*J2-1)/Ln(2));
    For K := 0 To E-1 Do
      Begin
        J1 := J2; S := PI*K*2/M;
        C := Cos(S); S := Sin(S);
        For J := 1 To J1 Do
          Begin
            L := 2*J-1;
            U[I] := Data[L+1]*C+Data[L+2];
            V[I] := Data[L+1]*S;
          End;
          S := 2*S*C; C := 2*C*C-1;
          For P := 1 To P1 Do
            Begin
              U[J1+1] := 0; V[J1+1] := 0;
              J1 := Trunc((J1+1)/2);
              For J := 1 To J1 Do
                Begin
                  L := 2*J-1;
                  U1 := U[L]*C-V[L]*S+U[L+1];
                  V[I] := U[L]*S+V[L]*C+V[L+1];
                  U[I] := U1;
                End;
                S := 2*S*C; C := 2*C*C-1;
                End;
                R(K) := (Data[I]+(U(1)*C+V(1)*S))/M;
                End;
                For K := 0 To E-1 Do
                  Begin
                    J1 := J2; S := 2*PI*K/M;
                    C := Cos(S); S := Sin(S);
                    For J := 1 To J1 Do
                      Begin
                        L := 2*J-1;
                        U[I] := -(Data[L+1]*S);
                        V[I] := Data[L+1]*C+Data[L+2];
                      End;
                      S := 2*S*C; C := 2*C*C-1;
                      For P := 1 To P1 Do
                        Begin
                          U[J1+1] := 0; V[J1+1] := 0;
                          J1 := Trunc((J1+1)/2);
                          For J := 1 To J1 Do
                            Begin
                              L := 2*J-1;
                              U1 := U[L]*C-V[L]*S+U[L+1];
                              V[I] := U[L]*S+V[L]*C+V[L+1];
                              U[I] := U1;
                            End;
                            S := 2*S*C; C := 2*C*C-1;
                            End;
                            I(K) := -(U(1)*C+V(1)*S)/M;
                            End;
                            GotoXY(8,5);Write(' Working on Inverse Transform .....');
                            For K := 1 To E-1 Do
                              Begin
                                T := R(K);
                                F1 := F1+T;
                                F2 := F2+K*T;

```

```

End;
XF(0) := R(0)+2*(F1-F2*(1/E/E));
XF(0) := XF(0)+8;
P1 := Trunc(Ln(2+E-3)/Ln(2));
For j := 1 To N-1 Do
  Begin
    T2 := E+E;
    For K := 1 To E-1 Do
      Begin
        F := 1-K*K/12;
        U(K) := R(K)*F; V(K) := -(1(K)*F);
      End;
    K1 := E-1;
    S := 2*P1/M;
    C := Cos(S); S := Sin(S);
    For P := 1 To P1 Do
      Begin
        U(K1+1) := 0; V(K1+1) := 0;
        K1 := Trunc((K1+1)/2);
        For K := 1 To K1 Do
          Begin
            L := 2*K-1;
            U1 := U(L)*C-V(L)*S+U(L+1);
            V1 := U(L)*S+V(L)*C+V(L+1);
            U(K) := U1;
            V(K) := V1;
          End;
        S := 2*S*C; C := 2*C*C-1;
      End;
    End;
    XF(j) := R(0)+2*(U(1)*C+V(1)*S);
    XF(j) := XF(j)+8;
    (End);
  End;
For j := 0 To N-1 Do
  Data[j] := Round(XF(j));
ClosedInRow;
End;

Procedure TitleText;
Var
  X : Integer;
Begin
  GotoXY(20,4);
  Write('----- ENISPEC-11 -----');
  GotoXY(20,5);
  Write('----- LUMINESCENCE DATA STATION -----');
  GotoXY(20,7);
  Write('Version 2.0');
  GotoXY(25,9);
  Write('Haidong Kim, S.R. Crouch'); GotoXY(15,10);
  Write('Department of Chemistry, Michigan State University');
  GotoXY(25,11); Write('Matthew Zabol'); GotoXY(15,12);
  Write('Pesticide Research Center, Michigan State University');
  For x := 3 To 77 Do
    Begin
      GotoXY(x,13); Write( chr(205) );
    End;
  GotoXY(13,15);
  Write('ENISPEC-11 is a data acquisition and analysis program');
  GotoXY(13,16);
  Write('designed for luminescence data (fluorescence, phosphorescence)');
  GotoXY(13,17);
  Write('and phosphorescence lifetime ). It requires IBM PC-XT with');
  GotoXY(13,18);
  Write('Hercules monographica card, Labmaster data acquisition');
  GotoXY(13,19);
  Write('board( 30 KHz ), Aminco SPF-500 spectrometer and HP7475A');
End;

GotoXY(13,20);
Write('Plotter or Epson dot matrix printer. See ENISPEC-11 manual');
GotoXY(13,21);
Write('for detailed system configuration. '); GotoXY(18,23);
Write('***** Press any key to continue *****');
Readln;
End;

Procedure RestoreCom1;
Begin
  Reg.DX := 0; ( Restore Com:1 )
  Reg.AX := 254; ( Com1:9600,E,2,7 )
  Intr(20,Reg);
  Close(Com1);
End;

Procedure InitPlotter;
( Sets up plotter and Com1 for plotting )
Var
  Com1Addr: Integer;
Begin
  If GotoPlotter And (PlotNum = 0)
  Then Begin
    Reg.DX := 0; ( Set up Com1: with BIOS service 20($14) )
    Reg.AX := 195; ( Com1:4800,None,8,1 )
    Intr(20,Reg);
    Com1Addr := Memw($40:0); ( Get COM1 port address )
    Portw(Com1Addr+4) := 3; ( Set DTR, RTS ON )
    AssignAux(Com1, 0, 195); Rewrite(Com1);
    Write(Com1, Chr(27), 'Y'); ( Wake up plotter )
    Write(Com1, 'M:');
    Write(Com1, Chr(27), 'M:');
    Write(Com1, Chr(27), 'M1000;5;6:');
    ( Data block size 1000 byte, Enq 5, Ack 6 ASCII )
    End
  Else Begin
    If PlotNum = 0
    Then Begin
      Assign(Com1, PlotFileName);
      Rewrite(Com1);
      End
    Else Append(Com1);
  End;
End;

Function FreeSpace: Boolean;
( Check plotter buffer space for data transmission. )
Var
  Enq, Ack : Char;
Begin
  FreeSpace := False;
  Enq := Chr(5);
  Write(Com1, Enq);
  Reset(Com1); Readln(Com1, Ack);
  Append(Com1);
  If (Ack = Chr(13)) or (Ack = Chr(6)) Then FreeSpace := True;
End;

Procedure Draw3Daxis;
( Draw 3-D axis for EEM plot )

```



```

;-----
;                               ADCR.ASM
;
;This is a assembly subroutine for EMISPEC.PAS.
;This assembly routine collects spectrum data and stores
;data into Buffer data array. Data type is specified by
;DataType(1=Fluorescence,2=Phosphorescence,3=Lifetime).
;-----

```

```

Code Segment
Assume CS:Code
Public ReadData

```

```

;Procedure ReadData(Var Buffer:IntBuff; Var Count,DataType:Integer);

```

```

ReadData Proc Near
    Push BP                      ;Save BP on stack
    Mov BP,SP                   ;Now BP has stack pointer
    Cli                         ;Disable interrupt.
    Les DI,[BP+8]                ;Get Count
    Mov CX,ES:[DI]               ;Store it into CX
    Les DI,[BP+4]                ;Get data type
    Mov AL,ES:[DI]               ;Move it into AL
    Les DI,[BP+12]               ;Get Buffer address
    Cmp AL,2                     ;If DataType=2 then
    Je Phs                      ;Jump to phosphorescence
    Cmp AL,3                     ;If DataType=3 then
    Je Ltm                      ;Jump to lifetime routine

Fls:
    Mov DX,1814                 ;Start conversion by
    Mov AL,0                    ;writting any byte to
    Out DX,AL                   ;A/D start port(1814)

FDone:
    Mov DX,1812                 ;Check if data ready
    In AL,DX                    ;by checking ADC control
    Cmp AL,128                  ;port.
    Jb FDone                   ;Wait until done.
    Inc DL                      ;Get lower byte first
    In AL,DX
    Mov ES:[DI],AL              ;Store it into Buffer
    Inc DI                      ;Next location
    Inc DL                      ;Get higher byte
    In AL,DX
    Mov ES:[DI],AL              ;Store it into Buffer
    Inc DI                      ;Next location
    Loop Fls                    ;Continue Count times
    Jump Exit

Phs:
    Mov DX,1817                 ;Start conversion by sending
    Mov AL,48                   ;arm counter5 command to
    Out DX,AL                   ;timer control port

PDone:
    Mov DX,1812                 ;Check if data ready
    In AL,DX                    ;by checking ADC control
    Cmp AL,128                  ;port.
    Jb PDone                   ;Wait until done.
    Inc DL                      ;Get lower byte first
    In AL,DX
    Mov ES:[DI],AL              ;Store it into Buffer

```

```

    Inc DI                ;Next location
    Inc DL                ;Get higher byte
    In AL,DX
    Mov ES:[DI],AL        ;Store it into Buffer
    Inc DI                ;Next location
    Loop Phs
    Jmp Exit

Ltm:
    Mov DX,1817           ;Initialize timer OUT4
    Mov AL,228            ;to low
    Out DX,AL
    Mov DX,1814           ;Send A/D start conversion
    Mov AL,0              ;command for reset
    Out DX,AL

Reset:
    Mov DX,1812           ;Check data ready
    In AL,DX
    Cmp AL,128
    Jb Reset              ;Wait until done
    Mov DX,1814           ;Reset by inputting
    In AL,DX              ;higher data byte
    Mov DX,1812           ;Now enable A/D external
    Mov AL,132            ;start conversion.
    Out DX,AL
    Mov DX,1817           ;Load and arm
    Mov AL,108            ;counter 3 and 4.
    Out DX,AL

LDone:
    Mov DX,1812           ;Check if data ready
    In AL,DX              ;by checking ADC control
    Cmp AL,128            ;port.
    Jb LDone              ;Wait until done.
    Inc DL                ;Get lower byte first
    In AL,DX
    Mov ES:[DI],AL        ;Store it into Buffer
    Inc DI                ;Next location
    Inc DL                ;Get higher byte
    In AL,DX
    Mov ES:[DI],AL        ;Store it into Buffer
    Inc DI                ;Next location
    Loop LDone            ;Repeat DF.NumData times
    Mov DX,1817           ;Disarm counter 3
    Mov AL,196
    Out DX,AL

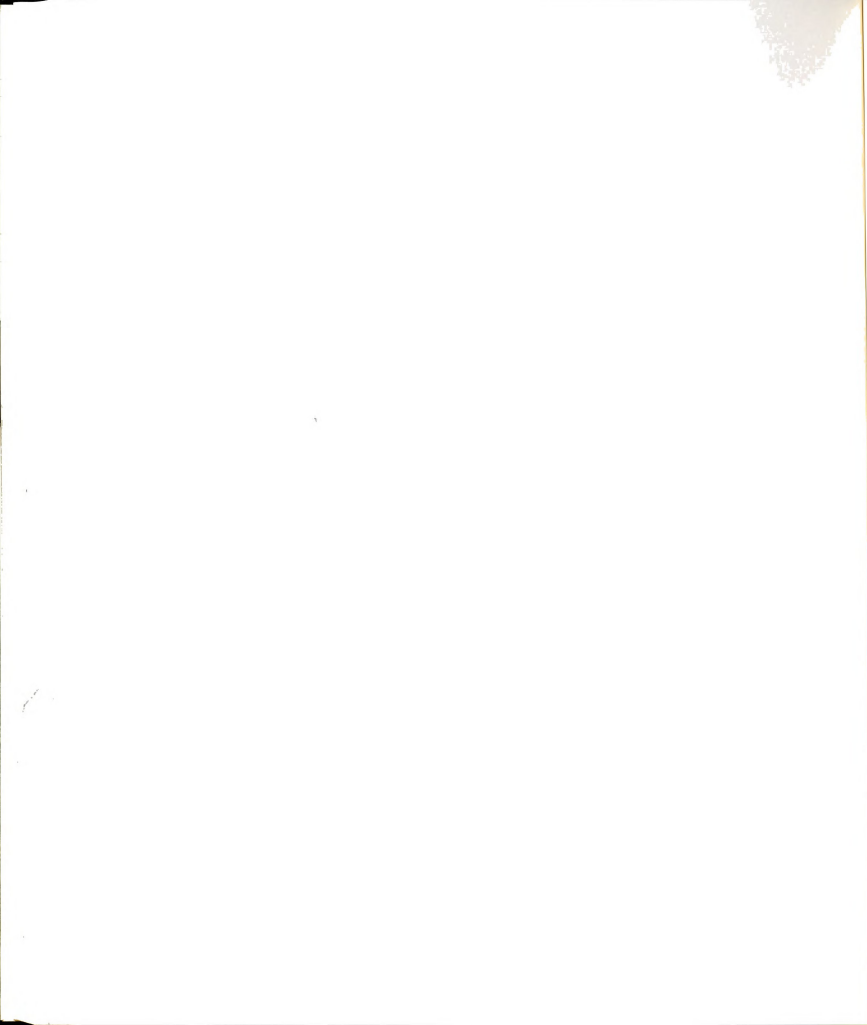
Exit:
    Sti                  ;Enable interrupts.
    Pop BP               ;Restore BP
    Ret 12               ;Three variables(3x4=12).

ReadData Endp
Code Ends
End

```

APPENDIX C

**SOURCE CODE OF
IBM-DACA**




```

05/31/1989 00:30      Filename: IBMDAC.PAS      Page
05/31/1989 00:30      Filename: IBMDAC.PAS      Page

Begin
  IoCode := IoResult;
  If IoCode <> 0 Then
    Begin
      If ScreenMode = 1 Then ExitProcess;
      OpenWindow(2);HeaderOn(2);
      Msg(3,3,Black,'*** Error! Press any key to continue. *');
      Ch := ReadKey;
      CloseWindow;
      End;
    CheckError := IoCode;
  End;

  Procedure TitleScreen;
  Var I,J : Integer;
  Begin
    CursorOn(false);ClrScr;
    GotoXY(1,1);
    For I := 1 To 79 Do
      Begin
        GotoXY(1,1); Write(Chr(177));
        GotoXY(1,25); Write(Chr(177));
        End;
      For I := 2 To 24 Do
        Begin
          GotoXY(1,1); Write(Chr(177));
          GotoXY(76,1); Write(Chr(177));
          End;
        DrawInBox(3,3,77,9,2,black,white);
        FullScreenBlack,Fill(5,false);LowVideo;
        DrawInBox(3,12,77,23,2,black,white);
        FullScreenBlack,Write(1,1, ' R E A L W O R L D ');
        GotoXY(25,4); Write(1,1, ' M - D A C ');
        GotoXY(33,6); Write('Version 1.0');GotoXY(8,8);
        Write('Copyright by Haidong Kim,Matthew Zabik,S.R. Crouch, 1988');
        GotoXY(15,9); Write('Department of Chemistry, Michigan State University');
        GotoXY(5,13);
        Write('IBMDAC REAL WORLD is a data acquisition and analysis program');
        GotoXY(5,14);
        Write('for IBM data acquisition board. For full operation and versat-');
        GotoXY(5,15);
        Write('ility the IBM data acquisition board must be modified according');
        GotoXY(5,16);
        Write('to the manual. All hardware set-up jumpers must be correctly');
        GotoXY(5,17);
        Write('installed before any data read and write operation. ');
        GotoXY(20,22); Write('*** Press any KEY to continue.....');
        Ch := ReadKey;
        CursorOn(true);
        End;
      Function DelayMseconds(Rate: Integer): Integer;
      (Calculate delay time interval(msec) between the data readings)
      Begin
        DelayMseconds := Round(58500/Rate);
        End;
      Procedure TimeDelay(Mseconds: Integer);
      (Delay seconds approximately.)
      Var I,J: Integer;
      Begin
        Delay(Mseconds);
        Procedure SetupDefault;
        Var I : Integer;
        Begin
          With SetupData Do
            Begin
              Mode := '0';
              NumData := 1000;InputSpan := '10';
              Bipolar := 'N';
              PttLabel := 'Data Number';
              PttLabel := 'Relative Intensity';
              Channel := '0';Rate := '60';
              Display := 'N';FastMode := 'N';
            End;
            For I := 1 To 3 Do
              With TimerData(I) Do
                Begin
                  TimerMode := '0';
                  Count := '0';
                End;
                FirstSetup := False;
            End;
            Procedure WriteSetup;
            Begin
              DrawInBox(2,12,76,24,2,Black,White);
              HeaderOn(7);
              With SetupData Do
                Begin
                  Msg(30,2,White,'Mode Number: ');Write(Mode);
                  Msg(5,1,White,'Number of Data : ');Write(NumData);
                  Msg(5,3,White,'Input Range(10/5 V) : ');Write(InputSpan);
                  Msg(5,5,White,'Bipolar Input(Y/N) : ');Write(Bipolar);
                  Msg(5,7,White,'Input Channel(0-3) : ');Write(Channel);
                  Msg(5,10,White,'Label(X-Axis) : ');Write(PttLabel);
                  Msg(5,11,White,'Label(Y-Axis) : ');Write(PttLabel);
                  Msg(40,4,White,'Fast Mode? (Y/N) : ');Write(FastMode);
                End;
            End;
            Procedure ListFile;
            Var
              Count,NextCol,NextRow: Integer;
              Filespec: String[12];
              DirInfo : SearchRec;
            Begin
              OpenWindow(1);HeaderOn(1);
              Msg(2,2,Black,'Enter the filespec. to (list: ');
              Readln(Filespec);
              ClosedWindow;
              OpenWindow(3);HeaderOn(3);
              If Filespec = '' Then Filespec := '*.*';
              FindFirst(Filespec,Archive,DirInfo);
              Count := 0; NextCol := 4; NextRow := 1;
              While DirError = 0 Do
                Begin
                  If NextRow = 14 Then
                    Begin
                      Writeln;Writeln;
                    End;
                End;
            End;

```

```

05/31/1989 00:30      Filename: IBM DAC.PAS      Page      6
      Write('Press any key for next page.....');
      Ch := ReadKey;
      NextCol := 4; NextRow := 1;
      Count := 0; ClrScr;
      End;
      Count := Count + 1;
      GotoXY(NextCol, NextRow); Write(DirInfo.Name);
      If Count = 5 Then
        Begin
          Count := 0;
          NextCol := 4; NextRow := NextRow + 1;
          End Else
            NextCol := NextCol + 14;
            FindIndex(DirInfo);
            End;
            WriteLn;
            Msg(20, 20, Black, 'Press ENTER to continue.....');
            ReadLn;
            CloseWindow;
            End;
            Procedure LoadData;
            Label Done;
            Var
              I : Integer;
              FileName: String(15);
              InFile: Text;
            Begin
              OpenWindow(1); HeaderOn(1);
              Msg(2, 2, Black, 'Enter the filename to be loaded: ');
              ReadLn(FileName);
              If FileName = '' Then Goto Done;
              Assign(InFile, FileName);
              Reset(InFile);
              If CheckError(0) < 0 Then
                Begin
                  Done: CloseWindow;
                  Exit;
                End;
              Msg(2, 5, Black, 'Loading data from disk.....');
              ReadLn(InFile, SetupData.Mode);
              Val(SetupData.NumData, NumData, ErrorCode);
              For I := 1 To NumData Do
                ReadLn(InFile, Data[I]);
              DataLoaded := True;
              Msg(4, 13, White, SetupData.Mode + ' ');
              Msg(6, 15, White, 'Number of Data');
              Write(NumData); Write(' ');
            End;
            {SI+}
            Procedure SaveData;
            Label Done;
            Var
              I : Integer;
              FileName: String(15);
              OutFile: Text;
            Begin
              OpenWindow(1);
              Msg(2, 2, Black, 'Enter the filename to be saved: ');
              ReadLn(FileName);
              If FileName = '' Then Goto Done;
              Assign(OutFile, FileName);
              Rewrite(OutFile);
              If CheckError(0) < 0 Then
                Begin
                  CloseWindow;
                  Exit;
                End;
              Msg(2, 5, Black, 'Saving data to disk.....');
              WriteLn(OutFile, SetupData.Mode);
              Val(SetupData.NumData, NumData, ErrorCode);
              For I := 1 To NumData Do
                WriteLn(OutFile, Data[I]);
              Close(OutFile);
              Done: CloseWindow;
              End;
              {SI+}
              Procedure Ask_Exit;
              Begin
                Define(Black, 13, 5, 60);
                Msg(25, 13, White, 'Do you want to save? (Y/N): ');
                Ch := ReadKey; Define(Black, 13, 5, 75);
                If Ch = 'Y' Then Change := False Else Change := True;
                If Change Then
                  Begin
                    GotoXY(5, 13); InVideo('F10'); Write(' Delete Entry');
                    GotoXY(33, 13); InVideo('Esc'); Write(' Exit Set-up');
                  End;
                End;
              Procedure Input_Setup;
              Label SetupAgain1, SetupAgain2;
              Var I : Integer;
              Begin
                WriteSetup;
                GotoXY(5, 13); InVideo('F10'); Write(' Delete Entry');
                GotoXY(33, 13); InVideo('Esc'); Write(' Exit Set-up');
                ExitOK := False; First_run := True;
                DefineSetupFields;
                SetupAgain1:
                  FieldInput('M0101', Escape);
                  SetupData.Mode := F1Var[1];
                  FieldInput('M0208', Escape);
                  Ask_Exit;
                  If Change Then Goto SetupAgain1;
                  With SetupData Do
                    Begin
                      NumData := F1Var[2]; InputSpan := F1Var[3];
                      Bipolar := F1Var[4]; Channel := F1Var[5];
                      P1LabelX := F1Var[6]; P1LabelY := F1Var[7];
                      FastMode := F1Var[8];
                    End;
                  With SetupData Do
                    Begin
                      If (FastMode='N') or (FastMode='W') Then
                        Begin
                          SetupAgain1;
                        End;
                    End;
                  End;
            End;

```

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```

Msg(40,5,White,'Real-time Display(y/n) : ');
Write(StdOut);
FieldInput('M0500',Escape);
Display := FIlVar(0);
End;
If (SetupData.Mode=0) And ((FastMode='n') or (FastMode='N'))
Then Begin
Msg(40,6,White,'Sampling Rate(Data/MIn): ');Write(Rate);
FieldInput('M1010',Escape);
Rate := FIlVar(10);
End;
End;
If Not (SetupData.Mode = '0') Then
Begin
OpenWindow(11);
HeaderOn(11);
Msg(2,2,Black,'Timer => Mode: ');
Write(TimerData[1].TimerMode);
Msg(25,2,Black,'Count Number(Max 65535): ');
Write(TimerData[1].Count);
Msg(2,3,Black,'Timer2 => Mode: ');
Write(TimerData[2].TimerMode);
Msg(25,3,Black,'Count Number(Max 65535): ');
Write(TimerData[2].Count);
Msg(2,4,Black,'Timer3 => Mode: ');
Write(TimerData[3].TimerMode);
Msg(25,4,Black,'Count Number(Max 65535): ');
Write(TimerData[3].Count);
DefineTimerSetup;
SetupGain2;
FieldInput('M0106',Escape);
Msg(20,6,White,'Save Data? (y/n): ');
Ch1 := ReadKey;
GotoY(20,6);InVideol;
If Not (Ch1 = 'y') Then Goto SetupGain2;
For i := 0 To 2 Do
Begin
TimerData[i+1].TimerMode := FIlVar(1+2+1);
TimerData[i+1].Count := FIlVar(1+2+2);
End;
End;
CloseWindow;
End;
Procedure SetTimer;
Var
i,TimerMode,CountNum : Integer;
TimerRegister : Word;
Begin
For i := 0 To 2 Do
Begin
Val(TimerData[i+1].TimerMode,TimerMode,ErrorCode);
Val(TimerData[i+1].Count,CountNum,ErrorCode);
Case TimerMode Of
0: Port(TimerCtrlReg) := (46+1+64);
1: Port(TimerCtrlReg) := (50+1+64);
2: Port(TimerCtrlReg) := (52+1+64);
3: Port(TimerCtrlReg) := (54+1+64);
4: Port(TimerCtrlReg) := (56+1+64);
(Save Data := Port(TimerCtrlReg+HigherByte));
End;
End;
End;
5: Port(TimerCtrlReg) := (58+1+64);
(Save Data := Port(TimerCtrlReg+HigherByte));
End;
End;
Case 1 of
0 : TimerRegister := Timer0Register;
1 : TimerRegister := Timer1Register;
2 : TimerRegister := Timer2Register;
End;
Port(TimerRegister) := Lo(CountNum);
Port(TimerRegister) := Hi(CountNum);
Delay(1);
End;
End;
Procedure InitDACA(DataType: lOType);
(Select Analog Device, and reset DACA)
Var
BaseAddr: Integer;
Begin
Port(IntrRegister) := 0; (Disable all Interrupts)
Delay(1);
Case DataType of
Analog: Begin
Port(DeviceRegister) := AnalogDevice;
Port(AICtrlRegister) := DisabledDACA;
Port(AICtrlRegister+HigherByte) := Channel;
Delay(1);
End;
Binary: Begin
Port(DeviceRegister) := BinaryDevice;
Delay(1);
End;
End;
End;
($L Freed)
Procedure GetFast(Var Data:IntArray; Var NumData,Channel:Integer);
External;
Procedure SoftTrigger;
Begin
Port(AICtrlRegister) := EnabledDACA;
Port(AICtrlRegister+HigherByte) := Channel;
End;
Function AnalogRead(DACmode: Integer): Integer;
Var
Done,LowByteData,HighByteData: Integer;
Begin
Case DACmode Of
0 : SoftTrigger;
1,2 : SetTimer;
End; (Reload counter)
Repeat
Done := Port(AICtrlRegister);
Until Done = 242;
Port(AICtrlRegister) := DisabledDACA;
LowByteData := Port(AICtrlRegister+HigherByte);
HighByteData := Port(AICtrlRegister+HigherByte);
End;

```

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<pre> If Not (DACMode = 0) Then (Set data read flag on. This sets) Begin Port[AICTrlRegister] := EnabledACA; Port[AICTrlRegister+ HigherByte] := Channel; End; AnalogRead := (HighByteData SHL 8) + LowByteData; End; Function ConvertVolt(RawData:Real; VoltSpan,Offset:Integer):Real; Begin ConvertVolt := (RawData*VoltSpan/4096) - Offset; End; Function ConvertData(RawData:Real; VoltSpan,Offset:Integer):Integer; Begin ConvertData := Round(((4096*(RawData+Offset))/VoltSpan)); End; Procedure StartRead; Label EscapeRead1,EscapeRead2; Var ScaleX,ScaleY,XInc:Real; Rate,dY,dYp,Xp,Yp,Ymin,Ymax,I:Integer; BipolarInput: Boolean; DataBuff: Integer; Begin I := 1; Val(SetupData.Rate,Rate,ErrorCode); Val(SetupData.Mode,DACMode,ErrorCode); Val(SetupData.Channel,Channel,ErrorCode); Val(SetupData.NumData,NumData,ErrorCode); If (SetupData.Bipolar='Y') or (SetupData.Bipolar='Y1') Then BipolarInput := True Else BipolarInput := False; InitDAC(Analog);Quit := False; If DACMode > 0 Then SetTimer; If (SetupData.FastMode = 'Y') or (SetupData.FastMode = 'Y1') Then Begin OpenWindow(1);HeaderOn(1); Msg(19,3,White,Reading Data); If DACMode = 9 Then GetFast(DataBuff,NumData,Channel) Else For I := 1 To NumData Do Data[I] := AnalogRead(DACMode); Msg(19,3,White,Processing Data); For I := 1 To NumData Do Begin If DACMode = 9 Then Data[I] := DataBuff[I]; Data[I] := ConvertVolt(Data[I],20,10); End; DataLoaded := True; ClosedWindow; End (not fast mode) Else If (SetupData.Display = 'N') Or (SetupData.Display = 'N1') Then Begin OpenWindow(1);HeaderOn(1); Msg(19,5,White,'To quit press Esc key. '); For I := 1 To NumData Do Begin RawData := AnalogRead(DACMode); </pre>	<pre> Data[I] := ConvertVolt(RawData,20,10); GotoY(23,3);Write(' '); GotoY(23,3);Write('Data ',I,' ',Data[I]:5:2); If KeyPressed Then Begin Ch1 := ReadKey; If (Ch1 = Chr(27)) Then Goto EscapeRead1; End; End; If DACMode = 0 Then TimeDelay(DelayInSeconds(Rate)); EscapeRead1: If I = NumData Then DataLoaded := True Else DataLoaded := False; ClosedWindow; End (4) Else (Display data on real time.) Begin (7) If BipolarInput Then Begin Ymin := -5; Ymax := 5; End Else Begin Ymin := -10; Ymax := 10; End Else Begin If SetupData.InputSpan = 'S' Then Ymax := 5 Else Ymax := 10; Ymin := 0; End; GrInitialSize; DrawPlotAxis(90,20,630,250,1,NumData,Ymin,Ymax,True); DrawText(15,140,1,0,1,White,SetupData.P[LabelY]); DrawText(250,285,0,0,1,White,SetupData.P[LabelX]); With AxisData Do Begin ScaleX := (ax2-ax1)/(axmax-axmin); ScaleY := (ay2-ay1)/(aymax-aymin); XInc := (ax2-ax1)/(NumData-1); If BipolarInput Then Yp := ay2-Round((ay2-ay1)/2) Else Yp := ay2; Xp := ax1; End; For I := 0 To NumData-1 Do Begin RawData := AnalogRead(DACMode); Data[I+1] := ConvertVolt(RawData,20,10); dY := Yp-Round(Data[I+1]*ScaleY); dx := Xp-Round(I*XInc); If Not BipolarInput And (dY > Yp) Then dY := Yp; If dY < 20 Then dY := 20; PutPixel(dx,dY,White); If KeyPressed Then Begin Ch1 := ReadKey; If (Ch1 = Chr(27)) Then Goto EscapeRead2; End; TimeDelay(DelayInSeconds(Rate)); End; Ch1 := ReadKey; If Ch1 = 'P' Then PrintScreen(False,6); EscapeRead2: If I = NumData-1 Then DataLoaded := True Else DataLoaded := False; </pre>	<pre> Page 10 </pre>

[illegible]

```

DataLoaded := True;
Plot(True);
Exit;
End;
Ch1 := ReadKey;
If Ch1 = "P" Then PrintScreen(False,6);
ExitProcess;
MainMenuScreen;
End;
($!+)

Procedure MainMenuScreen;
Begin
DrawInBox(2,3,78,9,2,Black,White); REAL WORLD';
GotoXY(22,2);Write('I & M - O A C');
GotoXY(34,4);Write('Version 1.0');GotoXY(10,6);
Write('Copyright 1988 by Maidong Kim,Matthew Zablit,S.R. Crouch');
GotoXY(13,7);Write('Department of Chemistry, Michigan State University');
If FirstSetup Then SetupDefault;
Writesetup;
End;

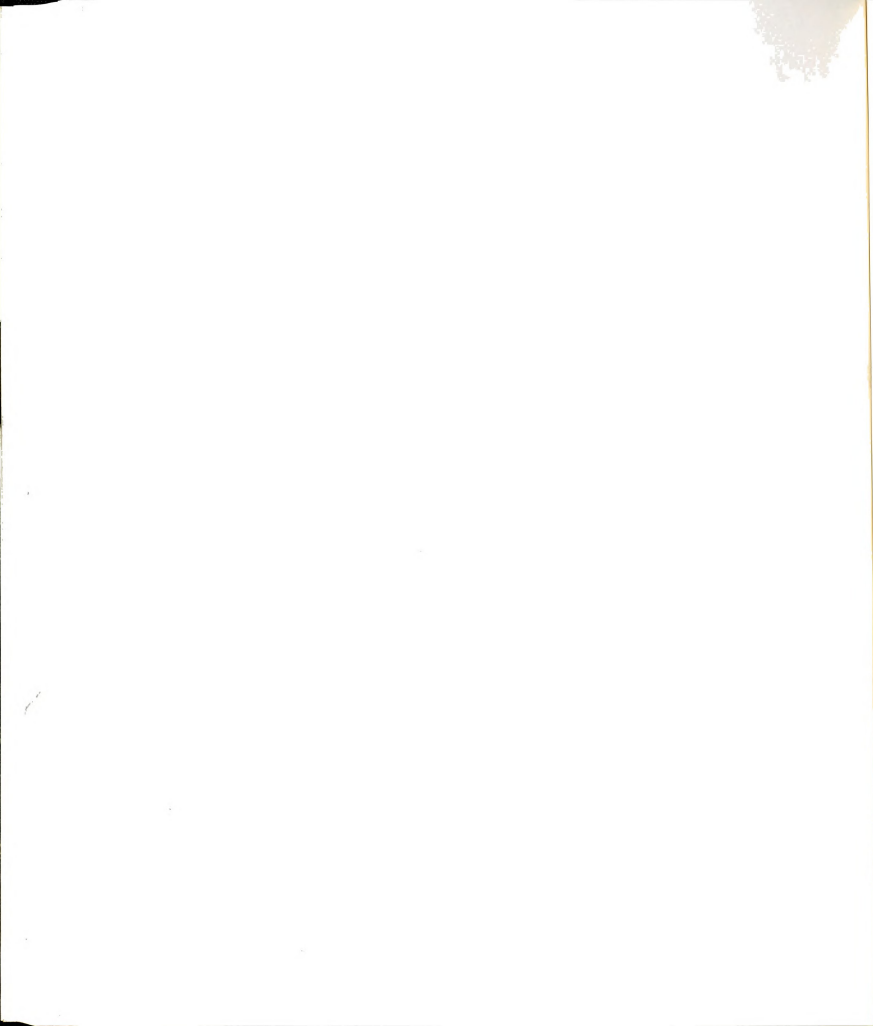
Procedure MainMenu;
Begin
MainMenuScreen;
Repeat
Action1 := BarMenu(1,5,White,black,'Data' Graph Exit');
Case Action1 Of
File Set-Up
1 : Begin
Action2 := Pulldownmenu(6,3,
'Load','Save','List File','',,,',,,,','',''),
Case Action2 of
1 : LoadDate;
2 : SaveDate;
3 : Listfile;
End;
End;
2 : Input_Setup;
3 : Begin
Action2 := PulldownMenu(8,3,
'Display','Start Read','Print','',,,',,,,','',''),
Case Action2 of
1 : DisplayDate;
2 : StartRead;
3 : PrintDate;
End;
End;
4 : Begin
Action2 := PulldownMenu(9,5,
'Screen Plot','Printer Plot','Plotted Plot',
'Plotted File','Multiple Plot','',,,',,,,','',''),
Case Action2 of
1 : ScreenPlot;
2 : PrinterPlot;
5 : Multiplot;
End;
End;
5 : Begin
OpenWindow(10);
Write('Are you sure? (y/n) ');
Ch1 := Readkey;
If Ch1 = 'Y'
Then Begin
FullScreen(Black,White,True);

```

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```
        ClrScr; Halt;
      End
    Else CloseWindow;
  End;
End;
Until Action1 = 6;
End;

Begin
  DefinedWindows;
  TitleScreen; ClrScr;
  FirstSetup := True; DataLoaded := False;
  MainMenu;
  ClrScr;
End.
```




```

;-----
; Procedure FastRead(Var Data: DataArray; Var NumData,
;                   Channel: Integer);
; Reads NumData with hardware A/D conversion trigger.
; This is a assembly subroutine for IBMDAC.PAS and
; LSDATA.PAS.
;-----

```

```

code      segment
          assume cs: code
          public getfast

getfast   proc near
          push bp
          mov bp,sp
          les di,[bp+4]      ;near call start at 4th byte
          mov bx,es:[di]     ;get channel in bx
          les di,[bp+8]     ;variable pointer is 4 bytes
          mov cx,es:[di]     ;get numdata in cx
          les di,[bp+12]    ;get data array address in es:di
          cli                ;clear interrupt

          conv:
          mov dx,02E2H       ;AI-ctrl register
          mov al,1           ;set new data flag to prevent overrun
          out dx,al          ;by software enabling A/D
          inc dx             ;now higher byte
          mov al,b1          ;get channel
          out dx,al          ;set channel
          dec dx             ;set dx to AI-ctrl register

          wait:
          in al,dx           ;read A/D status
          and al,1           ;mask out all except A/D done
          cmp al,0           ;check A/D conversion done
          jne wait          ;wait until done
          inc dx             ;point dx to AI-ctrl higher byte
          mov al,0           ;to skip channel set
          out dx,al          ;enable data read
          mov dx,22E2H       ;AI-read register
          in al,dx           ;read lower byte datum
          mov es:[di],al     ;store into data array
          inc di             ;next data location
          inc dx             ;next higher byte
          in al,dx           ;read higher byte datum
          mov es:[di],al     ;store into data array
          inc di             ;set next data array location
          loop conv          ;repeat NumData times
          sti                ;restore interrupt
          mov sp,bp
          pop bp
          ret 12

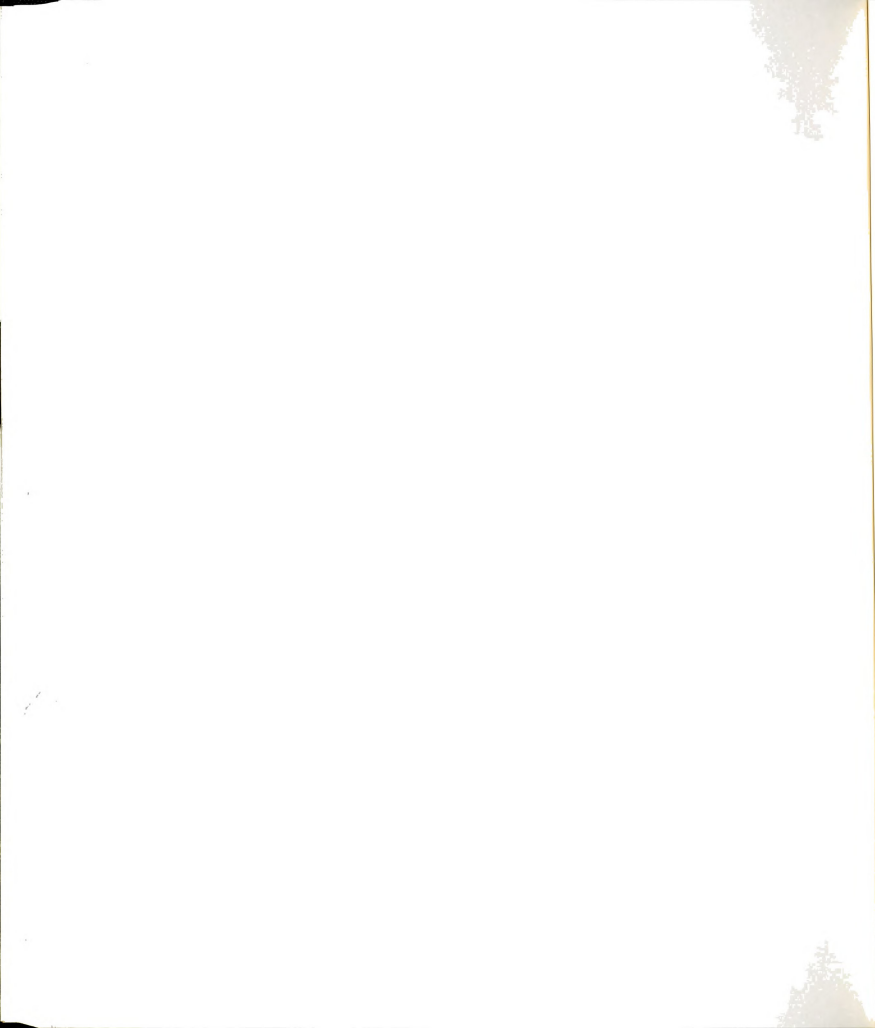
getfast   endp
code      ends
end

```

APPENDIX D

SOURCE CODE OF

LS-DATA



```
(-----)
(          L S D A T A          )
(          Version 1.5          )
(          )
(          Written by Waidong Kim )
(          Department of Chemistry, Michigan State University )
(          )
(-----)
```

```
Program L$Data;
```

```
Uses Crt,Dos,Printer,AuxInout,Graph,TpLib;
```

```
Type
```

```
Setup = Record
  LowX : String(4); HighX : String(4);
  Channel : String(1); NumData : String(5);
  InputSpan : String(2); Rate : String(5);
  Display : String(1); PtitLabelX : String(50);
  PtitLabelY : String(50); DataMode : String(1);
  ScanMode : String(1); ScanSpeed : String(1);
  Extn : String(3); Extn : String(3);
  Lowm : String(3); Highm : String(3);
  Gain : String(3);
End;
```

```
TimerSetup = Record
```

```
  TimerMode : String(1);
  Count : String(5);
End;
```

```
Str5 = String(5);
```

```
Str8 = String(15);
```

```
StrL = String(75);
```

```
IOType = (Analog,Binary);
```

```
IntArray = Array [1..3000] of Integer;
```

```
Const
```

```
BaseAddress = $02E2; (Base address of single DACA)
DeviceRegister = $02E2; (Device number register: No. 12)
IntrRegister = $02E2; (Register No. 13)
Timer0Register = $02E2; (Timer 0, Register No. 8)
Timer1Register = $02E2; (Timer 1, Register No. 9)
Timer2Register = $02E2; (Timer 2, Register No. 10)
TimerCtrlReg = $02E2; (Timer control reg. No. 11)
AltCtrlRegister = $02E2; (Device register 0, Read/Write)
AnalogDevice = $0009; (Device register 2, Read data)
BinaryDevice :Byte = 1;
EnabledACA :Byte = 0;
DisabledDACA :Byte = 0;
HigherByte
```

```
Var
```

```
Reg : Registers;
Coal : Text;
Action1.Action2 : Byte;
Ch1.Ch2.Ch3.Ch4 : Char;
Setup : Setup;
TimerData : Array[1..3] of TimerSetup;
Id.Id.Lat.Pit : Array[1..50] of Real;
Std : Array[1..50] of Str5;
Data : DataArray;
Display : String(1);
FileName,PlotFileName : Strs;
UnitX,PtitLabelX,PtitLabelY : StrL;
```

```
ReadData,Get,In,v1,v14,v15,v16,v17,v18,v19,v20,v21,
v22,v23,v24,v25,Lifetime,Background,Damp : Real;
LowX,HighX,Channel,NumData,Rate,InputSpan,ErrorCode,
DataMode,ScanMode,ScanSpeed,Extn,Extn,Lowm,Highm,
Gain,Rdata,PlotNum,CountNum,DACAnode,Lynax : Integer;
Change,Quit,FirstSetup,DataLoaded,GoPlotter : Boolean;
```

```
Procedure MainMenuScreen; Forward;
```

```
Procedure DefineWindows;
```

```
Begin
  DefWindow(1,12,4,68,8,2,White,Black);
  DefHeader(1,Black,White,'Dialog Box 1 ');
  DefWindow(2,20,6,60,6,2,White,Black);
  DefHeader(2,Black,White,'Dialog Box 2 ');
  DefWindow(3,2,10,78,24,2,White,black);
  DefHeader(3,Black,White,'List File ');
  DefWindow(4,10,10,70,24,2,White,black);
  DefHeader(4,Black,White,'Display Data ');
  DefWindow(5,15,9,65,20,2,White,black);
  DefHeader(5,Black,White,'Decay Kinetics '); (File Menu)
  DefWindow(6,5,3,15,5,1,Black,White);
  DefHeader(7,2,12,78,24,2,Black,White);
  DefWindow(7,White,Black,'Data Acquisition Board Set-up');
  DefWindow(8,28,3,39,6,1,Black,White); (Data Menu)
  DefWindow(10,48,1,61,1,Black,White); (Decay Menu)
  DefWindow(11,43,5,65,5,2,White,Black); (Plot Menu)
  DefWindow(12,32,5,78,15,2,White,Black); (Exit Menu)
  DefHeader(12,Black,White,'Plotter Plot '); (Plotter Menu)
End;
```

```
Procedure DefineSetupFields;
```

```
Begin
  With SetupData Do
```

```
    Begin
      DefineField(1,White,Black,LowX,'013202004M');
      DefineField(2,White,Black,HighX,'023203004M');
      DefineField(3,White,Black,NumData,'033204005M');
      DefineField(4,White,Black,Channel,'043205001M');
      DefineField(5,White,Black,InputSpan,'053206002M');
      DefineField(6,White,Black,Display,'063207001A');
      DefineField(7,White,Black,Rate,'073208005M');
      DefineField(8,White,Black,PtitLabelX,'082110050A');
      DefineField(9,White,Black,PtitLabelY,'092111050A');
      DefineField(10,White,Black,DataMode,'106702001M');
      DefineField(11,White,Black,ScanMode,'116703001M');
      DefineField(12,White,Black,ScanSpeed,'126704001M');
      DefineField(13,White,Black,Extn,'136705003M');
      DefineField(14,White,Black,Extn,'146706003M');
      DefineField(15,White,Black,Lowm,'156707003M');
      DefineField(16,White,Black,Highm,'166708003M');
      DefineField(17,White,Black,Gain,'176709003M');
    End;
End;
```

```
Procedure DefineTimerSetup;
```

```
Begin
  DefineField(1,White,Black,TimerData[1].TimerMode,'012002001M');
  DefineField(2,White,Black,TimerData[1].Count,'025002005M');
  DefineField(3,White,Black,TimerData[2].TimerMode,'032003001M');
  DefineField(4,White,Black,TimerData[2].Count,'045003005M');
```

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<pre> DefineField(5,White,Black,TimerData[3].TimerMode,'052004.001M'); DefineField(6,White,Black,TimerData[3].Count,'065004.005M'); End; Procedure ShowMessage; Begin OpenWindow(1);HeaderOn(1); Msg(19,3,Black,'Data array is empty! '); Ch := ReadKey; CloseWindow; End; Procedure RestoreCom1; Begin Reg.DX := 0; Reg.AX := 254; InTr(20,Reg); Close(Com1); End; Function Pow(a,b: Real): Real; (Returns a value of a raised to the power b) Begin Pow := Exp(b*Ln(a)); End; Function FreeSpace: Boolean; (Check plotter buffer space for data transmission.) Var Enq,Ack : Char; Begin FreeSpace := False; Enq := Chr(5); Write(Com1,Enq); Reset(Com1); ReadIn(Com1,Ack); Append(Com1); If (Ack = Chr(13)) or (Ack = Chr(6)) Then FreeSpace := True; End; Procedure ReadPrompt; Var ErrorNm: Integer; Begin Reset(Com1); Repeat Read(Com1,Ch4); Until (Ch4 = Chr(10)); Append(Com1); End; Function CheckError(ScreenMode: Integer): Integer; (ScreenMode: 0=text, 1=Graphics) Var IoCode: Integer; Begin IoCode := IoResult; If IoCode <> 0 Then Begin If ScreenMode = 1 Then ExitProcess; OpenWindow(2);HeaderOn(2); </pre>	<pre> Msg(3,3,Black,'*** Error! Press any key to continue. '); Ch := ReadKey; CloseWindow; End; CheckError := IoCode; End; Function DelayMsecnds(Rate: Integer): Integer; (Calculate delay time interval(msec) between the data readings) Begin DelayMsecnds := Round(58500/Rate); End; Procedure TimeDelay(Msec: Integer); (Delay seconds approximately.) Var I,J: Integer; Begin Delay(Msec); End; Procedure SetupDefault; Var I : Integer; Begin With SetupData Do Begin NumData := '300';InputSpan := '10'; LowX := '500';HighX := '800'; PtitLabelX := 'WAVELENGTH (NM)'; PtitLabelY := 'RELATIVE INTENSITY'; Channel := '0';Rate := '120'; Display := 'Y';DataMode := '1'; ScanMode := '2';ScanSpeed := '12'; Exrm := '300';Emra := '500'; Lowrm := '500';Highrm := '800'; Gain := '1'; End; For I := 1 To 3 Do With TimerData(I) Do Begin TimerMode := '0'; Count := '0'; End; FirstSetup := False; End; Procedure ExtractSetup; Begin Val(SetupData.LowX,LowX,ErrorCode); Val(SetupData.HighX,HighX,ErrorCode); Val(SetupData.NumData,NumData,ErrorCode); Val(SetupData.Rate,Rate,ErrorCode); Val(SetupData.Channel,Channel,ErrorCode); Val(SetupData.InputSpan,InputSpan,ErrorCode); Display := SetupData.Display; PtitLabelX := SetupData.PtitLabelX; PtitLabelY := SetupData.PtitLabelY; Val(SetupData.DataMode,DataMode,ErrorCode); Val(SetupData.ScanMode,ScanMode,ErrorCode); Val(SetupData.ScanSpeed,ScanSpeed,ErrorCode); Val(SetupData.Exrm,Exrm,ErrorCode); </pre>	4

```

Val(SetupData.Emm,Emm,ErrorCode);
Val(SetupData.Lowm,Lowm,ErrorCode);
Val(SetupData.Highm,Highm,ErrorCode);
End;

Procedure WriteSetup;
Begin
  DrawInBox(2,12,78,24,2,Blue,Yellow);HeaderOn(7);
  Msg(5,2,White,Low X-axis Label Number : ');Write(LowX);
  Msg(5,3,White,High X-axis Label Number : ');Write(HighX);
  Msg(5,4,White,Number of Data : ');Write(NumData);
  Msg(5,5,White,Input Channel(0-3) : ');Write(Channel);
  Msg(5,6,White,Input Range(10/5 V) : ');Write(InputSpan);
  Msg(5,7,White,Real Time Display(Y/n) : ');Write(Display);
  Msg(5,8,White,Sampling Rate(Data/Min) : ');Write(Rate);
  Msg(5,9,White,Label(X-Axis) : ');Write(PitLabelX);
  Msg(5,10,White,Label(Y-Axis) : ');Write(PitLabelY);
  Msg(5,11,White,Data Mode(1:EL,2:PH) : ');Write(DataMode);
  Msg(5,12,White,Scan Mode(1:EX,2:EN) : ');Write(ScanMode);
  Msg(5,13,White,Scan Speed(1:60,2:120) : ');Write(ScanSpeed);
  Msg(5,14,White,Excitation Wavelength : ');Write(Exm);
  Msg(5,15,White,Emission Wavelength : ');Write(Emm);
  Msg(5,16,White,Scan Lower Limit(nm) : ');Write(Lowm);
  Msg(5,17,White,Scan Higher Limit(nm) : ');Write(Highm);
End;

Procedure ListFile;
Var
  Count,NextCol,NextRow: Integer;
  FileSpec: String[12];
  DirInfo: SearchRec;

Begin
  OpenInBox(1);HeaderOn(1);
  Msg(2,2,Black,Enter the fileSpec. to list: ');
  ReadIn(FileSpec);
  CloseInBox;
  OpenInBox(3);HeaderOn(3);
  If FileSpec = '' Then FileSpec := '*.*';
  FindFirst(FileSpec,Archive,DirInfo);
  Count := 0; NextCol := 4; NextRow := 1;
  While DosError = 0 Do
    Begin
      If NextRow = 14 Then
        Begin
          WriteLn;
          Write('Press any key for next page.....');
          Ch := ReadKey;
          NextCol := 4; NextRow := 1;
          Count := 0; ClrScr;
        End;
      Count := Count+1;
      GotoXY(NextCol,NextRow);Write(DirInfo.Name);
      If Count = 5 Then
        Begin
          Count := 0;
          NextCol := 4; NextRow := NextRow+1;
          End Else
            NextCol := NextCol+14;
          FindNext(DirInfo);
        End;
      WriteLn;
      Msg(20,20,Black,Press ENTER to continue.....');
      ReadLn;

```

```

      CloseInBox;
    End;
  Procedure LoadData;
  End;
  ($I-)
  Label Done;
  Var
    i: Integer;
    FileName: String[15];
    InFile: Text;
  Begin
    OpenInBox(1);HeaderOn(1);
    Msg(2,2,Black,Enter the filename to be loaded: ');
    ReadIn(FileName);
    If FileName = '' Then Goto Done;
    Assign(InFile,FileName);
    Reset(InFile);
    If CheckError(0) <> 0 Then
      Begin
        Done: CloseInBox;
        Exit;
      End;
    Msg(2,5,Black,Loading data from disk.....');
    ReadIn(InFile,LowX); ReadIn(InFile,HighX);
    ReadIn(InFile,NumData); ReadIn(InFile,InputSpan);
    For i := 1 To NumData Do
      ReadIn(InFile,Data[i]);
    Close(InFile); CloseInBox;
    DataLoaded := True;
    WriteSetup;
  End;
  ($I+)

  Procedure SaveData;
  End;
  ($I-)
  Label Done;
  Var
    i: Integer;
    FileName: String[15];
    OutFile: Text;
  Begin
    OpenInBox(1);
    Msg(2,2,Black,Enter the filename to be saved: ');
    ReadIn(FileName);
    If FileName = '' Then Goto Done;
    Assign(OutFile,FileName);
    Rewrite(OutFile);
    If CheckError(0) <> 0 Then
      Begin
        CloseInBox;
        Exit;
      End;
    Msg(2,5,Black,Saving data to disk.....');
    WriteLn(OutFile,LowX); WriteLn(OutFile,HighX);
    WriteLn(OutFile,NumData); WriteLn(OutFile,InputSpan);
    For i := 1 To NumData Do
      WriteLn(OutFile,Data[i]);
    Close(OutFile);
    Done: CloseInBox;
  End;
  ($I+)

```

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File name: ISDATA.BAS

• • •

```

begin
  DelLine(Black, 13, 5, 60);
  Msg(25, 13, White, 'do you want to save? (y/n): ');
  Ch := KeyKey; DelLine(Black, 13, 5, 72);
  if Ch = 'y' then Change := false else Change := true;
end;

Procedure Ask_Exit;

```

```
(Select counter 1,LSB/MSB,mode 4,Binary: 001110000)
5: Port[TimerCtrlReg] := (58+164);
(Select counter 1,LSB/MSB,mode 5,Binary: 0011101010)
End; (End Case)
```

```

Ch1 := Reskey:DelLine(Black,13,5,75);
If Ch1 = 'Y' Then Change := False Else Change := True;
If Change Then

```

```

0 : TimerRegister := Timer0Register;
1 : TimerRegister := Timer1Register;
2 : TimerRegister := Timer2Register;

```

```
Msg(5, 13, White, 'F10 : Delete Entry');
Msg(33, 13, White, 'Esc : Exit Set-up');
```

```

Z := timerRegister := timer2Register
End;
Port{timerRegister} := Lo(CountNum);
Port{timerRegister} := Hi(CountNum);
Delay(1);

```

Procedure Input_Setup;

```
End;  
End;  
Procedure InitDACa(DataType: lOtype);  
(Select Analog Device, and reset DACa)
```

Label SetupAgain;

Var i : Integer;

Begin

```
Var
  BaseAddr: Integer;
```

```
WriteSetup;  
Msg(5,13,White,'F10 : Delete Entry');  
Msg(33,13,White,'Esc : Exit Set-up');  
ExitOK := False; First_run := True;  
DefineSetupFields;
```

```
baseaddr: integer;
begin
  port(IntrRegister) := 0; (Disable all interrupts)
  Delay(1);
```

DefineSetu

```

Port[DeviceRegister] := AnalogDevice;
Port[ALCtrlRegister] := Disab(0xACA);
Port[ALCtrlRegister+ HighByte] := Channel;
Delay(1);

```

FieldInput('N0116',Escape);

```
Port[DeviceRegister] := AnalogDevice;
Port[AICTrigger] := DisabledDACA;
Port[AICTrigger.HighestByte] := Channel;
Delay(1);
```

ASK EXIT;
IF Change, Then Goto SetupAgain;
With SetimData, No

```
Port[AIctr(Register+ HigherByte) := Channel;  
Delay(1);
```

Begin

```

End:
Binary: Begin
Port [DeviceRegister] := BinaryDevice:

```

Low

```

binary: begin
  Port[DeviceRegister] := BinaryDevice;

```

WUSD

```
For (i=deviceRegister) :- SerialYuvDevice;
Delay(1);
```

```
ImpulsePon := Ffiver[5]; Display := Ffiver[6];
Rate := Ffiver[7]; Labelk := Ffiver[8];
PtitLabely := Ffiver[9]; DataMode := Ffiver[10];
ScanMode := Ffiver[11]; ScanSpeed := Ffiver[12];
Ezra := Ffiver[13]; Ezra := Ffiver[14];
Lorm := Ffiver[15]; Highrm := Ffiver[16];
```

```

End;
End;
End;
(SSL Freed)
Procedure GetFast(Var Data:IntArray; Var NumData,Channel:Integer);
External;
End;

```

**End;
ExtractSetup;**

External:

Procedure SoftTrigger:

Procedure SetTimer;

```
Var
    i, TimerMode, CountNum : Integer;
```

```
Port[AICtrlRegister] := EnabledACA;
Port[AICtrlRegister+ HigherByte] := Channel;
End;
```

Timerregister : Word;

Function AnalogRead(DACmode: Integer): Integer;

Begin

```
begin
  for i := 0 to 2 do
```

```

Begin
  For i = 0 To 2 Do
    Begin
      Val(TimerData[i+1],TimerMode,TimerMode,ErrorCode);
      Val(TimerData[i+1],Count,CountLim,ErrorCode);
      Case TimerMode Of
        0: Port(TimerCtrlReg) = (28*164);
          (Select counter i,SG/M58 mode 0, s1serv: 00110000)

```

```

Begin
  Val(TimerData[i+1].TimerMode, TimerMode, ErrorCode);
  Val(TimerData[i+1].Count, CountLim, ErrorCode);
  Case TimerMode Of
    0: Port(TimerCtrlReg) := (48#164);
    1: (Select counter 1, LSB/MHS_mode, 0, Binary: 00110000)
      Port(TimerCtrlReg) := (50#164);
    2: (Select counter 1, LSB/MHS_mode, 1, Binary: 00110010)
      Port(TimerCtrlReg) := (52#164);
    3: (Select counter 1, LSB/MHS_mode, 2, Binary: 00110100)
      Port(TimerCtrlReg) := (54#164);
    4: (Select counter 1, LSB/MHS_mode, 3, Binary: 00110110)
      Port(TimerCtrlReg) := (56#164);
  End
End

```

```

Done, LowByteData, HighByteData: Integer;

Begin
  Case DACMode Of
    0 : SoftTrigger;
    1, 2 : SetTimer;
    End; (End Case)
  Repeat
    Done := Port(AICtrRegister);
  Until Done = 242;
  Port(AICtrRegister) := DisabledDACA;
  Port(AICtrRegister + HigherByte) := Channel;
  LowByteData := Port(AICrdsRegister);
  (Enable data read)
End;

```

```

05/31/1989 00:11      Filename: L$DATA.PAS      Page      10
HighByteData := Port(AICReadRegister+HigherByte);
If Not (DataCode = 0) Then (Set data read flag on. This sets)
Begin
  Port(AICReadRegister) := EnabledACA;
  Port(AICWriteRegister+HigherByte) := Channel;
End;
AnalogRead := (HighByteData SHL 8) + LowByteData;
End;
Function ConvertVolt(RawData:Real; VoltSpan,Offset:Integer):Real;
Begin
  ConvertVolt := (RawData*VoltSpan/4096) - Offset;
End;
Function ConvertData(RawData:Real; VoltSpan,Offset:Integer):Integer;
Begin
  ConvertData := Round(((4096*(RawData+Offset))/VoltSpan));
End;
Procedure ReadBACA;
Label
EscapeRead1,EscapeRead2;
Var
  ScaleX,ScaleY,Xinc : Real;
  DelayTime,dx,dy,Xp,Yp,Ymin,Ymax,I : Integer;
  DataBuff : IntArray;
Begin
  I := 1;
  DelayTime := DelayInSeconds(Rate);
  InitDAC(Analog,Quit := False;
  If (Display = 'n') Or (Display = 'M')
  Then
  Begin
    (4)
    OpenWindow(1);HeaderOn(1);
    Msg(19,5,White,'To quit press Esc key. ');
    For I := 1 To NumData Do
    Begin
      (5)
      RawData := AnalogRead(DACAnode);
      Data[I] := ConvertVolt(RawData,20,10);
      GotoX(23,3);Write(
      GotoX(23,3);Write('Data ',I,' ',Data[I]:5:2);
      If KeyPressed Then
      Begin
        (6)
        Ch1 := ReadKey;
        If (Ch1 = Chr(27)) Then Goto EscapeRead1;
      End;
      (6)
      TimeDelay(DelayTime);
      End;
      (5)
    End;
  End;
EscapeRead1;
If I = NumData
Then DataLoaded := True Else DataLoaded := False;
CloseWindow;
End
(4)
Else
  (7)
  Display data on real time.)
  Begin
    (7)
    If SetupData.InputSpan = 'S' Then
    Begin
      Ymin := 0; Ymax := 5;
    End Else
    Begin
      Ymin := 0; Ymax := 10;
    End;
  End;
End;
InitialSize;
DrawPlotAxis(90,20,600,250,LowX,HighX,Ymin,Ymax,True);
DrawGrid(15,140,1,0,White,PltLabelY);
DrawGrid(450,285,0,0,1,White,PltLabelX);
With AxisData Do
Begin
  (9)
  ScaleX := (ax2-ax1)/(xymax-xmin);
  ScaleY := (ay2-ay1)/(ymax-ymin);
  Xinc := (ax2-ax1)/(NumData-1);
  Yp := ay1; Yp := ay2;
  (9)
  For I := 1 To NumData Do
  Begin
    (10)
    RawData := AnalogRead(DACAnode);
    Data[I] := ConvertVolt(RawData,20,10);
    dx := Xp-Round(Data[I]*ScaleX);
    If (dx > Yp) Then dx := Xinc;
    If (dx < 20) Then dx := 20;
    PutPixel(dx,dy,White);
    If KeyPressed Then
    Begin
      Ch1 := ReadKey;
      If (Ch1 = Chr(27)) Then Goto EscapeRead2;
    End;
    TimeDelay(DelayTime);
    End;
    Ch1 := ReadKey;
    If Ch1 = 'P' Then PrintScreen(False,6);
  End;
EscapeRead2;
Then DataLoaded := True Else DataLoaded := False;
ExitProcess;
MainMenuScreen;
End;
(7)
(1)
End;
Procedure ReadS5;
(Read data from LS5)
Var I : Integer;
Hdata : Array[1..10] of String[7];
Begin
  Write(Com1,'$C 0'+Chr(13));ReadPrompt;
  Reset(Com1);
  For I := 1 to 10 Do (Read header information)
  Begin
    Read(Com1,Hdata[I]);
    WriteLn(Hdata[I]);readLn;
  End;
End;
Procedure SetLS5;
Var
  Smode,Scode: String[1];
  Sspeed,Sex,Sem,Sgain,Slow,Shigh: String[3];
Begin
  AssignMux(Com1,0,186); (Com1,2400,E,7,1)
  Rewrite(Com1);
  Write(Com1,'$E 0'+Chr(13));ReadPrompt;
  Case Smode Of
    1 : Smode := '0'; (Fluorescence mode)
    2 : Smode := '1'; (Phosphorescence mode)
  End;
End;

```



```

ClosedWindow;
GrInitialize;
If Not (NumFiles = 0) Then
  For i := 1 To NumFiles Do
    Begin
      Assign(InFile,FileName(i));
      Reset(InFile);
      If CheckError(1) <> 0 Then Begin
        MainMenuScreen;
        Exit;
      End;
      Readln(InFile,LowX); Readln(InFile,HighX);
      Readln(InFile,NumData);Readln(InFile,InputSpan);
      For j := 1 To NumData Do
        Readln(InFile,Data(j));
      Close(InFile);
      DataLoaded := True;
      Plot(True);
    End;
  End;
  Ch1 := ReadKey;
  If Ch1 = 'P' Then PrintScreen(False,6);
  ExitProcess;
  MainMenuScreen;
End;
($I+)

Procedure InitPlotter;
(Sets up plotter and Com1 for plotting)

Var
  Com1Addr: Integer;

Begin
  If GoPlotter And (PlotNum = 0)
  Then Begin
    AssignAux(Com1,0,195); Rewrite(Com1);
    Write(Com1,Chr(27),'.'); (Wake up plotter)
    Write(Com1,'.M:');
    Write(Com1,Chr(27),'.M:');
    Write(Com1,Chr(27),'.M1000;5;6:');
    (Data block size 1000 byte,Enq 5,Ack 6 ASCII )
    End
  Else Begin
    If PlotNum = 0
    Then Begin
      Assign(Com1,PlotFileName);
      Rewrite(Com1)
      End
    Else Append(Com1);
  End
End;

Procedure DrawFrame(UnitX,PlotItA,PlotItB: StrL);
(Draw graph frame with plotter for data plot)

Var
  Pld,PldX,X,Y,I: Integer;
Begin
  Pld := Round(NumData/10);
  Pld := Round((HighX-LowX)/10); (data increment)
  WriteLn(Com1,'M:SP:1P1850;2100 9250 7350:');
  WriteLn(Com1,':SCI:0:'); NumData,0,1000;);
  WriteLn(Com1,'PU:0:'); OPD NumData,0,NumData,
    1000,0,1000,0,OPU);
  WriteLn(Com1,':$1.23;.33;T1.1.7.0');
  X := 0;

```

```

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If GoPlotter Then Repeat Until FreeSpace;
For i := 0 To 10 Do
  Begin
    WriteLn(Com1,'PA',X,'0:XT:');
    WriteLn(Com1,'CP',1,'1:LB',Lowmm*Round((1*Pld),Chr(3)));
    X := X+PLX
  End;
WriteLn(Com1,'PA6.5,0:CP+27,-2.5;LB',UnitX,Chr(3));
WriteLn(Com1,'TL 1.2,0');
If GoPlotter Then Repeat Until FreeSpace;
Y := 0;
For i := 0 To 11 Do
  Begin
    WriteLn(Com1,'PA',0,'1:Y,YT:');
    WriteLn(Com1,'CP',4,'-25;LB',Y,Chr(3));
    Y := i+100;
  End;
WriteLn(Com1,'PA',1,'400:CP-6,-2:D10,1:LBRELATIVE',
  'IMERSIF',Chr(3),0,1,0,1);
If GoPlotter Then Repeat Until FreeSpace;
WriteLn(Com1,'PA',1,'-200;SI;LB',PlotfTB,Chr(3));
WriteLn(Com1,'PA',1,'-250;LB',PlotfTB,Chr(3));
End;

Procedure MultiPlotter(PlotStyle: Integer);
(Plots multiple data on the same graph frame)
Var
  X,Y: Integer;
Begin
  If GoPlotter Then Repeat Until FreeSpace;
  For X := 1 To NumData Do
    Begin
      Y := Round(100*Data[X]);
      If PlotStyle = 0
      Then WriteLn(Com1,'PA',X,'1:Y,PP:');
      Else WriteLn(Com1,'PA',X,'1:Y,PP,PU:');
      If GoPlotter Then Repeat Until FreeSpace;
    End;
    WriteLn(Com1,'PU:');
    WriteLn(Com1,'SP0,1');
    CloseWindow;
  End;

  Procedure StartPlot;
  (This routine plots data with HP7475A. Computer com1 used to
  communicate with plotter. Com1 DB25 connector must be configured
  as dual-terminal by connecting pin 20 to pin 6 and pin 4 to pin
  5,8. Only three lines(2,3,7) is used for communication. Data flow
  control is checked and controlled by software. HP7475A must
  be configured as 4800 baud Direct connection mode.)
  Var
    PlotStyle,PlotChoice: Integer;
    UnitX,PlotfTB,PlotfTB: String(80);
  Begin
    If PlotNum = 0
    Then Begin
      GoToXY(2,2);Write('Enter unit for X-axis: ');
      ReadLn(UnitX);GoToXY(2,3);
      Write('Enter the 1st line label(X-axis): ');
      GoToXY(2,4);ReadLn(PlotfTB);GoToXY(2,5);
      Write('Enter the 2nd line label(X-axis): ');
      GoToXY(2,6);ReadLn(PlotfTB);
      GoToXY(2,7);Write('Enter plot style(0: Line, 1: Dot)
      :');

```

```

      ReadLn(PlotStyle);
      GoToXY(12,10);Write('==== Working ===== ');
      IntPlotter;
      DrawFrame(UnitX,PlotfTB,PlotfTB);
      MultiPlotter(PlotStyle);
      PlotNum := PlotNum+1;
    End
  Else (If PlotNum > 0)
  Begin
    Msg(2,3,Black,'Enter plot style(0: Line, 1: Dot): ');
    ReadLn(PlotStyle);
    Msg(12,5,Black,'==== Working =====');
    WriteLn(Com1,'SP1,1');
    MultiPlotter(PlotStyle);
  End;
End;

Procedure PlotterPlot;
Label PlotAgain;
Var
  Ys : Char;
ExitPlot: Boolean;
Begin
  If Not DataLoaded Then
    Begin
      ShowMessage;
      Exit;
    End;
    PlotNum := 0; GoPlotter := True;
    OpenWindow(12); HeaderOn(12);
    Msg(2,2,Black,'Check plotter. If ready press any key. ');
    Ch := ReadKey;
    Msg(2,2,Black,'
    If (Ch = #0) Then
      Begin
        Ch := ReadKey;
        If (Ch = #27) Then CloseWindow;
        Exit;
      End;
    PlotAgain;
    StartPlot;
    OpenWindow(12); HeaderOn(12);
    Msg(2,3,Black,'Append another data to the plot? (Y/N): ');
    ReadLn(Ys); CloseWindow;
    Ys := UpCase(Ys);
    If Ys = 'Y' Then
      Begin
        LoadData;
        OpenWindow(12); HeaderOn(12);
        Goto PlotAgain;
      End;
    RestoreCom1;
  End;
End;

Procedure PlotterFile;
Label PlotAgain;
Var
  Ys : Char;
ExitPlot: Boolean;
Begin
  If Not DataLoaded Then
    Begin

```

```

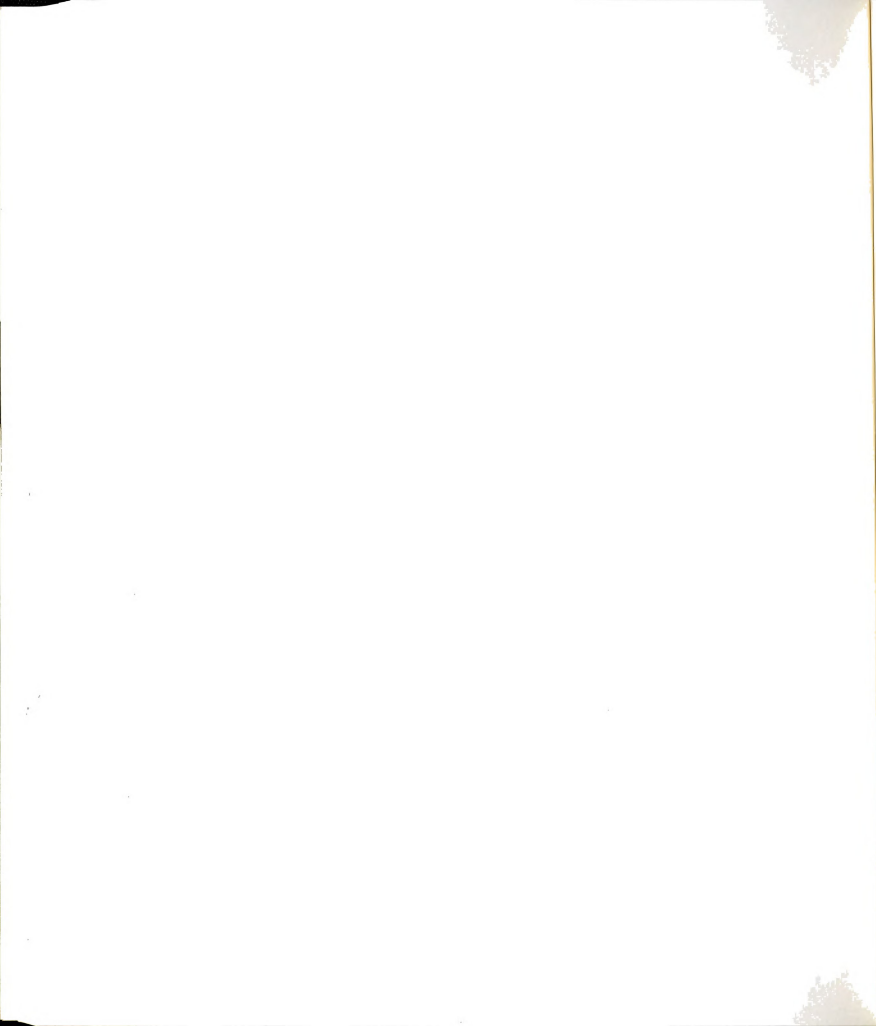
05/31/1989 00:11      Filename: LSDATA.PAS      Page      18
ShowMessage;
Exit;
End;
PlotNum := 0; GoPlotter := False;
OpenWindow(12); HeaderOn(12);
PlotAgain;
If PlotNum = 0 Then
  Begin
    Msg(2,2,Black,'Enter plot file name: ');
    ReadLn(PlotFileName);
    If PlotFileName = '' Then
      Begin
        CloseWindow;
        Exit;
      End;
    End;
  End;
StartPlot;
OpenWindow(12); HeaderOn(12);
Msg(2,3,Black,'Append another data to previous file? (y/n): ');
ReadLn(Ya); CloseWindow;
If Ya = 'y' Then
  Begin
    LoadDate;
    OpenWindow(12); HeaderOn(12);
    Goto PlotAgain;
  End;
End;

Procedure ReadDecay;
Label Start;
Var
  Dat,Drm: Real;
  Stg,Da: String(5);
  Sdat: String(4);
  Schm: String(1);
  Cr: Char;
  I,h,Cht: Integer;
Begin
  Start;
  OpenWindow(1); Cr := Chr(13);
  Msg(2,1,Black,'Number of data : '); ReadLn(NumData);
  Msg(2,2,Black,'Delay time(ms) : '); ReadLn(Dt);
  Tg := Tg*100; Str(Tg,Stg); (Time unit for LS-5 is 0.01 ms)
  Msg(2,4,Black,'Time interval(ms) : '); ReadLn(Gt);
  LowX := Round(Dt*1000); (Label in us)
  HighX := LowX+Round((NumData-1)*Gt*1000);
  For I := 1 To NumData Do
    Begin
      Id(I) := Dt + (I-1)*Gt;
      Ld(I) := Sqr(Id(I));
      V16 := V16 + Ld(I);
      V14 := V14 + Id(I);
      Id(I) := Id(I)*100;
      Lq := Data(I);
      Ldat(I) := Ln(Lq);
      Scl := Sqr(Ldat(I));
      V15 := V15 + Ldat(I);
      V17 := V17 + Scl;
      Plt(I) := Ldat(I)/(Id(I)/100);
      V18 := V18 + Plt(I);
    End;
  End;
  V19 := ((NumData*V18)-(V14*V15))/((NumData*V16)-(Sqr(V14)));
  V20 := -0.434/V19;
  V21 := V19*(V18-V14*V15/NumData);
  V22 := V17-Sqr(V15)/NumData;
  V23 := V22-V21;
  If V23 < 0 Then V23 := Abs(V23);
  V24 := V21/V22;

AssignLux(Com1,0,186); (Com1,2400,E,7,1)
Rewrite(Com1); **** Check Auto Zero and press Enter. ');
Msg(8,5,Black,'**** Setting up Instrument.....');
Ch := ReadyKey;
Msg(8,5,Black,'****');
Write(Com1,'SRE 0'+Cr); ReadPrompt;
Write(Com1,'SPH 1'+Cr); ReadPrompt;
Write(Com1,'SPG'+Stg+Cr); ReadPrompt;
Write(Com1,'SPD'+Std(1)+Cr); ReadPrompt;
Write(Com1,'SAC 10900'+Cr); ReadPrompt;
Write(Com1,'SFR 3'+Cr); ReadPrompt;
Msg(12,5,Black,'');
For I := 1 To NumData Do
  Begin
    Write(Com1,'SPD'+Std(I)+Cr); ReadPrompt;
    Write(Com1,'SAC 0'+Cr); ReadPrompt;
    Reset(Com1);
    Repeat
      Read(Com1,Ch4);
      If Ch4 = Chr(127) Then Read(Com1,Da);
      Until (Ch4=Chr(10)); ReadPrompt;
      Schm := Copy(Da,1,1); Sdat := Copy(Da,2,4);
      Val(Sdat,Dat,ErrorCode); Val(Schm,Drm,ErrorCode);
      Dnm := Pos(10,Drm);
      Data(I) := (Dat/Dnm)/10;
      GotoXY(15,5); Write('Data ',I,' ===== ',Data(I)*100);
      Rewrite(Com1);
    End;
    Write(Com1,'SMA'+Cr); ReadPrompt;
    DataLoaded := True; CloseWindow;
    Close(Com1);
  End;

Procedure CalcLifetime;
Var
  I: Integer;
  Lq,Scl: Real;
Begin
  OpenWindow(1);
  V16 := 0; V14 := 0; Dt := Round(LowX/1000);
  Gt := Round((HighX-LowX)/(NumData-1)/1000);
  Msg(15,1,Black,'Calculating lifetime.....');
  For I := 1 To NumData Do
    Begin
      Id(I) := Dt + (I-1)*Gt;
      Ld(I) := Sqr(Id(I));
      V16 := V16 + Ld(I);
      V14 := V14 + Id(I);
      Id(I) := Id(I)*100;
      Lq := Data(I);
      Ldat(I) := Ln(Lq);
      Scl := Sqr(Ldat(I));
      V15 := V15 + Ldat(I);
      V17 := V17 + Scl;
      Plt(I) := Ldat(I)/(Id(I)/100);
      V18 := V18 + Plt(I);
    End;
  End;
  V19 := ((NumData*V18)-(V14*V15))/((NumData*V16)-(Sqr(V14)));
  V20 := -0.434/V19;
  V21 := V19*(V18-V14*V15/NumData);
  V22 := V17-Sqr(V15)/NumData;
  V23 := V22-V21;
  If V23 < 0 Then V23 := Abs(V23);
  V24 := V21/V22;

```



```

If v24 < 0 Then v24 := Abs(v24);
v25 := Sqrt(v24);
v1 := Sqrt(v23/48);
Msg(5,3,Black,Lifetime);
Msg(5,3,Black,Lifetime);
Msg(5,3,Black,Correlation coeff. := v1);
Msg(5,3,Black,Std. deviation := v1);
Ch := ReadKey;
CloseWindow;

Procedure FldLifetime;
(Calculate lifetime, initial decay signal, and background.
See Cline Love et al, 1996, 56, 1984, Anal., Chem.)
Var
  Dtl: Integer;
  Sum1, Sum2, Sum3, Y, R: Real;
Begin
  Dtl := Trunc(NumData/3);
  Sum1 := 0; Sum2 := 0; Sum3 := 0;
  For I := 1 To Dtl Do
    Begin
      Sum1 := Sum1 + Data[I];
      Sum2 := Sum2 + Data[I+Dtl];
      Sum3 := Sum3 + Data[I+2*Dtl];
    End;
  Y := (Sum2 - Sum3)/(Sum1 - Sum2);
  Lifetime := -(Dtl*Dtl)/Ln(Y);
  R := Sqr((Sum1 - Sum2)/(Sum1 - 2*Sum2 + Sum3));
  Background := (Sum1 - R)/Dtl;
  Damp := R*(1-Pow(Y,Dtl))/(1-Y);
  GotoXY(14,15); Write(Lifetime);
  GotoXY(14,16); Write(Background);
  GotoXY(14,17); Write(Damp);
  GotoXY(14,17); Write('Decay Amplitude:');
  GotoXY(14,19); Write('Working .....');
  Datal := 0 To NumData-1 Do
    +BackGround);
  CloseWindow;
End;

Procedure DecayKinetics;
Var
  I: Integer;
  kpl, ks, kmp, kp, kmq, Ma, Maxquencher, q1: Real;
Begin
  OpenWindow(5); HeaderOn(5);
  Msg(2,2,Black,Number of Data: 1); ReadIn(NumData);
  Msg(2,3,Black,Maximum concentration of quencher: 1);
  ReadIn(Maxquencher); q1 := Maxquencher/NumData;
  Msg(2,4,Black,Micell concentration := 1); ReadIn(Ma);
  Msg(2,5,Black,Exit rate := 1); ReadIn(ks);
  Msg(2,6,Black,Entrance rate := 1); ReadIn(kpl);
  Msg(2,7,Black,Decay constant in micelle := 1); ReadIn(kmp);
  Msg(2,8,Black,Decay constant in water := 1); ReadIn(kp);
  Msg(2,9,Black,Quenching constant on micelle := 1); ReadIn(kmq);
  Msg(2,10,Black,Quenching constant on micelle := 1); ReadIn(kmq);
  For I := 1 To NumData Do Begin
    RData := 1/(ks+kmp+kmq)*q1-(kpl*ks*Ma)/(kq*(q1+kp+kpl*Ma));
    Datal := RData*1E+6; End; (Lifetime in microseconds)
  DataLoaded := True;
  HighX := Round(Maxquencher*10000); (X)=(Q)*x10000 form
  LowX := 0; LowLim := 0; HighLim := HighX;
  InputSpan := 1; Lymax := Round(1.1*Data[1]);
  PltLabelY := 'LIFETIME (Microseconds)';
  PltLabelX := 'QUENCHER (x10000)';
  CloseWindow;
End;

Procedure MainMenuScreen;
Begin
  DrawWindow(2,3,78,9,2,Black,White);
  Msg(24,2,White,1,5,5,0,1,1,1,0,0,0);
  Msg(34,4,White,Version,1,5); Msg(17,6,White,
  '(C) Copyright 1988 by Haidong Kim, Matthew Zablitt';
  Msg(15,7,White,Pesticide Research Center, Michigan State University);
  If FirstSetup Then
    Begin
      SetupDefault;
      ExtractSetup;
    End;
  WriteSetup;
End;

Procedure MainMenu;
Begin
  MainMenuScreen;
  Repeat
    Action1 := BarMenu(1,6,White,Black,DecayPlotExit);
    FileSetUpDataDecayPlotExit;
  Case Action1 of
    1: Begin
      Action2 := PulldownMenu(6,3,
      'Load','Save','List File',1,1,1,1,1,1,1,1);
      Case Action2 of
        1: LoadData;
        2: SaveData;
        3: ListFile;
      End;
    End;
    2: Input_Setup;
    3: Begin
      Action2 := PulldownMenu(8,4,
      'Display','Print','Read DATA','Start Scan',1,1,1,1,1,1,1,1);
      Case Action2 of
        1: DisplayData;
        2: PrintData;
        3: ReadData;
        4: StartScan;
      End;
    End;
  End;
End;

```

```

End;
4 : Begin
  Action2 := PulldownMenu(9,4,
    'ReadDecay','CalcLifetimes','SimulateDecay','DecayKinetics',
    '','','','','');
  Case Action2 of
    1 : ReadDecay;
    2 : CalcLifetimes;
    3 : SimulateDecay;
    4 : DecayKinetics;
  End;
End;

5 : Begin
  Action2 := PulldownMenu(10,5,
    'Screen Plot','Printer Plot','Plotter Plot',
    'Plotter File','Multiple Plot','','','');
  Case Action2 of
    1 : ScreenPlot;
    2 : PrinterPlot;
    3 : PlotterPlot;
    4 : PlotterFile;
    5 : ScrnMulPlot;
  End;
End;

6 : Begin
  OpenWindow(11);
  Write(' Are you sure? (y/n) ');
  Ch1 := ReadKey;
  If Ch1 = 'y'
  Then Begin
    FullScreen(Black,White,True);
    ClrScr; Halt;
  End
  Else CloseWindow;
  End;
End;

Until Action1 = 7;
End;

Begin
  DefinedWindows;
  FirstSetup := True; DataLoaded := False;
  MainMenu;
  ClrScr;
End.

```





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