

SOME STUDIES IN ANALYTICAL LUMINESCENCE SPECTROMETRY

by

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A B S T R A C T

The general theory and principles governing luminescence processes in solution are briefly reviewed. The instrumentation used for the studies described in this thesis is also discussed.

The advantages and disadvantages of the use of photon counting detection interfaced with a commercial spectrofluorimeter are presented. The general theory governing photon counting is discussed. The linear range of operation of the photon counting technique for some organic molecules is evaluated and the corresponding detection limits are also presented.

Quantum yields for Pb(II) and Tl(I) at low temperature and lifetime measurements of inorganic ions, in hydrochloric acid and lithium chloride media are presented.

A technique has been developed for the determination of submicrogram amounts of beryllium using its blue fluorescent complex formed with 2-hydroxy-3-naphthoic acid at pH 7.5. The selectivity of the technique prevents aluminium and other ions from interfering. Solvent extraction of the fluorescent complex was achieved with hexanol.

The fluorescence of the beryllium-2-hydroxy-3-naphthoic acid complex was used to investigate the formation constant of the weak beryllium-EDTA complex in a procedure in which EDTA competed with 2-hydroxy-3-naphthoic acid for beryllium in solution. The conditional

(iii)

stability constant of beryllium-EDTA was calculated by this method.

A brief preliminary study of the adsorption of organic molecules on zeolite matrices was conducted and suggestions for further work are made.

A C K N O W L E D G E M E N T S

The work described in this thesis is entirely original except where due reference is made. No part of the work has been previously submitted for any other degree. The work was carried out in the Chemistry Department, Imperial College of Science and Technology, between October, 1973 and November, 1976.

I wish to express my gratitude to my supervisor, Dr. G.F. Kirkbright, for his invaluable help, guidance and advice during this time. Thanks are also due to several of my colleagues for helpful discussions, and to Mrs. Carol Okech who typed this work.

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CHAPTER 1

1.1. INTRODUCTION

When molecule species are irradiated by photons of light, the light may be absorbed, transmitted or reflected. The light that is absorbed may stimulate electronic and vibration transitions in the molecule. Electronic excitation usually takes place from the lowest vibrational level of the ground state, E_0 . The light that is absorbed or emitted by a body is absorbed or emitted in discrete units termed quanta. The energy, E , carried by one quantum is proportional to the frequency of the radiation i.e.

$$E = h\nu = \frac{hc}{\lambda} \text{ ergs} \dots\dots\dots 1.1.$$

where h is Plank's constant (6.624×10^{-27} erg sec)

ν is frequency (sec^{-1})

λ is wavelength (cm)

c is the constant velocity of light in vacuo (cm sec^{-1})

Once the molecule has been excited to the vibrational levels of the first electronically excited state it rapidly loses its vibrational energy by collisions with neighbouring molecules. If the molecule has been excited to an even higher electronic excited state, E_2 and E_3 then it may undergo radiationless transition known as 'internal conversion' whereby transition to a high vibrational level of the first excited state, of the same energy as the lower vibrational level of the higher electronic excited state, occurs.

Collisional deactivation again causes loss of the resulting vibrational energy back to the E_1, V_0 state. From this level the molecule can return to any of the vibrational - rotational levels of the ground state by the emission of light of the corresponding frequency. These processes are illustrated in Figure 1.1. It is this emission process which is known as fluorescence.

If all the molecules which originally absorbed light underwent this mechanism completely then the solution would be said to have a quantum efficiency of unity where

$$\text{quantum efficiency} = \frac{\text{number of quanta emitted}}{\text{number of quanta absorbed}}$$

Other mechanisms however compete with the fluorescence process; they are internal conversion, collisional deactivation, conversion to lower triplet levels, and photochemical reactions such as dissociation. These processes may reduce the fluorescence efficiency markedly.

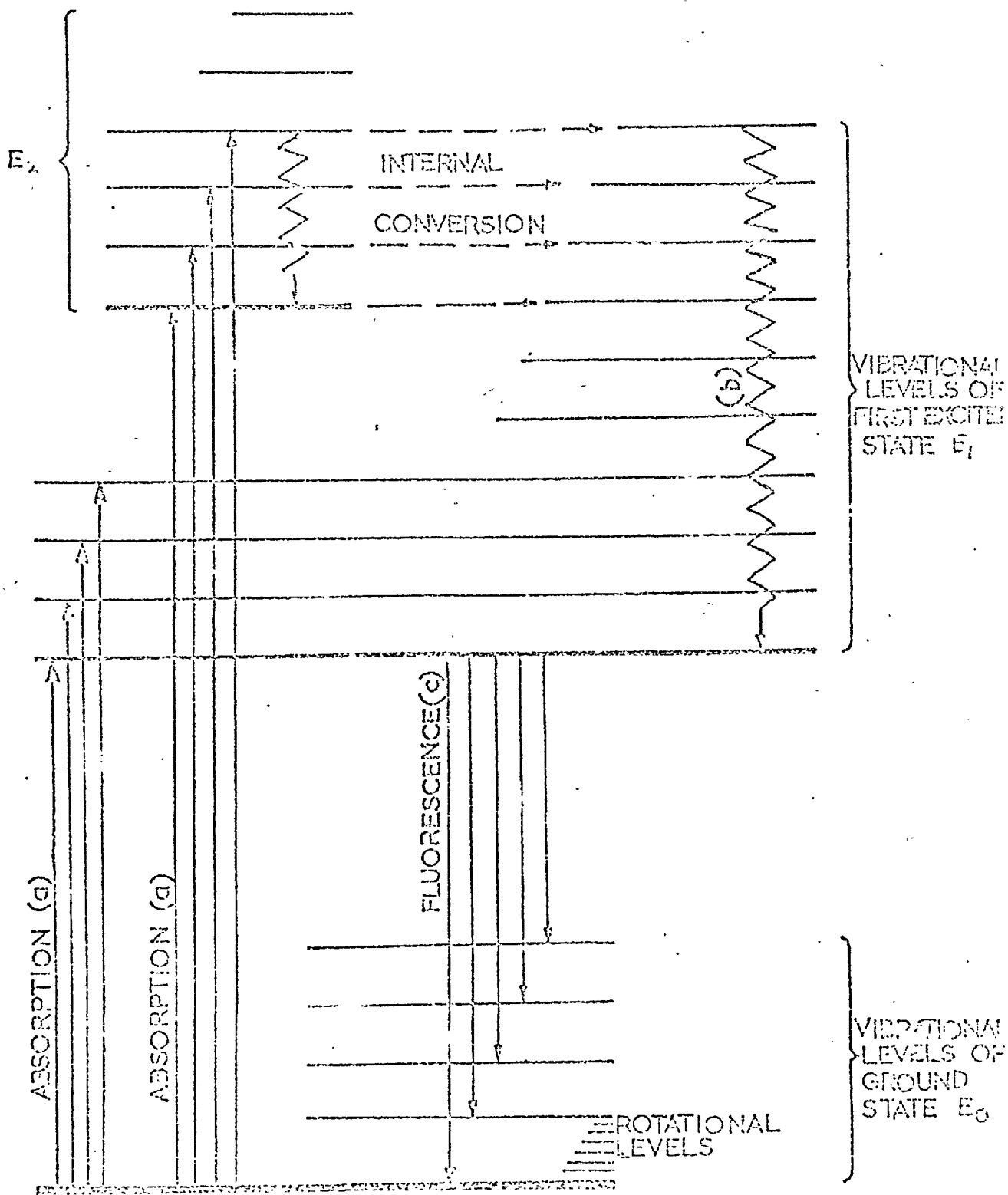


Fig 1.1

Absorption bands of equal intensity and emission maxima of equal intensity would be expected if the position of the energy bands as indicated in fig. 1.1. was the only factor to be considered. In fact the intensities of the different absorption bands vary greatly as do the fluorescence emission bands. The Frank-Condon principle explains this phenomenon. The principle states that absorption and hence excitation, takes place within 10^{-15} seconds and during this time the heavy nuclei of the atoms do not appreciably change their position or momentum, therefore, the nuclear configuration in the excited state is the same as that of the ground state prior to absorption. A simple diatomic molecule is taken to explain the Frank-Condon principle.

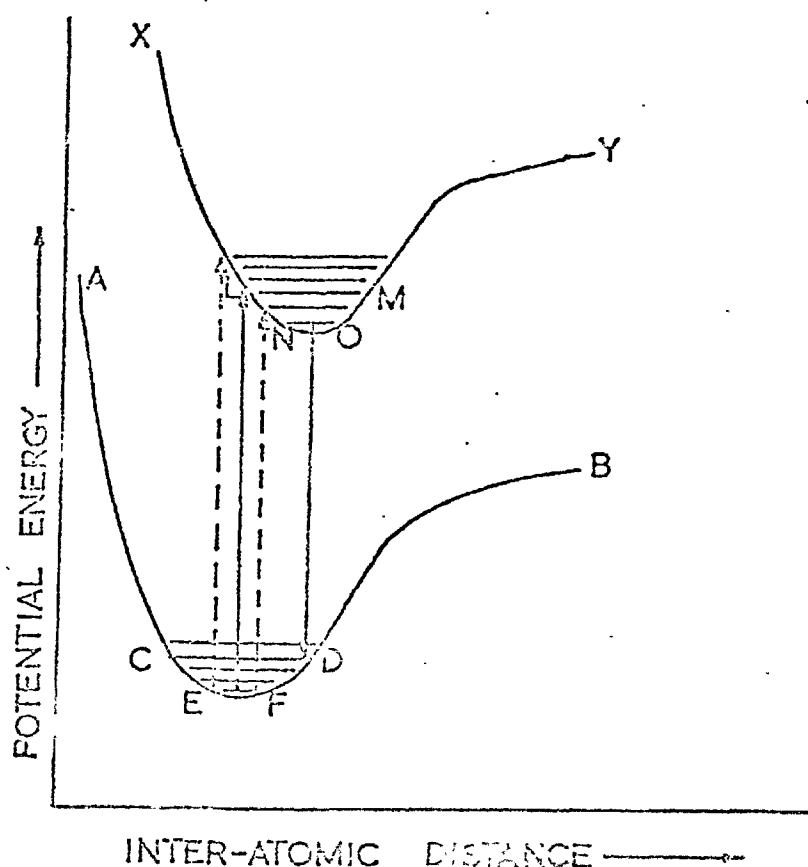


Fig 1.2

The curve AB represents the potential energy of the system in the ground state and the horizontal lines represent molecules in the ground state having different numbers of quanta of vibrational energy.

The excited state is represented by a similar curve XY situated higher up the diagram by a distance equal to the electronic excitation energy.

As no nuclear positional or momentum change occurs the transition can be represented by a vertical line between the two electronic states. For molecules which possess no vibrational energy the point from which transition is most likely to occur is considered. Other transitions however can occur from other positions towards the end of the line EF and from other vibrational levels, though the probability is much lower. Therefore one intense absorption band, together with several of lower intensity can be expected; this may be represented by the dotted lines drawn vertically from either end of the line EF.

Following excitation and subsequent loss of vibrational energy, the most probable transition will be that from the middle of the line NO back to the ground state at D. This will be the most intense band although other weaker ones can be seen for transitions to other vibrational levels of the ground state. The molecule will finally lose its excess vibrational energy by collisions with other molecules in solution.

It should be noted that the emission spectrum is frequently a mirror image of the primary absorption band. This is due to the fact that the distribution of vibrational levels in the first excited state, which determines the shape of the first absorption band, is approximately the same as those in the ground state which are responsible for the shape of the emission spectrum.

1.2. PHOSPHORESCENCE

Electrons in atomic and molecular orbitals are spin coupled, E_0 and E_1 Figure 1.3. representing the singlet states which correspond to these spin coupled states. Spin reversal of an electron may occur though according to the Pauli exclusion principle a new orbital must be formed for the uncoupled electron. This new configuration will show spectroscopically as a triplet because of the interaction of the spins and orbitals. Hund's rule states that spin multiplicity favours the ground state, as a result the excited triplet state T_1 is less energetic than the singlet E_1 from which it was derived. However, the energy difference separating the two states is normally small and exchange between the two can readily occur. This exchange process is known as intersystem crossing.

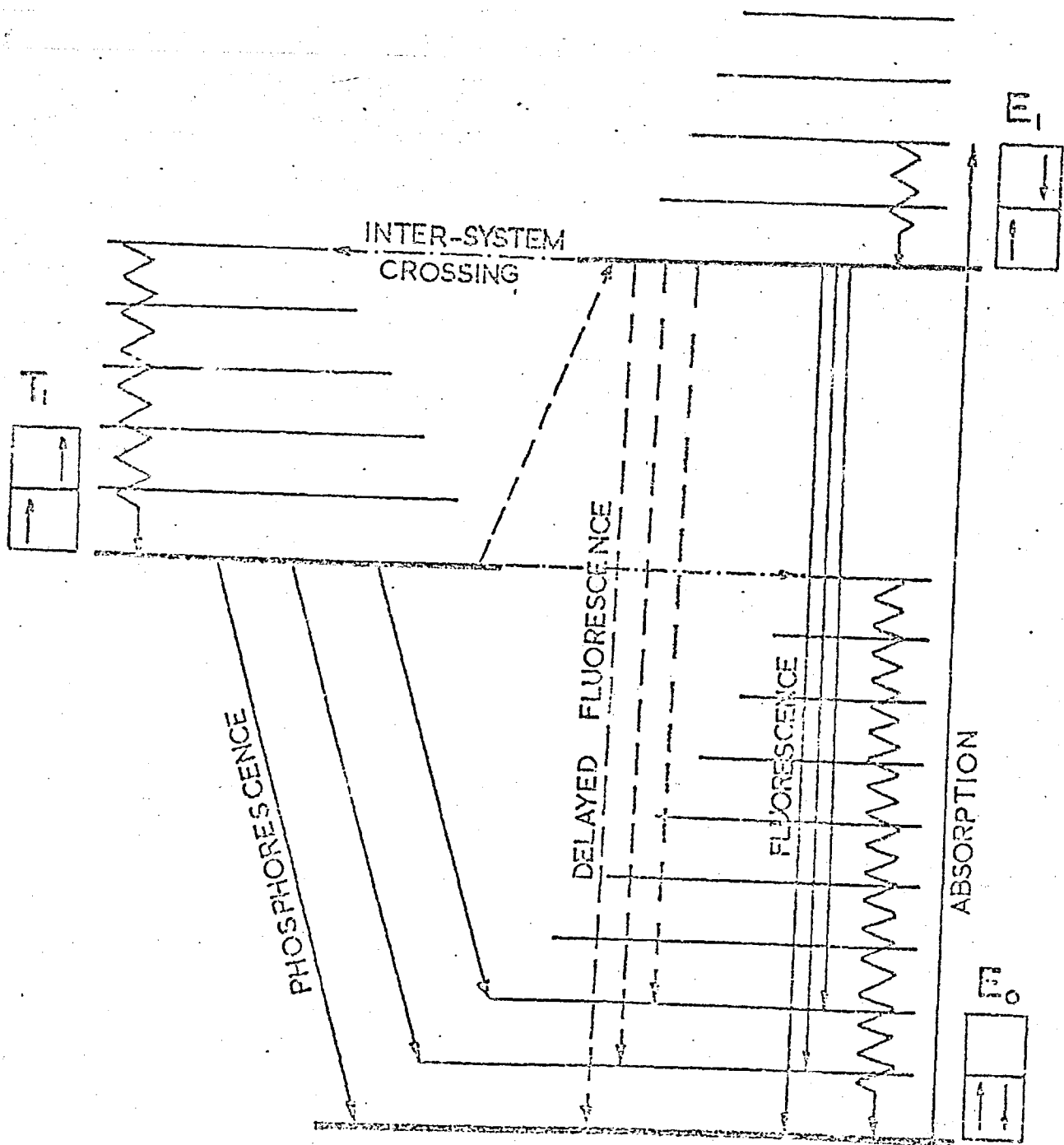


Fig 1.3

Processes in competition with normal fluorescence have similar rate constants i.e., ca. 10^{-8} secs; one such process is intersystem crossing. The lowest vibrational level of the T_1 state is situated somewhere below the lowest excited singlet state but its higher vibrational levels reach the level of the lowest excited singlet and transition between the two can occur. This mechanism is called intersystem crossing where a molecule crosses over from the excited singlet to one of the upper vibrational levels of the lowest triplet state. Consideration of the process is clarified if a potential energy diagram such as that shown in Fig. 1.4. is studied. This diagram refers only to a diatomic molecule, consideration of polyatomic molecules, however, would involve the same general principle.

Light absorption is represented by the vertical line CD. The molecule now in the excited singlet state rapidly loses its excess vibrational energy passing down the curve XY to the point M. At this point the positions and moments of the atomic nuclei are identical to those corresponding to a molecule in the lowest triplet state, and at this point the molecule can transfer to the corresponding vibrational level of this triplet state. As a consequence the molecule adopts the relatively long lived triplet state during the lifetime of the E_1 state.

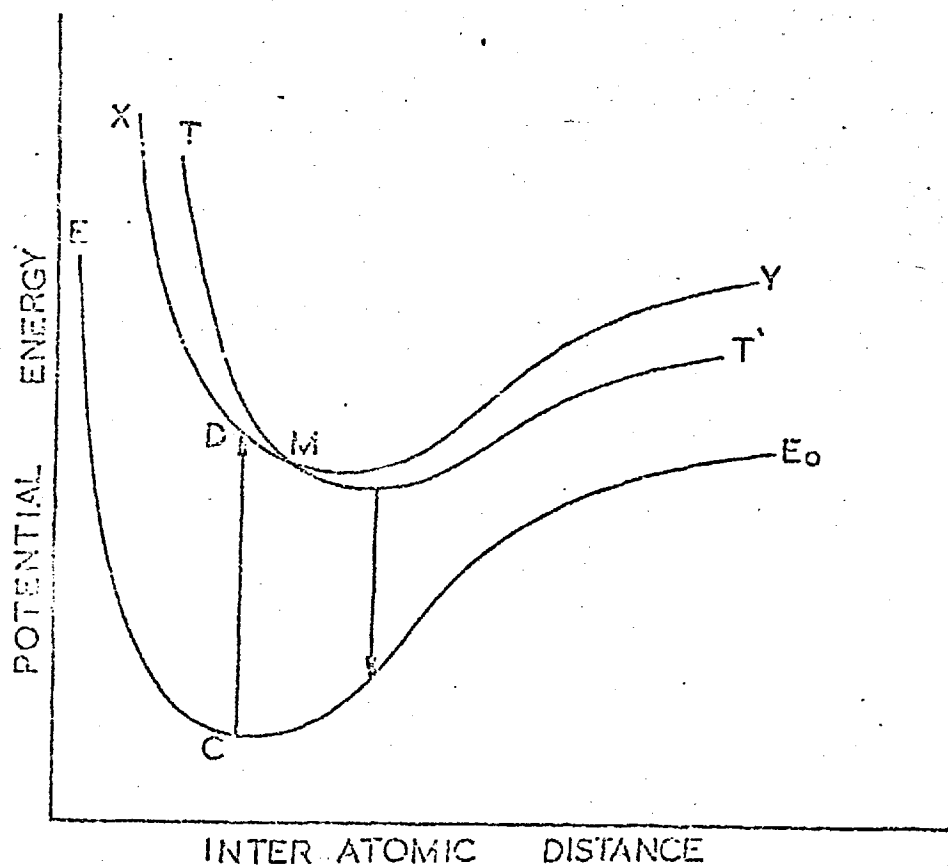


Fig 1.4. Potential Energy Diagram Illustrating Inter-system Crossing

Normal fluorescence occurs when the electron returns to the ground state E_0 from E_1 and the first excited state E_1 has a finite lifetime of $10^{-7} - 10^{-9}$ seconds. If the molecule possesses a triplet state T_1 below E_1 state then phosphorescence may occur. The molecule passes from the E_1 state to T_1 and phosphorescence emission occurs when the electron returns to the ground state E_0 . Transition between states of different multiplicity, however, are forbidden and consequently the direct promotion of an electron from E_0 to T_1 is forbidden as it would require simultaneous spin decoupling. Once the triplet state has been achieved indirectly, re-radiation will be difficult as spin de-coupling must occur; the lifetime of the triplet state is thus relatively long ($10^{-4} - 10$ seconds).

The difference between fluorescence and phosphorescence is that whilst the former involves a singlet-singlet transition the latter involves a triplet-singlet mechanism. As a result fluorescence appears to be an instantaneous process (ca. 10^{-8} seconds) whereas phosphorescence is a great deal slower (ca. $10^{-4} - 10$ seconds). An important consequence of this time factor is seen where the process of collisional deactivation is considered. Though collisions between solvent and solute molecule can be an important factor in decreasing the intensity of fluorescence, phosphorescence has been noted only rarely at room temperature where collisions occur readily, and most samples have to be frozen to form a glass so that the collision rate is drastically reduced.

Phosphorescence emission occurs at a longer wavelength than the fluorescence emission for the same molecule because of the small loss in energy in the transition from the E_1 to T_1 state. It will, however, have a similar spectral shape to the fluorescence band because the same vibrational levels of the ground state are involved for both mechanisms.

Delayed fluorescence can occur by several mechanisms, the two most important of which will be discussed briefly here. Firstly, E-type or thermally assisted delayed fluorescence^{3,2}. This occurs by a mechanism similar to that of normal phosphorescence. However, when the triplet state has been attained, thermal energy excites the molecule back to the first excited singlet state from where its transition back to the ground state gives rise to fluorescence emission.

P-type delayed fluorescence is the second of the more common mechanisms and this occurs by the collision of two excited triplet molecules, with the production of ground state molecule and one excited singlet molecule^{4,2}. The subsequent emission of delayed fluorescence occurs when the electron falls back from the excited singlet to the ground state.

Both these mechanisms give rise to emission of the same spectral characteristics as direct fluorescence though the intensity is much lower and the lifetime is characteristic of that of the triplet state.

For normal fluorescence the fluorescence intensity F is defined as the intensity of absorbed light,

measured in quanta per second, multiplied by the quantum efficiency, ϕ_f ; therefore

$$F = I_a \phi_f = (I_o - I_t) \phi_f \dots\dots\dots(1.2.)$$

I_a is intensity of light absorbed,

I_o is intensity of incident radiation and I_t is intensity of transmitted radiation. The Beer Lambert law, however, states that

$$\frac{I_t}{I_o} = e^{-kc^1l^1} \dots\dots\dots(1.3.)$$

where c^1 is the concentration of material (molecules/cm³) and k is the effective cross-sectional area of one molecule in cm².

Therefore $F = I_o (1 - e^{-kc^1l^1}) \phi_f \dots\dots\dots(1.4.)$

Expansion of this equation gives:

$$F = I_o \left[kc^1l - \frac{(kc^1l)^2}{2} + \frac{(kc^1l)^3}{6} - \dots\dots \frac{(kc^1l)^n}{n!} \right] \phi_f \dots\dots(1.5.)$$

Changing concentration to more practical units of moles/litre and calculating the molar extinction coefficient.

$$F = I_o \left[2.3ec l - \frac{1 - 2.3 ec l}{2} + \frac{(2.3ec l)^2}{6} - \dots\dots \right] \phi_f \dots\dots(1.6.)$$

Therefore for solutions which absorb only weakly (i.e. where the value $ec l$ is small) the above equation simplifies to:

$$F = I_o [2.3ec l] \phi_f \dots\dots\dots(1.7.)$$

Therefore the intensity of fluorescence is directly proportional to the concentration of the solute present, providing all other factors are kept constant. Also, for constant concentration, the fluorescence intensity varies directly with the incident radiation intensity. Thus if a very high intensity source is used very low concentrations of fluorescent solute can in theory be readily measured, and it is this point that illustrates the basic difference between molecular spectrofluorimetry and absorption spectrophotometry. In the latter technique the detection limit or sensitivity is governed by the minimum measurable difference between two signals of high intensity (I_0 and I_t) and the intensity of the source has no effect on the detection limit. In spectrofluorimetry, however, given a stable high intensity of exciting radiation, and a very sensitive and stable amplification and detection system, there should be in theory, no limit to the concentrations detectable. In practice this does not hold. The thermal noise of the photomultiplier tube and also source noise can be significant, but the factor which most often limits the sensitivity is the magnitude and variation in the background (blank) fluorescence. This may arise in one of two ways, either the organic reagent used under the conditions of the determination may exhibit a large fluorescence signal or from fluorescent impurities present in either the reagent or solvent. First or second order scattering of the incident light or Raman scattering by the solvent may also be a limiting factor.

The possibility of Raman scattering has been fully discussed by Parker⁵ who has thoroughly investigated the phenomena for several solutions. When light falls on a substance a proportion of it is scattered at the same wavelength, but a small percentage interacts with the vibrational and rotational modes of the solvent molecule and is re-emitted at a slightly longer wavelength. The difference in wavelength between the absorbed and emitted light corresponds to the energy absorbed during this interaction. Since the emission occurs at a longer space than the incident radiation, it is possible that this Raman emission may cause serious interference with a normal weak fluorescence signal if it is in the same wavelength region. Only the main Raman band need be considered as the others are of very low intensity. If water is the solvent then it will exhibit its main band at a frequency $0.3\mu^{-1}$ lower than that of the exciting light. Other factors that contribute to the magnitude of the blank signal can be partially removed by the purification of reagents and solvents. Raman scattering cannot be readily overcome.

1.3. QUENCHING PROCESSES

The fluorescence quantum efficiency for any compound, ϕ , should ideally have a value of unity, and in fact values of 0.97 for Rhodamine B in ethanol and 0.92 for fluoresceine in 0.1 m sodium hydroxide solution have been reported⁶. Several factors, however, can cause a reduction in this value.

These quenching effects can take several forms, (a) those which directly affect quantum efficiency and (b) those which interfere indirectly with the quantum efficiency, e.g. the inner-filter effect.

1.4. LIFETIME OF LUMINESCENCE

The luminescence intensity decays according to a first-order rate equation when the incident radiation is interrupted i.e. exponentially with time

$$-dI = k.I.dt \dots\dots\dots(1.8)$$

$$\text{Therefore } I = I_0 e^{-kt} \dots\dots\dots(1.9)$$

Where I_0 is the initial intensity at an arbitrary time zero, I is the intensity at a time t , and k is the rate constant for the process. This rate constant is related to the average, or mean, lifetime of decay of luminescence, τ , by the equation.

$$k = \frac{1}{\tau} \text{ or } = 1/k \dots\dots\dots(1.10)$$

substituting for k , the equation 2 becomes

$$I = I_0 e^{-t/\tau} \dots\dots\dots(1.11)$$

From this equation, it can be derived that the time t , over which the decay is measured, becomes equal to τ , when intensity has fallen to $\frac{1}{e}$ of its initial value.

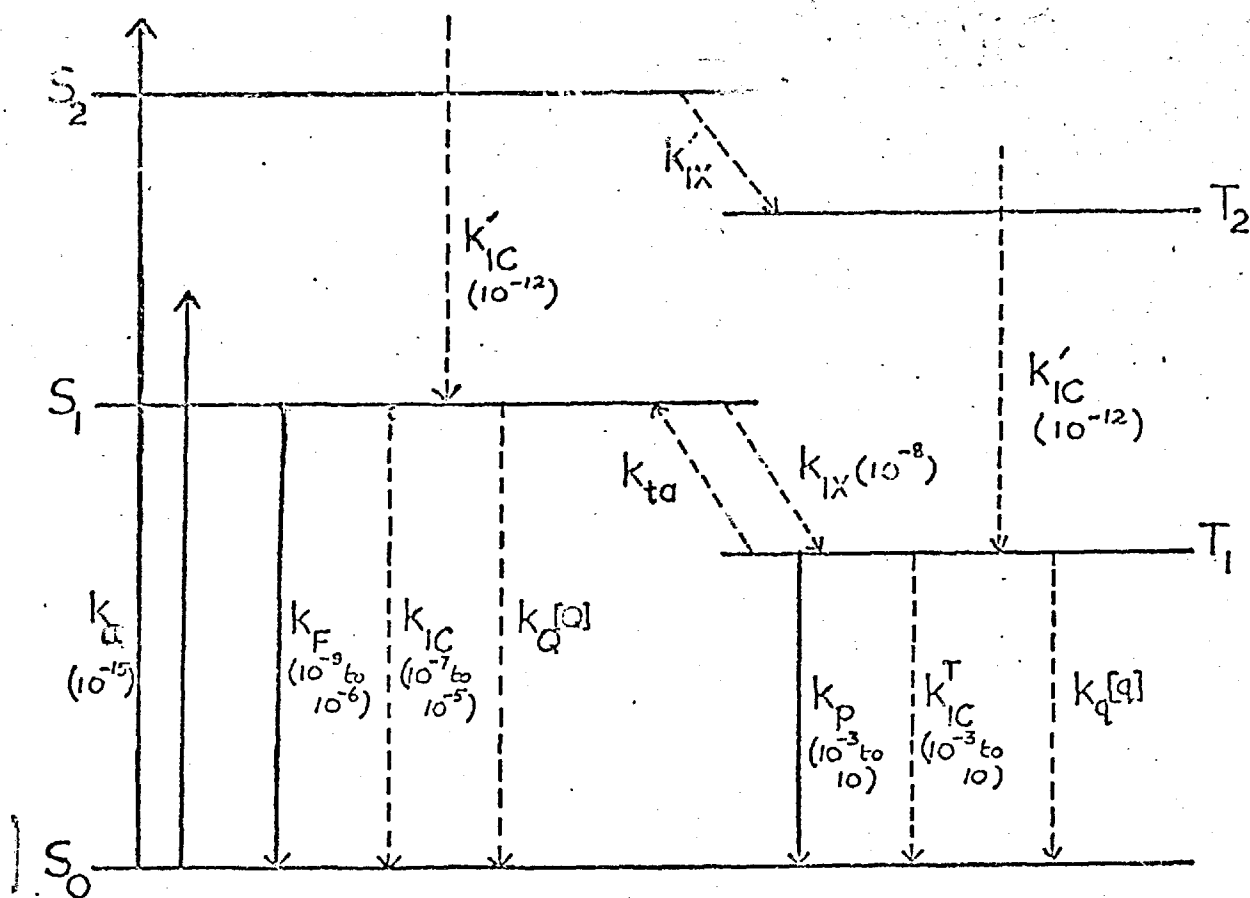
The intrinsic or natural radiative lifetime for fluorescence emission is related to the rate constant for fluorescence k_F by the equation

$$\tau_0 = \frac{1}{k_F} \dots\dots\dots(1.12)$$

Similarly, for phosphorescence, the corresponding relationship is

$$\tau_0 = \frac{1}{k_p} \dots\dots\dots(1.13)$$

Where k_p is the rate constant for phosphorescence emission. The observed lifetime, τ , will be equal to the natural lifetime only in the absence of any deactivating process, both internal and external (i.e., when $\phi = 1$).



Rate constants of Transitions (with approximate meantime in sec.).

Fig 1.5

Absorption of light takes place within the period of a light wave, i.e. within 10^{-15} sec. The rate of light absorption

is denoted by a k_a . k_f is the rate constant for fluorescence emission ($S_1 \rightarrow S_0$), k_{ic} for internal conversion from the lowest singlet excited state S_1 , k_{ix} for intersystem crossing ($S_1 \rightarrow T_1$), k_p for phosphorescence emission ($T_1 \rightarrow S_0$) and k_{ic} for internal conversion from the triplet state to the ground state singlet ($T_1 \rightarrow S_0$). The rate constant for the overall process of thermal activation followed by intersystem crossing from T_1 to S_1 is denoted by k_{ta} . Luminescence quenching by solute molecules are second-order processes, with bimolecular rate constants k_Q and k_q . As the concentration quenching of the solute is often much greater than that of the excited molecules, however, the processes can be represented as first-order reactions with rate constants $k_Q^1 [Q]$ and $k_q [q]$ when Q and q are the concentrations of quenching molecules, for quenching of excited singlet and triplet respectively. The internal conversion, with the rate constant k_{ic} , from upper excited states for both singlet and triplet levels is large; the mean time for these processes is about 10^{-12} sec. There is little evidence for intersystem crossing from upper excited states, which is denoted with a rate constant k_{ix} . If this process occurs, it must be fast to compete with the internal conversion from these states, i.e. it must take place within about 10^{-12} sec.

The observed lifetime, τ , is determined by all the deactivation processes, and for a fluorescence emission, it is given by the equation

$$\frac{1}{\tau} = k_f + k_{ix} + k_Q [Q] \dots\dots\dots (1.14)$$

The fluorescence efficiency, ϕ_f , is given by the equation

$$\phi_f = k_f / k_f + k_{ic} + k_{ix} + k_Q [Q] \dots\dots\dots (1.15)$$

From equations 5, 7, and 8 it follows that

For phosphorescence emission, the measured lifetime, τ_1 is given by:

$$\tau_1 = (k_p + k_{ic} + k_q [q] + k_{ta}) \dots\dots\dots (1.17)$$

The efficiency of formation of the triplet, ϕ_T , defined as the number of triplet molecules formed per quantum of exciting light absorbed, is given by:

$$\phi_T = k_{ix} / (k_f + k_{ic} + k_{ix} + k_q [q]) \dots\dots\dots (1.18)$$

In the absence of added singlet quenchers, the triplet formation efficiency, ϕ_T is:

$$\phi_T = k_{ix} / (k_f + k_{ic} + k_{ix}) \dots\dots\dots (1.19)$$

The phosphorescence efficiency, ϕ_p , is then given by:

$$\phi_p = k_p \cdot \phi_T / (k_p + k_{ic} + k_q [q] + k_{ta}) \dots\dots\dots (1.20)$$

From equations 1.13, 1.17 and 1.20 it can be shown that

$$\phi_p / \phi_T = \frac{k_p}{k_p + k_{ic} + k_q [q] + k_{ta}} \dots\dots\dots (1.21)$$

At low temperatures k_{ta} becomes negligible, and in rigid media diffusion is very small so that the term $k_q [q]$ also becomes negligible. In the absence of a quencher, the quantum efficiencies are denoted as ϕ_f , ϕ_p and ϕ_T and the equation (1.15) can be written as:

$$\phi_f = k_f / (k_f + k_{ic} + k_{ix}) \dots\dots\dots (1.22)$$

From equations (1.19) and (1.22)

$$\frac{\phi_T}{1 - \phi_f} = \frac{k_{ix}}{k_{ic} + k_{ix}} \dots\dots\dots (1.23)$$

Hence the relation between $\bar{\tau}$ and $\bar{\tau}_0$ for phosphorescence can be derived from equations (1.21) and (1.23)

$$\frac{\bar{\tau}_0}{\bar{\tau}} = \frac{1 - \phi_f}{\phi_p} \cdot \frac{k_{ix}}{k_{ic} + k_{ix}} \dots\dots\dots (1.24)$$

If it is assumed that the internal conversion occurs only from the triplet state then the equation (1.24) simplifies to:

$$\frac{\bar{\tau}_0}{\bar{\tau}} = \frac{1 - \phi_f}{\phi_p} \dots\dots\dots (1.25)$$

In order to determine the value of $\bar{\tau}_0$ or k_p for phosphorescence, which are fundamental characters of the substance and are independent of temperature, it is necessary to determine ϕ_p , and also ϕ_T . The parameter ϕ_T is somewhat difficult to determine, but it has been assumed by many workers that because the lifetime of the first excited singlet state (S_1) is short, the internal conversion of S_1 is negligible. Therefore the equation (1.19) simplifies to:

$$\phi_T \approx k_{ix} / (k_f + k_{ix}) \dots\dots\dots (1.26)$$

and equation (1.22) to:

$$\phi_F \approx k_f / (k_f + k_{ix}) \dots\dots\dots (1.27)$$

Hence from equations (1.26) and (1.27)

$$\phi_T \approx (1 - \phi_f) \dots\dots\dots (1.28)$$

The above relation has been found to be approximately valid for a number of compounds ^{7, 8} over a range of temperatures ⁹.

1.5. INSTRUMENTATION

Commercially available instruments for measuring luminescence are rather similar and a description of the Aminco-Bowman spectrofluorimeter will serve as an example of a typical instrument. This instrument has been used by the author during the work described in this thesis.

The Aminco-Bowman double monochromator spectrofluorimeter consists of an optical unit, photomultiplier, microphotometer, power supply and necessary components. The optical unit includes an electrical panel, xenon arc lamp with housing and blower, two monochromators, two slit holders (installed in monochromators), cell compartment, photomultiplier housing with manually operated rotary slit-turret and filter holder with shutter control. This unit, with the exception of the xenon lamp and sweep circuit, operates on 115-Volt 50 or 60-cycle single-phase A.C. (approximately 100 Watts). The xenon arc lamp power is supplied from the d.c. power supply, and the sweep power circuit is energised by a mercury battery contained within the optical unit.

Light from the source is dispersed by the excitation monochromator to enable selection of the wavelength of the light incident on the sample. The light emitted from the sample is viewed at right angles to the exciting beam and dispersed by a similar monochromator to analyse the spectrum of the radiation incident on the detector. The radiation is transformed by the detector into a weak electrical signal and fed to the photometer where it is amplified.

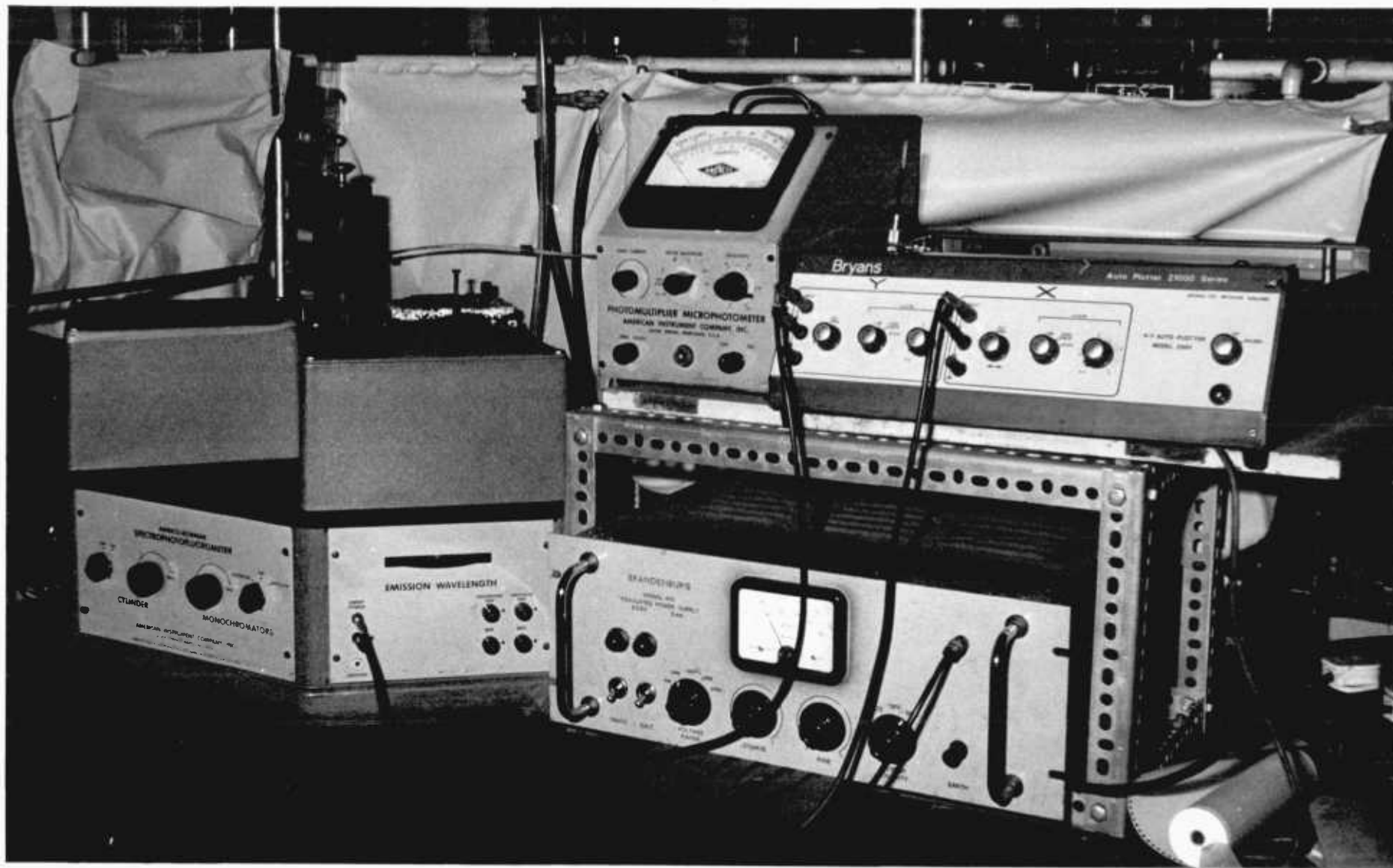


Fig 1.6

Aminco-Bowman double monochromator spectrofluorimeter

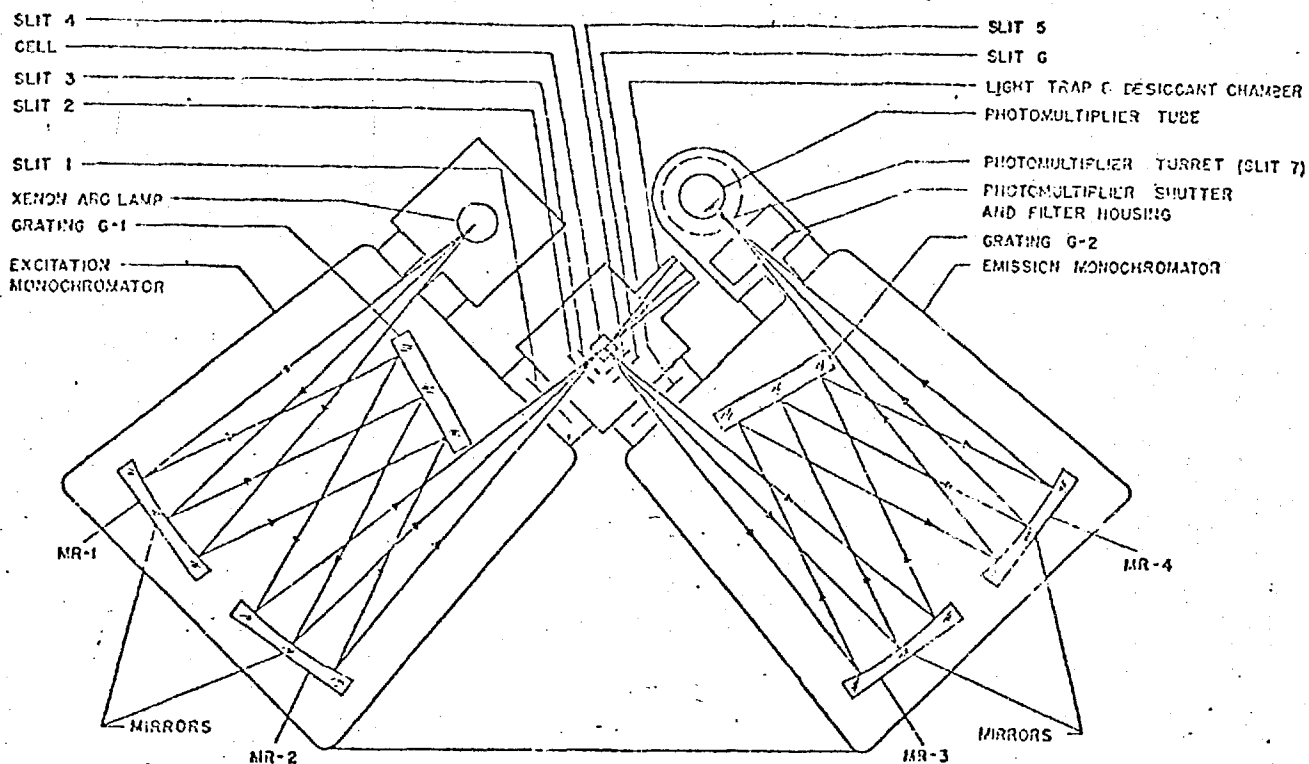


Fig 1.7. Optical Diagram of Aminco-Bowman Spectrofluorimeter

TABLE 1.1

SIZE, ARRANGEMENT AND PLACEMENT OF SLITS

(Width of Slits in Millimeters)

(Slit positions shown in Fig. 1.4)	Mono-chromator Slit 1	Cell Slit 2	Cell Slit 3*	Cell Slit 4*	Cell Slit 5	Mono-chromator Cell Slit 6	Photo-multiplier Slit 7
Arrangement No. 1 [†]	1	0.5	3	3	0.5	1	0.5
Arrangement No. 2	2	1	3	3	1	2	1
Arrangement No. 3	3	2	3	3	2	3	2
Arrangement No. 4	4	3	4	4	3	4	3
Arrangement No. 5 ^{**}	5	4	5	5	4	5	5

*These slits serve as baffles to reduce instrumental scatter.

†Highest resolution, recommended for identification.

**Highest sensitivity, recommended for trace analysis.

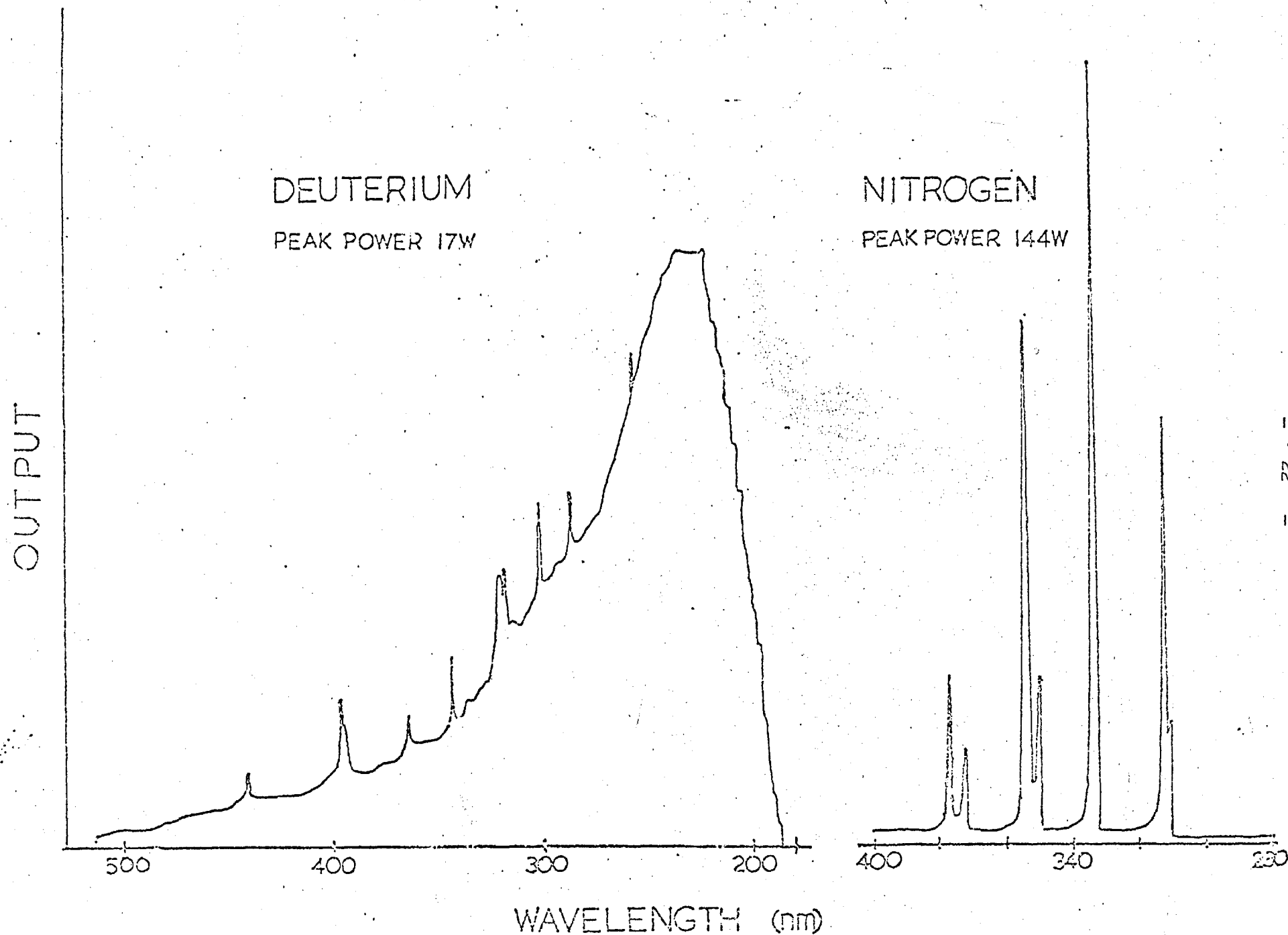


Fig 1.8. Spectra Characteristics of TRW Nanosecond Source Lamps Employed

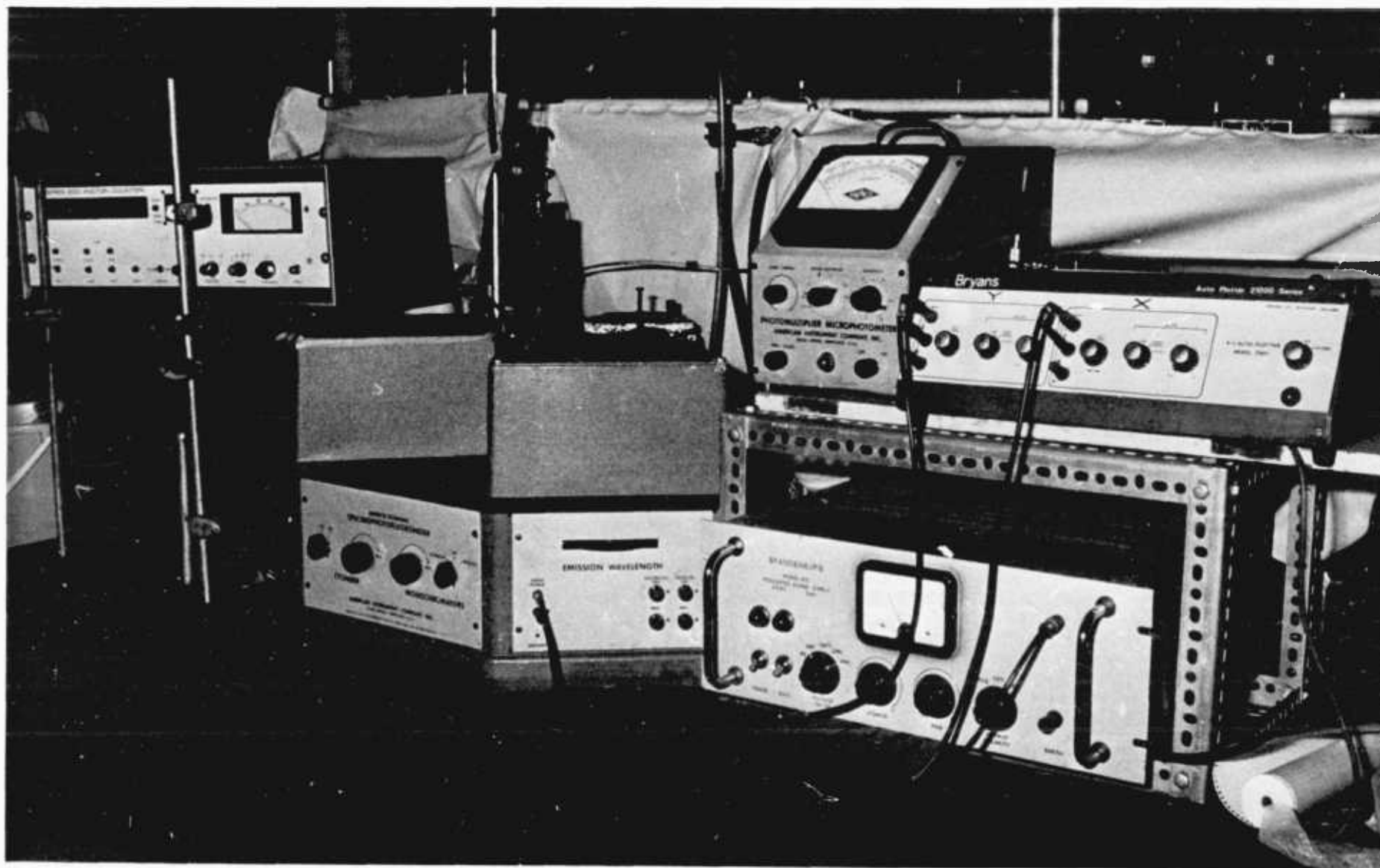


Fig 1.9 Aminco-Bowman double monochromator spectrofluorimeter with photon counting modular detector

An X-Y recorder connected to the photometer enables the photomultiplier output to be displayed on the Y-axis the X-axis input being connected to the wavelength scanning device of the monochromator. The monochromators are fitted with plane gratings each having 600 grooves/mm and 52 x 52 mm ruled area. The axes of the four spherical mirrors and the normals to both plane gratings are coplanar. The two monochromators are optically identical except that the gratings of each are blazed at different wavelengths. The excitation grating is blazed at 300 nm (1st order) in order to strengthen the output of the xenon lamp which decreases below 400 nm. The emission grating is blazed at 500 nm (1st order) to improve the response for fluorescence at long wavelengths. Each monochromator has a wavelength range of 200 to 800 nm. The gratings of the monochromators are moved by motor-driven cans to which are coupled graduated discs for visual observation and wavelength adjustment.

The fluorescence signal passes from the sample to the analysing monochromator and falls on to the photocathode of either an RCA 1 P28 or EMI 9601 photomultiplier tube. The EMI 9601 photomultiplier was used in the photon counting work and subsequent work to be described. The 1 P28 was used for all other studies. The EMI 9601 was supplied by a Brandenburg power supply and the signal recorded by either a photon counter or the photomultiplier microphotometer with its power supply disconnected.

The photomultiplier microphotometer unit consists of the associated circuitry for the photomultiplier tube and an amplifier which responds to the small current produced by the photomultiplier tube and registers the amplified current on the meter. The unit also possesses circuitry for stabilizing the anode voltage at 0.1V

for normal line voltage variations. This is required as the dynode current of the photomultiplier tube is highly sensitive to variations in dynode voltage and therefore requires a stable power supply. The line voltage stabilizer also provides stabilized power to the amplifier. A control for off-setting any photomultiplier dark current caused either by thermionic emission or small light leaks is placed on the microphotometer. The zero adjust control permits zeroing of the meter. The meter multiplier control allows the removal of high voltage from the photomultiplier tube, provides a zero reference voltage required for zeroing the meter and permits sensitivity variation in seven fixed steps 1.0, 0.3, 0.1, 0.03, 0.01, 0.003 and 0.001 over a range of 1000 to 1 (relative intensity of signal on the meter is a product of the transmittance scale reading and the multiplying factor used). At the rear of the unit are outlets for a recorder and oscilloscope.

The recorder used for plotting all the spectra was a Bryans X-Y recorder Auto plotter series 21000 (Bryans Limited, Mitcham, England). This required 200 - 250 volts a.c. at 50 - 60 Hz, single phase and provided spectra 7 x 10 in. in area. As previously described the X-axis is directly linked to the wavelength scale of the optical unit, and the Y-axis to the rear of the microphotometer unit.

The Aminco-Bowman spectrophotofluorometer is a single beam instrument and provides spectra that are uncorrected for the variation in intensity of the xenon arc output the monochromators characteristics are those of the photomultiplier tube with wavelength. Any fluorescence signal produced is therefore only a

product of those parameters and has little absolute significance. Parker and Rees ¹⁰, along with Chen ¹¹ and other workers, ^{12,13,14,15} have described a method for the correction of the excitation and emission spectra in order to obtain the "true" excitation and emission spectra. The corrected excitation spectra are obtained by correction of the observed spectra for any variation in the xenon arc lamp spectral distribution and the transmission characteristics of the excitation monochromator. The corrected fluorescence spectra produced by allowing for the transmission characteristics of the analysing monochromator and the variation with wavelength of the detector sensitivity. Such procedures, however, involve tedious point-by-point correction and unless a corrected spectra attachment is available (American Instrument Co., Inc., Aminco-Bowman spectrofluorometer, Bulletin 1968, 2392-F 14) for rapid production of those spectra this procedure does not appear worthwhile for analytical requirements; though a certain amount of standardisation in this field of analysis would be of no little significance. Though it would obviously be of value to know the true position and intensity of the excitation and emission maxima of any system, the apparent maxima are of a more practical significance, i.e. the maximum response on the operator's own instrument and therefore providing reference is made the uncorrected spectra as obtained directly from the instrument are used in all work described here except for quantum yield determinations. In this latter work an effort is made to obtain the corrected area under the spectrum so that it may be compared with that of a standard substance.

The interference from the cell walls caused by self-fluorescence¹⁶ scatter or internal reflection is also minimised. Another important factor in deciding upon a suitable instrumental geometry is clarified when overall sensitivity is being considered (figure 1.9).

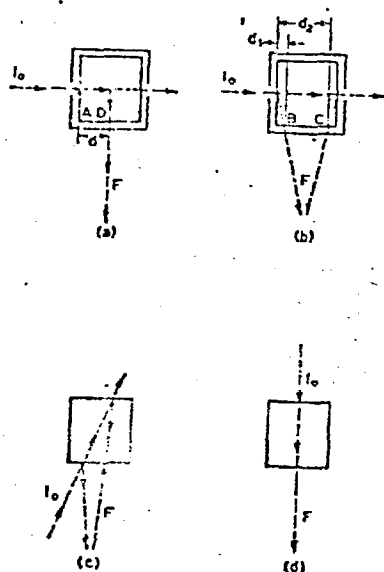


Fig 1.9 Methods for illumination and measurement

(a) and (b) right angle methods; (c) frontal illumination;
(d) in line method.

Though the double monochromator system used here was very satisfactory, several other types are available and they may be more practical in certain cases. The use of an instrument for employing a filter in place of the excitation monochromator appears very attractive for trace determinations because it allows much greater intensity and a wider spectrum of irradiation while a monochromator on the emission side allows more precise resolution of the fluorescence radiation. The light losses inside the grating monochromators used in the Aminco-Bowman system are rather great, and therefore a large increase in sensitivity may be obtained by substituting a filter. However, fluorescence signals received from filter instruments invariably include spurious signals from light scatter, Raman emission, etc., which cannot be differentiated. Therefore the choice of instrument depends on the compromise required between sensitivity, selectivity, resolution and precision.

The reason for measuring the fluorescence at right angles to the incident beam is to reduce any scattering of the incident radiation which would otherwise augment the fluorescence signal and hence prevent accurate trace analysis. Even though most instruments possess efficient monochromators, scattered radiation can still be a serious problem as the excitation and emission peaks usually overlap or are very close to one another.

Instruments that measure the fluorescence signal at angles other than 90° to the incident radiation also have advantages. Frontal illumination is required for the study of solid samples and very strong solutions. A straight through system can be used for phosphorescence measurements; Parker and Rees^{17,10} have

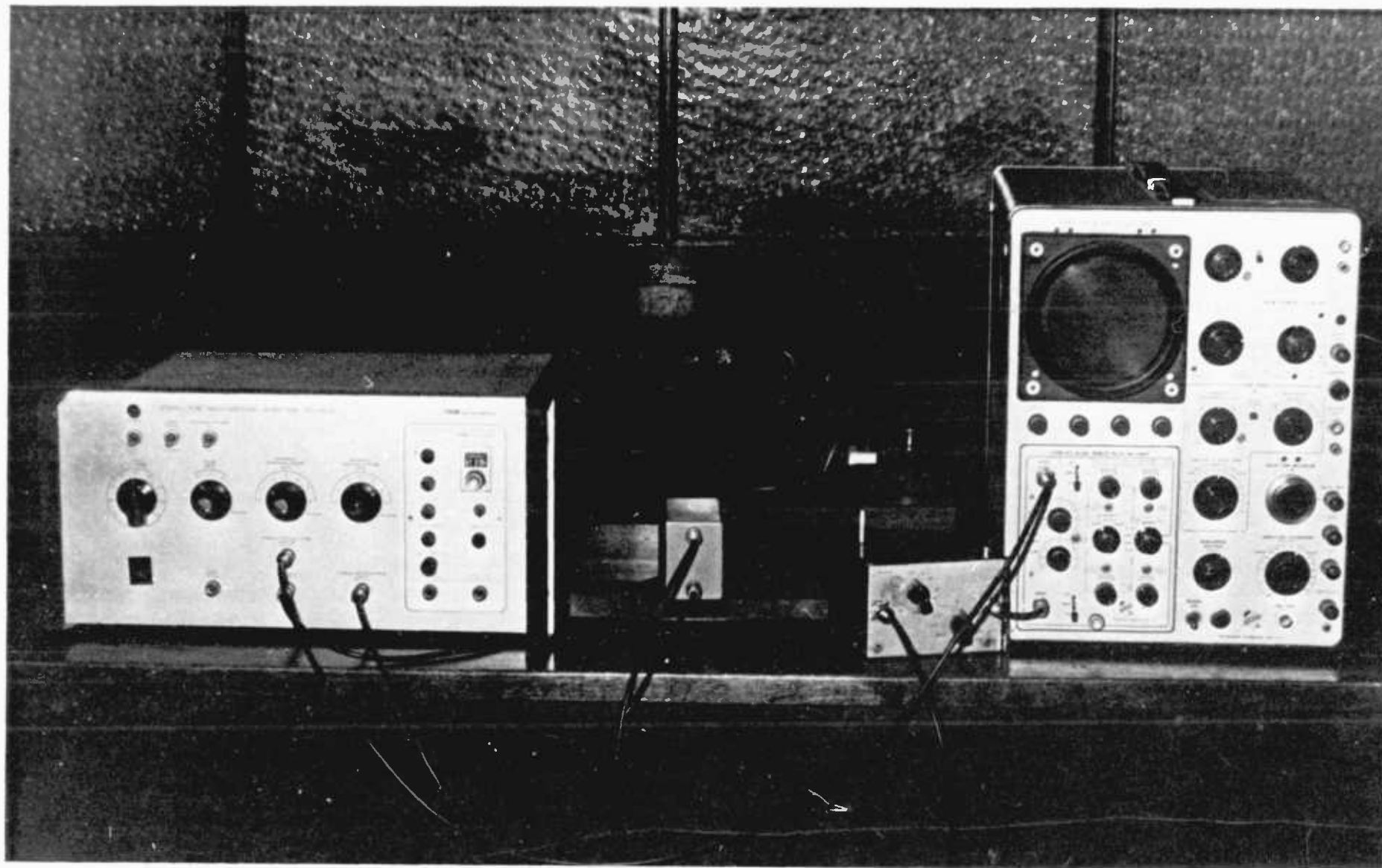


Fig 1.11

TRW nanosecond source unit and fluorimeter

indicated certain advantages of this system when ordinary fluorescence systems are being studied.

Several workers have produced critical reviews of particular types of commercially available instruments ^{18,19,20,21,22,23} and before purchase of any apparatus is made it is advisable to consult these reviews.

1.6 THE TRW-NANOSECOND SPECTRAL SOURCE SYSTEM

The TRW Model 31B Nanosecond Spectral Source (NSS) system, shown in Fig 1.11, consists of the source, lamp housing and control unit and power supply which provides repetition rate control and high and low voltage. The TRW Model 38A relay lens assembly with a pair of quartz condenser lenses in the excitation and emission optical paths, provides chromatic focussing of light over the range 200 to 1000 nm from lamp housing to the sample and from the sample to the detector. A tunable interference filter monochromator (Model 39A) in the path of emitted light allows precise wavelength selection and maximum separation of the fluorescent pulse. Model 40A filter mount carrier in conjunction with model 37A filter. Filter mount adaptor accommodates 2 x 2 inch glass or interference filters. A mount carrier constructed by Narayanaswamy²⁴ accommodates 2 x 2.5 inch Locarte filters and also, with slight alteration of the relay lens assembly, a liquid filter. The detector is an RCA Type 931A photomultiplier tube (S-4 response) incorporated in a TRW Model 33A photomultiplier housing unit. The optical arrangement of the TRW apparatus is shown below in Fig 1.12.

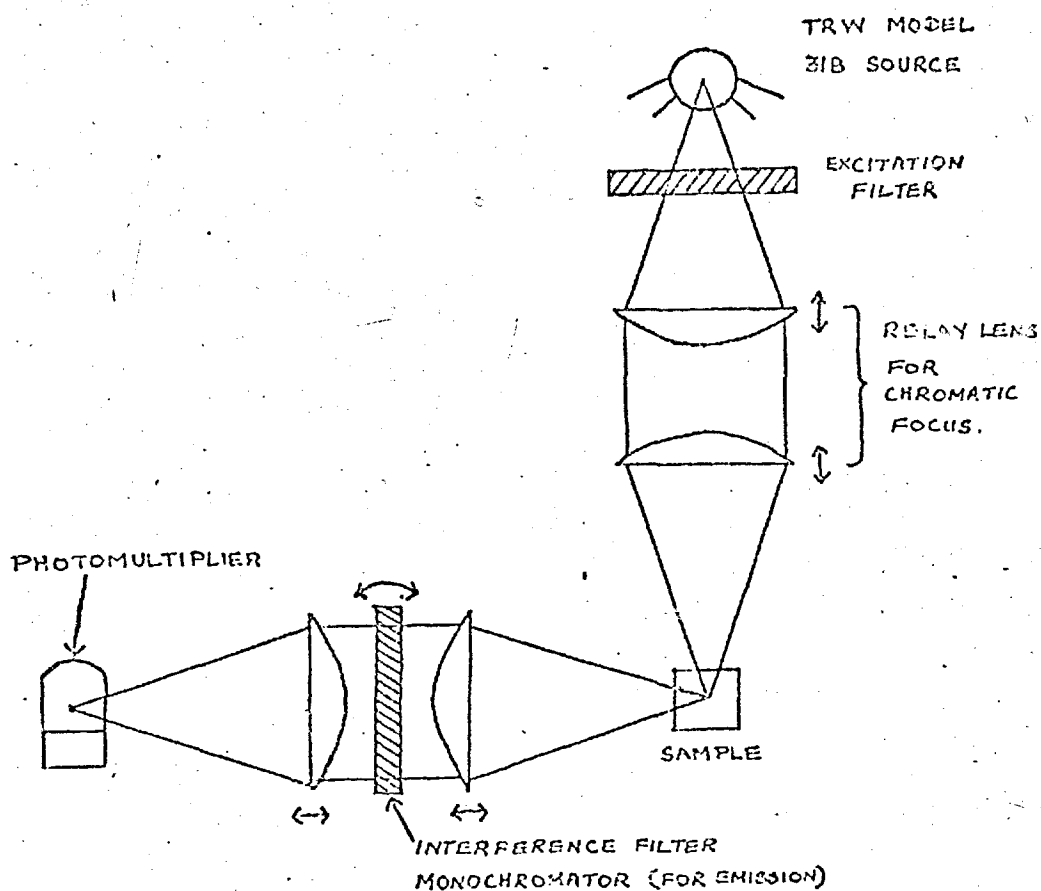


Fig 1.12. Optical Arrangement of the TRW model 31B decay time fluorimeter

The sample chamber (Model 34A) accommodates the sample holder (Model 35A) for liquids contained in a cylindrical fused quartz tube 75mm long, i.d. 8 mm, and wall thickness 1 mm. A slight modification of the sample holder permits use of the quartz Dewar flask fig1.12 for measurements at liquid nitrogen temperature.

The source used to excite the sample, depending on the region of wavelength of excitation required was either a deuterium (No. 268) or nitrogen (No. 267) arc lamp. The spectral characteristics of these lamps are shown in Fig 1.8.

A tektronix oscilloscope, type 585A, with a type 82 dual trace plug-in unit, was used in conjunction with the TRW instrument, to display the decay traces. The monitor output of the internal photomultiplier from the NSS system and the output of the external photomultiplier are connected to the input (A and B respectively) of the oscilloscope, both terminated in 50 ohms for measurement of decay time up to about 100 nanoseconds. Alteration of the time constant of the measuring circuit, by increasing the termination resistance (at the input B), enables measurement of decay times of the order of microseconds or greater. Table 1.2 shows the maximum termination resistance that can be used in measuring a particular decay time, and also the time constant (τ_{rc}) of the measuring circuit.

The time constant of the decay signal should be ten times that of the measuring circuit.

The decay traces were photographed using a Hewlett Packard oscilloscope Polaroid camera (Model 196B) with type 107 polaroid film. The lifetimes were calculated from these traces in the photographs as described in Chapter 3.

TABLE 1.2 Maximum time constants of measuring circuit with TRW source for particular termination resistances employed to measure decay times of luminescence

Load Resistance (ohms)	τ_{Decay}	τ_{RC}
50	$\geq 10 \text{ ns}$	—
50	$\geq 20 \text{ ns}$	—
50	$\geq 50 \text{ ns}$	—
50	$\geq 100 \text{ ns}$	—
100	$\geq 200 \text{ ns}$	$20 \text{ ns} = (100 \Omega) \times (2 \times 10^{-10} \text{ pf})$
270	$\geq 540 \text{ ns}$	54 ns
510	$\geq 1.02 \mu\text{s}$	102 ns
1 K	$\geq 2 \mu\text{s}$	200 ns
2.2 K	$\geq 4.4 \mu\text{s}$	440 ns
4.7 K	$\geq 9.4 \mu\text{s}$	940 ns
10 K	$\geq 20 \mu\text{s}$	2 μs
15 K	$\geq 30 \mu\text{s}$	3 μs
27 K	$\geq 54 \mu\text{s}$	5.4 μs
47 K	$\geq 94 \mu\text{s}$	9.4 μs
100 K	$\geq 0.20 \text{ ms}$	20 μs
270 K	$\geq 0.54 \text{ ms}$	54 μs
470 K	$\geq 0.94 \text{ ms}$	94 μs
1 Meg.	$\geq 2 \text{ ms}$	200 μs

1.7. TRACE METAL ANALYSIS BY FLUORIMETRY

In a general review of fluorescence analysis of inorganic substances Shcherbov²⁵ lists 20 elements for which sensitivities in the range of 1 to 0.1 $\mu\text{g}/\text{ml}$ may be obtained.

Additional data on the fluorescence excitation spectra and absorption spectra of the chelates of Al, Be, Th, Zr, B, and Li show the same close correlation of these two types of spectra as is evidenced in the case of pure organic compounds²⁶. The emission spectra of these chelates are also given in this article. Turkevich²⁷ has reported on a study of the reaction of 50 Rhodanines and 10 thiozolidine -2, 4- diones as spot test reagents with various metal ions. Filter paper was impregnated with the reagents and spotted with the metal ions. He concludes that rhodanines are group reagents for Ag^+ , Hg^{2+} , Cu^+ , Cu^{2+} , Au^{3+} , Pd^{2+} and Pt^{4+} and the thiozolidines are group reagents for La^{3+} and UO_2^{2+} in addition to the ions listed above. Stolyarov and Grigorev have applied luminescence methods to the microchemical detection of a number of elements with a sensitivity of 0.1 to 0.0005 μg in 0.003 ml²⁸. The methods are based on the formation of fluorescent products by introduction of microquantities of the elements into compounds or crystals such as CaO or KI, Ga with rhodamine B, Pb with pyridine and KI, and arsenic with ferrocynide. Fluorescent reactions are also given for Sb and Sn.

The reaction products of 8-quinolinol with some 30 ions have been surveyed²⁹ with respect to their fluorescence colours, extraction with CCl_4 , the proper pH for extraction and spot reactions on paper. Popovych and Rogers³⁰ continued their study of the metal 8-quinolates

as to the function of solvents and substitutes on the fluorescence. The studies of Umland and Meckenstock^{31, 32} on the partition of the metal complexes of 8-quinolinols, substituted in the 7-position, between water and chloroform have shown some interesting effects such as the quantitative extraction of the calcium complex when the chloroform contains 2% n-butylamine. The widely distributed blue fluorescence between 430 and 480 nm which is found in many minerals and commercial chemicals has been shown to be caused by traces of organic materials probably from atmospheric dust^{33, 34} and has made metal chelates with 12 salicylaldehyde condensation products and determined some factors influencing the fluorescence intensity. He also studied the metal chelates of the condensation products of resorcyaldehyde with hydrazine and aromatic amines with 11 metal ions³⁵. The sensitivity of the fluorescence reactions of quercetin with salts of Al, Be, Ga, In, Y, La, Mg and Zn has been determined and the optimum pH ranges have been established³⁶.

Bishop³⁷ has reported on the pH requirements, complex formation, and use of a titration indicator³⁸ of 8-quinolinol-sulfuric acid with the metals usually determined with 8-quinolinol. He has also investigated the fluorescence properties³⁹ of the salicylate complexes of Cu, Ce, Ni, Zn, Mg, Be, Fe and Al.

Luminescence in the system $\text{Sb}_2\text{O}_3\text{-Sb}_2\text{O}_5$ can be used to detect small amounts (0.1 ppm) of Mn. The ions of Ag, Sn, Ca, Tl, Mo, U, Ti and Ce do not activate the luminescence. Boron and arsenic increase the luminescence intensity when Pb, Cd, Fe, Ni, Ce and Cu have a quenching effect. Ba and Be produce new luminescent substances⁴⁰.

The fluorescent materials zinc sulphide and fluorescein have been used as tracers on solids released in the atmosphere in fallout studies ⁴¹.

An investigation of the reactions of five salicylamines shows that these reagents may be used as acid-base indicators and also form fluorescent complexes with Be, Sc, Zr, and Ga ^{43, 44}, Kirkbright and Stephen have reported on some metallofluorescent derivatives of 2,2'-diamino phenyl N, N', N' - tetraacetic acid and shown that of 32 ions tested Cu, Co, Ni, Mn, Pb and Zn do not form fluorescent chelates.

A general discussion of spectrofluorimetry as a trace technique in inorganic analysis which includes the requirements for apparatus, reagents, procedures, and 31 references has been given by West ¹⁸.

Anderson and Hercules ⁴⁵ have determined the fluorescence spectra and quantum efficiencies of the thioxoxine chelates of Cd and Zn. Davies ⁴⁶ used chloroform to extract the 8-thio quinolates of In, Ga, Cd and Tl and demonstrated their possibilities. Blyum and Pavlova ⁴⁷ have extracted 24 complex and simple salts of Rhodanine B, Rhodanine S, and Crystal Violet with benzene and benzene-acetone mixtures and have determined their optical properties. These authors also measured the fluorescence intensities of the rhodanine complexes. The higher sensitivity of the fluorescence methods compared to absorption methods was ascribed to the lower blank values.

A review of fluorescence analysis of inorganic substances for the years 1962 to 1968 which covers 36 elements, CN^- and N_2H_4 and lists 298 references has appeared in a Russian publication ⁴⁸.

Analytical applications of Schiff's bases are reviewed with 66 references. The factors influencing the stability and colour of the bases and chelates are discussed ⁴⁹. The compound 3,5 -bis (dicarboxyaminomethyl)- 4,4'-dihydroxy stilbene was found to form fluorescent complexes with Al, Be, Sr, Ba, Zr, Co, Y, La, Ga and Lu. A review of the luminescence characteristics of the halogen salts of Bi, Sn, Sb, Te, Ga, Ge, As, Si, CN, In, Au and Ag at 185°C shows the application of this luminescence method of analysis ⁵⁰. An excellent article on the fluorescence properties of 2,3-, 2,4-, and 2,5,- dihydroxybenzylidene-o-aminophenol and their complexes with a number of di- and trivalent elements shows a number of applications to analysis of metal ions. The 2,4- compound seems to produce the most intense fluorescence in the chelates and the complex with Al in 70% DMF at a pH of 6.1-6.2 has the highest intensity ⁵¹. The analytical applications of the flavonols as fluorimetric and colorimetric reagents have been the subject of reviews by two authors: in one case 180 references are listed ⁵² and in the other 118 ⁵³. The author of the latter article has also published a review on the analytical applications of the hydroxycoumarins ⁵⁴.

Hafnium complexes with morin in NH_2SO_4 and upon addition of methyl alcohol it is possible to detect 25 ng of Hf in 5 ml of solution. In HClO_4 Hf complex of quercetin fluoresces without interference from Zr and forms the basis for the fluorimetric determination of Hf with a limit of detection of 10ng/ml ⁵⁶. 8-quinolinol-5-sulphonic acid was used to mask Cu, Ni, Al, Fe and U interferences and as an indicator in the zinc-ferrocyanide titration ⁵⁷.

Fluorescence spectra were recorded for Cu (I) complexes with pyridinecarboxylic acids ⁵⁸ and N-methyl-piperamine ⁵⁹.

2-Hydroxy-1-naphthaldehyde forms a fluorescent complex with Sc. and a nonfluorescent complex with Cu to form the basis for the determination of copper (1) by fluorescence titration⁶⁰. Tungsten may be determined by formation of a fluorescent complex with morin with a sensitivity of 15 ng of W. The triphenyltin content in potatoes has been determined fluorimetrically after complex formation with 3-hydroxyflavone.

1.8. METHODS FOR THE DETERMINATION OF TRACES OF BERYLLIUM

Recent advances in the design of nuclear reactors have led to a demand for higher working temperatures. The use of beryllium as a cladding material for the fuel elements is attractive because it is strong, light (sp. gravity: 1.784), has a low thermal neutron capture cross-section and a high melting point (1283°C)⁶¹.

Beryllium disease has received extensive study since 1943 and the literature has been reviewed by Tepper et. al.⁶². No cases of beryllium poisoning have been observed in miners handling beryl ore. A variety of ailments, however, ranging from dermatitis to severe respiratory illness, have been reported among workers engaged in the extraction of the ore and others handling fluorescent powders containing beryllium⁶³.

Because of the above reasons the determination of traces of beryllium is of paramount importance. The following general methods are commonly employed for the determination of microquantities of beryllium: Colorimetry, fluorimetry and emission spectrography.

The colour reactions of beryllium are of two types. In one type the pH of the solution is usually adjusted to a value which will cause precipitation of beryllium hydroxide, which then absorbs the reagent with a change in colour. This is lake formation. The other type involves reaction between the dye and beryllate, the pH of the medium high enough to dissolve beryllium hydroxide. On the whole, this type of reaction is to be preferred as being more specific ⁶⁴.

Beryllium can also be determined by spectrographic methods; one such method a commercial plasma jet was modified by replacement of the direct-injection system by a nebulizer chamber to give improved stability and permit instantaneous photoelectric read-out when operated in conjunction with a grating monochromator with non-integrated photoelectric detection similar to that used in flame spectrometry. For 67 elements detection limits are reported and some are compared with the corresponding values for a N_2O -acetylene flame. The plasma jet is also useful for the determination of Zr, Be, Si, B, Ti ⁶⁵.

Silver, Be, Cu, Ge, Mn, Mo, Ni, Pb and Ti were detected by emission spectrography after evaporation of the sample in a platinum basin on a sand bath or in vacuo at 100° . Optimum operating conditions and a procedure for the determination of Mn were established ⁶⁶. A method for the simultaneous spectrographic determination of Zn, P, S, B, Be and F with a plasma current for excitation has been described by the Russian workers Savimova et. al. ⁶⁷.

Along with spectrographic methods atomic absorption spectrophotometric methods are also applied. Nakahara et. al. have determined beryllium in aqueous solution by measurement of absorption

at the 234.9 line in the N_2O -acetylene flame; the calibration graph was rectilinear over the range 0 to 5 ppm⁶⁸. They reported the effects of 33 elements at 100-fold concentration, of acids and 11 water miscible organic solvents (ketones and alcohols). They also described the application of the method to the determination of 0.01 to 0.5% of Be in alloys based on Al, Cu or Mg. There are many such methods for the determination of beryllium in alloys⁶⁸. Fluorimetric methods will be reviewed elsewhere.

There are other not so common or frequently used techniques for Beryllium: Thus, eg. Gallai et. al. described an amperometric method where optimum conditions for the amperometric determination of Be by titration with 5-(bis-(Carboxymethyl) amino) barbituric acid using an anodically polarised graphite electrode are given. The titration was carried out in acetate buffer at pH 5 - 6 at a potential of 0.6 to 0.8V. The method is suitable for determining Be in the range 0.11 μ m to 0.11 mM in the presence of Al, Ca, Mg, Fe(III), Fe(II), Mn(II), Ni, Co, Zn and Cu(II) provided the EDTA is used as a masking agent. The method was used for the rapid determination of Be in alloys containing Al, Mg and Zn⁷¹.

Other methods include coulometric, chromatographic and photon activation techniques; these, however, have not been widely adopted for trace analysis.

CHAPTER 2

CHAPTER II

2.1. PHOTON COUNTING

When light interacts with matter energy is absorbed or released in quanta or photons. Each photon can give up an energy equal to $h\nu$ where ν is the frequency of the light and h is Planck's constant. The output signal of a photomultiplier tube is inherently digital as a result of the quantum nature of the photoelectric effect. According to Franklin, Horlick and Malmstadt⁷⁵ the advantages of photon counting are direct digital processing, a decrease of effective dark current by order of magnitude, improvement of signal-to-noise (SNR) ratio, accurate signal integration, improved precision of analytical results and elimination of reading error.

Single photon counting (SPC) has been utilised in astronomical studies and Raman spectroscopy to detect and measure very weak signals. The technique finds more application today due to the development of fast electronics.

In studying biological systems by methods that employ light energy, there are dangers that intense sources will cause photo-decomposition processes in the molecules. The alternative then is to use moderate sources and develop sensitive detection systems that will respond to the low light levels that result from molecules or atoms, after illumination. Nondispersive systems useful in multichannel analysis would benefit much from such detection systems.

Since Mainstadt⁷⁵ presented a paper evaluating photon counting as a detection technique in absorption and emission spectroscopy, a fair amount of work has been performed and presented exploiting its inherent advantages. Morton⁷² had already presented the fundamentals of photon counting and analysed basic systems, i.e. signal-to-noise treatment of data; detector selection and discrimination of useful pulses, those pulses that originate from the photocathode as a result of incident photons. Sharp⁷⁴ has employed SPC in atomic spectroscopy. He demonstrated the advantages of using a small time constant and discriminator levels as low as possible all aimed at resolving photon generated pulses.

SPC, because of its superiority and advantages over analogue techniques at low light levels due to low sample concentration or high instrumental resolution, is increasingly being applied to molecular fluorimetry, kinetic studies and other photochemical processes. Tuan and Wild⁷⁶ have utilised the advantage of using long count times. They have also proceeded to interface their system with a computer and have been able to process their spectral data corrected for dark count and wavelength drift. They have therefore obtained quantum yields in good agreement with earlier literature values.

2.2. PHOTOMULTIPLIERS

At present the most commonly encountered detector used for the photon counting in the UV/visible region is the photomultiplier⁷².

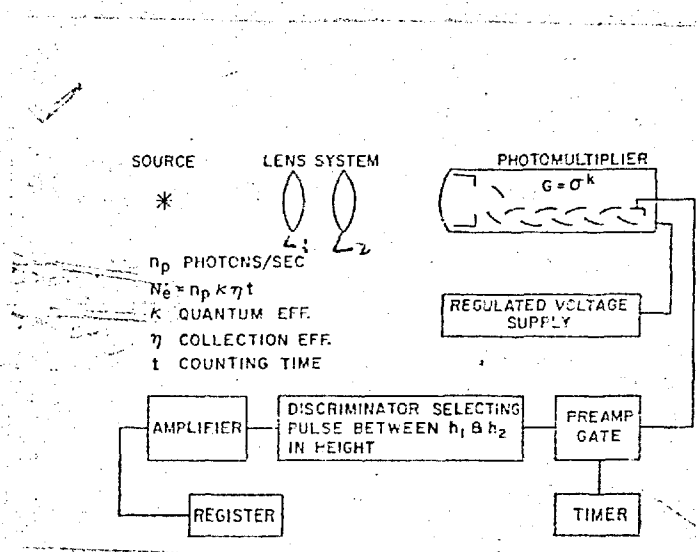


Fig 2.1 Schematic Diagram of a Photon Counting Detection System

Figure 2.1 shows a schematic diagram of a photon counting system using a photomultiplier as the detector. Light coming through the optical system via the lenses L_1 and L_2 falls on the multiplier photocathode. Each photon has a probability K (frequently called quantum efficiency) of causing the emission of a photoelectron from the photocathode. This quantum efficiency depends upon the material of the cathode and the wavelength of the incident light. The photomultiplier consists of a series of dynodes. The first dynode is biased positive with respect to the photocathode so that the emitted electron is accelerated towards the first dynode. At the same time, the first dynode is biased negative with respect to the 2nd dynode while the third dynode is biased positive with respect to the 2nd and negative with respect to the fourth. Of the electrons emitted by the cathode only a certain fraction η reaches the first dynode and produces output pulses of electrons. Obviously, the photomultiplier designer and manufacturer attempt to make η the collection efficiency of the device as close to unity as possible. If the arrival rate of photons

at the cathode is designated by n_p , the number of pulses N_e appearing at the output of the tube during the time t is given by $N_e = n_p k_i t$

$$N_e = n_p k_i t \dots\dots\dots (2.1)$$

After appropriate electronic processing, these pulses are recorded in a counting system.

Less information is lost by handling the information digitally, since this removes to a first approximation the effects of gain instability which are marked in analogue uses of photomultipliers ⁷³. Also treating data digitally has an advantage because the counter can be interfaced with modern computerised data handling systems ⁷⁴.

Typical photomultipliers have from 9 - 20 dynodes providing gains in the range of $10^5 - 10^8$. The gain is also critically dependent on the voltage applied across the dynode chains. The power supply voltage fluctuation is important to the photon counting method as the fluctuation, although it does not affect the count number, will affect the pulse amplitude.

Consider a PMT with a collection efficiency = ϵ
 Transfer efficiency = η
 Secondary emission coefficient = δ
 Number of dynodes in chain = n

then the overall gain $G = \epsilon(\eta\delta)^n \dots\dots\dots (2.2)$

usually expressed as $G = \frac{M}{S} \times 10^6 \dots\dots\dots (2.3)$

where M = overall PMT sensitivity (A/lumen)

S = cathode sensitivity (μ A/lumen)

The electrons which traverse the dynode chain do so over different paths and therefore arrive at the anode at different times. It has been shown that the time distribution of electron arrival at the anode is Gaussian so that the current pulse can be expressed in the form

$$i_a = \frac{Ge}{\sigma\sqrt{2\pi}} \exp\left(-\frac{t^2}{2\sigma^2}\right) \dots\dots\dots (2.4)$$

where e = charge on the electron

σ = the standard deviation

t = time

The width of the pulse is an important consideration in photon counting, because the technique depends on time resolving individual pulses when the average rate of pulse arrival is high. Tubes producing narrow pulses are therefore superior for use in photon counting systems.

2.3 PHOTOMULTIPLIER DARK CURRENT

A photomultiplier placed in total darkness in the absence of incident light will still generate current pulses. These pulses give rise to the "dark current" observed. This dark current varies from tube to tube. The dark current may be the result of the following causes (a) thermionic emission from the cathode or other internal parts of the device (b) leakage currents over the envelope material or through tube base, socket, or circuit insulation (c) pulses originating from radioactive sources (d) cold field emission and residual gas ionisation. These causes will be discussed here below.

(a) Thermionic emission

For most photomultiplier tubes thermionic emission from the photocathode is the major contributor to dark current. Thermal dark current approximately obeys the Richardson law

$$I = 21 \times 10^2 T^2 \exp - \frac{(1.16 \times 10^4 \phi_T)}{T} \dots\dots\dots (2.5)$$

Where T is the temperature

l = length

and ϕ_T is the thermal work function.

The temperature occurs twice in the expression and it is generally found that cooling the PMT to about -40°C is sufficient to reduce the thermal dark current effectively to zero. Another way to reduce the thermionic emission current is to reduce the area of the cathode. However, provision must be made to focus all the light to be measured onto the small cathode, otherwise the signal current will be reduced proportionately. Thermionic emission can also occur from the dynodes although with less frequency, because the thermionic work function of the metal dynode surfaces is higher than that of the semiconductor cathode surface. Thermionic pulses from the dynodes can be distinguished from photon pulses because in general they experience less gain and are therefore smaller.

(b) Leakage currents

The leakage current over the surface of the phototube envelope may often be reduced by carefully cleaning and drying the surface to reduce conducting contaminants. Low leakage base and socket materials

should be used. One excellent method of eliminating the effect of leakage currents in the output is to apply a circular band of electricity conductive paint surrounding but not touching the point at which the anode lead passes through the envelope.

(c) Pulses originating from radioactive sources

The envelope glasses used in photomultiplier manufacture contain residual amounts of natural K^{40} isotope which emits particles that result in bursts of Cerenkov photons from the glass. Cosmic particles can also cause dark pulses by secondary emission from the photo-cathode and dynode surfaces. Cerenkov radiation and residual gas ionisation. Finally, glass fluorescence may occur when the tube is used in the presence of radiation sources. Generally pulses derived from radioactive sources are larger than photon pulses because a burst of photons or electrons is generated in a time that is short compared with the anode pulse-width.

(d) Cold field emission and residual gas ionisation

When the photomultiplier is operated at the recommended voltage, pulses from these sources are not important. However, they are the major dark current component at higher voltages.

2.4. PULSE PILE-UP IN PHOTON COUNTING

Two effects are possible due to pulse 'pile-up' at a fixed discriminator level. Two pulses which should each be counted can 'pile-up' so that only one count is recorded⁷⁷. This usually occurs

at high light levels when the pulse resolution of the counting system is inadequate to deal with the pulse arrival rate. A net loss of one count results. Two smaller pulses can pile up and sum to produce a pulse large enough to be counted when neither pulse should have been counted, thus a net excess of one count is recorded. The discriminator level determines the relative importance of these two effects on the recorded count. At low discriminator levels, when a large fraction of the theoretical count is recorded, the major effect is the loss of pulses due to pile-up.

2.5. SIGNAL-TO-NOISE RATIO CONSIDERATIONS IN SPC

When the photomultiplier is open the signal N observed on the output (digital or analogue) is a measure of both pulses due to photons and pulses due to dark current. Hence the signal required is

$$S = N - D \quad \dots\dots\dots (2.6)$$

where N = Total signal count

D = Dark current count

To determine S an additional measurement of D must be made with the photomultiplier closed. Hence we introduce errors in both the values of N and D . These errors will add independently in the form of their standard deviations and the total error will be given by

$$E = (\sigma_N^2 + \sigma_D^2)^{\frac{1}{2}} \quad \dots\dots\dots (2.7)$$

The signal-to-noise ratio will then be given by

$$\frac{N - D}{(\sigma_N^2 + \sigma_D^2)^{\frac{1}{2}}} = \frac{S}{(\sigma_N^2 + \sigma_D^2)^{\frac{1}{2}}} \quad \dots\dots\dots (2.8)$$

where S is the mean of a number of readings taken over an interval

(count period) of time at a preset time.

Using a similar argument the standard deviation of $N = (N)^{\frac{1}{2}}$ and $D = (D)^{\frac{1}{2}}$. Both measurements are independent and the standard deviation, σ_s , is given by $(N + D)^{\frac{1}{2}}$ and the signal-to-noise ratio is given by

$$\frac{S}{\sigma_s} = \frac{N - D}{(N + D)^{\frac{1}{2}}} \dots\dots\dots (2.9)$$

In the instrumental system used in the present work the signal-to-noise ratio for the analogue system was measured connecting the photomultiplier output to the Aminco microphotometer and then to a chart recorder. The signal was taken to be the mean difference between the reading when the FMT is open and reading when it is closed, while noise was taken as the fluctuation in the signal.

2.6 NOISE IN PHOTON COUNTING

Poisson Noise

Poisson Noise is a fundamental source of noise associated with all random processes. The measured variance results from finite period counting of events randomly distributed in time. Thus the variance in the count rate derived from a photon arrival rate of N_s is given by

$$(\sigma^2 N_o)_p = N_o_s = N_s \phi \eta A \dots\dots\dots (2.10)$$

where A = fraction of photon derived pulses

N_o_s = observed count rate

η = first dynode collection efficiency

Similar expressions can be written for the background and dark count. In d.c. systems Poisson noise is usually referred to as 'shot noise' and is expressed in terms of the mean anode current and the noise equivalent bandwidth Δf

$$(\sigma_{d.c.})_p = \sqrt{2e g_m \Delta f} \dots\dots\dots (2.11)$$

Johnston Noise

Electrons moving through a conductor continually exchange energy with the thermal vibrations of the lattice ions. As a result, random fluctuations in the charge density occur causing variations in the potential drop across the conductor. Johnston noise is therefore another fundamental source of noise and its variance is given by

$$\sigma_j^2 = \frac{2kTR}{t} = \frac{kP}{t} \dots\dots\dots (2.12)$$

where k is the Boltzmann constant

T is the absolute temperature

R is the resistance of the conductor

and t is the observed or integration period.

This equation shows that Johnston noise is most likely to occur in the load resistor, R_L , where it may result in the generation of spurious pulses. These pulses may either be counted directly (at very low discriminator levels), or can take part in producing pulse pile-up by either of the two mechanisms. Generally, however, the variance term due to Johnston noise will be much less than terms due to Poisson and flicker noise.

Flicker Noise

The previous noises are fundamental but flicker noise is caused by non-ideal behaviour of the experimental system ⁸³⁻⁸⁴.

Signal and Background Radiation Flicker Noise

Signal and background flicker in molecular luminescence can result from lamp flicker and general external influence on the experimental system such as vibration. The variance due to signal flicker may be written $(\sigma^2 N_o)_f = E_s^2 N_o_s^2 = (N_s E_s A \phi \eta) \dots \dots \dots (2.13)$

where E_s is the dimensionless 'flicker-factor' for the signal ⁸⁵.

Likewise, the variance due to the background flicker is given by

$$(\sigma^2 N_o)_f = E_b^2 N_o_b^2 = (N_b E_b A \phi \eta)^2 \dots \dots \dots (2.14)$$

Flicker in the Counting System

Flicker in the PMT gain, amplifier, discriminator level and photo-cathode quantum efficiency etc., result in a broadening of the pulse height distribution. This does not in itself constitute noise, particularly when a low discriminator is used. However, as the discriminator level is raised the noise component due to the variations in pulse amplitude increases and reaches a peak dN_o/dA , i.e. the rate of change of the observed count with the discriminator level is at a maximum.

Read-out Noise

For the digital read-out there is always an uncertainty of ± 1 in the last figure, because the counting circuits are not synchronised

with the photon derived pulses. This variance of one may be of importance at low count rates, but in general will be of no consequence in analytical spectrometry.

2.7 COMPARISON OF SPC AND DIRECT CURRENT MEASUREMENT TECHNIQUES

Photon counting has been proposed as a measurement technique for several analytical spectrometric applications^{75, 78, 80}. There are several reasons why photon counting technique appears to have inherent advantages over the conventional d.c. method of measurement of light intensities. First, radiant flux information is processed in a discrete manner, reducing the number of domain conversions⁸¹ necessary to obtain a number related to the incident light level. Second, the processing of information by digital circuitry makes photon counting detection less susceptible to the long time drift and 1/f noise which often limits analogue systems. This feature enables the spectrometrists to utilize much longer averaging times than would be feasible with d.c. detection where low frequency noise components can predominate at narrow measurement system bandwidths. Other advantages of the photon counting technique include the ability to discriminate against photo-multiplier dark current originating down the dynode chain and the virtual elimination of the reading error which can limit analogue systems⁷⁵.

Despite these attractive features, there are drawbacks to photon counting, particularly at the moderate to high light levels encountered in many types of analytical spectrometry. Pulse overlap limits photon counting to relatively low light levels unless deadline compensation or mathematical correction techniques are used^{79, 82}. Light levels which result in an anode pulse rate of 10^6 Hz represent the usual upper limit of photon counting systems using present 'state-of-the-art'

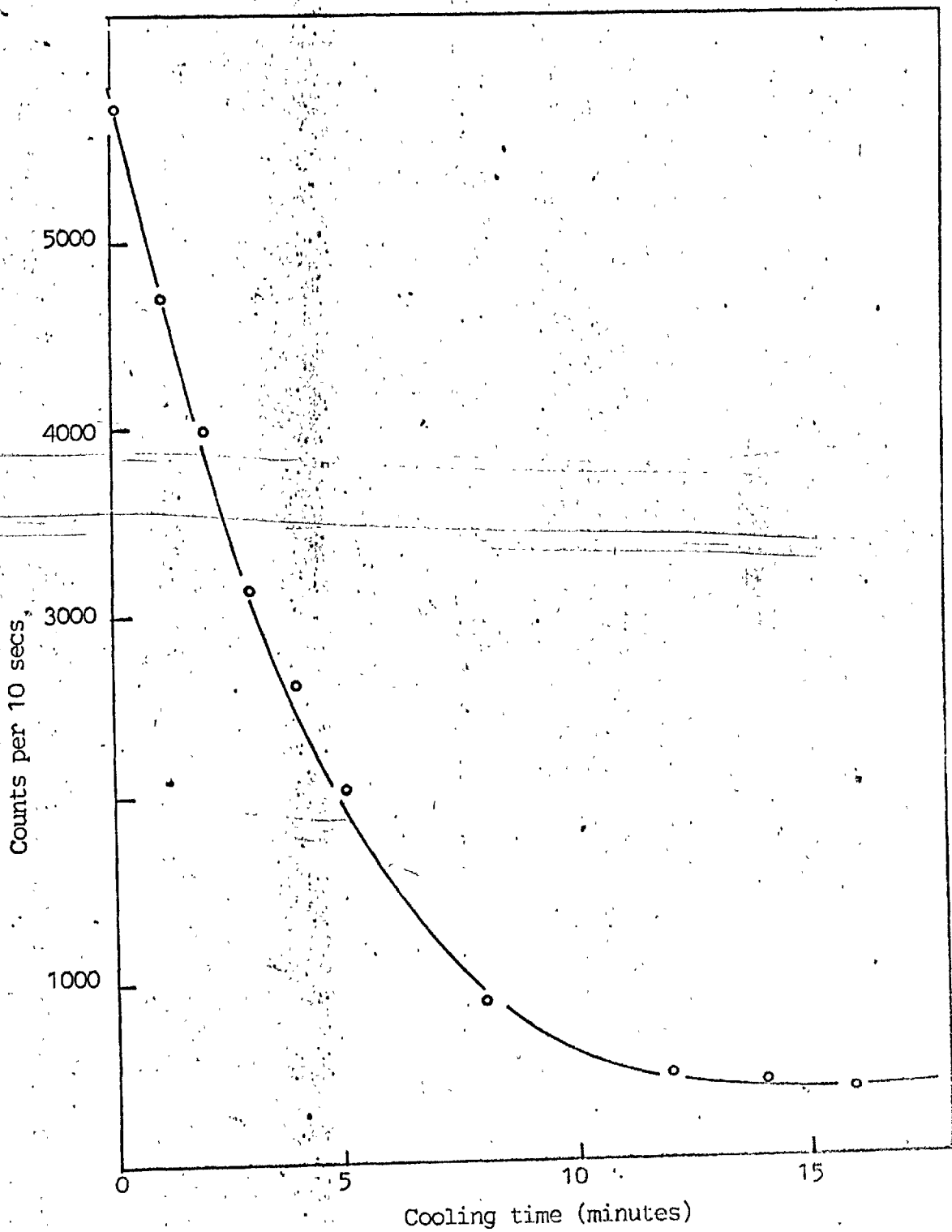


Fig 2.2. Variation of dark Current with Temperature

electronic circuitry. At normal photomultiplier gains of 10^5 to 10^6 this corresponds to an anodic current of only 10^{-8} - 10^{-7} A which is several orders of magnitude less than the maximum anodic current for linear operation. Thus using a photomultiplier in the d.c. mode allows the measurement of light intensities several orders of magnitude greater than can be measured with linearity in the photon counting mode. In short-noise limited operation, where the signal-to-noise ratio increases with the square root of the incident photon flux, use of photon counting may lead to decreased SNR's if the light level is purposely reduced for good linearity. In many analytical spectrometric applications, measurements must be made in the presence of background radiation. Thus even when signal strengths are low, the presence of large amounts of background may cause pulse rates to be higher than can be measured without pile up, another drawback to photon counting at present is the complexity required for processing the data to obtain numerical readout in the appropriate units. Such operations as taking the logarithm, the ratio, or the reciprocal are common in spectrometric applications and require computer data processing for photon counting with a single beam spectrometer unless a conversion back to the analogue domain is performed with its consequent errors and non-linearities. This latter drawback is becoming obsolete with the increased availability of small dedicated laboratory computers.

2.8. ANALYTICAL RESULTS WITH THE COUNTING TECHNIQUE

(a) Effect of cooling the photomultiplier

The RCA IP28 photomultiplier (side-window) supplied with the commercial double monochromator spectrofluorimeter (American Instrument Company Silver Spring Maryland USA) was replaced by the

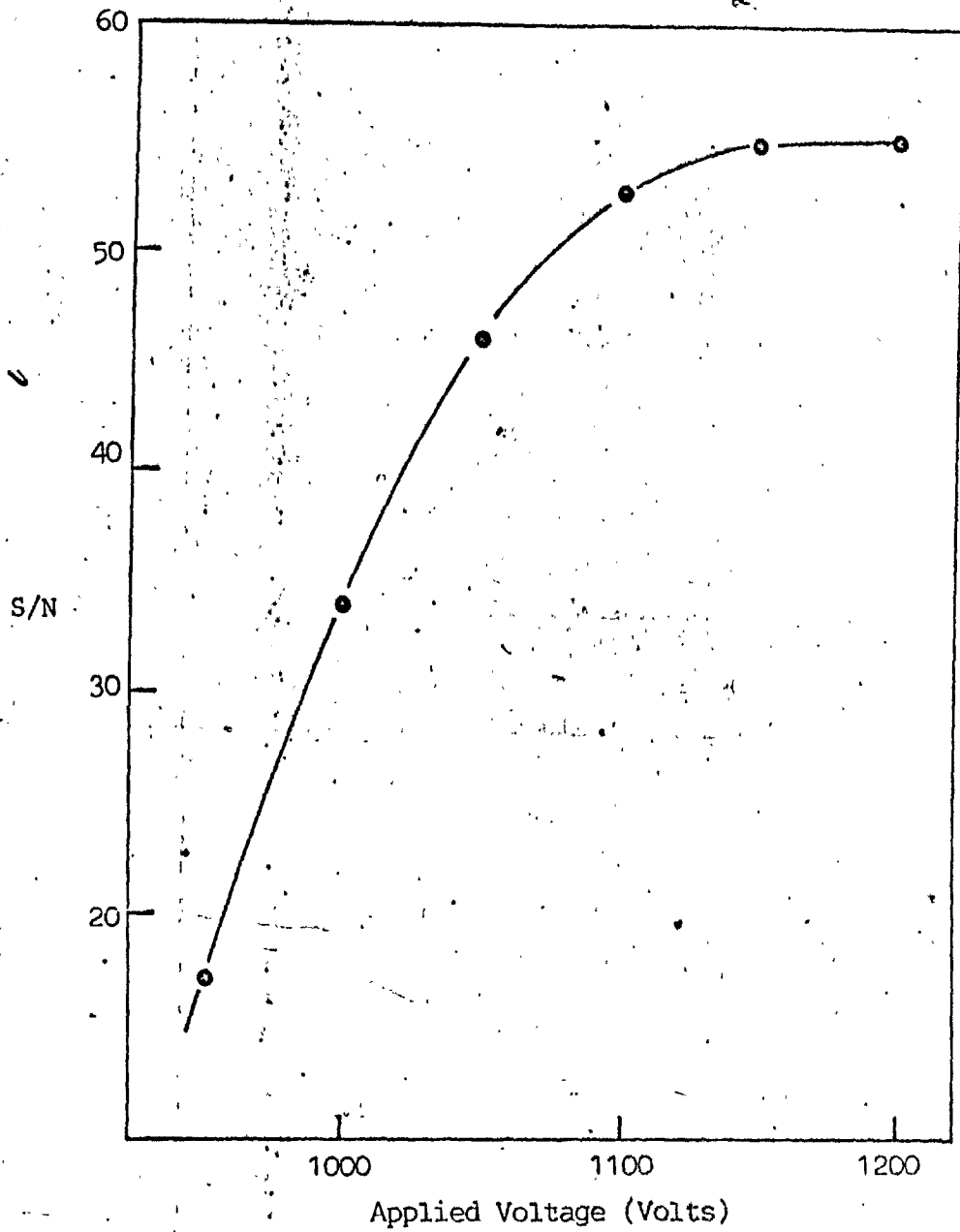


Fig 2.3 Variation of signal-to-noise ratio with applied voltage for EMI 9601B photomultiplier tube

EMI 9601 B (end-window with a response S-11). This photomultiplier was placed in a housing that had a cooling facility. Dry ice was used for cooling.

In the experiment to cool the PMT, dry ice was placed in the space outside the PMT housing and cooling was caused by conduction, Quinine sulphate (10^{-7} M) was used to produce a luminescence signal at 450 nm for the uncooled and cooled tube. The results can be seen in fig 2.2. Counts for every 10 secs were recorded and plotted against time (minutes). It can be seen that at time 0 minutes the counts were about 4500 per 10 secs but after 15 minutes the curve had reached its lowest. At this stage the dark current was virtually zero. The tube was therefore used with the dark current reduced in this way. The luminescence background was not a problem, hence cooling the tube was sufficient to reduce noise significantly. It is to be expected that in situations where high background radiation is present, cooling of the tube will be less advantageous, because the noise contribution of the dark current is then relatively small.

(b) Voltage Optimisation

In optimising spectrophotocchemical systems, various procedures can be used. To optimise voltage in our work signal-to-noise ratio was used. Once an expression for S/N is known, then optimum values of each experimental parameter can be calculated by the standard maximisation technique of taking the partial derivative of S/N with respect to the parameter of interest, X, setting the partial derivative equal to zero and assume that all instrumental parameters are

independent of each other then

$$\left(\frac{\delta_{S/N}}{\partial x} \right)_{y,z \text{ etc}} = 0 \quad \dots\dots\dots (2.15)$$

where y and z are other experimental variables held constant during the optimisation process.

To optimise voltage the spectrometer slit-width (the only other variable parameter) was held constant while voltage was varied from 1000V to 1400V, at intervals of 50 Volts. At each interval the signal produced due to 10^{-6} M quinine sulphate solution and the dark count were taken. Ten readings of each were taken and the average plotted versus voltage. The optimum voltage was obtained at 1200V. This was fixed for all other measurements. Fig 2.3 shows the plot of S/N versus voltage. It has a plateau from 1100V onwards; all further work was undertaken using a voltage of 1200V.

(c) Slit Optimisation

Two expressions have been developed (equations 2.8 and 2.9). Equation 2.8 is derived from actual standard deviations of a sample of readings. Equation 2.9 derived from the assumption that the standard deviation of a single count is approximately equal to its square root. The former equation takes into consideration the lamp flicker contribution to total noise while the second does not. It was argued that when S/N values were worked out by both methods they are approximately equal then it was observed, (2.8) was tending to (2.9) and thus flicker noise was a minimum.

It was found that the wider the slit the greater the S/N observed. A value was therefore selected that gave values of S/N which were approximately equal. Slit arrangement 2 (half-band width of 2 nm throughout), on the excitation side and slit arrangement 4 (half-band width 4 nm) on the emission side were chosen and kept constant in all subsequent work.

Selection of Optimum Slit Width

Slit Arrangement 2

<u>Sample Signal</u>	<u>Background noise signal</u>	<u>Blank signal</u>
21,596	7181	8766
22,746	6978	8572
21,779	7245	8835
22,299	7283	8808
21,725	7275	8718

$$S/N = 76.06$$

Slit Arrangement 3

<u>Sample Signal</u>	<u>Background noise signal</u>	<u>Blank signal</u>
121045	7955	20413
120149	7805	21000
120963	7956	18253
117930	7853	17869
117355	8061	17890

$$S/N = 163.59$$

Slit Arrangement 4

<u>Sample Signal</u>	<u>Background noise signal</u>	<u>Blank signal</u>
281750	7886	57993
280865	7932	57283
280054	8077	52080
280155	7820	51989
280550	7924	49967

$$S/N = 399.18$$

Slit Arrangement 5

<u>Sample Signal</u>	<u>Background noise signal</u>	<u>Blank signal</u>
507257	8062	110854
501302	8035	113075
502139	8172	116562
497550	8049	117544
500745	8050	116406

$$S/N = 494.00$$

It will be noticed from the above results that although the signal-to-noise ratio increases with wider slits, the blank contributes to the noise significantly. This may include stray light. It was therefore decided that arrangement 2 on the excitation side would channel enough lamp radiation while arrangement 4 on the emission side would transmit significant amount of fluorescent radiation to give linear calibration graphs.

2.9. EXPERIMENTAL COMPARISON OF SFC AND ANALOGUE SYSTEMS

Samples and Methods Used

Thiamine:

Thiamine or vitamin B1 can be analysed by various methods classified as microbiological and chemical. The chemical ones include fluorimetry which provides high sensitivity. It was decided to use this system to demonstrate that photon counting can be used to analyse organic systems with low background fluorescence. This procedure followed was that recommended by Ohnesorge and L.R. Rogers⁸⁹ where thiamine is oxidised to thiochrome.

REAGENTS

Thiamine (aneurine chloride) hydrochloride $C_{12}H_{17}N_4OSCl.HCl$ (BDH laboratory reagents) was dissolved in 0.01 N HCl to make a $10^{-2}M$ stock solution which was stored in a dark brown bottle in a refrigerator. Dilutions were made as required.

Buffer solution (pH 7.0) was prepared from analytical reagents grade citric acid and dibasic sodium phosphate.

3.6N H_2SO_4 , and 15% NaOH solutions were prepared from reagent grade chemicals; 1% $K_3Fe(CN)_6$ was prepared and stored in the dark.

PROCEDURE

A sample of up to 8ml, containing up to 100 ppm of thiamine, was pipetted into a 25ml glass stoppered volumetric flask. 2ml of 15% NaOH solution and 2 drops of 1% $K_3Fe(CN)_6$ were added and the

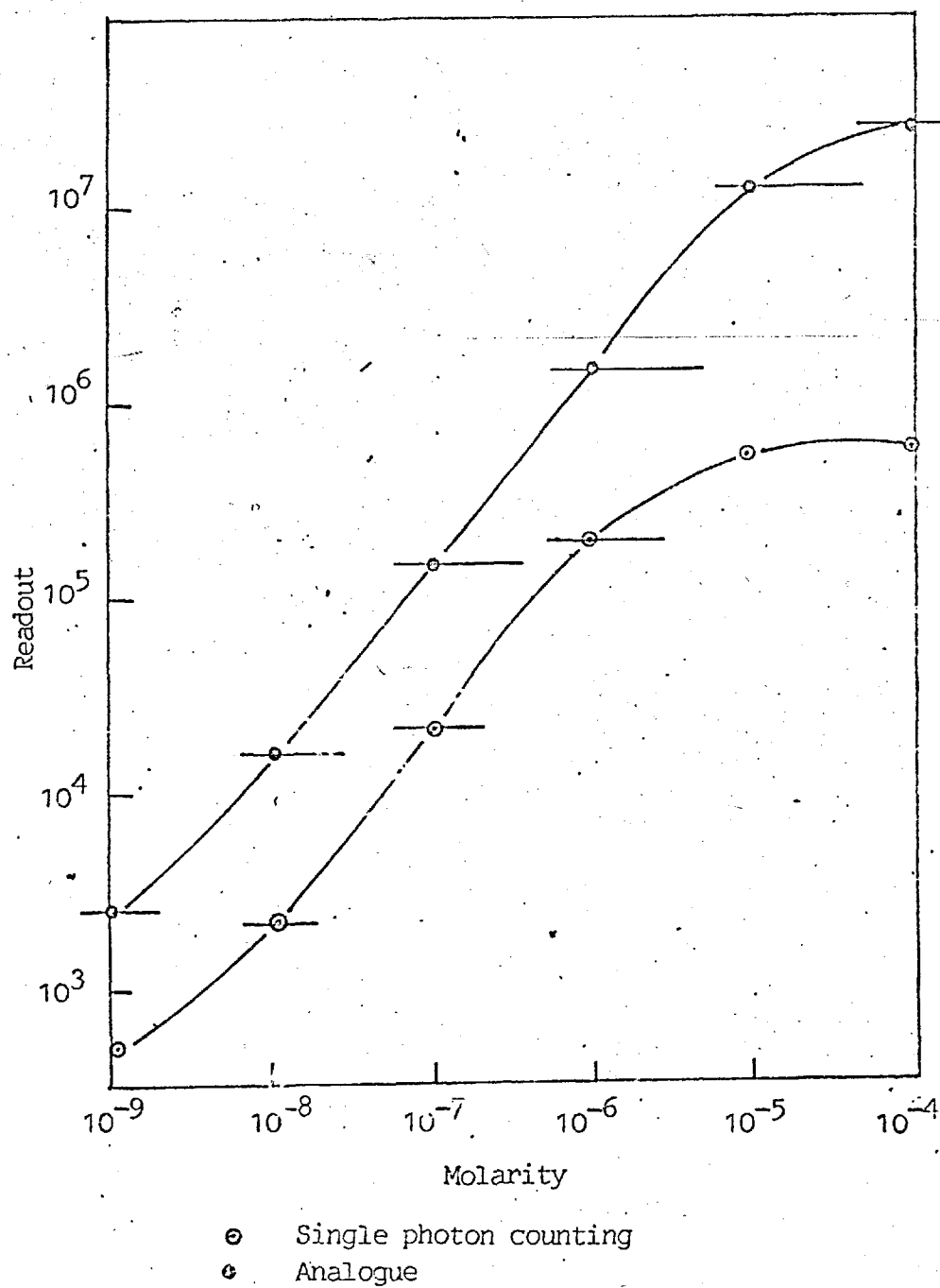


Fig 2.4. Comparison of linear range for SPC and Analogue systems using Quinine Sulphate.

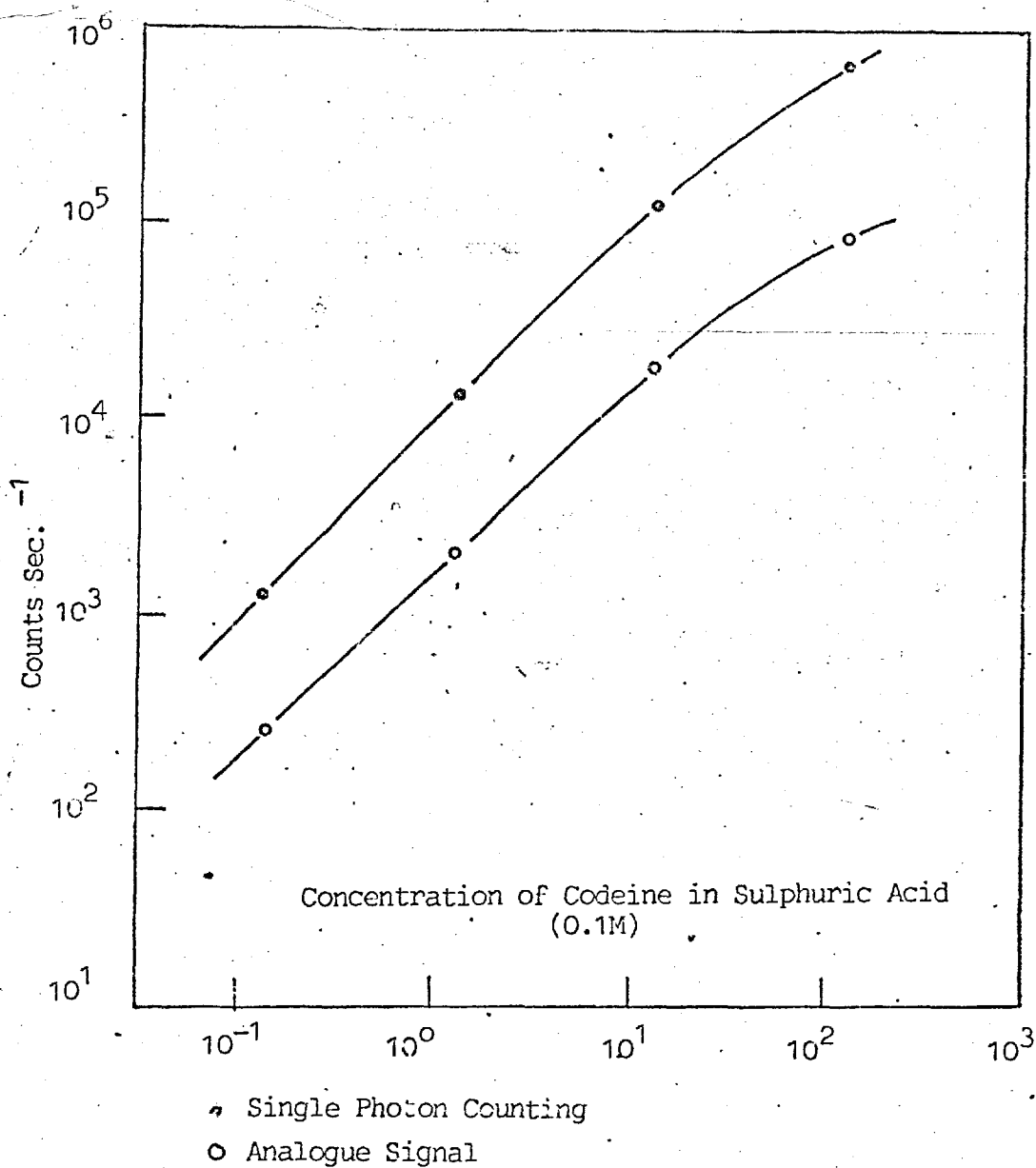


Fig 2.5. Comparison of the linear range for SPC analogue systems using Codeine

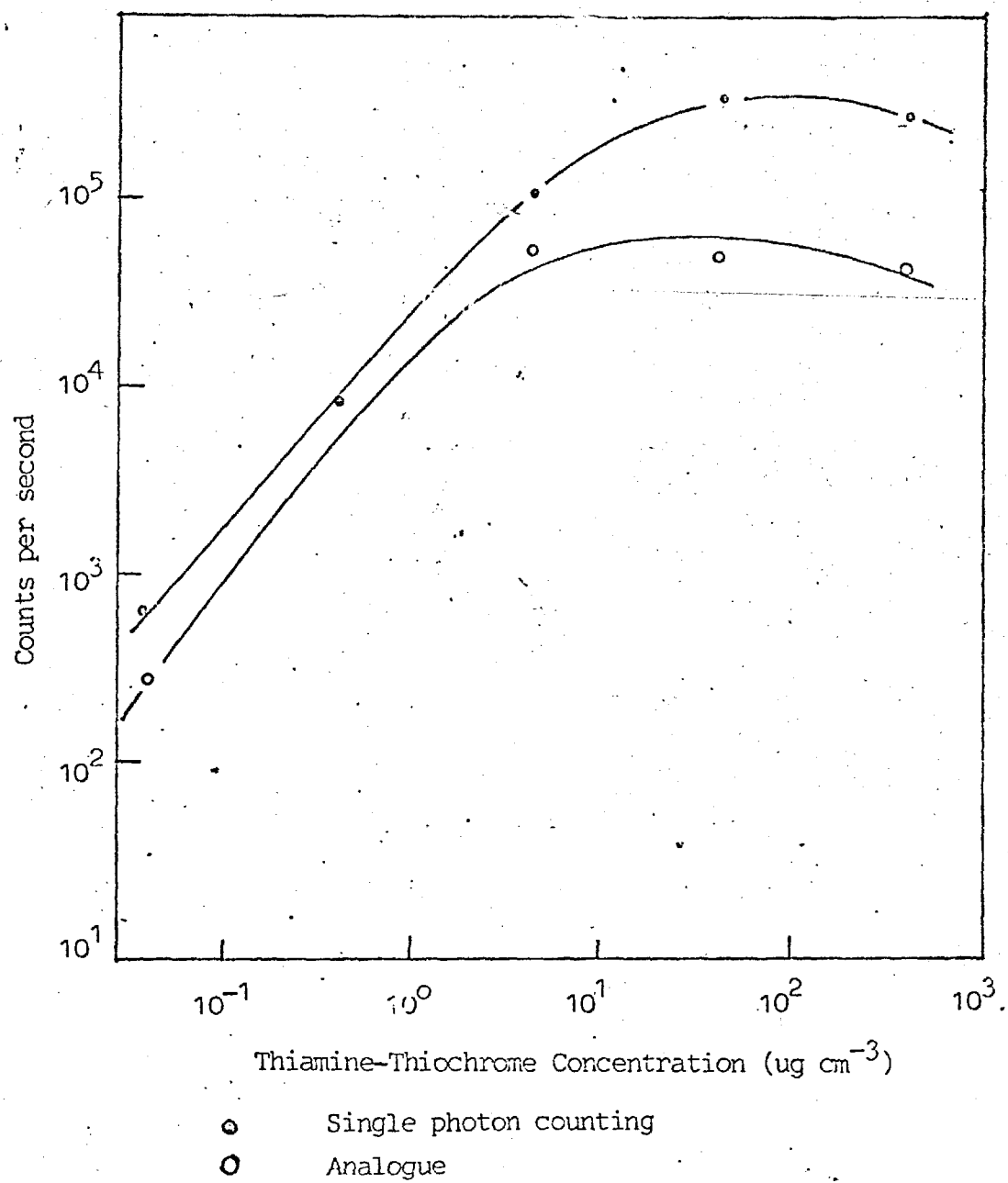


Fig 2.6. Comparison of the linear range for SPC and analogue systems using Thiamine-Thiochrome.

flask was shaken before being placed in the dark. After 10 minutes in contact with the alkaline oxidant 2.0ml of 3.6N H_2SO_4 was added with shaking to neutralise the base and the sample was diluted to volume with pH7.6 buffer solution. After the solution had been mixed and allowed to stand in the dark for another 10 minutes, the intensity of the fluorescence measured at 460 nm with excitation at 360 nm.

CODEINE:

Codeine was dissolved in 0.1N H_2SO_4 and treated as such, i.e. dilutions made as required.

QUININE SULPHATE

Quinine sulphate was also dissolved in 0.1N H_2SO_4 . It was used in standard work and later used for this comparison of the digital versus analogue detection system.

COMPARISON OF LINEAR RANGE - TABLE

For Analogue and SPC Techniques

<u>Compound</u>	<u>Analogue Detection</u>	<u>SPC Detection</u>
Quinine Sulphate	7 ppm - 9×10^2 ppm	5 ppm - 6×10^3 ppm
Codeine	same	same
Thiamine	0.4 - 4.8 ppm	0.014 - 6.8 ppm

COMPARISON OF DETECTION LIMITS - TABLE

For Analogue and SPC Techniques

<u>Compound</u>	<u>Analogue Detection</u>	<u>SPC Detection</u>
Quinine Sulphate	2.7 ppm	3.6×10^{-2} ppm
Codeine	2.8×10^{-1} ppm	3.5×10^{-2} ppm
Thiamine	4×10^{-1} ppm	1.5×10^{-2} ppm

The linear ranges mentioned above can be clearly seen as plots in Figs 2.4, 2.5, 2.6 for Q.SO₄, codeine and thiamine respectively.

CONCLUSION:

It is evident that the SPC Detection limits are better than those of the analogue system. The SPC signal is not affected by background noise as much as the analogue signal since the former filters in shorter circuitry. Due to the same reason, the linear range for SPC system is more expanded.

CHAPTER 3

3. LUMINESCENCE LIFETIMES AND QUANTUM YIELDS OF INORGANIC IONS

3.1. INTRODUCTION

The luminescence of aqueous solutions of halide salts containing heavy metal ions has been studied by several workers. The fluorescence observed from solutions of thallium (I)⁹⁰ and lead⁹¹ in the presence of excess of chloride has been used for their determination. The violet fluorescence observed in both cases has been attributed to complex anionic $TlCl_3^{2-}$ and $PbCl_4^{2-}$ species⁹². The low temperature luminescence characteristics of several heavy metal ions have been investigated and used for the determination of some of their media in hydrochloric and hydrobromic acid⁹³⁻¹⁰¹.

The luminescence quantum yield of thallium(I) in aqueous solutions has been found to decrease slightly with increase in concentration of the chloride ion¹⁰² and that of aqueous HCl, HBr, and LiCl solutions in the presence of thallium(I) and lead (II) ions have been found to increase considerably with decrease in temperature¹⁰³, and¹⁰⁴. Similarly the luminescence lifetimes of thallium(I), lead (II) and bismuth(III) ions in lithium chloride solutions have been found to increase with decrease in temperature¹⁰⁵.

3.2. MEASUREMENT OF LUMINESCENCE LIFETIMES

The experimental procedures for measuring luminescence lifetimes vary according to the order or magnitude of the decay period. Phosphorescence lifetimes are almost always much longer

than fluorescence lifetimes and are therefore easier to measure. The simplest method for measuring long lifetimes is by recording the luminescence emission signal as a function of time after complete termination of the excitation radiation by means of a rapid mechanical shutter. For lifetimes longer than about one second, a strip-chart recorder may be used. For faster decay times (e.g. milliseconds), an oscilloscope with a response time much smaller than the sample decay time can be employed to record luminescence intensity as a function of time. If the luminescence persists for a period up to 10^{-4} sec. after the removal of exciting radiation, mechanical shutters consisting of slots in a rotating phosphorescope can or chopper disc are used¹⁰⁶.

The decay time of prompt fluorescence is generally of the order of several nanoseconds, and for such decay periods mechanical shutters are replaced by electro-optical shutters. The methods used for measurements of fluorescence lifetimes include phase-shift techniques¹⁰⁷⁻¹¹⁵.

3.3. MEASUREMENT OF LUMINESCENCE QUANTUM YIELDS

Several types of method have been described for the determination of the quantum yields of solutions. These include the use of standard scatterers, such as magnesium oxide¹¹⁶ or colloidal solutions,¹¹⁷ or relative methods which substitute a compound of known quantum yield in place of a standard scatterer as a reference. Calorimetric methods have also been described as procedures for measuring quantum yields, which are calculated from the energy yields obtained from temperature changes during irradiation¹¹⁸⁻¹²⁰. In the work reported the relative method

utilizing benzophenone as a reference for luminescence quantum yields has been employed.

Reagents:

Metal Salts:

Potassium antimonyl tartrate, Bismuth pentahydrate Cerium Chloride and Uranyl nitrate hexahydrate were used to prepare 10^{-2} M solutions in 6M HCl. Copper iodide was dissolved in lithium chloride (7.4M). Thallium chloride and lead chloride solutions were prepared in lithium chloride. Analytical reagent grade concentrated hydrochloric acid (Hopkins and Williams Limited) was used. This was prepared by dissolving benzophenone in EPA (5:5:2 V/V mixture of diethyl ether, isopentane and ethanol) Benzophenone (AR grade) was used as a standard at 77°K.

3.4. MEASUREMENT OF LUMINESCENCE LIFETIMES

The apparatus used for measuring the lifetimes was the TRW nanosecond spectral source system described in Chapter 1. The signal was measured across a suitable load resistor and preamplified and displayed on the oscilloscope. The luminescence decay traces were photographed with the Polaroid oscilloscope camera from the face of the oscilloscope. Fig 3.1 shows the decay trace obtained for a 10^{-3} M solution of Thallium (I) in 7.4M LiCl at Liquid N₂ temperature.

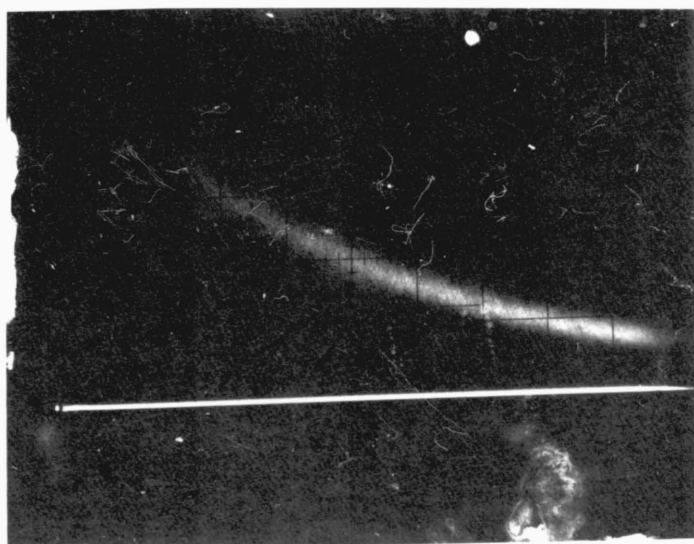


Fig 3.1 Fluorescence decay Thallium(I) at 77°K 10^{-3}M solution of Thallium(I) in 7.4M LiCl, lamp used in Dueterium No.268. Filters: Chlorine gas on excitation side (256 nm) and Locarte Filter on the emission side (380 nm).

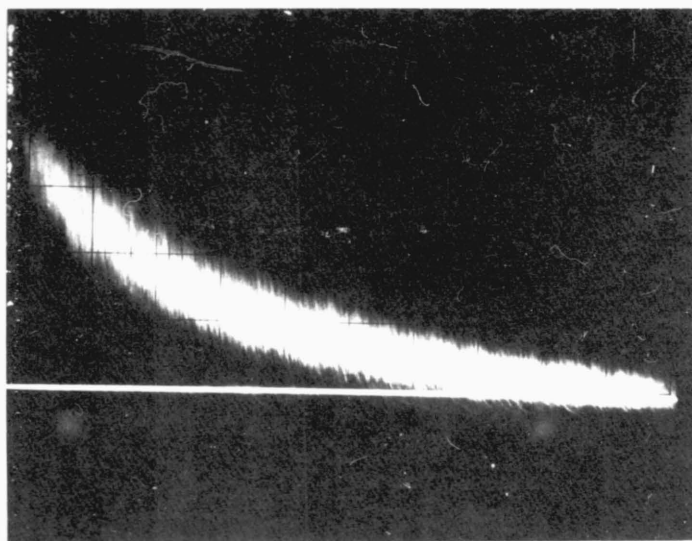


Fig 3.2 Luminescence decay of lead complex in LiCl at liquid nitrogen temperature (77°K), 10^{-3}Pb(II) solution in 7.4M LiCl termination 10,000 ohms.

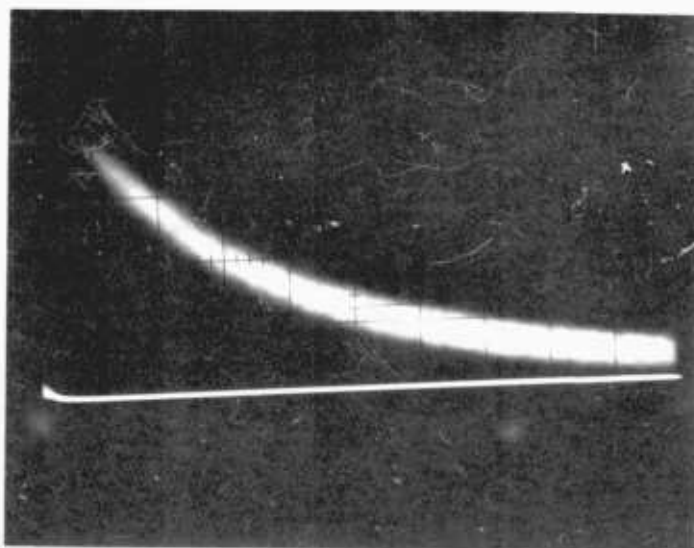


Fig 3.3 Luminescence decay of Telurium $10^{-3}M$ in $6M$ HCl at liquid nitrogen temperature $77^{\circ}K$. Nitrogen lamp No.267 used.

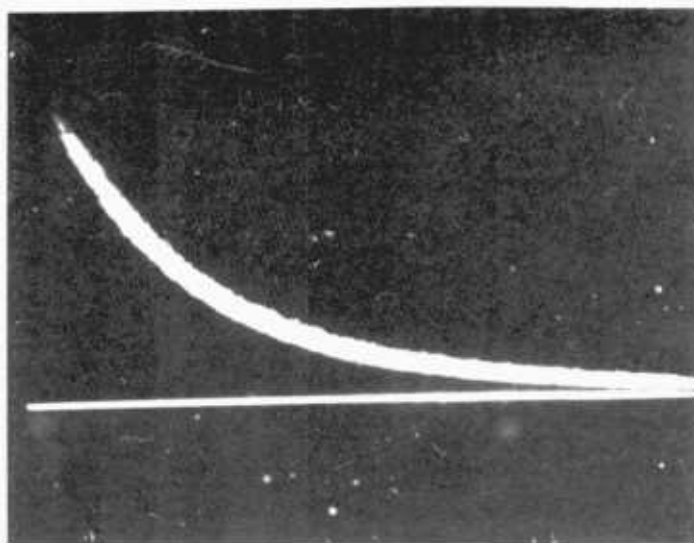


Fig 3.4 Fluorescence decay of Uranium $U(VI)$ $10^{-3}M$ in $7.4M$ HCl at liquid nitrogen temperature. Nitrogen lamp No.213.

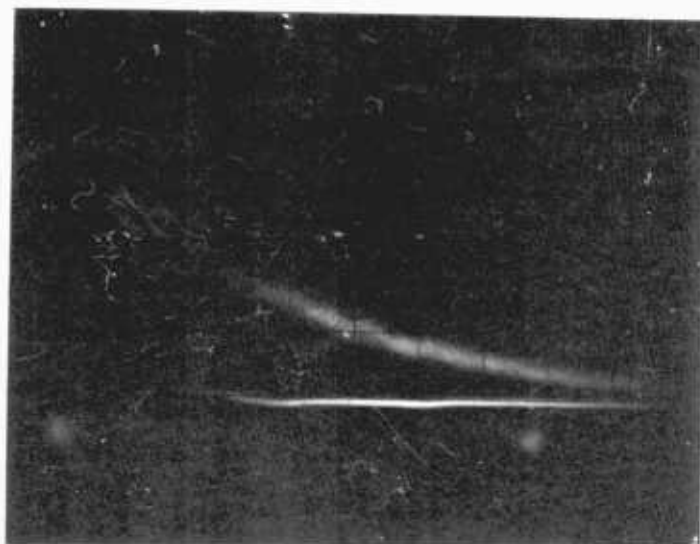


Fig 3.5 Fluorescence decay of Cerium(III) $10^{-3}M$ in $7.4M LiCl$ at liquid nitrogen temperature. Deuterium Lamp No.268.

The traces displayed above are typical of others obtained from inorganic ions.

All solvents used were analytical reagents grade purity.

Filters

Filters with high transmission characteristics were used for selecting luminescence excitation and emitted radiation. They were Corning, interference (Baird Atomic Co.) or Locarte filters. A solution filter 5 cm thick prepared from saturated $Ni SO_4$ solution in a silica cell was used to select the exciting radiation for $Tb(III)$ (below 300 nm). A chlorine gas filter contained in the same 5 cm cell was employed to select the excitation wavelength for $Ti(II)$ and $Ce(III)$ (below 300 nm).

Procedure for calculating lifetimes

Simple log intensity vs time Plots were used to calculate the lifetimes from the decay trace photographs. The traces obtained with almost all the ions were quite diffuse, and had an appreciable thickness due to signal noise. Therefore for each trace two readings of intensities were made from the photograph, one from the base line to the bottom of the trace. Plots of log intensity vs time were linear obtained from each photograph, and correspondingly two lifetime values were calculated from their slopes. The plots of log intensity vs time were linear in all instances. Each of the lifetime values shown in table 3.1 and 3.2 is the average of the two values obtained as described above with an error of half the difference. The oscilloscope time base was assumed to be correct.

3.5. MEASUREMENT OF QUANTUM YIELD

The procedure employed for obtaining the quantum yields of luminescence at room temperature was the relative method. The area under the luminescence emission spectrum from a sample solution was compared with the emission from a substance of known quantum yield. The reagents were excited at the wavelength of their excitation maximum and the ratio of the areas under the luminescence emission spectra enables a determination of the quantum yield of the unknown to be made. Benzophenone was taken as the standard at low temperature (77°K) and its quantum yield was taken as 0.67¹²².

The total fluorescence emission F from a solution is given by the equation:

$$F = kI_0 (1 - 10^{-A}) \quad \dots\dots\dots (3.1.)$$

where I_0 is relative intensity of exciting light.

For standard and unknown sample irradiated at the wavelength of the excitation maximum the equation becomes:

$$\frac{F_x}{F_s} = \frac{I_o^x (1-10^{-A_x l}) \phi_x}{I_o^s (1-10^{-A_s l}) \phi_s}$$

$$\phi_x = \phi_s \frac{F_x}{F_s} \cdot \frac{I_o^s (1-10^{-A_s l})}{I_o^x (1-10^{-A_x l})} \dots\dots\dots (3.3.)$$

where F is the area under the corrected fluorescence emission spectrum, I_o is the relative intensity of the exciting light at the wavelength of excitation, ϕ is the quantum yield, A is the absorbance of the solution at the wavelength of excitation, and l is the path length of the solution subscripts x and s refer to the unknown and reference solutions respectively. For dilute solutions, for which only a small fraction light is absorbed equation 3.1. reduces to

$$\phi_x = \phi_s \frac{F_x}{F_s} \cdot \frac{I_o^s}{I_o^x} \cdot \frac{A_s}{A_x} \dots\dots\dots (3.4.)$$

This equation was used for calculating the quantum yields of fluorescence of some standard compounds.

The quantum yields (ϕ) were corrected for the different refractive indices of the solvents by multiplying ϕ by the ratio $(\eta_x/\eta_s)^2$ where η is the refractive index of the solvent. In our case the refractive indices of EPA and lithium chloride had to be determined at 77°K. The refractive index of EPA was available in literature¹²² (1.48) but that of lithium chloride was not available at 77°K but at temperatures near room temperature. Hence the index had to be extrapolated down to 77°K. The value obtained was 1.436.

Luminescence measurements were made with the aminco-Bowman spectrophotofluoremeter (Chapter 1). Silica tubular cells (dimensions 200 mm length, i.d. 3 mm and wall thickness 1 mm) immersed directly in liquid nitrogen in a Dewar flask were used.

Procedure

The recorded luminescence emission spectra of the solutions of unknown and of the standard benzophenone in EPA were corrected using the correction factors obtained by Narayanaswamy²⁴. The areas under the curves were then calculated by using Simpson's Rule¹²¹. Lead and thallium were determined in this way using benzophenone as the standard. Cleanliness of the sample tube and the Dewar flask (to reduce bubbling of liquid nitrogen) was very important. The duration between successive runs needed to be very short to ensure that there was no drift in the instrument and that conditions were identical for both the standard and the sample. The two spectra were obtained on the same graph paper on which the wavelengths had been marked.

Results and Discussion

The measured lifetimes of inorganic ions in 6M HCl and LiCl at liquid nitrogen temperature (77°K) are given in table 3.1. Each mean lifetime shown in table 3.1. is the average of at least two determination and was calculated as described earlier in this chapter.

The log intensity vs time plots for all decay traces were found to be linear, which indicates that the luminescence measurement from all the ions decays exponentially.

From the work described here and that of other authors, it is evident that absorption and luminescence observed from heavy metal ion solutions in 6M HCl and LiCl correspond to the electronic transitions of the respective free ions and correspond to the transitions between the lowest excited ground and excited electronic states. The luminescence correspond to spin-forbidden $^3P \longrightarrow ^1S_0$ transitions and the lifetimes shortened due to the heavy atom effect of the metal.

It should be pointed out that on the basis of heavy atom effect on the luminescence, it is difficult to explain the long lifetimes of Bi(III) ion luminescence in both 6M HCl and LiCl. In Ce(III) ion, the luminescence is probably caused by the transition of the 4f electron to higher energy levels.

TABLE 3.1 Measured Luminescence Lifetimes of Inorganic Ions in Hydrochloric Acid and Lithium Chloride

Luminescence lifetime μ seconds

Ion	Salt Used	6M HCl	7.4M LiCl
Tl(I)	TlCl	4.18 $\pm$ 0.76	5.20 $\pm$ 0.28
Pb(II)	Pb(NO ₃) ₂	34.0 $\pm$ 4.0	31.5 $\pm$ 9.5
Te(IV)	Te powder	7.22 $\pm$ 0.77	w
Bi(III)	Bi(NO ₃) ₃ .5H ₂ O	5990 $\pm$ 440	4800
Ce(III)	Ce(NO ₃) ₃ .6H ₂ O	0.040 $\pm$ 0.005	0.033 $\pm$ 0.006
U(VI)	UO ₂ (NO ₃).6H ₂ O	590 $\pm$ 30	590 $\pm$ 10

w denotes weak luminescence signal that decay trace could not be obtained.

TABLE 3.2 Luminescence Quantum Yields and Lifetimes at 77°K
in LiCl Quantum Yield of benzophenone is 0.67¹²²

Ion	Quantum Yields	Lifetime Measured $\tau_m, \mu \text{ sec}$	Radiative Lifetime $\tau_0, \mu \text{ sec}$
Tl(I)	(a)	(b)	(c)
Tl(I)	0.60 $\pm$ 0.04	5.20 $\pm$ 2.8	8.67 $\pm$ 0.24
Pb II	0.25 $\pm$ 0.025	31.5 $\pm$ 9.5	126 $\pm$ 9.475

- (a) Each result is the average of at least three determinations
- (b) Each result is the average of at least two determinations
- (c) Calculated using the equation $\tau_0 \phi = \tau_m$

The radiative lifetime (τ_0) is related to the measured lifetime (τ_m) and the luminescence quantum yield, ϕ , by the equation $\tau_m = \tau_0 \phi$

Measurement of quantum yields at 77°K now reveals that the luminescence is enhanced at low temperature while the lifetime measurement shows a decreasing trend. It may be possible that increased luminescence is due to reduced radiationless loss. The solutions were deoxygenated before measurement so that the quenching action of oxygen should be minimized.

CHAPTER 4

4. DETERMINATION OF BERYLLIUM BY SPECTROFLUORIMETRY

4.1. Introduction

Sill and Willis ¹²³ have published an extensive treatise on the determination of submicrogram quantities of beryllium with morin in dust, bones, smears steels etc., (3, 5, 7, 2, 4-pentahydroxyflavone). They also describe the preparation of glass standard. In the morin method for beryllium, Venosi ¹²³ removed interferences by distillation from hydrochloric acid and extraction. Fedorchuk ¹²⁵ used a mercury pool cathode for the removal of interfering ions. Additional reports on the determination of beryllium with morin in biological materials ¹²⁶, sea water ¹²⁷ and alloys ¹²⁸ have appeared. A comparison with colorimetric reagents and the spectrographic method for the determination of beryllium in air samples showed that the morin fluorimetric method is superior to the others ¹²⁸.

Korenman and Grishin ¹³⁰ have tested about 150 anthraquinone and azo dyes for their reactions with Be and Al. About 50 gave positive reactions. Salts of Sc, In, Th, Zr, Cr, Zn and Mg also gave fluorescence reactions; those of Fe^{3+} , Ca and Ba did not. The United States Bureau of Mines Reports give two fluorescence field tests for beryllium; one used morin ¹³¹ and the other uses quinizarin ¹³². The determination of Be with morin in low grade ores ¹³³, in bronzes ¹³⁴ and in the air ¹³⁵ has also been described. In each case directions are given for removal of likely interferences. In a micro method sensitive to 5×10^{-4} g of Be per ml with morin, Brill and Fruvot ¹³⁶ advocate the use of additional alcohol and show that the complex consists of 3 atoms of Be to 2 moles of morin. 2-Hydroxyquinoline is recommended

for the gravimetric, volumetric, spectrometric or fluorimetric determination of Be; aluminium does not interfere in this method ¹³⁷.

Several authors have reported improvements on the morin method for beryllium. These include the use of complexing agents to exclude catalysts for the oxidation of morin ¹³⁸, complexing agents to mask interfering ions ¹³⁹, proper buffers ¹⁴⁰ separation by the titanium phosphate method ¹⁴¹ and the use of Sn^{2+} to prevent oxidation ¹⁴². Interferences are noted by the above and also by other authors ¹⁴³. Efim'ychev ¹⁴⁴ has shown that salicylaldehyde can be used as an effective reagent for beryllium in acetone or amyl alcohol but not in water. Several of the azomethines have been treated as reagents for Al and Be. Beryllium can be determined in bronze without the removal of Al, Fe, or Cu with 3-hydroxy-2-naphthoic acid, provided these metals are combined with a complexing agent ¹⁴⁶.

3-hydroxy-2-naphthoic acid was a new reagent by the 1960's. It was observed to enable the detection of 0.2p.p.b. of Be by measurement of its blue fluorescent complex.

Fletcher has made a theoretical study of the Be-morin system and determined the ionisation constants and formulas of the chelates formed under different conditions ¹⁴⁷. Morin is shown to be a good indicator for Be in its titration with sulphosalicylate ¹⁴⁸. The principal factors capable of disturbing the Be determination with morin have been outlined by Francois and Grandclement ¹⁴⁹. A procedure for the determination of Be in silicate minerals is given by Rutkowski ¹⁵⁰. The Be-morin method has been compared to the

much longer beryllium colorimetric method on samples containing 30 to 640 p.p.m of BeO found to be approximately equal ¹⁵¹.

A new reagent has been developed that is reported to be highly selective and sensitive for Be, La and Lu. The name of the compound is 1-(dicarboxyaminomethyl)-2-hydroxy-3-naphthoic acid. The Be complex shows its maximum intensity at pH 6.8 and its excitation and fluorescence maxima are 360 and 450 nm respectively. Linear analytical curves for Be are obtained in the range 0.09 to 1.8 ug. Interferences have also been determined ¹⁵². The inhibition by Be on the catalytic effect of calf intestinal alkaline phosphatase on the hydrolysis of 2-naphthyl phosphate which is monitored by measuring the fluorescence of the 2-naphthol has been reported as another method of analyses of Be. This method has been used to determined between 18 and 90 ng of Be and 0.6 - 6 ug of Zn ¹⁵³. An excellent review of available fluorescence reagents for Be has been compiled with 33 references ¹⁵⁴.

Tetracycline, along with 5,5-diethyl -2-thio barbituric acid, gives a blue-violet fluorescence with Be at pH 9 in borate buffer. A linear response is produced with 5 to 15 ug of Be per 50 ml. In the complex, Be and the two organic compounds have a 1:1:1 ratio. The ions Fe(III), Al, Ca and Be in 2-fold amounts relative to Be interfere. The fluorescence is measured at 506 nm with an excitation at 406 nm ¹⁵⁵. The compounds salicylidene-2-amino-3,5-dimethylarsonic acid and salicylidene-2-aminophenylarsonic acid have been studied in reactions with 28 metal ions. The first of these compounds has been described as a reagent for Be ¹⁵⁶. In a study of the metal chelates of flavone derivatives it was found that the Be complexes with 3-hydroxy-4-methoxy- and 3,5,7-trihydroxy flavone gave greater intensity of emission than the others ¹⁵⁷.

forms a fluorescent complex with Be which has its maximum excitation at 403 nm and emission at 483 and is extracted by CCl_4 at pH 6.5-10; phosphate, fluoride and several metal ions interfere. Another extraction method for Be in natural water at a level of ca. 1 μg of Beo/ml uses the acetylacetone-trylon B complex by CHCl_3 extraction ¹⁵⁸.

Complexation with o-(salicylideneamino)-phenylarsonic acid at pH 6 was found to give a sensitivity of 1 ng/ml and to be free of interferences by many other metals ¹⁵⁹. Another procedure for Be uses 6-methyl-1-hydroxyxanthone to form a fluorescent complex in 1:1 methanol-water containing pH 8 buffer; the complex is extracted with CCl_4 which is then dried thoroughly and the fluorescence measured at 470 nm with 435 nm excitation ¹⁶⁰ of 13 derivatives of 5-hydroxy-chromone tested as fluorimetric reagents for Be, the 2-ethyl-3-methyl and the 2-methyl-7-methoxy-5-hydroxycrchromones were judged suitable and the fluorescence was found to be most intense in CCl_4 ¹⁶¹.

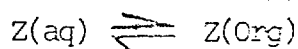
Morisige ¹⁶² has presented an extensive compilation of the fluorescence characteristics of aromatic Schiff base complexes for aluminium, gallium, beryllium and scandium analysis. The optimum conditions for fluorimetric analysis were discussed. Eriochrome Red B was used as a fluorochrome to detect beryllium and other ions ¹⁶³. 3-amino-5-sulfosalicyclic acid forms intensely fluorescing complexes with aluminium and beryllium. The presence of volatile beryllium compounds in orchard leaves has been detected by the morin fluorimetric method ¹⁶⁴. Dinaphthoylmethane (1,3-di (2-naphthyl)-1,3-propanedione) has been used as a reagent to both coprecipitate and form a luminescent product with beryllium for in situ fluorimetric detection of the precipitate ¹⁶⁵.

4.2. SOLVENT EXTRACTION

Solvent extraction is used for the isolation or enrichment of certain compounds or groups of compounds in chemical mixtures. For instance, trace organic compounds present in a public water supply can be concentrated and isolated by means of the extraction of large volumes of water with relatively small volumes of non-polar solvents. Trace metal impurities in important industrial products can be separated from overwhelming major constituents by selective extraction with specific reagents. Complex mixtures of organic compounds can be simply classified into acids, bases, and neutral species by means of two extraction steps.

Phase Equilibria Thermodynamics

Consider some solute Z that is equilibrated between an aqueous phase and an organic solvent phase. The equilibrium can be simply represented as



The free-energy change for the process can be expressed thus $\Delta G = \Delta G^{\circ} + RT \ln (A_z)_{org} - RT \ln (A_z)_{aq} \dots\dots\dots (4.1)$

$$G = G^{\circ} + RT \ln \frac{(A_z)_{org}}{(A_z)_{aq}} \dots\dots\dots (4.2)$$

Since $\Delta G = 0$ at equilibrium, we obtain the relationships shown below:

$$\Delta G^{\circ} = RT \ln \frac{(A_z)_{org}}{(A_z)_{aq}} \dots\dots\dots (4.3)$$

$$\ln \frac{(A_z)_{org}}{(A_z)_{aq}} = - \frac{\Delta G^{\circ}}{RT} \dots\dots\dots (4.4)$$

$$\frac{(A_z)_{org}}{(A_z)_{aq}} = e^{-\Delta G^{\circ}/RT} \dots\dots\dots (4.5)$$

Thus the ratio of the activities of the solute in the two phases turns out to be a constant determined by G° and T . We term this activity ratio the thermodynamic partition coefficient, α_p :

$$\alpha_p = \frac{(Az)_{org}}{(Az)_{aq}} \dots\dots\dots (4.6)$$

The Partition Coefficient

The required activity coefficients are rarely known, so that an approximation based on concentrations alone is commonly employed. This approximate relation is

$$K_p = \frac{[Z]_1}{[Z]_2} \dots\dots\dots (4.7)$$

Where K_p is called the partition coefficient and subscripts refer to the concentrations of the solute Z in phases 1 and 2 ¹⁶⁶.

Extraction

Woodward ¹⁶⁷ determined beryllium by spectrofluorimetry using 2-hydroxy-3-naphthoic acid in aqueous medium. The procedure he developed is here being taken further by extracting the complex so formed into *n*-hexanol. This way the complex is separated from excess reagent and foreign ions, the fluorescence emitted by the hexanol solution is that of the complex and the background fluorescence should be much reduced.

Many extracting reagents were investigated, none, however, proved as worthwhile as hexanol. 2-Hydroxy-3-naphthoic acid dissolved in benzene and chloroform to produce a violet and lilac fluorescence, respectively. In ethanol, and after extraction into higher-*n*-alcohols

(butanol, amyl alcohol and hexanol), it showed the same green fluorescence as in aqueous medium. The beryllium complex therefore was extracted from aqueous solution at pH 7.5 into n-butanol, amyl alcohol, and hexanol, but not into benzene, chloroform nor amyl acetate. After extraction into these alcohols, the complex showed a blue fluorescence similar to that in aqueous solution. Hexanol was chosen for the extraction from the three successful alcohols because it was least soluble in water.

Woodward ¹⁶⁷ removed foreign ions by masking them with EDTA and CDTA (trans-1,2diaminocyclohexane tetracetic acid) and calcium to complex the excess EDTA or CDTA. This procedure was cumbersome and slow and it was hoped that the extraction procedure would accomplish three things, (a) it would eliminate the masking step, (b) it would be more selective with respect to foreign ions, and (b) it would result in greater analytical sensitivity.

4.3. EXPERIMENTAL

Spectral Characteristics

The spectra were taken with the solutions extracted into n-hexanol. Figure 4.1 shows the excitation and emission spectra of the complex and reagent respectively.

- A. Be 10^{-4} M + reagent 10^{-4} M emission is set at 485 nm.
- B. Reagent alone, emission set at 492 nm.
- C. Be + reagent, excitation set at 382 nm.
- D. Reagent alone, excitation set at 365 nm.

These spectra are uncorrected for variations in lamp intensity and the response characteristics of the photomultiplier. The reagent

exhibits its green fluorescence at 365 and its fluorescent emission maximum at 492 nm. The excitation shifts 5 nm to higher wavelength while emission shifts from 510 nm in aqueous phase to 492 nm in the organic phase. The blue fluorescent complex exhibits its excitation maximum at 382 nm while its emission maximum is at 485 nm. There is also a shift here in the organic phase. The excitation shifts 2 nm to higher wavelength while the emission shifts from 460 to 485 nm.

Optimum pH for Extraction

Fig. 4.2 shows a plot of fluorescence intensity versus pH. Extraction of a fixed amount of complex was affected at different pH values and the optimum was determined as ca 8, as can be seen in Fig 4.2. However, at high pH's the reagent was extracted along with the complex. It was decided therefore to use pH 7.5 which had been recommended previously¹⁶⁷.

Effect of pH on Formation

Figure 4.3 shows a plot of fluorescence intensity versus pH. Here optimum pH for complex formation was determined. All other factors were kept constant but pH was varied. The optimum pH did not occur at 7.5 but this pH was used for reasons given in the previous section.

Variation of the Volume of Hexanol Extractant

The volume of the aqueous layer was kept constant at 50 ml and that of the organic layer was varied. As expected the fluorescence intensity decreased as the volume of hexanol increased, as can be seen in figure 4.5. The optimum volume taken was that volume

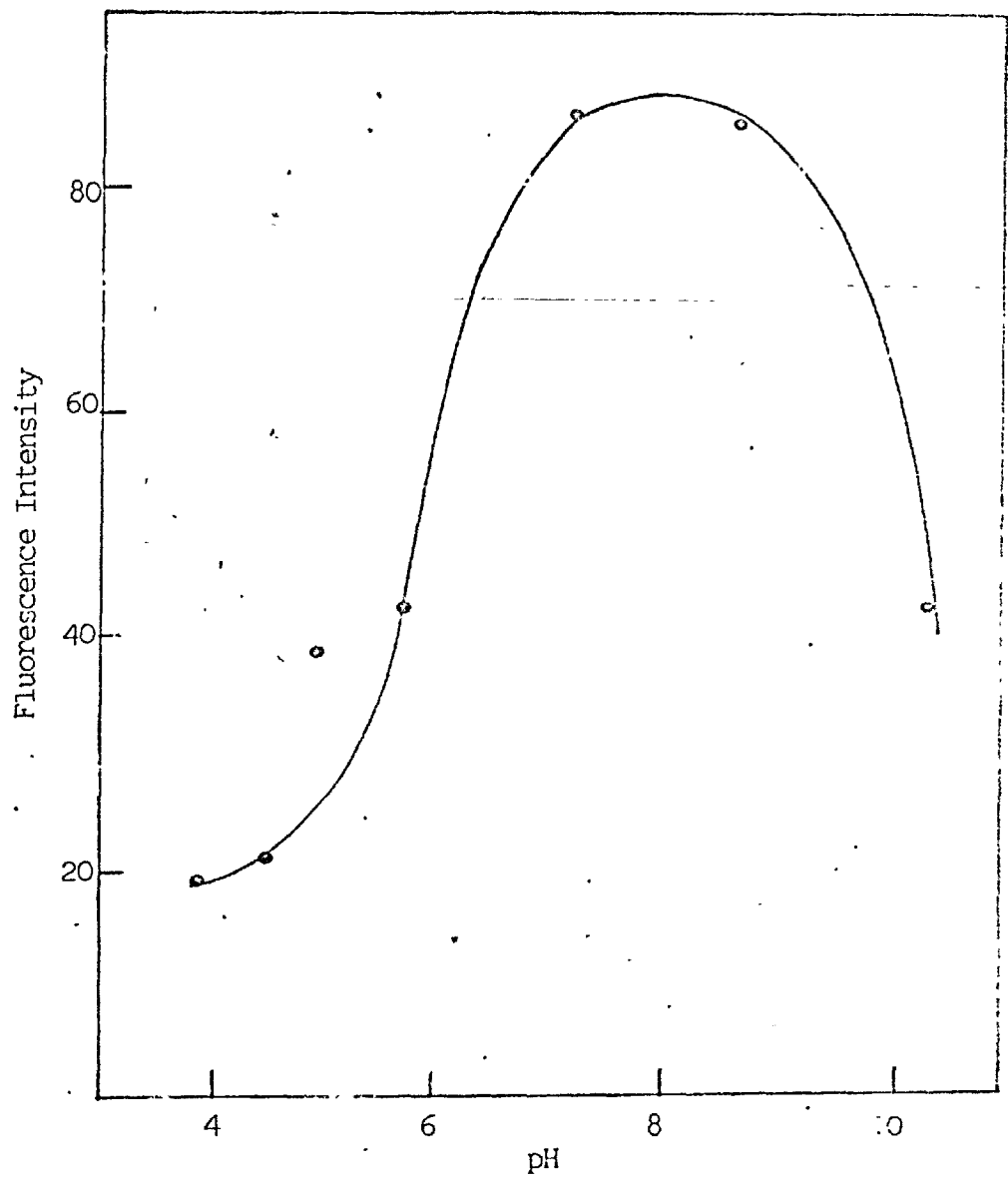


Fig4.2.. Variation of fluorescence intensity with pH during extraction

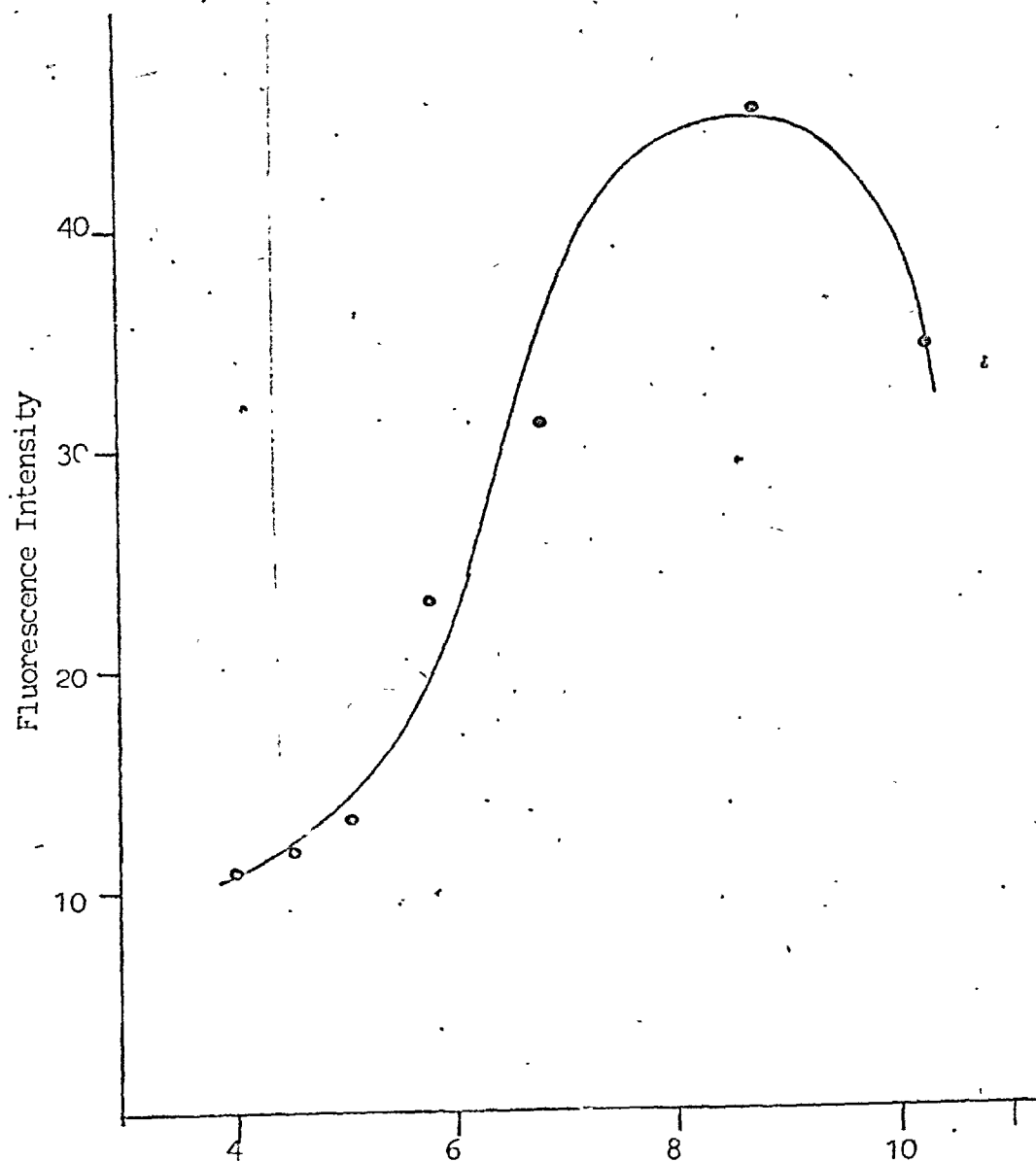


Fig4.3. Variation of fluorescence intensity with pH during complex formation

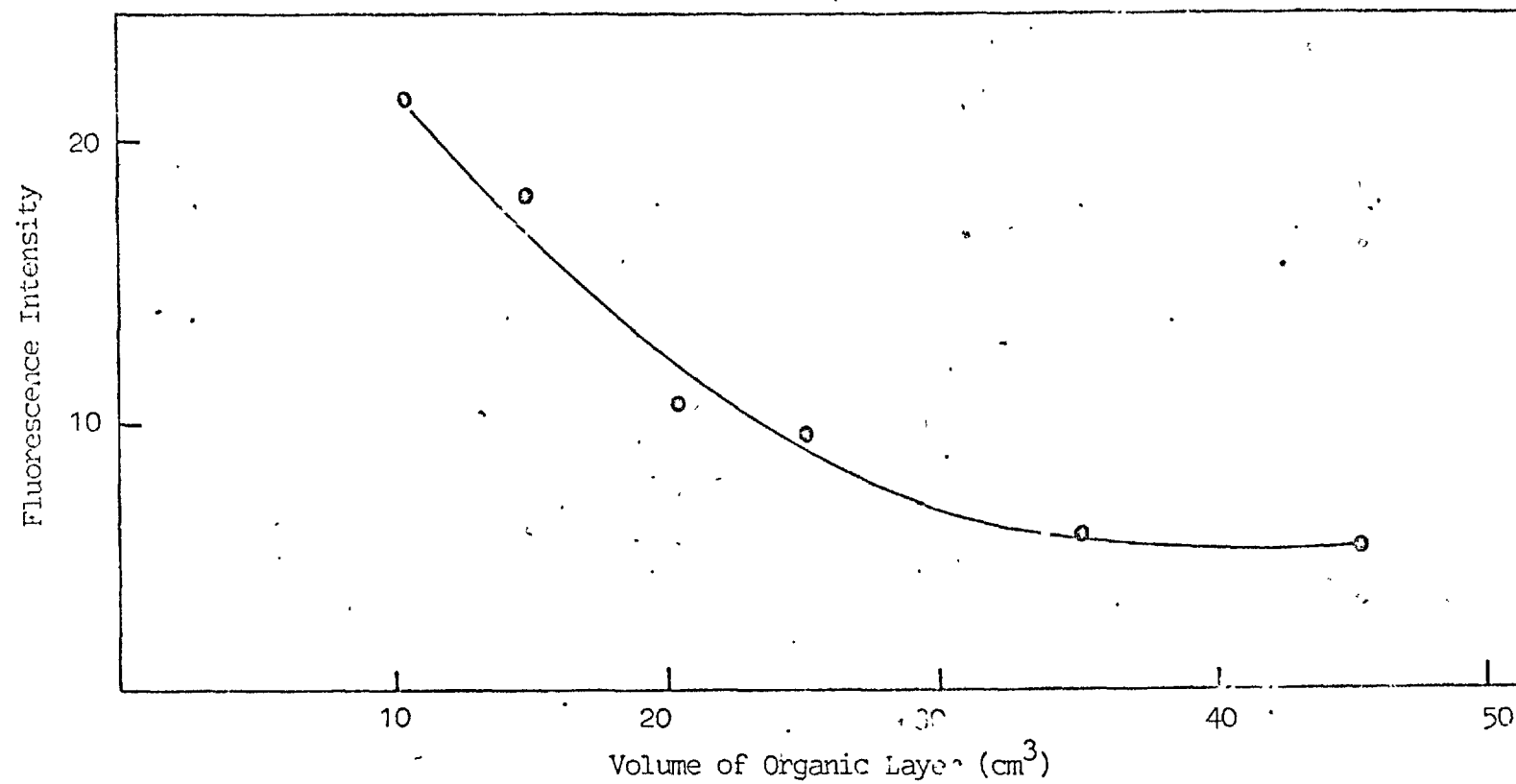


Fig 4.4. Variation of fluorescence intensity with the organic layer volume for extraction.

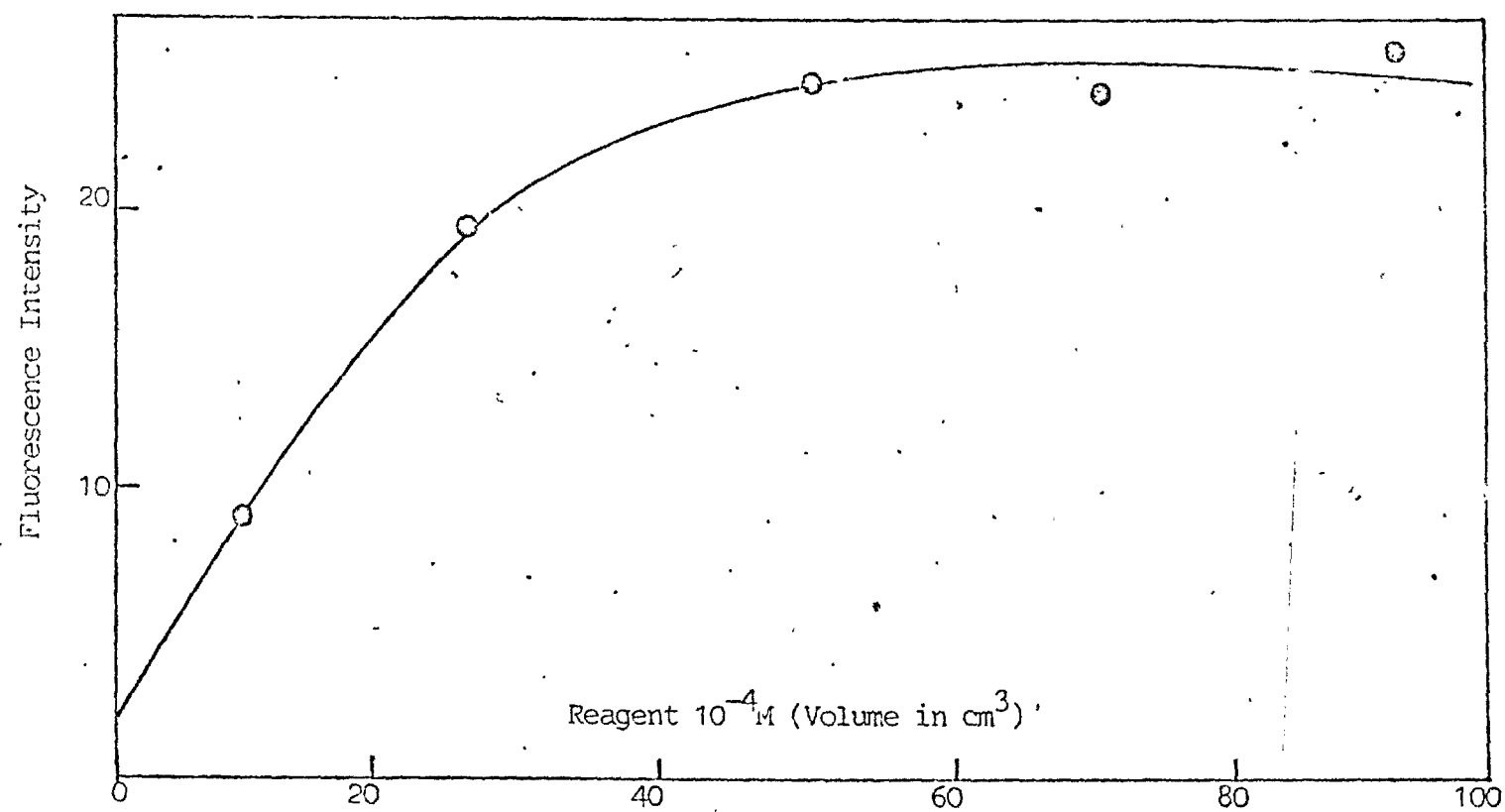


Fig 4.5. Variation of fluorescence intensity with reagent concentration

which extracted the complex and remained as small as possible. 20 ml was taken and this and the 50 ml aqueous layer volume were maintained throughout the work on beryllium.

Reagent Concentration

A varying excess of 10^{-4} M sodium 2-hydroxy-3-naphthoate solution was allowed to react for 30 minutes with 1 ml of 10^{-4} beryllium solution buffered at pH 7.5. The intensity of the blue fluorescence of the beryllium complex was measured as usual (excitation at 380 nm and emission at 460 nm). As shown in Figure 4.6 about 50-fold molar excess of reagent was required to develop maximum fluorescence. This graph was corrected for the blank contributions to the fluorescence at 460 nm of the varying amount of reagent employed.

Effects of foreign ions on the determination of Beryllium

There are various masking procedures available for interfering ions in the beryllium method as described earlier in this chapter. Woodward¹⁶⁷ masked interfering ions by EDTA and Calcium or by one of CDTA (trans-1,2diaminocyclohexane- N, N, N', N'-tetraaceticacid) also along with calcium to complex the excess CDTA. Masking by EDTA and calcium was only partial for aluminium but in this procedure it was hoped that the beryllium complex would be extracted leaving the foreign ions into solution. This proved successful for aluminium and many other ions.

The interference of 27 cations and 12 anions were investigated using 0.9 ug of beryllium in the presence of 1000-fold molar excesses of the foreign ions under investigation. The pH of each solution was

adjusted to ca. 8 before buffering to pH 7.5. 50 ml of this solution was then taken and extracted with 20 ml of Hexanol. The investigation revealed that, under the conditions described above, the following ions did not interfere:

tungstate, calcium, cadmium, zinc, nitrate, aluminium, perchlorate, bromide, molybdate, yttrium, boron, acetate, nitrite, nickel, uranium(VI) and cyanide. Manganese(II) and lead(II) did not interfere at 100-fold and fluoride did not interfere at 10-fold excess. The following ions did interfere, however; thorium(IV), chromium(III), phosphate, lead, iron(II), iron(III), fluoride, sulphate, silicate, manganese(II), copper(II), cerium(IV), titanium(IV), vanadium(V), indium, sulphide, sulphite.

Tartrate was employed for masking the interfering ions and masked copper(II), manganese(II), lead(II) successfully all at 10,000-fold molar excess. Thorium(IV), vanadium(V) and chromium(III) were masked at 1000-fold molar excess and titanium at 100 and iron(III) at 50-fold molar excess.

TABLE 4.1 Data for Precision, Study for extraction procedure

Fluorescence Intensity	Deviation $(x-\bar{x})$	Variance $(x-\bar{x})^2$
20	-2	4
23	1	1
22.5	.5	.25
25	3	9
21	-1	1
22	0	0
22.5	.5	.25
22.5	.5	.25
23.5	1.5	2.25
21.4	-.6	.36

Mean 22	Total	18.36
---------	-------	-------

$$\text{Standard deviation} = \frac{(x-\bar{x})^2}{n-1} = \frac{18.36}{9} = 1.43$$

$$\text{Coefficient of variation} = \frac{1.43}{22} \times 100 = 6.5\%$$

TABLE 4.2

Fluorescence Intensity	Deviation $(x-\bar{x})$	Variance $(x-\bar{x})^2$
73	3	9
73	3	9
63	-7	49
74	4	16
69	-1	1
67	13	9
68	-2	4
70	0	0
70	0	0
69	-1	1

Mean 70	Total	98
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$$\text{Standard deviation} = \frac{(\sum x - \bar{x})^2}{n-1} = \frac{98}{9} = 3.30$$

$$\text{Coefficient of variation} = \frac{3.30}{70} \times 100 = 4.7\%$$

4.1
Table I is the precision data for extracted solution while
4.2
table II is the unextracted solution. Extraction is accomplished with 20 ml of Hexanol and 50 ml of aqueous solution which comprised 1 ml of 10^{-4} M beryllium 10 ml reagent (2-hydroxy-3-naphthoic acid) and buffer pH 7.5, 5 ml.

Tartrate was investigated as a masking agent and was partially successful because the precision deteriorated when tartrate was used in the extracted phase.

Calibration Curves

1.0 to 10 ml aliquots of standard beryllium solution were transferred by pipette into 100 ml volumetric flasks. The pH was adjusted to ca. 8 with dilute ammonia solution. Five ml of pH 7.5 buffer solution and 10 ml of 10^{-4} M reagent solution were added and the solution was diluted to 100 ml with water. The intensity of the fluorescence of the solutions was measured after 30 minutes at 460 nm with an excitation wavelength of 380 nm or instead of measuring directly after 30 minutes, the solution was extracted with 20 ml of hexanol. Fig 4.7 is the corresponding graph obtained using the extraction procedure.

In the extracted phase the detection limit is slightly better (1.8×10^{-9} g/ml) than in the unextracted phase (9×10^{-8} g/ml).

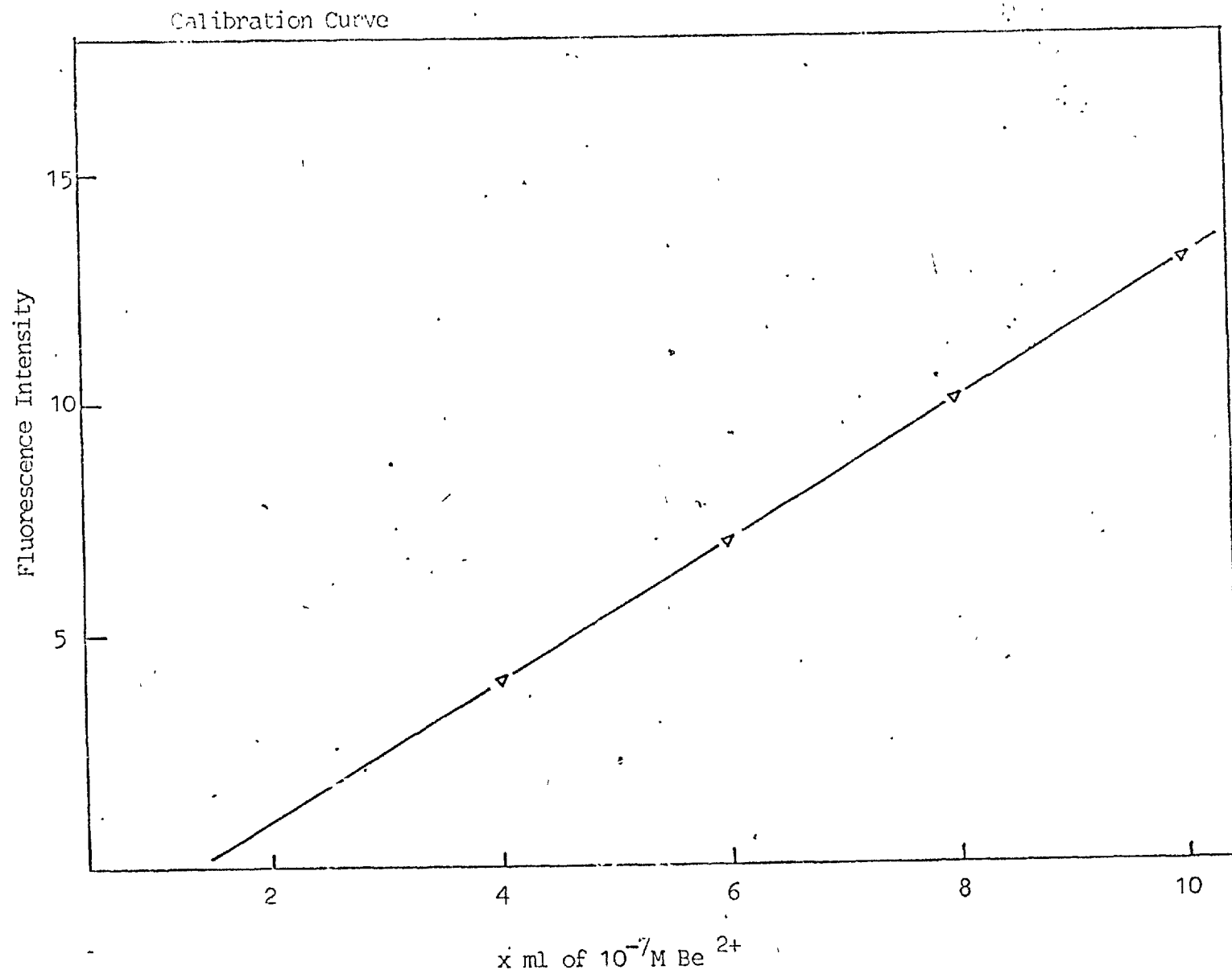


Fig 4.6 Calibration graph for the determination of detection limit of the extracted complex.

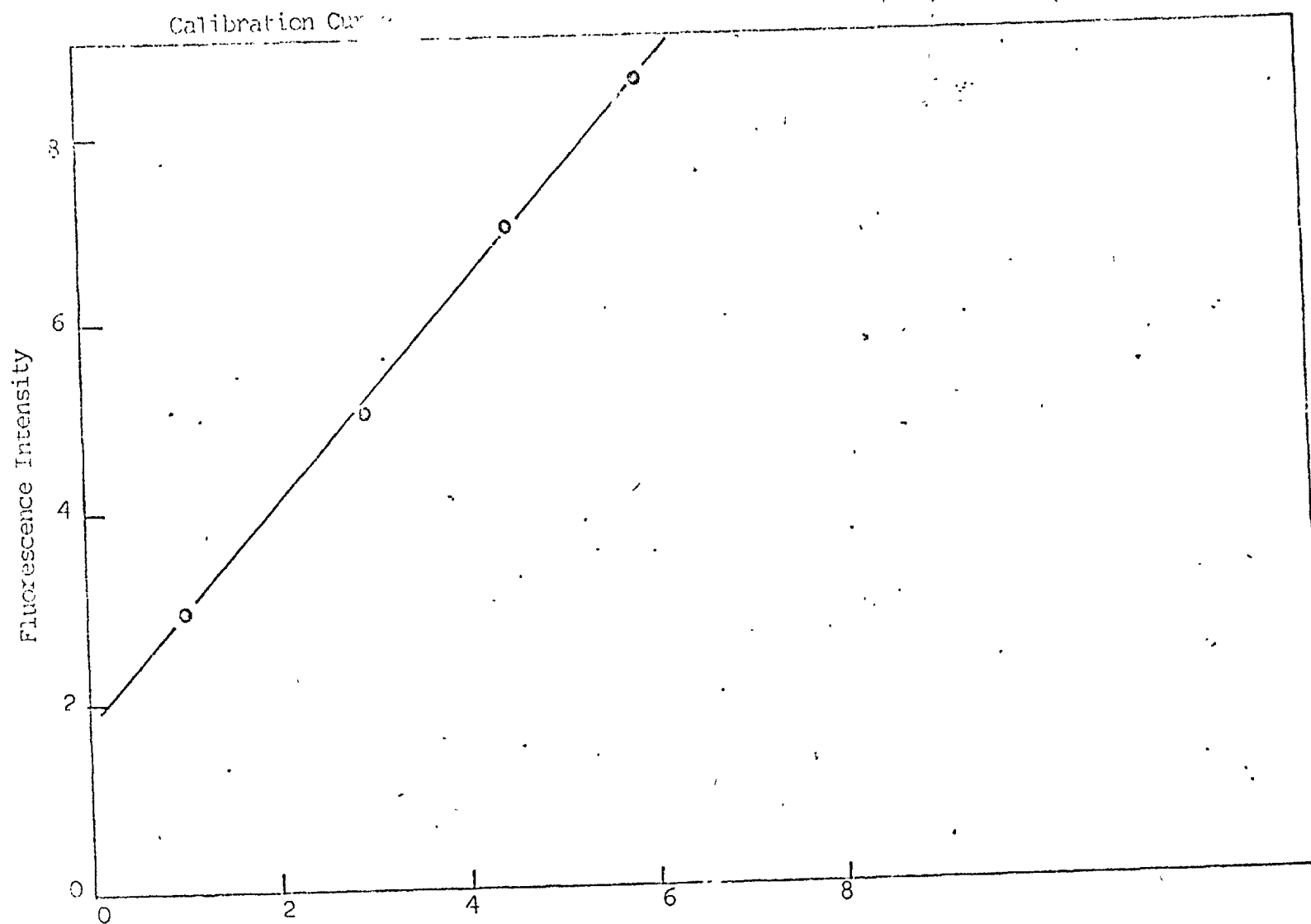


Fig. 4-7 Calibration curve for the determination of detection limit of the unextracted complex.

CHAPTER 5

THE DETERMINATION OF THE CONDITIONAL STABILITY CONSTANT OF THE
BERYLLIUM-2-HYDROXY 3-NAPHTHOIC ACID AND EDTA-BERYLLIUM COMPLEXES

5.1. Introduction

There are many solution properties which change measurably as a result of complex formation. In principle all these measurements can give information relating to the existence and stability of different complex species. By a careful selection one may find one or more suitable experimental methods for the investigation of all kinds of complexes. It must be emphasised, however, that a careful consideration is necessary as to whether the effects of complex formation are quantitatively reflected in the measured property. To choose an adequate method is a prerequisite for obtaining reliable equilibrium constants. Besides the classical methods such as spectrophotometry and potentiometry, which are usually available in every chemical laboratory, the armoury of experimental methods has been enriched by a number of new approaches, such as nuclear magnetic resonance and electron spin resonance spectroscopy. Sometimes a linear relationship is assumed between the price of the instrument and the value of information obtained by its means. Although there is no doubt that the new methods requiring very expensive instruments may make it possible to determine otherwise inaccessible equilibrium constants, this view is completely wrong. Research in the field of equilibrium chemistry is relatively inexpensive, and in most cases reliable stability constants can be obtained by using simple and easily available methods and instruments.

Optical Methods

Frequently one of the most spectacular effects of complex formation is the change of spectral properties which may occur. The

reasons for light absorption by the complexes are as follows:

The excitation of the electrons of both the metal ions and the ligand(s) is influenced by their interaction. The electrons of transition metal ions are easily excited and consequently absorb in the visible region, that is these ions give coloured compounds. The electron systems of the non-transitional metal ions and the ligands are much more stable. The excitation of the electrons requires much greater energy, so these species absorb radiation in the ultraviolet range of the spectrum. Owing to the interaction of the central ion and the ligand a charge transfer from the ligand to the metal ions (and vice versa) may occur on irradiation. This phenomenon is the reason for the so-called charge transfer spectra in the visible or the near-ultraviolet region. As a matter of course, complex formation characteristics of the ligands, leading to the shift of peaks in the infrared spectrum. All these changes may be employed to study complex formation. The relationship between the absorbance of the composition of a given solution is given in the ideal case by the Beer-Lambert law:

$$\log \frac{I_0}{I} = A = d \sum \epsilon_i C_i \dots\dots\dots (5.1)$$

where d is the absorption length, A is the absorbance of the solution, ϵ_i the molar absorptivity of the i th species (which has concentration C_i); A and ϵ_i refer to a given wavelength. The intensive factor, the molar absorptivity, depends on the medium and on the temperature. The molar absorptivities of the metal ion, of the ligand and - in most cases - of the coordinatively saturated complex can be determined directly, but those of the intermediary complexes are just as unknown as their stability constants. If two species in the solution have at a certain wavelength the same molar absorptivity, the series of the spectra of solutions, when the sum of the concentrations is constant but the ratios of their concentrations are changed, exhibits

a common crossing point, the so-called isosbestic point. Because it is very unlikely that a third species has the very same molar absorptivity at this wavelength, the occurrence of isosbestic point(s) in the formation of a series of complexes gives evidence for the simultaneous existence of two absorbing species. It is a great advantage of the spectrophotometric method that the measurements can be made at many different wavelengths. In selecting the wavelength it is helpful to use different bands of the spectrum. The agreement of stability constants obtained from measurements at different wavelengths is strong evidence for their reliability.

Spectrophotometric measurements in the ultraviolet, visible and infrared regions can easily be made with commercial instruments.

The error of spectrophotometric measurements is determined by chemical and by instrumental factors¹⁶⁸. The molar absorptivities refer to a certain medium. In the study of very weak complexes, high ligand concentrations must be applied which in fact means a change in the medium. Price¹⁶⁹ has pointed out that even without any specific interaction between particles, purely topological reasons may lead to a change in the charge transfer spectra. From this change an apparent association constant of the order of 0.2 litre/mole can be calculated. Therefore this is the lower limit of the stability constants which can be obtained spectrophotometrically.

5.2. THE METHOD OF CONTINUOUS VARIATION

Though the principle of continuous variation was first applied by Ostromisslensky¹⁷⁰ nevertheless the discovery of the method is frequently attributed to Job¹⁷² who applied it to complex systems.

CHAPTER 6

It was very popular between the mid-forties and mid-fifties and is still frequently applied. The principle of the method is simple. Suppose that the metal ion and the ligand form one complex



A series of solutions is prepared in which the sum of the total concentrations of Me and L is constant, but their proportions are continuously varied:

$$T_{\text{Me}} + T_{\text{L}} = C \quad \dots\dots\dots (5.3)$$

$$x = \frac{T_{\text{L}}}{T_{\text{Me}} + T_{\text{L}}} \quad \dots\dots\dots (5.4)$$

The equilibrium concentrations of the species Me, L and Me_mL_n are given by the equations:

$$[\text{Me}] = C(1 - x) - m[\text{Me}_m\text{L}_n] \quad \dots\dots\dots (5.5)$$

$$[\text{L}] = Cx - n[\text{Me}_m\text{L}_n] \quad \dots\dots\dots (5.6)$$

$$[\text{Me}_m\text{L}_n] = \beta_m n [\text{Me}]^m [\text{L}]^n \quad \dots\dots\dots (5.7)$$

Evidently, the concentration of the complex reaches a maximum at a certain value of χ . This value (χ_{max}) can be obtained by differentiating equations 5.5 to 5.7 with respect to χ , setting $d[\text{Me}_m\text{L}_n]/dx = 0$ and eliminating $[\text{Me}]$ and $[\text{L}]$

$$m(Cx - n[\text{Me}_m\text{L}_n]) = n(C(1 - \chi) - m[\text{Me}_m\text{L}_n]) \quad \dots\dots\dots (5.8)$$

$$\text{That is } m\chi_{\text{max}} = n(1 - \chi_{\text{max}}) \quad \dots\dots\dots (5.9)$$

$$\text{and } \frac{n}{m} = \frac{\chi_{\text{max}}}{1 - \chi_{\text{max}}} \quad \dots\dots\dots (5.10)$$

The method of continuous variation is most frequently applied in the

evaluation of spectrophotometric data but in principle it can be applied in connection with polarimetry, NMR, magnetic susceptibility measurements, fluorimetry, etc.

The simplicity of this technique makes it very attractive, but before it is applied the following limitations have to be considered:

- (i) The change in the activity coefficients with changes in concentration may influence the molar ratio at which the concentration of the complex is of maximum value. To eliminate this effect the measurements have to be carried out at constant and sufficiently high ionic strength^{173, 174}.
- (ii) The measured property must be a strictly linear function of the concentration of the complex¹⁷³.
- (iii) If the conjugate acid of the ligand is weak, the position of the extremum depends on the pH^{173, 175}.

5.3. THE MOLE-RATIO METHOD

In the case of successive complex formation, more reliable information can be obtained by the mole-ratio method¹⁷⁶. Here the total concentration of the metal ions is kept constant while the total concentration of the ligand is increased. The absorbance is plotted as a function of the total ligand concentration. If only one complex of high stability is formed, the graph consists of two linear intersecting parts. The ratio of total concentrations of metal and ligand at the intersection gives directly the ligand to metal ion ratio in the complex. The stability constant in this case cannot be obtained. If the complex is moderately stable a steep curve is obtained and the

intersection of the slopes of the two linear parts of the curve gives the molar combining of ligand to metal ion ratio. In favourable cases the stability constant can be derived simply from the difference between the measured curve and that constructed from the slopes. In the case of weak complexes this simple method fails to give any reliable information.

If N complexes are formed and each of them is stable enough to avoid overlapping equilibria, the curve consists of N sections. The slopes of the sections are determined by the molar absorptivities of the complexes.

Harvey and Manning¹⁷⁷ have pointed out that the composition can also be obtained from the slopes of the curves of absorbance versus the total ligand concentration. They observed that the break-points on the curves become more pronounced at higher ionic strength. This means that the stability of the complexes increases with increasing ionic strength. This, however, is not a general phenomenon.

5.4. CALORIMETRY

The heat evolved when a ligand is added to a solution of a metal ion is given by:

$$Q = \sum_{i=1}^n \left[\frac{M_i L_i}{e_i} \right] \cdot \Delta H_i + \Delta H_{dil} \dots\dots\dots (5.11)$$

where Q is the total heat evolved (or absorbed), V is the volume ΔH_i is the molar formation enthalpy of the ith species and ΔH_{dil} is the heat of dilution. All the methods for the mathematical treatment of spectrophotometric data should be applicable to calorimetric data.

However, modern instrumental methods¹⁸⁵ especially those developed by Eigen, make possible the study of the fastest chemical reactions.

These methods enable us to distinguish between co-ordination in the inner and outer sphere¹⁸⁶. Many reactions are catalysed by ligands, metal ions and metal complexes. The study of these catalytic reactions, can also provide information on the stability constants of the complex species¹⁸⁷.

If the reaction is first-order for the species of which the stability constant is to be determined the rate can be described by an expression strictly analogous to equation (5.1), the corresponding intensive factors being the elementary rate constants, so for the mathematical treatment of data the methods already discussed can be applied. However, some important differences have to be borne in mind. The rate constants are much more sensitive to temperature variations than are the molar absorptivities. In spectrophotometry, the measurement can be made at different wavelengths but in kinetics a reaction can be characterised by a single intensive factor. Especially in the case of catalytic reactions it must be remembered that thermodynamically less stable species may contribute to the observed rate, because it can be expected that the value of the intensive factor will exhibit a large value in just that case. This makes possible the detection of such species and in favourable cases even the determination of their stability constants.

5.6. LIQUID-LIQUID PARTITION

The partition of solute between immiscible liquids may be used to investigate complex equilibria. The distributed solute may be the

ligand or a species containing metal ion. The condition that the two solvents - one of which is usually an aqueous solution - should be immiscible, is often not completely fulfilled. If the mutual solubility of the two liquids is appreciable, the change of the medium and its effect on the complex equilibria have to be considered. In the study of complex equilibria in an aqueous solution, the other liquid used is an organic solvent. The extraction of the species can be followed by many methods. Most generally, radioactive isotopes are applied. This method makes it possible to work at very low metal ion concentration, permitting the study of mono-nuclear complexes even though the tendency to form polynuclear complexes is strong.

5.7. POTENTIOMETRIC METHODS

The concentration changes caused by complex formation may be monitored potentiometrically. In fact, most recorded stability data has been determined by potentiometric methods. By potentiometric measurements both the metal ion and the ligand concentration can be determined. It is a prerequisite of the application of potentiometric methods that the electrode reaction be reversible. In this case the activity (concentration) in question can be calculated from the measured potential by means of the Nernst equation.

There are two possibilities for the performance of the experiments: either a solution is prepared for each potential measurement or one solution is titrated with another and the potential is measured after each addition of titrant. The latter method is evidently much more rapid and with careful performance of the experiments is almost as exact as the more laborious first method, so it is much more popular.

For the potentiometric measurements an electrode must be selected, the potential of which is a well-defined function of the concentration of the ion to be determined, and also a reference electrode. The electrode, together with the solution in which it is immersed, comprises a halfcell. For the measurement of potential this has to be combined with another halfcell containing the reference electrode. If the two electrodes are in contact with the same solution and respond to different constituents of the liquid the electromotive force of the cell is the difference between the potentials of the two halfcells. If, however, the two electrodes are immersed in two different solutions, an electrolytic connection must be made between the two halfcells. This liquid has the result that the measured potential is not equal to the difference of the potentials of the two halfcells. The liquid junction potential is caused by the different mobilities of the different ions. Therefore potassium chloride solutions are frequently used to minimise the liquid junction potential, since the mobilities of the potassium and chloride ions are similar. If this is impossible, it is necessary to use well-constructed salt bridges. The so-called 'Wilhelm' reference halfcell¹⁸⁸ with liquid junction is certainly one of the best.

5.8. THE Be-EDTA COMPLEX

Woodward¹⁶⁷ masked interference in the fluorimetric determination of beryllium with 2-hydroxy-3-naphthoic acid from 1000-fold molar excesses of the foreign ion under investigation with a 1250-fold excess of EDTA. It was observed, however, that the presence of excess EDTA in the solution caused a reduction in the fluorescence intensity due to the beryllium-2-hydroxy-3-naphthoic acid complex. A 6000-fold excess calcium was therefore added in order to complex any unused excess EDTA. It thus appeared that at low concentrations of beryllium,

EDTA formed a weak complex at pH 8 with beryllium ions. There is very little information on the Be-EDTA complex in the literature and the existence of this weak complex of EDTA with a small divalent metal ion was considered to be noteworthy. Having developed the 2-hydroxy-3-naphthoic acid method for the determination of beryllium, it seemed worthwhile to investigate the stability of the Be-EDTA complex employing this technique.

The competitive chelation reactions between 2-hydroxy-3-naphthoic acid and EDTA for beryllium in aqueous solution at pH 8 were studied fluorimetrically. The fluorescent complex (beryllium-2-hydroxy-3-naphthoic acid) was formed in the usual manner and EDTA several times more concentrated than 2-hydroxy-3-naphthoic acid was added to the complex. A reduction in fluorescence intensity results via formation of the Be-EDTA complex.

Woodward¹⁶⁷ proposed the formation of a beryllium-reagent complex 1:1 molar proportions. Figures 5.1 and 5.2 are confirmation of the same proposal. These curves were obtained using 10^{-4} M solutions of beryllium and reagent, buffered at pH 7.5 and measuring the fluorescence intensity at 380/460 nm. Figure 5.1 was obtained in the aqueous media but for Fig 5.2 the solution was extracted after buffering and allowing to stand for 30 minutes. The extraction was accomplished with 20 ml of Hexanol and 50 ml of the solution. In both cases the solution fluorescence was corrected for the reagent blank. The total amount of beryllium plus reagent solutions was 20 ml of 10^{-4} M per 100 ml for curves C in both figures, and 15 ml of 10^{-4} M per 100 ml for curves B. Curves A in both figures, was obtained by adding 20 ml of 10^{-3} M EDTA to solutions similar to those of curves C. In the aqueous phase the value obtained for the stability constant of the Be-2-hydroxy-3-naphthoic acid complex was 1.58×10^5 from the Job's plots.

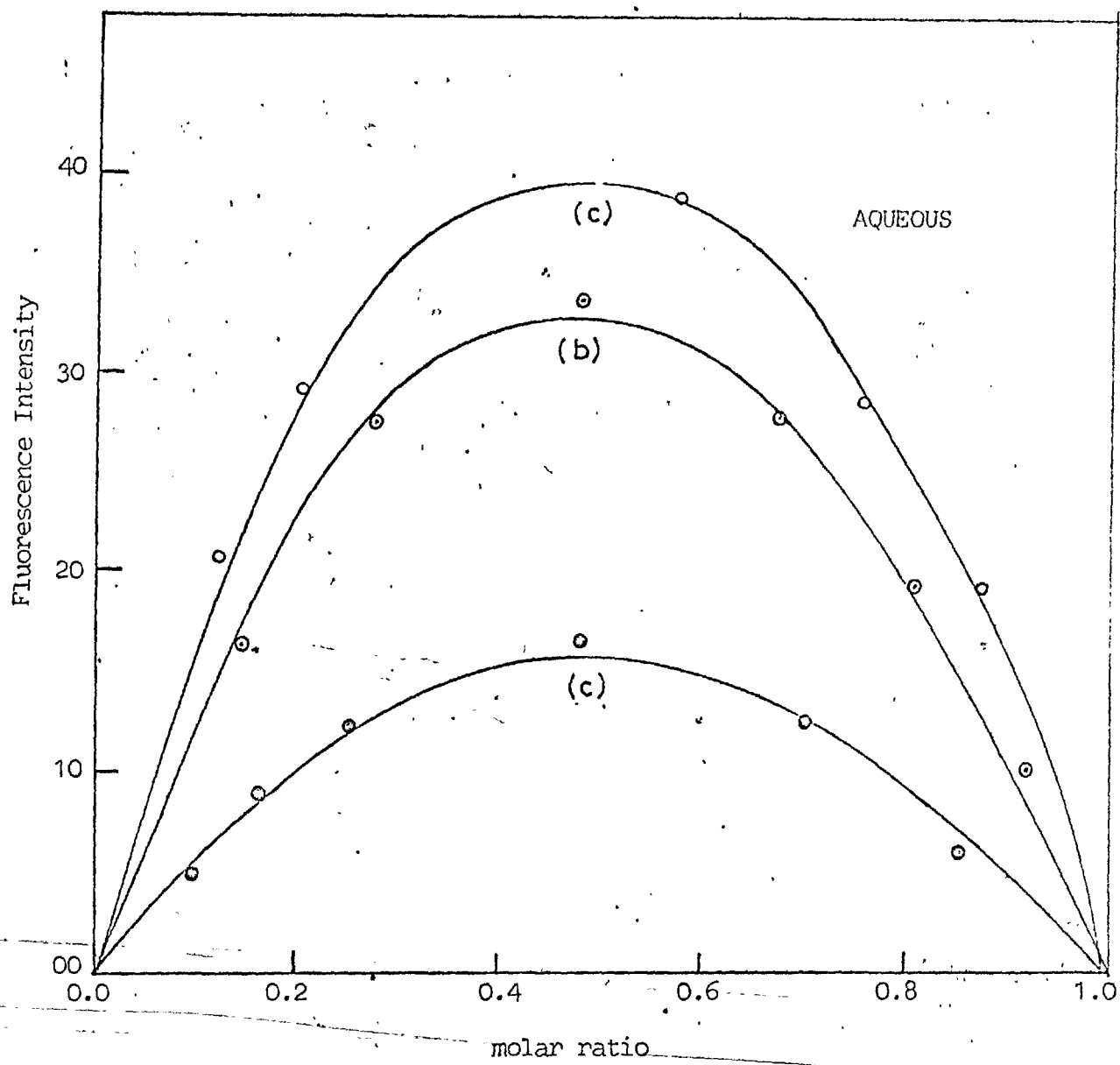


Fig 5.1. Variation of fluorescence intensity with molar ratios of beryllium and reagent in the aqueous media.

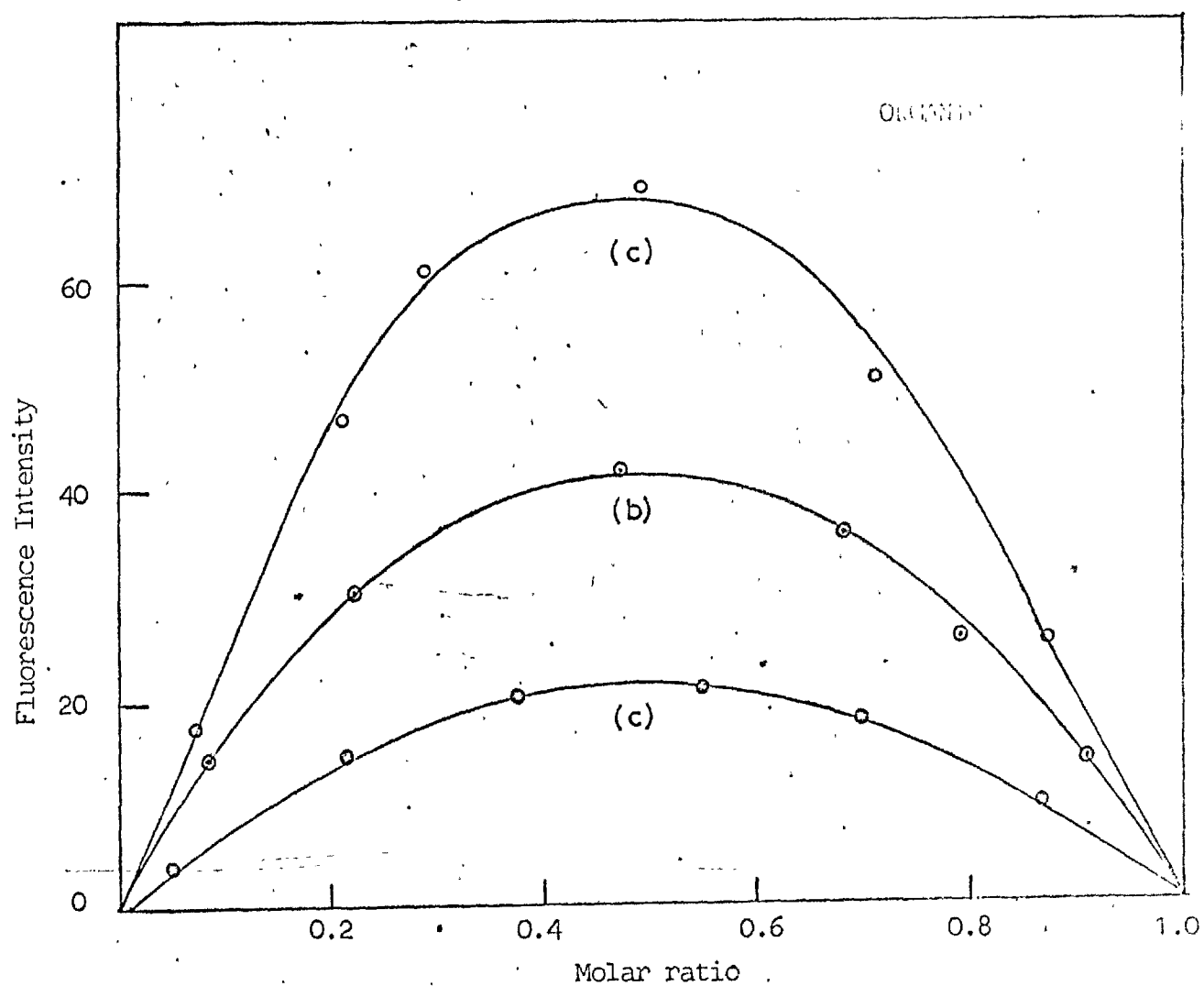


Fig 5.2. Variation of fluorescence intensity with molar ratios of beryllium and reagent in the organic phase.

while in the organic phase the conditional stability was calculated as 4.3×10^4 . This shows that the Beryllium-2-hydroxy-3-naphthoic acid complex is relatively stable. The stability constant was calculated by taking a point on one side of the maximum of curve B or C in Fig 5.1. Take another point on the same side of the other curve at a point . Both of these points are at the same fluorescence intensity value.

$$\text{Then } K = \frac{x}{(M-x)(R-x)} = \frac{x'}{(M'-x')(R'-x')}$$

where M and R represent total molar concentration of M (metal) and R (reagent) if no reaction had taken place between them.

Of course $x = x'$

$$\text{from which } = \frac{MR - M'R'}{(M+R)-(M'+R')}$$

substitution of x in the expression for K, the conditional stability constant provided the numerical values given above.

Experiment

5 ml of 10^{-4} M Beryllium was complexed with 10 ml of 10^{-4} 2-hydroxy-3-naphthoic acid. This was adjusted to ca. pH 8 with ammonia solution before adding 5 ml buffer solution of pH 7.5. Then in successive flasks increasing volumes of EDTA (10^{-3} M) was added and diluted to 100 ml. The fluorescence was developed for 30 minutes and then the solution (50 ml) was extracted with hexanol (20 ml). The fluorescence intensity was measured at 380/460 nm. Fluorescence intensity at 460 nm was plotted against increasing amounts of EDTA.

Results and Discussion

The graph obtained from the above experiment is an exponential

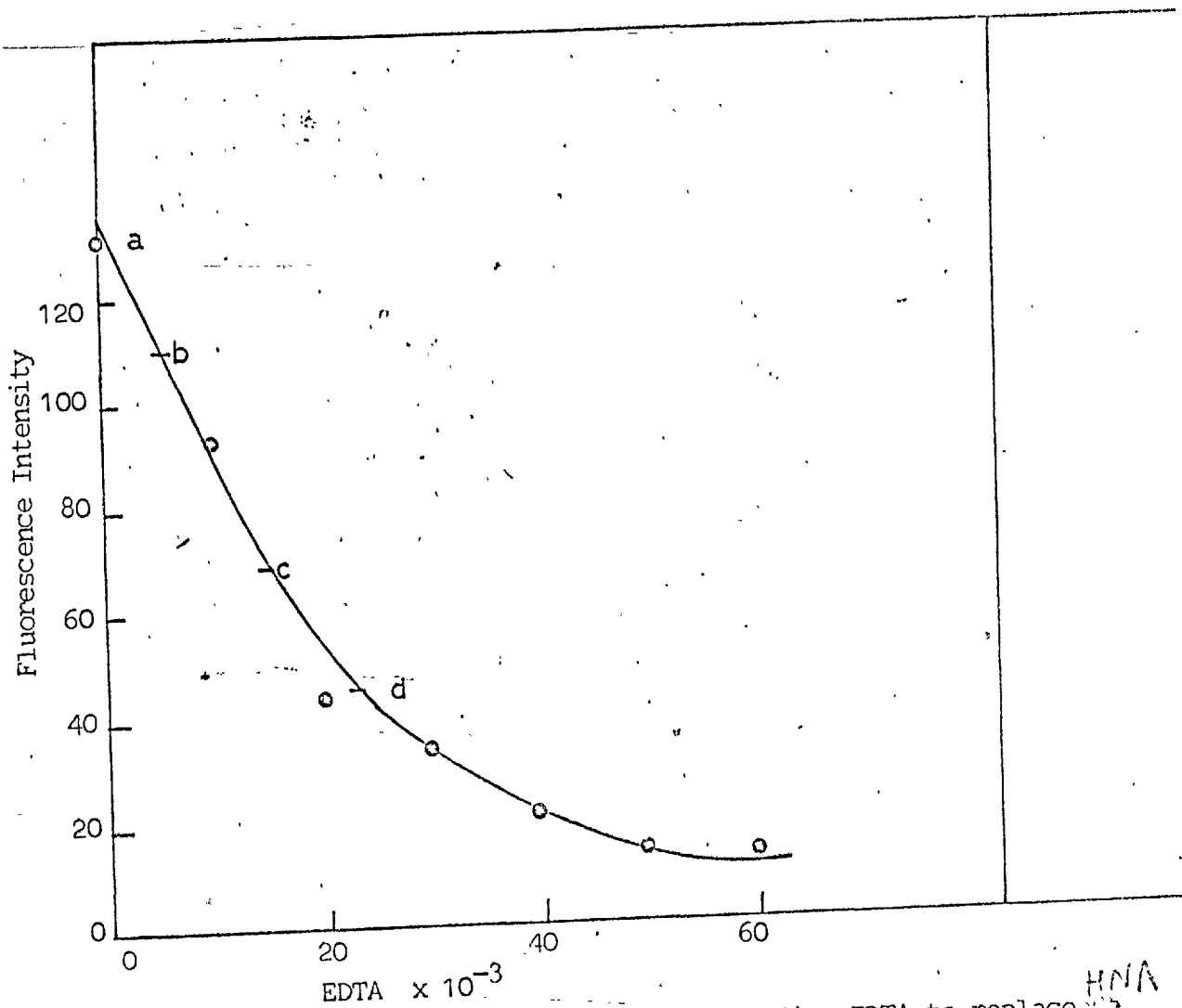
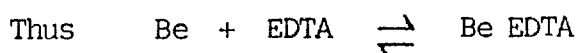


Fig 5.3. Reduction of fluorescent complex by adding EDTA to replace ^{HNA}

curve, as can be seen from Fig 5.3. The fluorescence intensity recorded provides a direct linear measure of the residual concentration of the Be-2-hydroxy-3-naphthoic acid complex left in the solution in the presence of different intensities of EDTA. This data may be employed to obtain an approximate value for the stability constant of the beryllium-EDTA complex at pH 7.5.

The general principle of the method used is to calculate the amount of EDTA which is required to reduce the fluorescence intensity, and thus the concentration of Be-2-hydroxy-3-naphthoic acid complex, (Be-HNA) to 50% of its original value. The assumption is then made that the reduction in fluorescence intensity results from the binding of beryllium from the 2-hydrox-3-naphthoic acid complex into the Be-EDTA complex.



$$K_{\text{Be-EDTA}} = \frac{[\text{Be EDTA}]}{[\text{Be}][\text{EDTA}]}$$

The concentration of EDTA which must be used to have the fluorescence from various values on the graph of Figure 5.3 may be used. determined.

Thus, between for example points A and C on Fig 5.3 15 ml of 10^{-3}M EDTA in 100 ml final solution (i.e. a concentration of $1.5 \times 10^{-4}\text{M}$) is required to reduce the fluorescence intensity from 94 to 47 units. At point B, 50% of the beryllium present in the solution (i.e. $\frac{1}{2} \times 5 \times 10^{-6}\text{M}$) is bound as the Be-EDTA complex rather than Be-HNA complex. To a first approximation we may assume that the value

of the free beryllium concentration will be very low and consider the participation only of the 'free' concentration value for beryllium at equilibrium from that 50% of the beryllium liberated from the Be-HNA complex on addition of EDTA. Thus, all the beryllium for the Be EDTA complex will be assumed to come from the Be-HNA complex.

Thus on the addition of EDTA



Initially

$$5 \times 10^{-6} \text{M} \quad 1.5 \times 10^{-4} \text{M}$$

At equilibrium

$$(5 \times 10^{-6} - 2.5 \times 10^{-6}) \quad (1.5 \times 10^{-4} - 2.5 \times 10^{-6}) \quad (2.5 \times 10^{-6})$$

$$\begin{aligned} \text{Thus } K_{\text{BeEDTA}} &= \frac{\text{Be EDTA}}{\text{BeHNA EDTA}} = \frac{2.5 \times 10^{-6}}{(5 \times 10^{-6} - 2.5 \times 10^{-6})(1.5 \times 10^{-4} - 2.5 \times 10^{-6})} \\ &= \frac{6.6 \times 10^3}{1} \end{aligned}$$

values for the Be-EDTA conditional stability constant may also be calculated similarly between other points on the graph of Fig 5.3.

Thus to reduce the fluorescence by one-third of its original value to point B 5.5 ml of 10^{-3}M EDTA is required (i.e. a concentration of $0.55 \times 10^{-4} \text{M}$ EDTA). At this point one-third of the Be is bound as Be-EDTA (i.e. $\frac{1}{3} \times 5 \times 10^{-6} \text{M}$).

At this point K may again be calculated as :

$$\begin{aligned} K &= 1.66 \times 10^{-6} / (5 \times 10^{-6} - 1.66 \times 10^{-6})(0.55 \times 10^{-4} - 1.66 \times 10^{-6}) \\ &= \frac{9 \times 10^3}{1} \end{aligned}$$

similarly at point D on Figure 5.3 to reduce the fluorescence by two-thirds, 24 ml of 10^{-3}M EDTA is required. At this point two-thirds of the Be is bound as Be-EDTA.

At this point K may be calculated as:

$$K = \frac{3.3 \times 10^{-6}}{(5 \times 10^{-6} - 3.3 \times 10^{-6})(2.4 \times 10^{-4} - 3.3 \times 10^{-6})}$$

$$= \underline{8.1 \times 10^3}$$

In this way an approximate average value for the conditional stability constant of the Be-EDTA complex may be obtained of $7.9 \pm 1.5 \times 10^{-3}$.

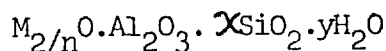
From this value it will be seen that, as expected, only a weak complex is formed between beryllium and EDTA at pH 7.5. In the method employed to obtain the value of the conditional stability constant the 2-hydroxy-3-naphthoic acid acts as an 'indicator' in a manner similar to a metallofluorescent indicator used in EDTA titrations of metal ions. Indeed, the use of 2-hydroxy-3-naphthoic acid as a metallofluorescent indicator has been reported¹⁹¹ for titration of aluminium with EDTA. As one of the well established criteria for obtaining successful sharp end-points in EDTA titrations, however, is that the stability constant of the metal-indicator complex should be at least 10^4 lower than the stability of the metal-EDTA complex, 2-hydroxy-3-naphthoic acid is not suitable for the titration of Be with EDTA. Had time permitted, it would have been instructive to investigate the variation of the conditional Be-EDTA stability constant with pH of the medium employed, and also the determination stability constant of the corresponding Al-EDTA complex by this method.

6. OTHER STUDIES

A study was undertaken to investigate if fluorescence or phosphorescence would be enhanced at room temperature for molecules adsorbed on zeolite. It was also aimed to study the possibility of producing sharp spectra at room temperature similar to those that would be obtained at low liquid nitrogen temperature. It had been reported that by using adsorption on paper, silica, alumina, etc., followed by thorough drying, in the case of acid or amine derivatives of polynuclear compounds, it was found possible to obtain strong triplet-state phosphorescence at room temperature avoiding the usual need for low temperature¹⁸⁹. This study was to examine the suitability of a zeolite for use in this way.

6.1. CRYSTALLINE ZEOLITE

Zeolites are crystalline, hydrated aluminosilicates of group I and group II elements, in particular, sodium, potassium, magnesium, calcium, strontium and barium. Structurally the zeolites are "framework" aluminosilicates which are based on an infinitely extending three-dimensional network of AlO_4 and SiO_4 tetrahedra linked to each other by sharing all of the oxygens. Zeolites may be represented by the empirical formula::



In this oxide formula, x is generally equal to or greater than 2 since AlO_4 tetrahedra are joined only to SiO_4 tetrahedra, n is the cation valence. The framework contains channels and interconnected voids which are occupied by the cation and water molecules. The cations are

quite mobile and may usually be exchanged to varying degrees, by other cations. Intracrystalline "zeolitic" water in many zeolites is removed continuously and reversibly. In many other zeolites, mineral and synthetic, cation exchange or dehydration may produce structural changes in the framework. Ammonium and alkylammonium cations may be incorporated in synthetic zeolites. In some synthetic zeolites, aluminium cations may be substituted by gallium ions and silicon ions by germanium or phosphorus ions. The latter necessitates a modification of the structural formula.

Although there are 34 species of zeolite minerals and about 100 types of synthetic zeolites, only a few have practical significance at the present time. Many of the zeolites, after dehydration, are permeated by very small channel systems which are not interpenetrating and which may contain serious diffusion blocks. In other cases, dehydration irreversibly disturbs the framework structure and the positions of metal cations, so that the structure partially collapses and dehydration is not completely reversible. To be used as a molecular sieve, the structure of the zeolite after complete dehydration must remain intact.

Molecular sieve action has been reported for other solids, crystalline and noncrystalline. These include coal, special active carbons, porous glass, microporous beryllium oxide powders and layer silicates modified by exchange with organic cations¹⁹⁰.

The work reported here was conducted using Linde molecular zeolite type 13X and 13Y.

6.2. ADSORPTION

Spectra were observed when compounds were adsorbed on zeolite. Compounds were dissolved in solution and then left overnight in contact with either zeolite 13Y or 13X. Then the wet zeolite was scooped on a silica slide and dried in the oven at 100°C for 1 hour. This was to make sure that the spectra were not those of solution in contact with the zeolite. Then the slide carrying the zeolite was placed in the cell compartment of the Aminco spectrofluorimeter with suitable filters. Spectra were recorded with the Bryans potentiometric recorder (see chapter 1).

However, it was possible that the spectra were those of molecules sitting on the zeolite and not actually adsorbed. It was therefore necessary to wash the adsorbing zeolite with the solvent many times. The spectra were still produced even after such washing. Concentrated hydrochloric acid dissolves zeolite. The adsorbing zeolite was dissolved in concentrated HCl and the released compound was separated using the solvent. The amount of the released compound increased when the amount of HCl was increased indicating that there was real adsorption.

A typical spectrum is shown in Fig 6.1. Pyrene dissolved in hexane was adsorbed overnight. The wet zeolite was spread on a silica slide (2cm by 5cm) and then the slide placed at 45° to the incident beam of light. A filter to allow excitation at 265 nm was used. Another filter (LF3) was used on the emission side to reduce scattered radiation.

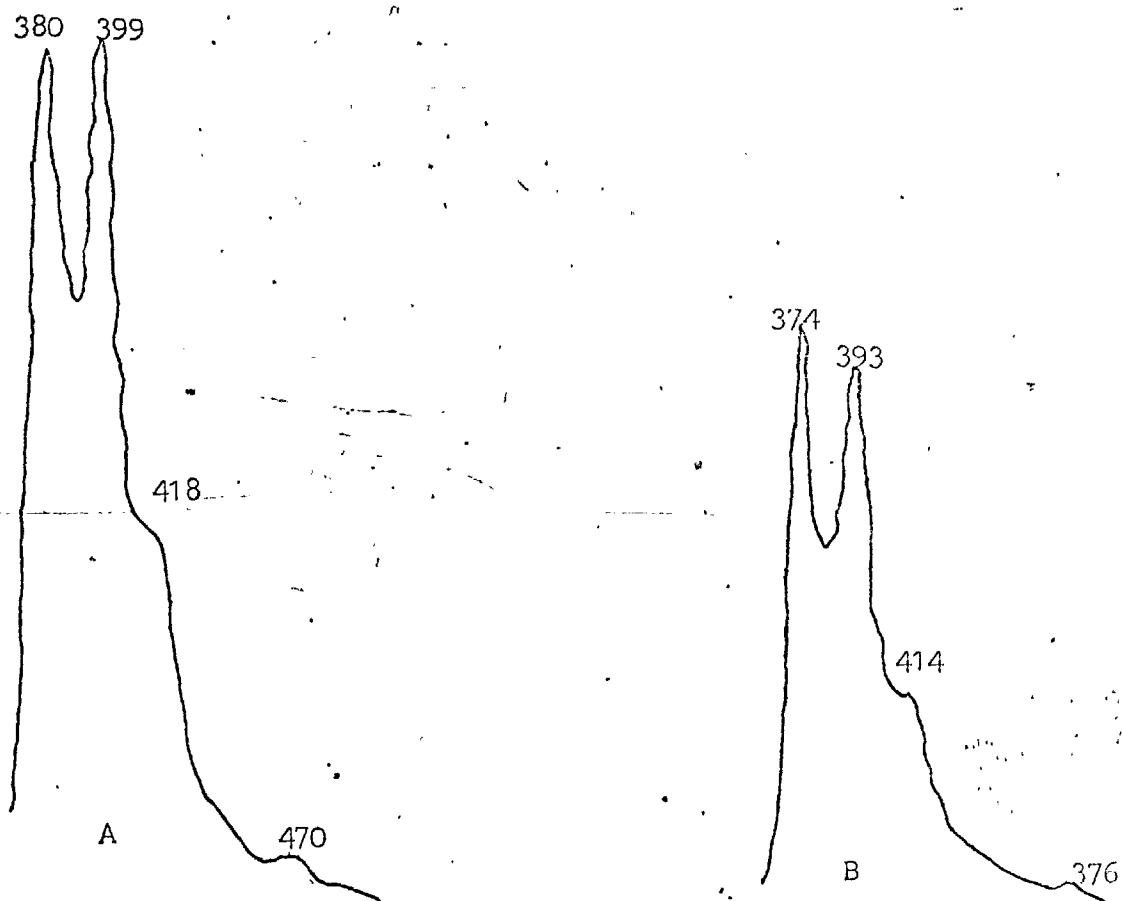


Fig 6.1. Emission Spectra:
(a) Pyrene in solution at 77°K
(b) Pyrene adsorbed in Zeolite at 298°K

CHAPTER 7

7.1. CONCLUSIONS

The studies conducted and reported in this thesis have been accomplished using the Aminco spectrofluorimeter (see Chapter 1) and have all derived from the fact that when a molecule absorbs energy, it gives out some or all of that energy in some form or other. Here energy given out as light has been the main concern.

Photon counting has been reported in chapter 2. It is obvious that photon counting is advantageous when used in conjunction with spectrofluorimetry. The Aminco spectrofluorimeter is a low resolution instrument and would be improved much if interfaced with the photon counter. Nonetheless, the tedious job of working out the statistics involved lessens the desirability of such a venture. But if extensive study is going to be conducted requiring low levels of detection it is advisable to use computers that are now available to cope with the statistics. Then photon counting would seem justifiable.

Chapter 3 reports the measurement of quantum yield and lifetime of luminescence of heavy metal ions in aqueous solutions of hydrochloric acid and lithium chloride. From the data presented in chapter 3, it is difficult to elucidate the mechanism of luminescence of the ions. The work carried out in this study was not extensive enough to elucidate mechanisms and further work would be worthwhile in this area.

The fluorescence of beryllium-2-hydroxy-3-naphthoic acid complex has been the subject described in chapter 4. The complex was extracted with hexanol. Beryllium is fatally toxic and a proper

method of analysis is imperative. In the extraction method the problem was lack of precision and accuracy which could be attributed to the method itself. However, the sensitivity and selectivity were improved and the method is useful.

The Beryllium-EDTA complex is described in chapter 5. This work derives from the fact that the EDTA at several times more concentrated than 2-hydroxy-3-naphthoic acid can replace the ligand that gives the fluorescent complex. A conditional stability constant was calculated from the results. Further work to investigate the variation of the conditional stability of the Be-EDTA complex with pH would be worthwhile.

Chapter 6 reports the adsorption of molecules by zeolites. It was envisaged that molecules adsorbed in the zeolite cavities might produce spectra similar to those produced by molecules frozen at 77°K. However, it was not possible to produce these spectra, but it was established that hydrocarbon molecules were adsorbed. The mechanism of adsorption is not understood, but it may be possible that they line side by side in the cavities so that their electron clouds interact with each other producing the broad band spectra observed.

There is not much future to this kind of work, but if undertaken again, it might be worthwhile to try ionic organic molecules and preferably try enhancing phosphorescence instead of fluorescence. Other adsorbing sites could be studied, e.g. silica, paper, etc.

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