SOME STUDIES USING MOLECULAR FLUORESCENCE AND PHOSPHORESCENCE TECHNIQUES IN TRACE ANALYSIS

By

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ABSTRACT

The general theory and principles governing luminescence processes in solution are briefly reviewed. The instrumentation used for the works described in the thesis is also discussed and observations on useful available literature are made. The procedure for obtaining factors for correction of luminescence spectra and the correction curves obtained are described.

The room-temperature fluorescence, low-temperature luminescence and phosphorescence ($77^{o}K$) characteristics of twenty nine antioxidants and ultra-violet absorbers commonly added to polymers have been examined. The spectral data and calibration curves for the luminescence and phosphorescence are described. Detection limits and lifetime measurements for the phosphorescence of fifteen additive compounds are also presented.

Sensitive spectrofluorimetric methods for the determination of phosphorus as orthophosphate are described, which are based on the formation of an ion-association complex between the molybdophosphoric acid and (i) rhodamine B, (ii) quinine. Both procedures described are highly selective and large amounts of silicates do not interfere. The structure of these complexes has also been examined.

Luminescence lifetimes of several inorganic ions in aqueous solutions of hydrochloric and hydrobromic acids measured at room temperature and at $77^{o}K$ are presented together with the quantum yields of luminescence of Tl(I), Pb(II) and Ce(III) ions in hydrochloric acid and of Tl(I) and Ce(III) in hydrobromic acid measured at room temperature. Possible mechanisms for the luminescence observed in these ions are discussed. Finally, the implications of the type of work described in the thesis are reviewed and suggestions for further work in this field are made.

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CHAPTER 1

THEORY AND INSTRUMENTATION OF LUMINESCENCE

1.1 Introduction

The absorption of energy by a molecular species takes place in discrete units called quanta. The quanta-energy relationship can be written as :

$$E = h\nu \quad , \quad \text{or} \quad E = hc/\lambda \qquad (1.1)$$

where E is the energy, h the Planck's constant, c the velocity of light, ν the frequency, and λ the wavelength. By absorption of energy the molecule is raised to an excited state. On returning to the normal or ground state, the molecule may re-emit a fraction of the absorbed radiation as a quantum of lower energy. The process by which the energy is emitted as light is called luminescence (which may be fluorescence or phosphorescence). The change in photon energy causes a shift of the luminescence emission spectrum to longer wavelengths relative to absorption spectrum, and is referred to as the "Stokes Shift". Fluorescence emissions have relatively short lifetimes (10^{-7} to 10^{-9} sec), while phosphorescence emissions have relatively long lifetimes (10^{-3} to 10 sec). Fluorescence and phosphorescence can also be distinguished by their spectra. The phenomena of fluorescence and phosphorescence are widely used in analytical techniques because of their potentially high selectivity and sensitivity. Theoretical and experimental aspects of fluorescence and phosphorescence in solution have been dealt with by a number of authors (Becker[1], Bowen[2], Guilbault[3], Hercules[4], Konstantinova-Shlezinger[5], Parker[6], Udenfriend[7], White and Argauer[8], and Zander[9]). An excellent coverage of spectrofluorimetric procedures and other matters pertaining to fluorimetric analysis has been given for many years in the biennial reviews of White and Weissler[10]. References for the analysis of elements and

compounds are also given in the text-books of Weissler and White[11], Berlman[12] and Passwater[13].

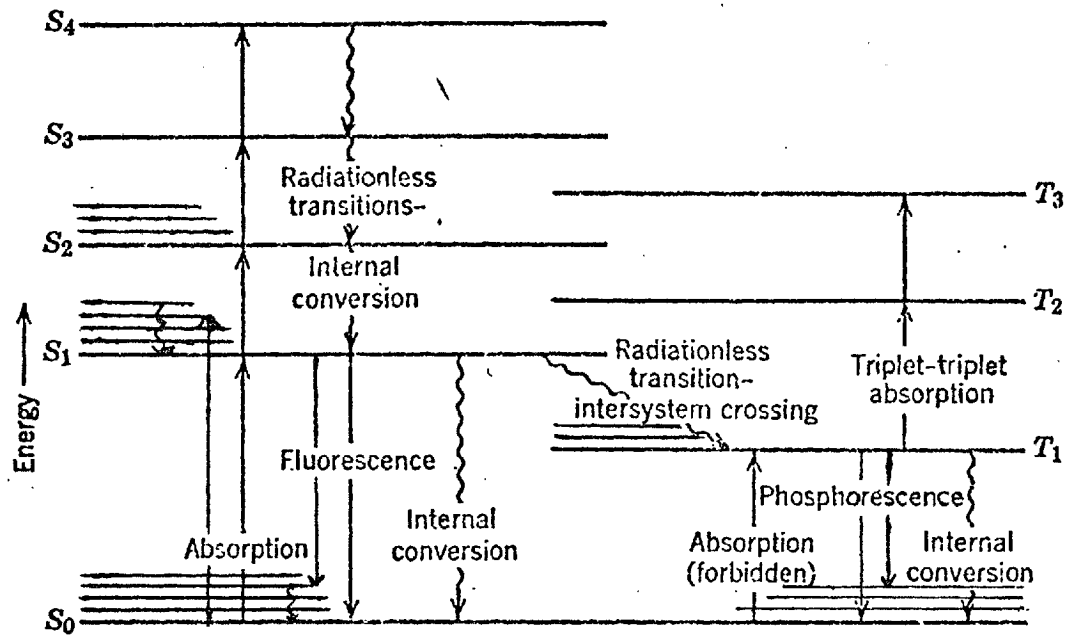
The general underlying theory of the phenomena of re-emission of absorbed radiation as fluorescence or phosphorescence, and instrumentation employed by the author in the studies reported in this thesis, are discussed in the following sections.

1.1.1 Theory

When a molecule in the ground state is irradiated with ultraviolet or visible light of known wavelength, electronic excitation from the ground state (S_o) to an excited state may take place. Depending on the nature of the molecule, there can be several energy levels of the excited state. If the electronic excitation takes place to any state above the first excited singlet (S_1), usually de-excitation occurs to the lowest excited singlet state. Photon absorption, generally, transforms a molecule in the ground state, in its lowest vibrational energy level to an electronically excited species which is also vibrationally excited. When the electron returns to the ground state from the first excited singlet state, the energy released may be dissipated in one of several ways : (a) as fluorescence emission, (b) as radiationless transitions with no emission, (c) when crossing to the triplet state (T_1), or (d) any combination of these processes (Figure 1.1). The radiationless transitions may involve conversions into vibrational, rotational or kinetic energy, i.e. heat or chemical energy.

A molecule in an excited singlet state is highly energetic and exists only for a short period of time. The process of fluorescence is seen within 10^{-7} to 10^{-9} sec, and usually occurs from the lowest vibrational level of the first excited singlet state. Within the lifetime of an electronically excited molecule, two radiationless processes can take place. One is 'vibrational relaxation',

FIGURE 1.1



Schematic state energy level diagram: S is singlet and T is triplet. The S_0 state is the ground state and the subscript numbers identify individual states.

approximate lifetimes

$S_0 \longrightarrow S_n$ (absorption)

$S_n \xrightarrow{10^{-11}\text{–}10^{-14}\ \text{sec}} S_1$ (internal conversion)

S_1 —
- $\xrightarrow{10^{-7}\text{–}10^{-9}\ \text{sec}} S_0 + h\nu$ (fluorescence)
- $\xrightarrow{10^{-8}\ \text{sec}} T_n$ (intersystem crossing)
- $\xrightarrow{10^{-5}\text{–}10^{-7}\ \text{sec}} S_0$ (internal conversion)

T_1 —
- $\xrightarrow{10\text{–}10^{-3}\ \text{sec}} S_0 + h\nu$ (phosphorescence)
- $\xrightarrow{10\text{–}10^{-3}\ \text{sec}} S_0$ (internal conversion)

which is the deactivation from a higher to the lowest vibrational level without change of electronic energy, and the other 'internal conversion', which is the deactivation of the molecule from a higher to a lower electronically excited state without change of spin multiplicity. Usually, the energy lost in these processes is converted into thermal energy. The process by which the molecules migrate from the first excited singlet state (S_1) to the triplet state (T_1) is referred to as 'intersystem crossing'. This transition may take place within 10^{-8} sec and is a spin-dependent internal conversion process (Figure 1.1). If the triplet state is occupied, phosphorescence emission, or de-excitation to the ground state, or both, can occur. The lowest excited triplet state (T_1) is most frequently of lower energy than the lowest excited singlet state, and therefore, phosphorescence occurs at longer wavelength than does fluorescence. Because phosphorescence originates from the lowest triplet state, it will have a decay time approximately equal to the lifetime of the triplet state (ca 10^{-3} to 10 secs).

Fluorescence and phosphorescence are in principle clearly distinguishable forms of emission, (e.g. compared to Tyndall and Rayleigh scattering), by the fact that in order to fluoresce and phosphoresce, the substance must first absorb some of the incident radiation. The scattering processes are of interest in spectrofluorimetry because they can interfere with measurements of fluorescence of extremely dilute solutions. Tyndall and Rayleigh scattering produces light which has the same frequency as that of the incident light, although different frequencies will be scattered to different degrees. Raman scattering appears in a region of lower frequency and this process in spectrofluorimetry has been discussed by Parker[14].

Each of the radiative transitions $S_1 \rightarrow S_0$ and $T_1 \rightarrow S_0$ gives rise to a characteristic emission spectrum by means of which the excited state concerned can be identified. All luminescence phenomena arising from the $S_1 \rightarrow S_0$

transition are generally termed 'fluorescence' and those arising from the $T_1 \rightarrow S_0$ transition, 'phosphorescence'. There are several ways in which the excited singlet S_1 can be populated and these give rise to different kinds of fluorescence. The simplest kind is that already discussed, which is also called 'prompt fluorescence'. A variety of processes exist, however, by which a molecule can pass to excited singlet state from the triplet state to result in an emission characteristic of the $S_1 \rightarrow S_0$ transition but with a lifetime much longer than that characteristic of the S_1 state. This emission, called 'delayed fluorescence', has been observed by Parker and co-workers for several organic molecules[15-18]. Delayed fluorescence has a lifetime between that of fluorescence and that of phosphorescence, and has the same fluorescence excitation and emission characteristics as normal (or prompt) fluorescence. Delayed fluorescence has been further classified into 'E-type' and P-type' delayed fluorescence according to the mechanism by which the process $T_1 \rightarrow S_1$ occurs[6].

Finally, there are other forms of long-lived luminescence, whose lifetime does not depend directly on the lifetime of the triplet state. These emissions are observed in rigid medium where the irradiation causes the solute, directly or indirectly, to decompose into fragments which recombine slowly with the formation of an excited singlet or triplet state, and classified as 'recombination delayed fluorescence' and 'recombination phosphorescence'.

1.1.2 Quantum Yield of Luminescence

The fraction of excited molecules undergoing luminescence emission determines the quantum yield or quantum efficiency of luminescence. In general, the quantum yield of an emission is defined as the fraction of molecules that emit a photon after direct excitation by the source. That is, the quantum yield of fluorescence, ϕ_F, is given by :

$$\phi_F = \frac{\text{Number of quanta emitted as fluorescence}}{\text{Number of quanta absorbed to an excited singlet state}} \quad (1.2)$$

and the quantum yield of phosphorescence, ϕ_P, is defined as :

$$\phi_P = \frac{\text{Number of quanta emitted as phosphorescence}}{\text{Number of quanta absorbed to an excited singlet state}} \quad (1.3)$$

In the case of phosphorescence, excitation usually takes place via a singlet state, because the direct absorption $S_0 \rightarrow T_1$ is usually forbidden.

If all the molecules excited by absorption of light emit fluorescence when returning to the ground state, then the quantum efficiency of fluorescence is unity. But in normal practice, the quantum efficiency for all the molecules is less than one, because a proportion of the excited molecules may return to the ground state by other processes, for example, by conversion to the triplet state (Figure 1.1), by collision with solvent molecules or with molecules of another solute, or some may undergo photochemical reaction. However, few organic compounds, such as rhodamine B[19,20], fluorescein,[19,20], 9-amino acridine[19], rubrene[20,21], and 9,10-diphenyl anthracene[22] have quantum yields that are close to unity in solution.

Several methods have been described for measurement of luminescence quantum yields in solution[23]. Absolute quantum yields in solution[19,24-29] are somewhat difficult to measure, whereas it is quite simple to obtain information on relative quantum yields[19,20,26,30].

1.1.3 Fluorescence Intensity and Concentration

The magnitude of the total fluorescence intensity will depend on the population of ground and excited state molecules in the solution. This forms the basis of the quantitative use of fluorescence measurements for analysis. Absorption of radiation of the wavelength of excitation precedes fluorescence emission. Therefore, the laws which govern the relationship between the

concentration of a fluorescent species in solution and fluorescence intensity arise out of, and depend on, those relating to absorption.

Beer's Law states that in a solution of absorbing substance, the absorbance is directly proportional to the concentration. If I_0 is the intensity of the incident light, and I_t that of the transmitted light, both measured in quanta per unit time, then the fraction of light transmitted is

$$\frac{I_t}{I_0} = 10^{-\epsilon c l} \qquad (1.4)$$

where ϵ is the molar absorptivity of the substance at the given wavelength of absorption, c is the concentration of the absorbing species and l is the optical pathlength of solution. The corresponding fraction of light absorbed is then given by :

$$\left(1 - \frac{I_t}{I_0}\right) = (1 - 10^{-\epsilon c l}) \qquad (1.5)$$

That is, the amount of light absorbed is :

$$I_0 - I_t = I_0 \ (1 - 10^{-\epsilon c l}) \qquad (1.6)$$

By definition, the total fluorescence intensity is equal to the rate of light absorption (in quanta per unit time) multiplied by the quantum efficiency of fluorescence. That is ,

$$F = (I_0 - I_t) \cdot \phi \qquad (1.7)$$

where F is the total fluorescence intensity (in quanta per unit time) and ϕ the quantum efficiency of fluorescence. By substituting equation (1.6), the equation (1.7) becomes

$$F = I_0(1 - 10^{-\epsilon c l}) \cdot \phi . \qquad (1.8)$$

Ideally, for very dilute solutions in which only a small fraction of light is absorbed, i.e. the term $\epsilon c l$ is not greater than about 0.05, the equation

(1.8) reduces to :

$$F = k.I_0.\phi \cdot (2.303\ \epsilon c\ l) \qquad (1.9)$$

where k is an instrumental constant which takes into account the instrumental artifacts and the fact that fluorescence will be emitted in all directions but will be viewed only over a relatively small solid angle. Thus, for any given substance and instrumental geometry, if the frequency and intensity of the exciting light are kept constant, the response of the detector is directly proportional to the concentration. With modern photomultipliers and high-gain amplifiers, very low fluorescence intensities can be measured. It is possible, by using large excitation intensity, to detect low concentrations of fluorescent substances. This is the fundamental difference from absorption spectrometry, in which the detection limit is set by the minimum detectable difference in intensity between the incident and transmitted light.

The intensity of fluorescence (or phosphorescence) emitted by a substance is also directly proportional to the intensity of the exciting light and to the detector sensitivity. For any particular instrument, the limit of detection of a fluorescent substance is established by the signal-to-noise ratio[31]. In principle, it should be possible to improve the detection limit of the substance indefinitely, by using large exciting intensity of light and sensitive and stable detection systems. The lower limit of detectability for a given substance may be taken as that amount of substance which will give a signal-to-noise ratio of unity, i.e. the point at which the fluorescence signal becomes equal to the inherent noise of the instrument. In the types of instruments used, the noise arises mainly from the instability of zero point, noise within electronics, and from variations in the photomultiplier tube current[32]. However, the factor limiting sensitivity is often not the electrical signal-to-noise ratio, but the overall fluorescence blank. A variety of factors may contribute to the

luminescence blank, including contributions from the fluorescence of the cuvette and impurities in the solvent or reagents, scattered exciting light, or even the Raman emission from the solvent[6].

Factors which reduce the fluorescence may be classified as quenching processes, i.e. which result in the true fluorescence efficiency being reduced, or as inner-filter effects, i.e. those which do not have any effect on the fluorescence efficiency, but reduce the intensity of the observed fluorescence by absorption of the exciting light or of the fluorescence light of the substance under investigation.

1.1.4 Quenching Processes

One of the very important quenching effects is the chemical reactivity of molecules in the excited states. Usually the chemical reaction is promoted by continuous irradiation. In practice, under the conditions of analysis by spectrofluorimetry the irradiation time is usually small, and quenching by this process, therefore, is of little significance.

Collisional deactivation of molecules in the excited state with other molecules, such as the solvent molecules, is of importance in quenching of fluorescence. This process involves the transfer of singlet energy from an excited molecule (a donor) to a nearby molecule (an acceptor). If this transfer takes place between molecules of the same solute, the effect is known as concentration quenching or self-quenching. Self-quenching is a relatively minor problem in fluorimetric analysis since it can be overcome by dilution of the sample. If the fluorescent molecule and the acceptor in solution are different, the quenching of fluorescence and concentration of the quencher are given by the Stern-Volmer equation[6,33]:

$$\phi_f^o / \phi_f = 1 + k_Q [Q] \cdot \qquad (1.10)$$

where ϕ_f^o is the fluorescence efficiency of solution in the absence of quencher, ϕ_f is the fluorescence efficiency in the presence of quencher of concentration Q, and k_Q is the quenching constant.

Generally it is found that an increase in temperature very markedly decreases the fluorescence intensity as a result of increase in the frequency of collisions between molecules, and at room temperatures, negative temperature coefficients of 1 - 2% are observed. This effect is not very serious as it appears to be, because in analytical spectrofluorimetry, in submicrogram range, an error of up to 5% is considered to be insignificant. Because of longer lifetime of the excited triplet state, the temperature effect is much more important in phosphorescence than in fluorescence,[22,34]. Intermolecular collisions can be minimised by increase of the viscosity of the medium. One of the methods used to increase viscosity is by cooling the medium to low (liquid nitrogen) temperatures, and usually phosphorescence can be observed under these conditions.

The rates of the processes of internal conversion and intersystem crossing, which compete with fluorescence, are markedly affected by the environment of the luminescent molecule. For example, substituents such as chloro, bromo, iodo and nitro groups in aromatic molecules, enhance phosphorescence and reduce fluorescence[35-38]. This phenomenon, described as the 'heavy-atom effect' is attributed to an increase in spin-orbit coupling. With increase in spin-orbit coupling in an organic molecule, the probabilities of intersystem crossing ($S_1 \rightarrow T_1$), of phosphorescence ($T_1 \rightarrow S_0$), and of radiationless transition to the ground state ($T_1 \rightarrow S_0$) are all increased. The coupling effects can be subdivided into 'internal' and 'external' effects. The term 'internal' refers to the effect when the 'heavy' atom is a constituent element of the fluorescing molecule; in 'external' effects it is present in the surrounding medium as constituent of the solvent or of some third component.

For example, the addition of ethyl iodide to solutions of aromatic hydrocarbons causes an increase in the ratio of the phosphorescence to fluorescence quantum yield[39-40]. Similar effects have been observed in the presence of heavy metal ions[41-45].

The presence of dissolved oxygen in solution has a pronounced action of quenching of fluorescence of many organic compounds. It shows little effect on very many inorganic-organic chelate systems. In most of the cases, the reduction in fluorescence intensity due to quenching, is the result of photo-oxidation of the compound by oxygen ; in many instances, however, there is no chemical reaction. The problem of oxygen quenching can be overcome by excluding the gas from the solution, and one of the efficient methods for removal of dissolved oxygen is by cooling to liquid nitrogen temperatures.

1.1.5 Inner Filter Effects

Inner filter effects, which cause a decrease in intensity of fluorescence, can be caused by one of two factors ; absorption of the fluorescence emission by a species in solution, or absorption of a fraction of the exciting radiation by some species in solution, which is usually non-fluorescent[46,47]. Inner filter effects can be recognised by following observations : alteration of excitation and emission spectra, or non-linearity of a fluorescence vs. concentration calibration curve at high concentrations. The fluorescent molecule can also act as its own inner-filter, and the effect is observed, usually at high concentrations. This is referred to as 'self-absorption' and it can be eliminated by dilution of the solution.

1.1.6 Lifetime of Luminescence

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

$$-dI = k.I.\,dt \qquad (1.11)$$

Therefore $$I = I_0.e^{-kt} \qquad (1.12)$$

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 :

$$k = \frac{1}{\tau} \quad \text{or} \quad \tau = \frac{1}{k} \qquad (1.13)$$

Substituting for k, the equation (1.12) becomes :

$$I = I_0.e^{-t/\tau} \qquad (1.14)$$

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

The intrinsic or natural radiative lifetime, τ_0, for fluorescence emission is related to the rate constant for fluorescence, k_F, by the equation :

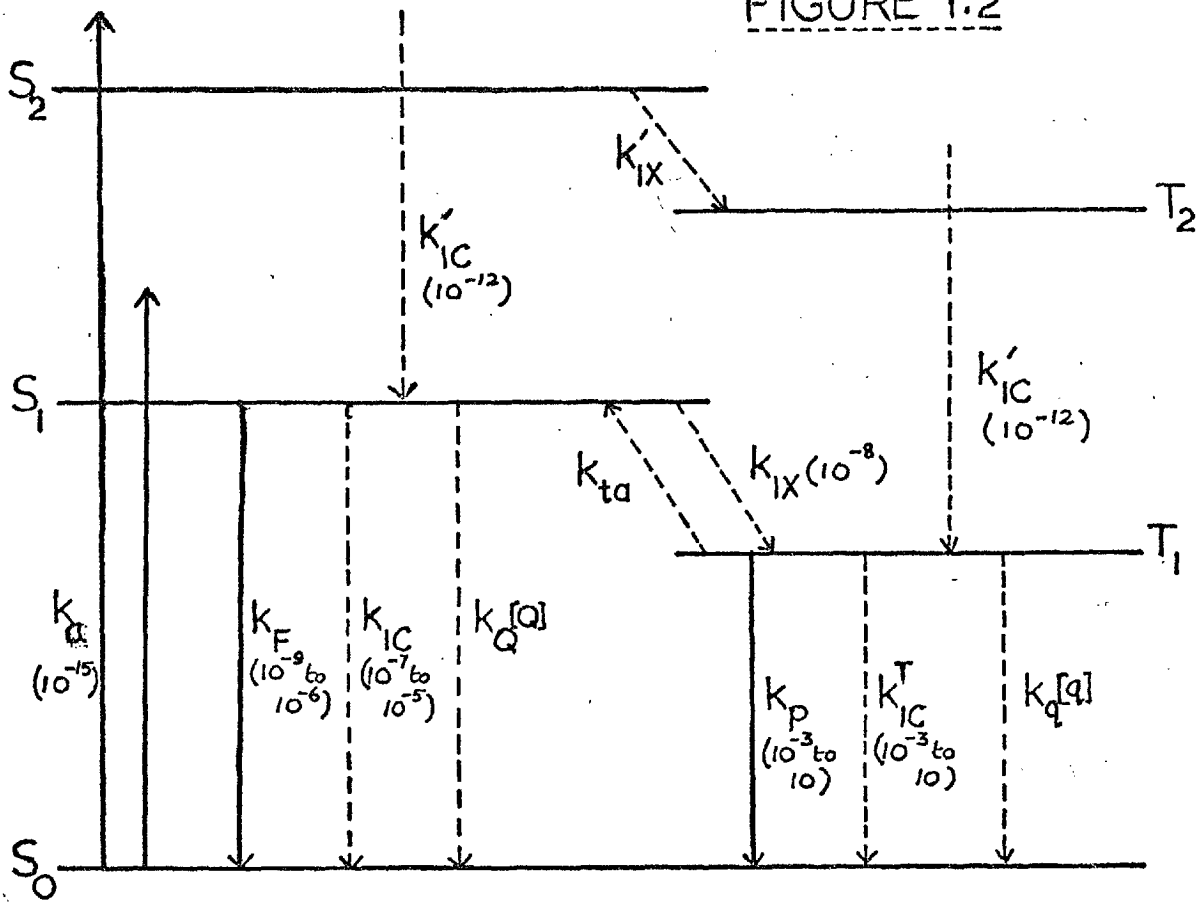
$$\tau_0 = \frac{1}{k_F} \qquad (1.15)$$

Similarly, for phosphorescence, the relation is

$$\tau_o = \frac{1}{k_P} \qquad (1.16)$$

where k_P is the rate constant for phosphorescence emission. The observed lifetime, τ , will be equal to the natural lifetime τ_0, only in the absence of any deactivating processes, both internal and external (i.e. $\phi = 1$). Figure 1.2 summerises the kinds of transitions, each denoted with the appropriate rate constant and with approximate mean time in seconds for the occurrence of the process.

FIGURE 1.2



Rate constants of Transitions (with approximate mean time in sec.).

Absorption of light takes place within the period of light wave, i.e. within about 10^{-15} sec. The rate of light absorption is denoted by k_a, in the figure 1.2, 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}^T for internal conversion from the triplet state to 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. But as the concentration quenching of the solute is often much greater than that of the excited

molecules, the processes can be represented as first-order reactions, with rate constants $k_Q \cdot [Q]$ and $k_q \cdot [q]$, where $[Q]$ 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 :

$$\frac{1}{\tau} = k_F + k_{ic} + k_{ix} + k_Q [Q] \qquad (1.17)$$

The fluorescence efficiency, ϕ_F, is given by the equation :

$$\phi_F = k_F / (k_F + k_{ic} + k_{ix} + k_Q [Q]) \qquad (1.18)$$

From equations (1.15), (1.17) and (1.18), it follows that :

$$\phi_F = \tau / \tau_o \qquad (1.19)$$

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

$$\frac{1}{\tau} = (k_P + k_{ic}^{\tau} + k_q [q] + k_{ta}) \qquad (1.20)$$

The efficiency of formation of triplet, ϕ_T, defined as the number of triplet molecules formed per quantum of exciting light absorbed, is given by[6] :

$$\phi_T = k_{ix} / (k_F + k_{ic} + k_{ix} + k_Q [Q]) \qquad (1.21)$$

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

$$\phi_T^o = k_{ix}/(k_F + k_{ic} + k_{ix}) \qquad (1.22)$$

The phosphorescence efficiency, ϕ_P, is then given by

$$\phi_P = k_P \cdot \phi_T/(k_P + k_{ic}^{\tau} + k_q [q] + k_{ta}) \qquad (1.23)$$

From equations (1.16), (1.20) and (1.23), it can be derived that

$$\phi_P / \phi_T = \tau / \tau_o \qquad (1.24)$$

At low temperatures k_{ta} becomes negligible, and in the rigid media the diffusion is very small so that the term $k_q [q]$ also becomes negligible. In the absence of quencher, the quantum efficiencies are denoted as ϕ_F^o, ϕ_P^o and ϕ_T^o, and the equation (1.18) can be written as :

$$\phi_F^o = k_F/(k_F + k_{ic} + k_{ix}) \qquad (1.25)$$

From equations (1.22) and (1.25),

$$\frac{\phi_T^o}{1-\phi_F^o} = \frac{k_{ix}}{k_{ic} + k_{ix}} \qquad (1.26)$$

Hence, the relation between τ and τ_o for phosphorescence can be derived from equations (1.24) and (1.26)

$$\frac{\tau_o}{\tau} = \left(\frac{1-\phi_F^o}{\phi_P^o}\right) \cdot \left(\frac{k_{ix}}{k_{ic} + k_{ix}}\right) \qquad (1.27)$$

If it is assumed that the internal conversion occurs only from the triplet state, then the equation (1.27) simplifies to :

$$\frac{\tau_o}{\tau} = \frac{1-\phi_F^o}{\phi_P^o} \qquad (1.28)$$

In order to determine the values of τ_o or k_P for phosphorescence, which are fundamental characteristics of the substance and are independent of temperature,

it is necessary to determine ϕ_P, τ and also ϕ_T[6]. The parameter ϕ_T is somewhat difficult to be determined, 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 (1.22) simplifies to :

$$\phi_T^o \approx k_{ix}/(k_F + k_{ix}) \qquad (1.29)$$

and equation (1.25) to :

$$\phi_F^o \approx k_F/(k_F + k_{ix}) \qquad (1.30)$$

Hence, from equations (1.29) and (1.30),

$$\phi_T^o \approx (1 - \phi_F^o) \qquad (1.31)$$

The above relation has been found to be approximately valid for some compounds[48,49] and for eosin in glycerol over a range of temperatures[50].

The absolute probabilities of electronic transitions, which depend on the nature of electronic states, determine the overall intensity of the corresponding absorption bands. Optical transitions that are probable in absorption are also probable in emission, and a direct relationship exists between the probability of fluorescence emission and the molar absorptivity for the corresponding absorption band. The fundamental relationship, mentioned above, was derived by Einstein[51], and the result showed that the probability of emission was directly proportional to the corresponding absorption probability and to the third power of frequency of the transition. Einstein's equations were later expressed in terms of commonly measured quantities[52,53] and a refractive index correction was added for cases where the absorbing systems were in solution[54]. Among the formulae derived to relate the radiative lifetime, τ_o, to the molecular extinction coefficient (ϵ), the simplest was that given by Bowen and Wokes[55]. The equation

given by Förster[56] was a somewhat more complex version, and it can be written as :

$$\frac{1}{\tau_o} = 2.880 \times 10^{-9} \cdot n^2 \int \frac{(2\bar{\nu}_o - \bar{\nu})^3 \cdot \epsilon \, d\bar{\nu}}{\bar{\nu}} \qquad (1.32)$$

where τ_o is expressed in seconds, $\bar{\nu}_o$ is the wave number (in cm^{-1}) about which the fluorescence and absorption bands bear a mirror-image relationship, n is the refractive index of the solvent, and the integration is carried out over the entire absorption band. Strickler and Berg[57] developed a modified formula, which should be valid for broad molecular bands when the transition is strongly allowed, and the equation is

$$\frac{1}{\tau_o} = 2.880 \times 10^{-9} \cdot n^2 \langle \bar{\nu}_f^{-3} \rangle_{Av}^{-1} \cdot \frac{g_l}{g_u} \cdot \int \epsilon \cdot d\ln\bar{\nu} \qquad (1.33)$$

where the reciprocal of the mean value of $\bar{\nu}_f^{-3}$ in the fluorescence spectrum is

$$\langle \bar{\nu}_f^{-3} \rangle_{Av}^{-1} = \int F \cdot d\bar{\nu} \Big/ \int F \cdot \bar{\nu}^{-3} \cdot d\bar{\nu}$$

F is the intensity of fluorescence in units of relative numbers of quanta at each frequency, and g_l and g_u are the multiplicities of the ground and excited states. Birks and Dyson[58] replaced the term n^2 in equation (1.33) by (n_f^3/n_a), where n_f is the mean refractive index of the solvent over the fluorescence band and n_a the mean value over the absorption band. The fluorescence lifetimes calculated using these equations, have been compared with those observed for a number of organic molecules in solution[57,59]. A knowledge of luminescence quantum yields is necessary for such comparison.

1.2 INSTRUMENTATION

The author's work involved the use of two different instrumental assemblies. The first was used for the measurements of fluorescence and phosphorescence spectra and for the development of the analytical methods reported. The second apparatus was used for the measurement of the lifetimes of luminescence of the systems investigated.

1.2.1. The Aminco-Bowman Spectrophotofluorometer

The Aminco-Bowman spectrophotofluorometer, which consists of an optical unit, a photomultiplier microphotometer and a power supply, is shown in Figure 1.3. Light from the source, which is a high intensity xenon arc lamp (150 watt), is dispersed by the excitation monochromator into monochromatic radiation, 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 into monochromatic 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. 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 optical diagram of the spectrophotofluorometer is shown in Figure 1.4. The monochromators have 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 longer wavelengths. Each

FIGURE 1.3

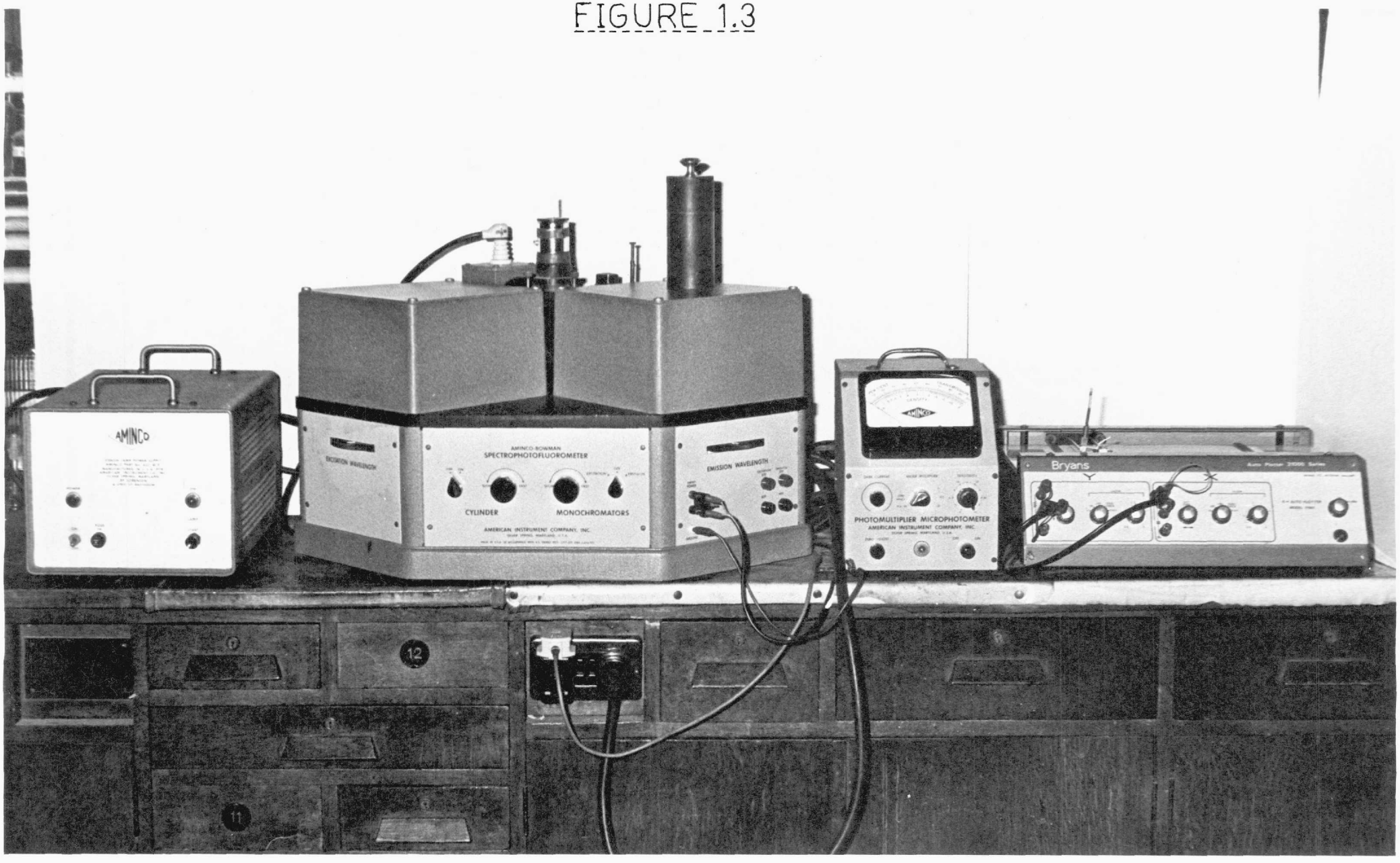

AMINCO
AMINCO-BOWMAN
SPECTROPHOTOFLUOROMETER
EXCITATION WAVELENGTH
EMISSION WAVELENGTH
CYLINDER
MONOCHROMATORS
AMERICAN INSTRUMENT COMPANY, INC.
PHOTOMULTIPLIER MICROPHOTOMETER
AMERICAN INSTRUMENT COMPANY, INC.
Bryans

FIGURE 1.4 Optical Diagram

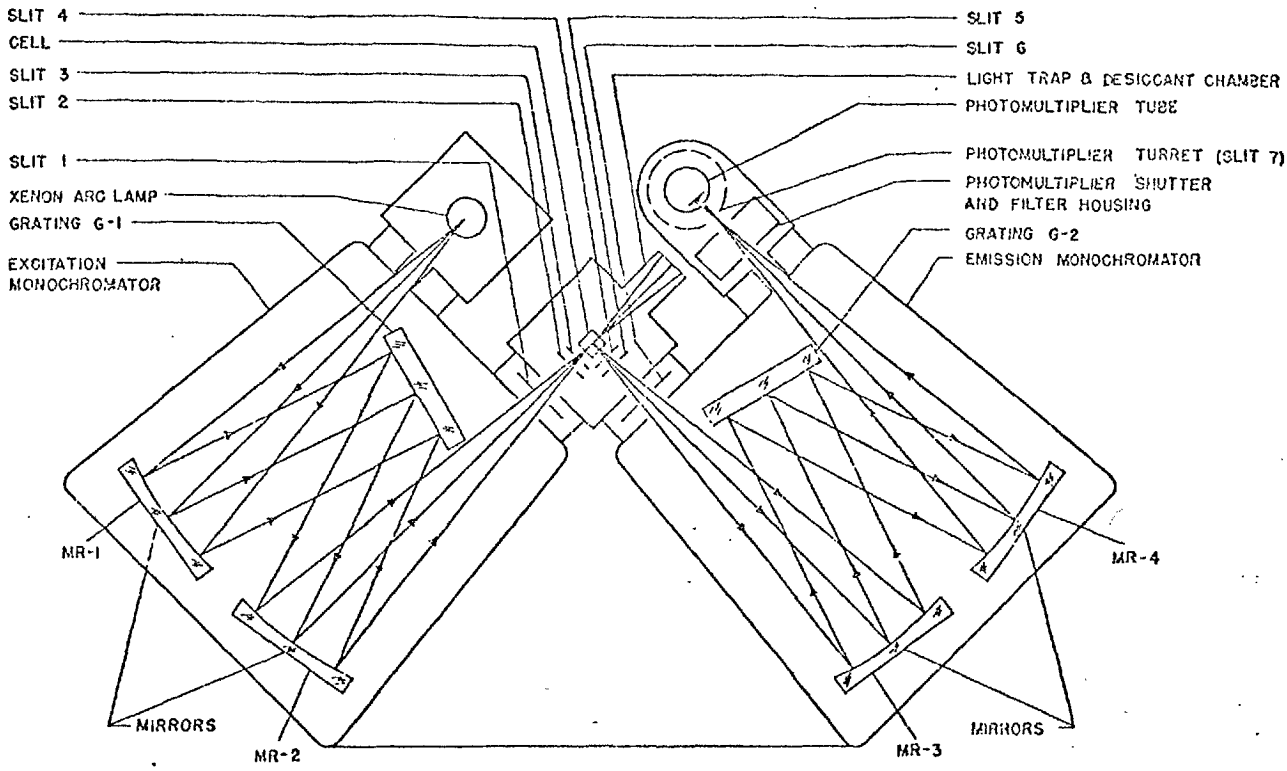


TABLE 1.1

SIZE, ARRANGEMENT AND PLACEMENT OF SLITS

(Width of Slits in Millimeters)

(Slit positions shown in Fig. 1.4)	Monochromator Slit 1	Cell Slit 2	Cell Slit 3*	Cell Slit 4*	Cell Slit 5	Monochromator Cell Slit 6	Photomultiplier Shutter 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.

monochromator has a wavelength range of 200 to 800 nm. The gratings of the monochromator are moved by motor-driven cams to which are coupled graduated discs for visual observation and wavelength adjustment.

The resolution of spectra is determined by the width of the slits. The spectral bandwidth of the excitation monochromator is determined by the width of the Slit 2 (Figure 1.4). Slit 1 acts as a baffle, and Slit 3 limits the size of the beam entering the cuvette. On the emission side of the instrument, resolution is obtained by using Slits at 4 and 7. Table 1.1 gives various slit arrangements which provide approximately equal resolving power for excitation and emission spectra. The slits provided with the instrument have widths from 0.5 mm to 5.0 mm and the dispersion of the monochromators is 10nm/mm. In most of the studies described in the thesis slit arrangement 3 was used.

Fused quartz cells, 10 x 10 mm square inside, 12 x 12 mm square outside and 50 mm high, were used in all measurements at room temperature. For measurements of luminescence (fluorescence and phosphorescence) at low temperature, an Aminco-Keirs Phosphoroscope was used. A cross-sectional diagram of the Phosphoroscope is shown in Figure 1.5. The sample in the holder is mounted inside a quartz Dewar flask filled with liquid nitrogen to lower the sample temperature. The two-port rotating shutter rotates at high speed around the Dewar, alternatively passing the light from the excitation monochromator into the sample and the light emitted from the sample into the emission monochromator. The speed of the rotating shutter of the Phosphoroscope can be controlled by a power-stat located on the front panel of the optical unit of the spectrophotofluorometer. This arrange ment permits measurements of phosphorescence, and the removal of the rotating shutter allows one to measure the fluorescence or total luminescence at low temperature.

FIGURE 1.5

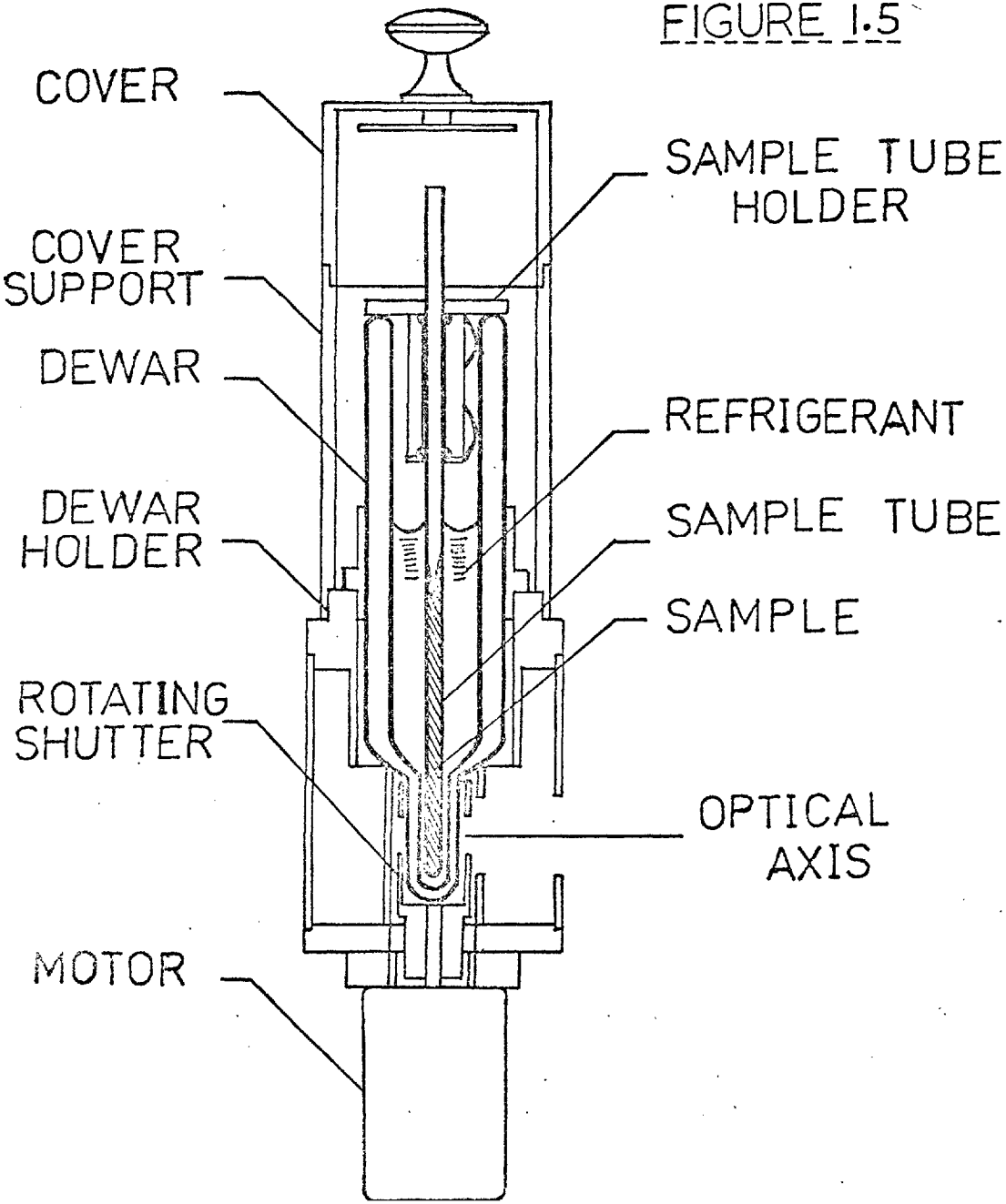
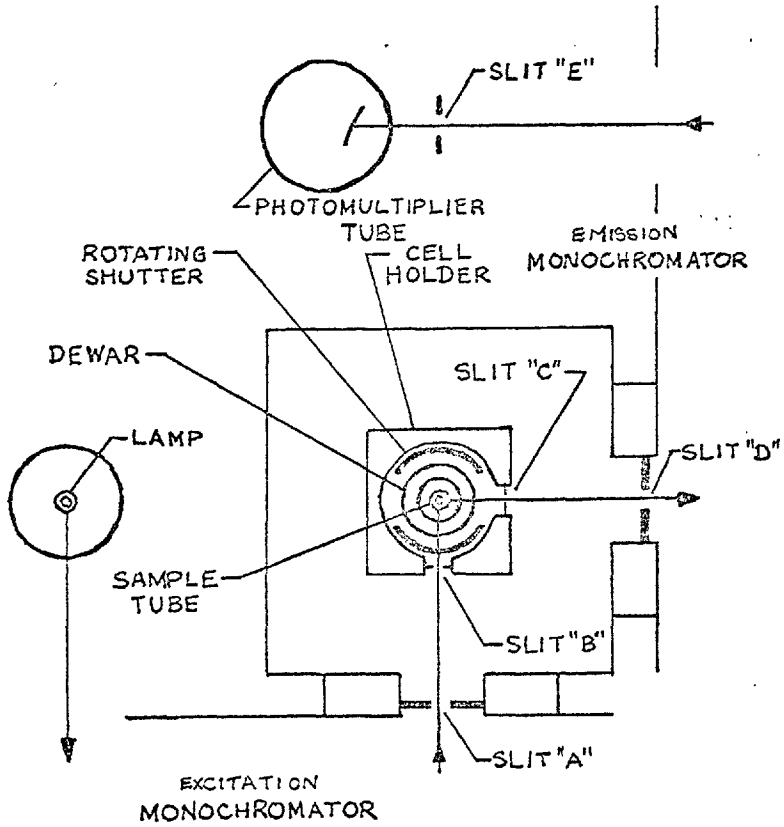


FIGURE 1.6



Positions of Slits in the Sample Vicinity when Using Dewar Flask Assembly.

The arrangements of slits for low temperature luminescence measurements are different to those at room temperature. Figure 1.6 shows the position of slits and Table 1.2, the different slit arrangements.

TABLE 1.2

Size, Arrangement and Placement of Slits for Phosphorescence

(Width in Millimeters)

(Slit positions shown in Fig. 1.6).	Monochromator Slit A	Dewar Slit B	Dewar Slit C	Monochromator Slit D	Photomultiplier Slit E
Arrangement No. 1*	1	0.5	0.5	0.5	0.5
Arrangement No. 2	2	1	1	2	1
Arrangement No. 3	3	2	2	3	2
Arrangement No. 4	4	3	3	4	3
Arrangement No. 5	5	4	4	5	4
Arrangement No. 6**	5	5	5	5	5

* Highest resolution
** Highest sensitivity

In the author's work, sample tubes made from precision-bore transparent silica tubing (Jencons Ltd.) of length 200 mm, i.d. 3 mm and wall thickness 1 mm were used. A sample volume of 0.5 ml was sufficient to fill these tubes to a suitable depth for use in the quartz Dewar phosphoroscope attachment.

An RCA type 1P28 photomultiplier tube (S-5 response), was used in the detector system. The photomultiplier housing includes a manually operated rotary slit-turret and filter holder with shutter control.

The sensitivity in the photomultiplier microphotometer is controlled by a meter-multiplier switch and sensitivity potentiometer. The meter-multiplier

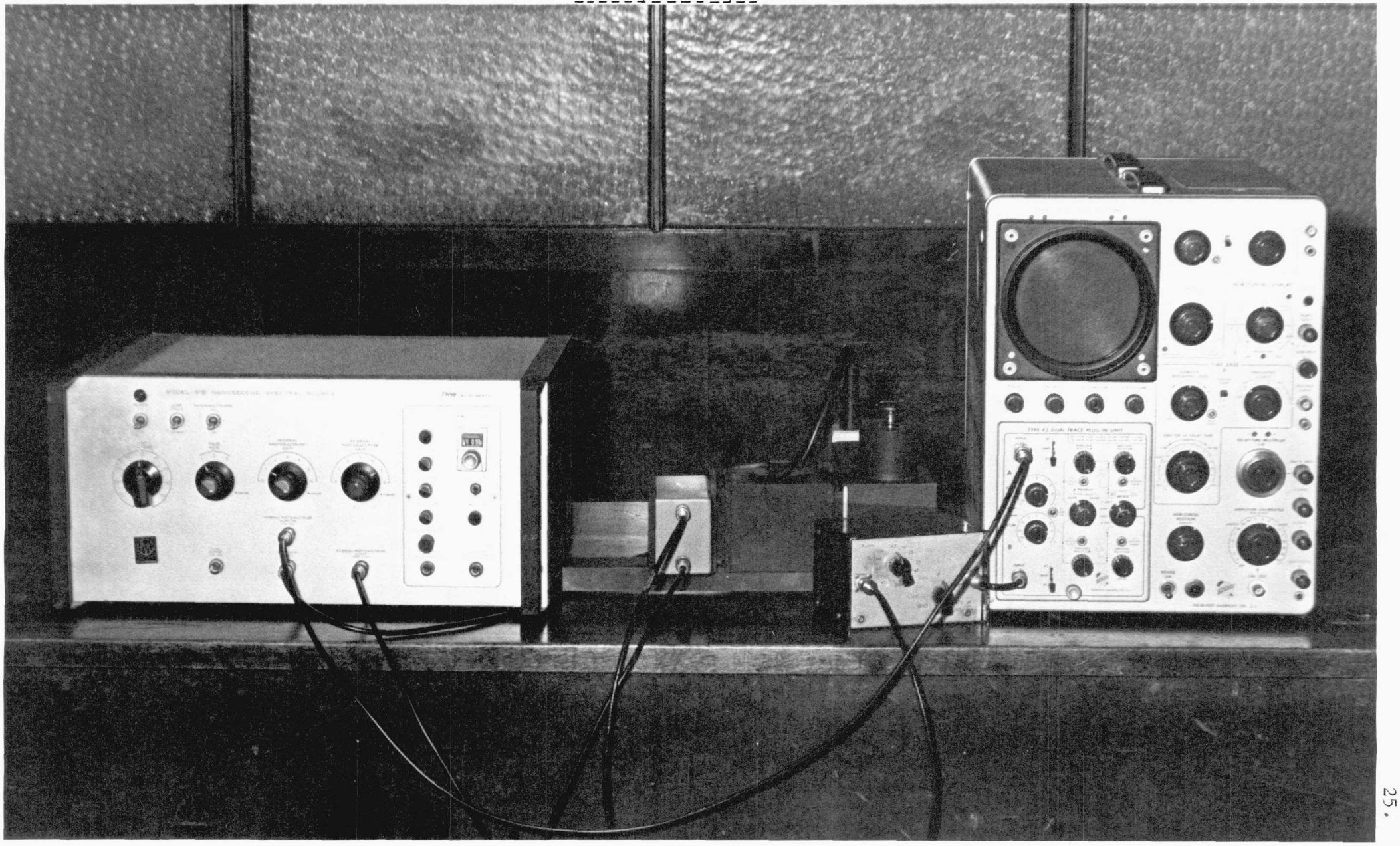
positions are numbered 1, O.3, O.1, O.O3, O.O1, O.OO3 and O.OO1 in order of increasing sensitivity. The sensitivity potentiometer is used for fine adjustments of sensitivity.

A Bryans X-Y recorder (Model 21OOl) was used to record the excitation and emission spectra. The excitation and emission spectra recorded directly from the spectrofluorometer are not corrected for variations in detector sensitivity, xenon arc lamp emission or grating emission with wavelength. The details of correction of spectra have been discussed in Chapter 2. Although true spectra are necessary for comparison of results obtained with other instruments, the uncorrected or apparent spectra, i.e. those recorded with the instrument under a given set of experimental conditions, are more useful for analytical purposes as the spectra obtained with the operator's own instrument will be reproducible. However, the fluorescence signals obtained from a given solution may vary due to variation in the sensitivities of instrumental components such as xenon arc. This variation will also be reflected in the analytical signal. The use of a standard substance of known quantum yield, will overcome this difficulty and permit comparison of fluorescence signals obtained with the same or similar instruments. Several substances have been studied as possible standards for measurement of fluorescence[23,30,46]. A solution of Quinine Sulphate in O.1 or 1.O N Sulphuric acid appears to be a nearly perfect standard[23,26].

1.2.2 The TRW-Nanosecond Spectral Source System

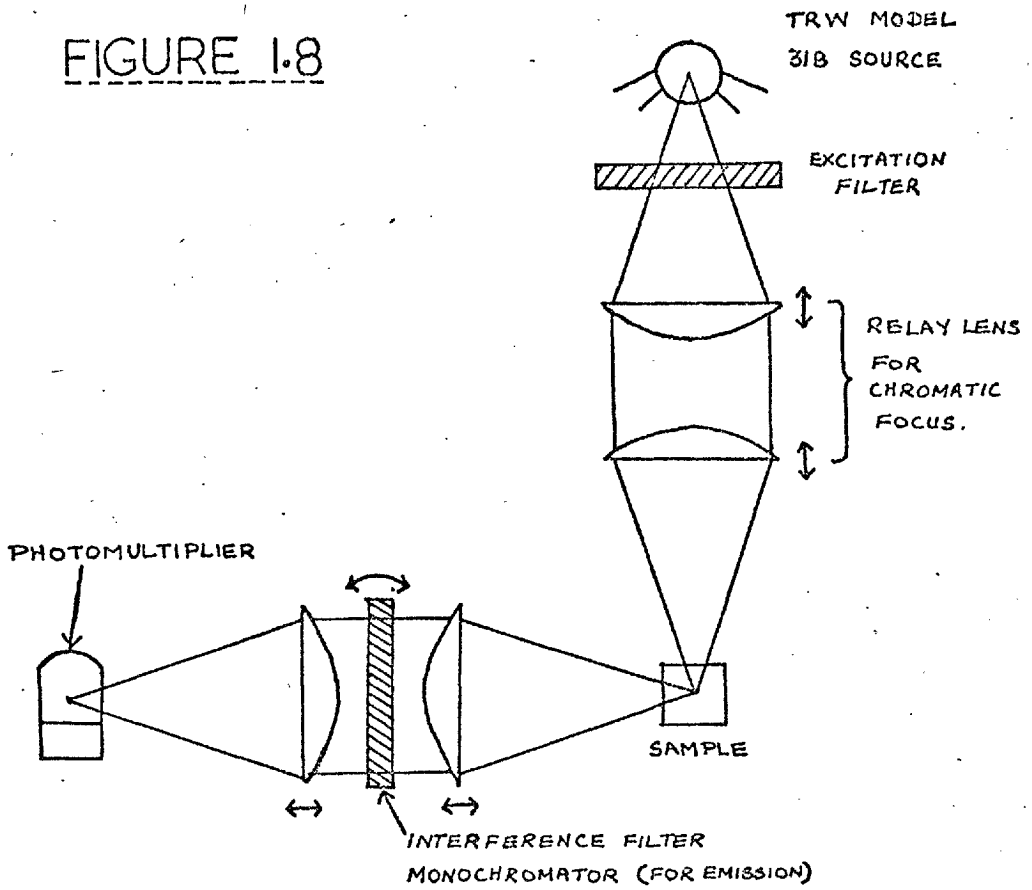
The TRW Model 31B Nanosecond Spectral Source (NSS) system, shown in Figure 1.7, consists of the source, lamp housing and the control unit and power supply which provides repetition rate control and high and low voltage. The TRW Model 38A Relay lens assembly with a pair of quartz condenser lenses in the excitation and emission optical paths, provides chromatic focussing of light

FIGURE 1.7



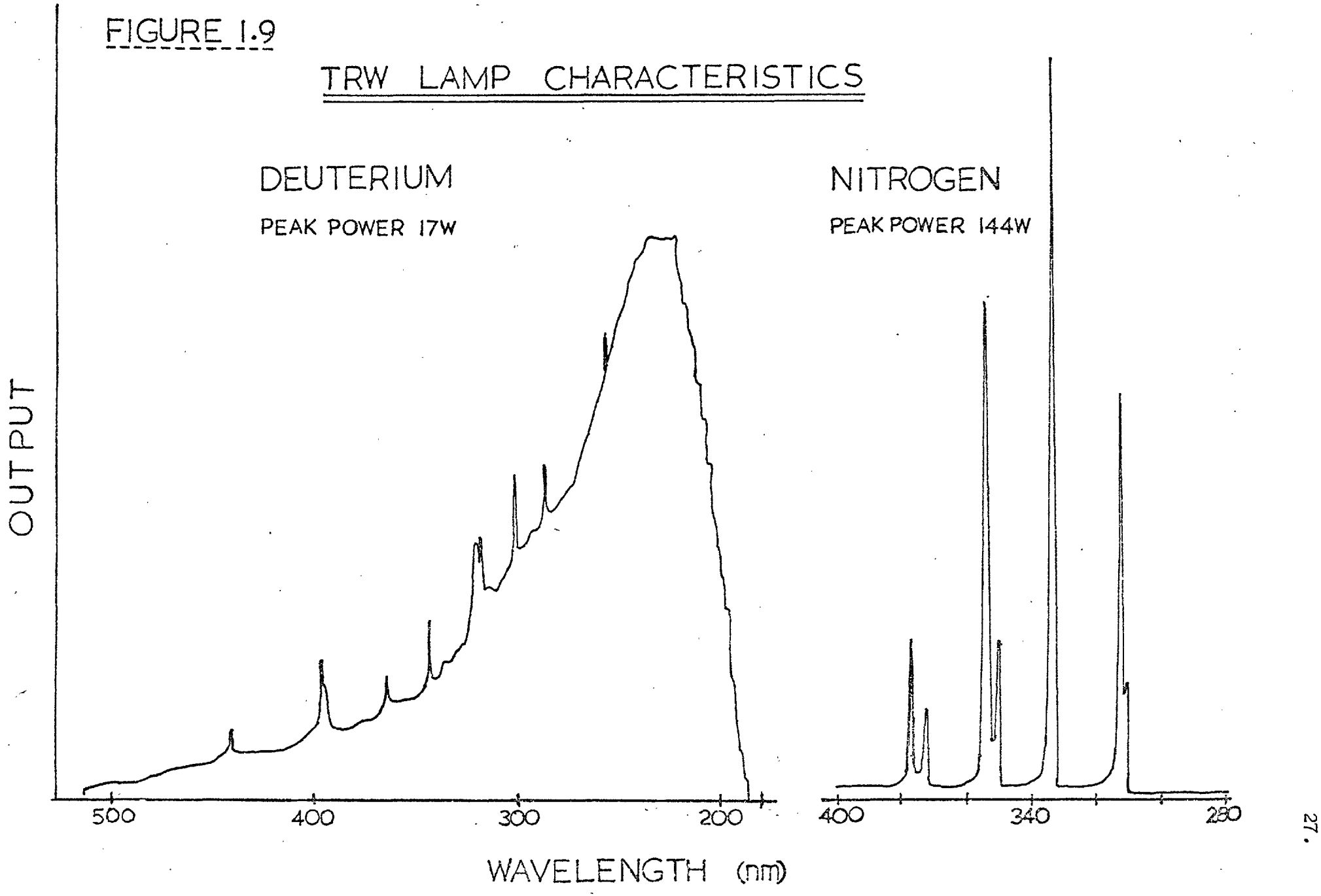
over the range 200 to 1000 nm from the lamp housing to the test sample and from test sample to the detector. A tunable interference filter monochromator (Model 39A) in the path of emitted light allows precise wavelength selection and maximum separation of the fluorescent pulse. Model 40A Filter mount carrier in conjunction with Model 37A Filter mount adaptor accommodates 2 x 2 inch glass or interference filters. A mount carrier constructed by the author 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 in Figure 1.8

FIGURE 1.8



OPTICAL SCHEMATIC OF THE TRW MODEL 31B DECAY TIME FLUOROMETER.

FIGURE 1.9


TRW LAMP CHARACTERISTICS
DEUTERIUM
PEAK POWER 17W
NITROGEN
PEAK POWER 144W
OUTPUT
500
400
300
200
400
340
280
WAVELENGTH (nm)

The sample chamber (Model 34A) accommodates the sample holder (Model 35A) for liquids, contained in a cylindrical fused quartz tube of 75 mm long, i.d. 8 mm, and wall thickness 1 mm. A slight modification of the sample holder permits the quartz Dewar flask (Figure 1.5) for measurements at liquid nitrogen temperature.

The source used to excite the sample, depending on the region of wavelength of excitation, was either a Deuterium (No.268) or Nitrogen (No.267) arc lamp. The spectral characteristics of these lamps are shown in Figure 1.9.

The NSS system also accommodates a TRW Model 32A decay time computer for measuring decay times up to 10 milliseconds. This computer simulates the pulse shape of the light source and integrates it to the shape of the fluorescence decay of the sample. The decay time values are displayed digitally. This unit was not used by the author as it requires the use of a dual-beam oscilloscope[60].

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 inputs (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 one to measure decay time of the order of microseconds or greater. Table 1.3 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.

TABLE 1.3

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

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 6.

CHAPTER 2

CORRECTION OF LUMINESCENCE SPECTRA

2.1 Calibration of Aminco-Bowman Spectrophotofluorometer

The excitation and emission spectra recorded with most commercially available spectrofluorimeters are uncorrected for the variation of either the photon output of the light source or the detector response sensitivity with wavelength. However, it is sufficient and convenient to calibrate the lamp and excitation monochromator as one unit and the detector and emission monochromator as another. Several methods have been suggested for calibrating lamps and detectors and these are summarised in the next few sections.

2.1.1 Excitation Monochromator Calibration

To obtain corrected excitation spectra the relative output from the source via the excitation monochromator as a function of wavelength must be known or kept constant. The method of calibration for a chosen monochromator depends on the detector, which can be a thermopile, quantum counter, actinometer, or calibrated emission monochromator.

When thermopiles are employed the relative energy is measured instead of the desired units of quanta per second at a given wavelength, and relatively high light fluxes and expensive readout systems are necessary[6,61]. Use of quantum counters requires a minimum of ancillary equipment and is the most popular method for calibration. The counters can be either optically dense, so that more than 99% of the incident light is absorbed, or optically dilute, in which case less than 10% of the incident light is absorbed. The optically dense method[6,62,63] is simple, rapid and most reliable. Use of optically dilute counters[28,64] requires the absorption spectrum of the compound to be

known accurately and the method is rarely used. Most of the optically dense quantum counters employ a concentrated solution of Rhodamine B in ethylene glycol.

One of the classical methods of measuring light intensity is by using chemical actinometry. Substances used as actinometers include Uranyl oxalate[61], potassium ferrioxalate[65-67], and, for response in the red region, some chromium complexes[68]. A calibrated emission monochromator has been used to calibrate the excitation monochromator over the 250 - 600 nm range[69,70]. This method is applicable to those systems which emply two grating monochromators.

2.1.2 Emission Monochromator Calibration

In order to obtain true emission spectra, the observed emission spectra requires correction using factors defined by the equation :

$$I(\bar{\nu}) = C(\bar{\nu}) \times O(\bar{\nu}) \qquad (2.1)$$

where $I(\bar{\nu})$ is the corrected relative emission intensity, $C(\bar{\nu})$ is the correction factor, and $O(\bar{\nu})$ is the observed intensity of emission, at a given frequency $\bar{\nu}$. Correction factors are usually obtained by reference to standard lamp sources, to compounds with known distributions of their spectral emissions, or to calibrated monochromator-light source combinations.

Standard tungsten-filament lamps are widely used due to the fact that the calibration procedure is simple, very easy, rapid, and very reliable. The lamp is operated at a known colour temperature, and the light from the lamp is passed through the emission monochromator-detector combination. From the apparent spectrum recorded, the correction factors, $C(\bar{\nu})$, are calculated using the equation (2.1)[6,30,69,70]. For calibration down to 250 nm, a quartz iodine lamp may be useful[71].

A number of compounds have been suggested for use as spectral emission standards to calibrate the emission monochromator[72]. Of these compounds only quinine sulphate has been checked by a number of workers[67,72-75], but there is considerable disagreement over the corrected emission spectrum.

The methods of using a calibrated xenon lamp and monochromator for the calibration of emission monochromators were developed by Parker[76] and Melhuish[62]. This method of calibration is relatively rapid, easy and reliable, and can be used for calibrating emission monochromators in the ultraviolet region. Comparison of the calibration curves obtained by using calibrated xenon arc-monochromator combination with those obtained by using standard lamps show that the agreement is quite satisfactory in the region of overlap (400 - 600 nm)[62,76,77].

Methods have also been described for obtaining correction factors, specifically, for Aminco-Bowman Spectrophotofluorometer[62,64,67,69,77]. Several self-correcting instruments, which record the corrected excitation and emission spectra directly, have also been described[3,78-80].

2.2 Experimental

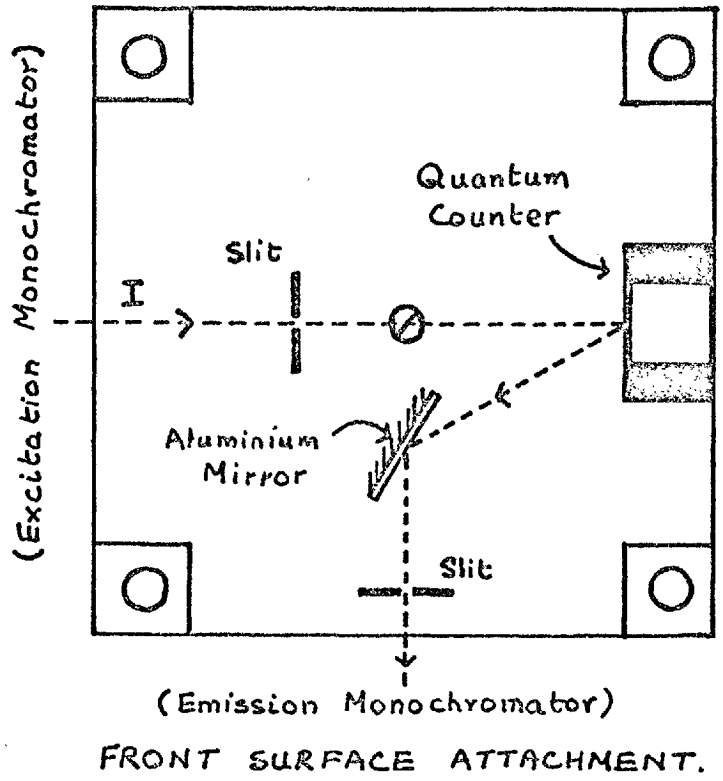
2.2.1 Apparatus

The Aminco-Bowman Spectrofluorimeter, described in section 1.2.1, with slit arrangement No.2 (Table 1.1), was used throughout.

An attachment (similar to that available from the American Instrument Co.[78] as a 'solid sample accessory') as shown in Figure 2.1 was constructed from aluminium sheets of thickness ~ 2 mm, and coated with matt black paint. This was used instead of the usual cuvette holder to contain the cuvette holding the quantum counter solution.

A standard tungsten ribbon lamp (Type W2 KGV 22) of Philip Research Laboratories, Netherlands, was used to calibrate the photomultiplier tube -

FIGURE 2.1



FRONT SURFACE ATTACHMENT.

emission monochromator combination. This lamp was operated at a constant current and voltage (10.05 amps, 12 V) to yield a colour temperature of 2130° K. (The author wishes to thank Dr. J. Wheaton of the Physics Department, Imperial College, for the loan of the lamp and power supply unit).

2.2.2 Materials

Rhodamine B (BDH Chemicals) was purified by dissolving in ethanol, filtering the solution from impurities, and recrystallised by the addition of diethyl ether. 1-Dimethylaminonaphthalene-5-sulphonate was synthesised by Laurance's method[81]. The standard scattering surface was prepared by depositing magnesium oxide (1 - 2 mm thickness) onto an etched aluminium plate by burning magnesium ribbon.

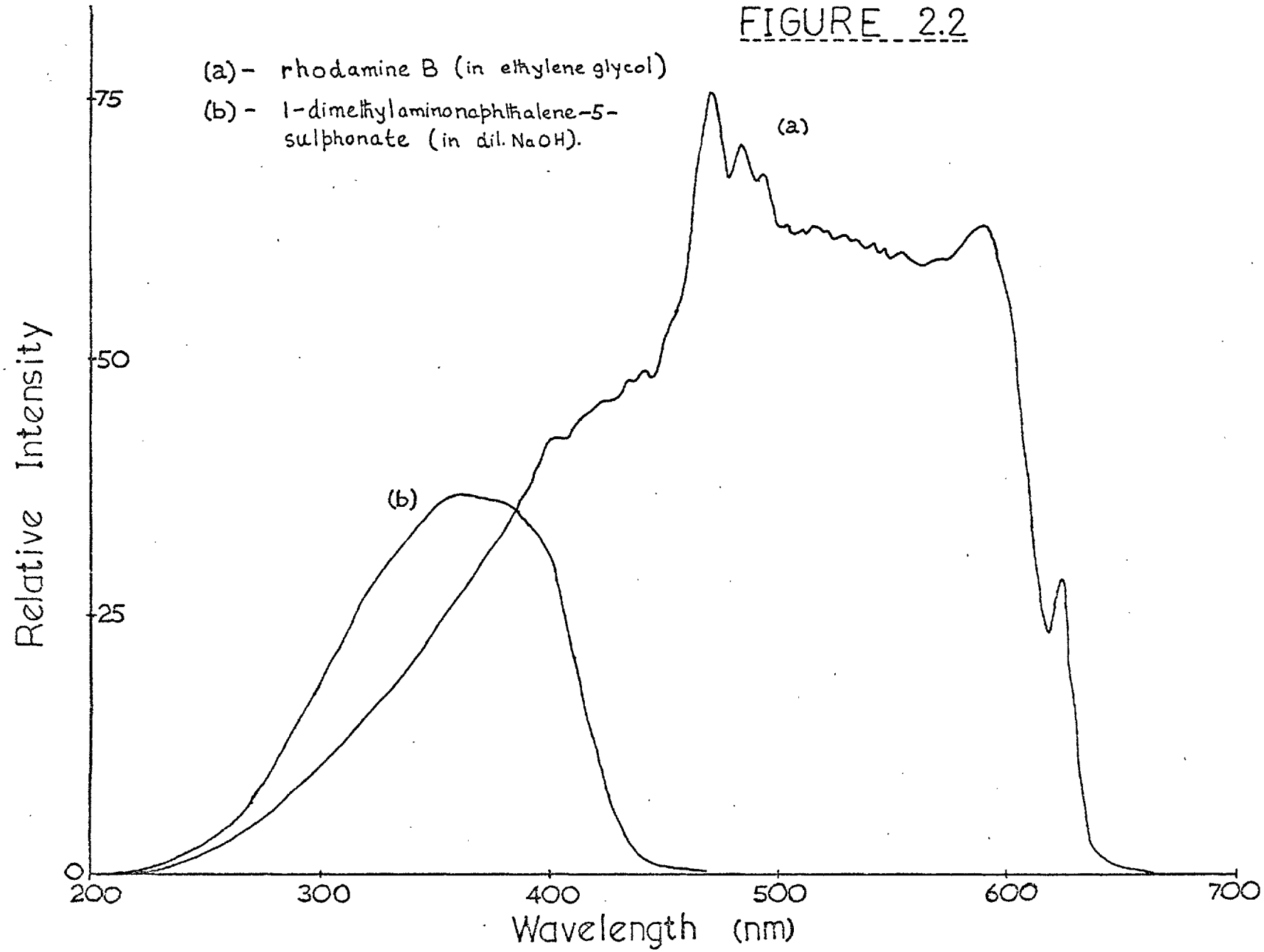
Quinine Sulphate (BDH Chemicals) was used as supplied by the manufacturers and Anthracence (BDH Chemicals) was recrystallised from ethanol.

2.2.3 Calibration using Quantum Counters

For wavelengths below 450 nm, the spectrofluorimeter was calibrated by the quantum counter or the fluorescent screen method. A concentrated solution of rhodamine B (3 g/l, 6.2 x 10^{-3}M in ethylene glycol), was placed in a fused quartz cell (10 x 10 x 50 mm) in the cuvette holder (Figure 2.1), and the fluorescence was observed from the front surface at an angle (~ 30^{o}) to the incident beam. The method relies on the assumptions that the rhodamine B solution has sufficiently high absorbance at wavelengths up to 600 nm to absorb essentially all of the excitation energy in the thin solution layer at the surface of the cell, that the quantum yield of rhodamine B is independent of excitation wavelength, and that the fluorescence is therefore proportional only to the number of quanta incident on the fluorescent screen. These assumptions appeared to be realistic as Melhuish[62] found that the calibrations carried out with this method agreed with those obtained using the thermopile method. Slits of width 1mm were used in the positions shown in Figure 2.1. The emission of rhodamine B was measured at a constant setting of 640 nm of the emission monochromator through a Locarte LF10 filter, which eliminates second-order scattered light, and the excitation monochromator was scanned. The signal recorded at the detector via the second monochromator is shown in Figure 2.2a, which is the output of the xenon lamp monochromator combination, in relative intensity units of quanta/sec. nm. For calibration of the lamp in the ultraviolet, the fluorescent screen used was a solution of 1-dimethylamino-naphthalene-5-sulphonate (0.02 M in dilute NaOH)[19], and the emission was observed at 550 nm through a Locarte LF6 filter. The signal recorded is shown in Figure 2.2b.

Comparison of calibration curves using rhodamine B and 1-dimethylamino-naphthalene-5-sulphonate shows agreement within 3%. The calibration with 1-dimethylamino naphthalene-5-sulphonate, however, is considered to be more reliable than that obtained with rhodamine B in the UV region[19,77].

FIGURE 2.2

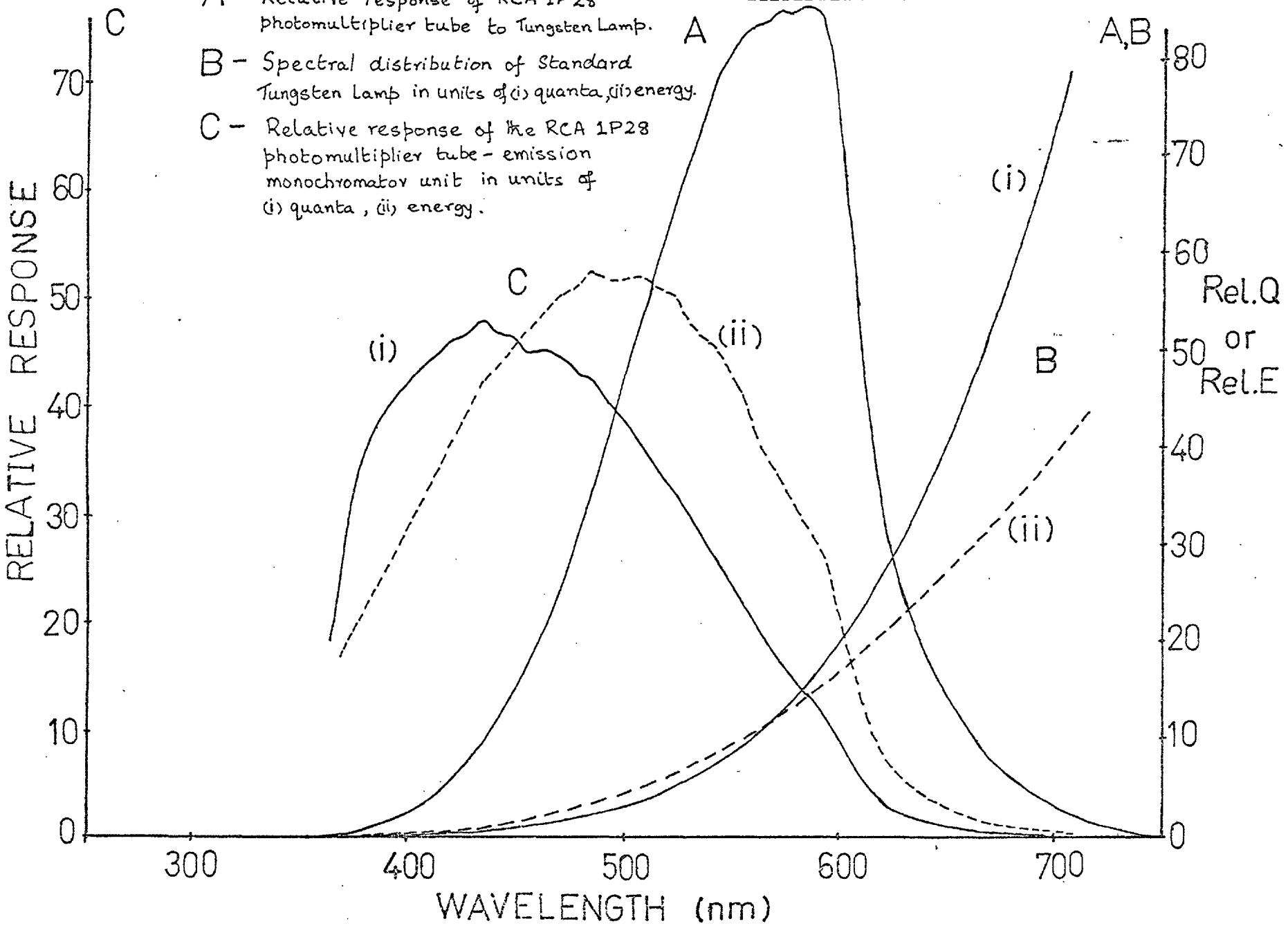

(a) - rhodamine B (in ethylene glycol)
(b) - 1-dimethylaminonaphthalene-5-sulphonate (in dil. NaOH).
(a)
(b)
Relative Intensity
75
50
25
0
200
300
400
500
600
700
Wavelength (nm)

The detector-emission monochromator combination was calibrated by using a xenon arc lamp calibrated as described above. The output of the lamp was directed into the emission monochromator by placing the magnesium oxide scattering plate at 45^{o} to the incident beam in the standard cuvette holder. The exciting radiation was attenuated by placing two blackened 40 mesh copper screens just ahead of the second concave mirror in the excitation monochromator to act as neutral density filters. Aminco slit arrangement No.2 was used. The detector response to the xenon arc output was measured by varying the excitation monochromator in steps of 5 nm and the emission monochromator was adjusted manually to give the maximum reading at each step. From these peak readings, using the previously determined output of the lamp, the correction factors are calculated. The dotted lines in Figure 2.4, show the relative factors for correction determined using the quantum counter.

2.2.4 Calibration using Standard Lamp

A standard tungsten ribbon lamp was used to calibrate the emission monochromator-photomultiplier combination. It was placed at the entrance of the monochromator at a distance of approximately 25 cms. The emission from the lamp was passed through a pair of 0.5 mm slits into the emission monochromator, and the slit at the exit of the monochromator (photomultiplier slit) was also set at 0.5 mm. The output of the lamp was attenuated by placing one blackened 40 mesh copper screen between the lamp and the monochromator. The spectral distribution of the lamp was measured by varying the emission monochromator in steps of 5 nm and reading the recorded intensity. The recorded relative response of the photomultiplier tube-emission monochromator combination to the lamp is shown in Figure 2.3A. Knowing the colour temperature of the lamp, the spectral distribution curve was calculated by means of Wien's Law. The equation, when substituted for the colour

FIGURE 2.3


A – Relative response of RCA 1P28 photomultiplier tube to Tungsten Lamp.
B – Spectral distribution of Standard Tungsten Lamp in units of (i) quanta, (ii) energy.
C – Relative response of the RCA 1P28 photomultiplier tube – emission monochromator unit in units of (i) quanta, (ii) energy.
RELATIVE RESPONSE
WAVELENGTH (nm)
Rel.Q or Rel.E
A,B
C
(i)
(ii)
B
0
10
20
30
40
50
60
70
80
300
400
500
600
700

temperature and other constants, takes the form :

$$E_\lambda = 3.73 \times 10^4 \times \frac{1}{\lambda^5} \times e^{-\frac{6.75}{\lambda}} \qquad (2.2)$$

where E_λ is the energy flux per unit wavelength interval (in watts/cm^2), and λ is the wavelength in μ (microns). Figure 2.3B shows the spectral distribution of the tungsten lamp in units of (i) quanta, and (ii) energy. In Figure 2.3C, the curves represent the relative response of the RCA IP 28 photomultiplier tube-emission monochromator combination to the tungsten lamp, in units of (i) quanta, and (ii) energy. These curves were obtained by dividing ordinates of curve A by those of curve B at a given wavelength.

The correction factors were calculated at intervals of 5 nm, by dividing the calculated relative energy by the recorded output of the photomultiplier, that is,

$$K_\lambda^E = E_\lambda / R \qquad (2.3)$$

where R is the recorded response of the monochromator-photomultiplier combination, E_λ is the energy calculated from equation (2.2), and K_λ^E the correction factor at a given wavelength λ. Thus multiplication of the recorded intensity at a given wavelength by K_λ^E converts the apparent intensity to a true relative energy. Correction factors for conversion to relative quanta per unit wavelength (K_λ^Q) was obtained by multiplying by λ. That is :

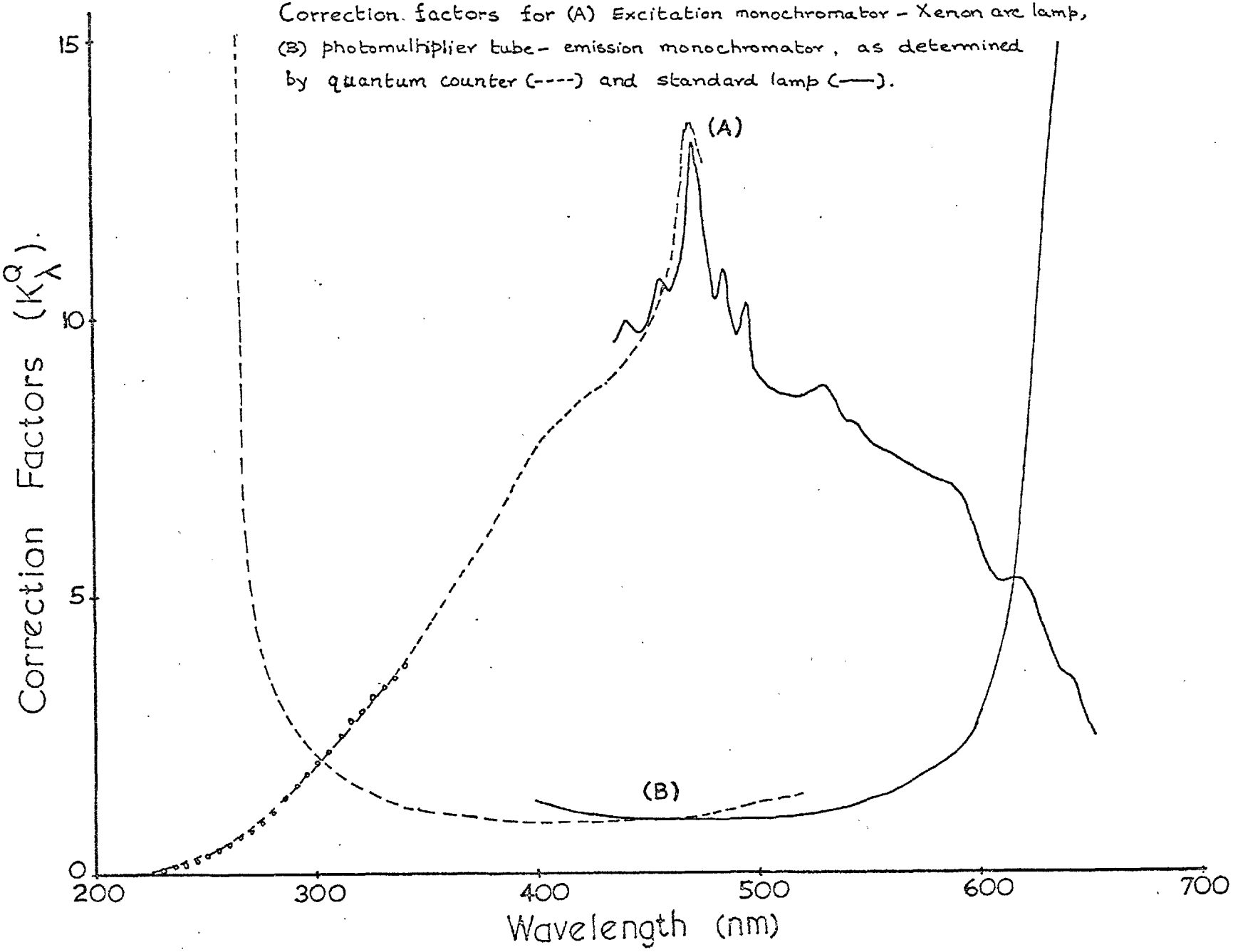
$$K_\lambda^Q = K_\lambda^E \cdot \lambda \qquad (2.4)$$

A spectra presented with an abscissa linear in frequency is preferred, as the energetic relationship between individual bands becomes linear. Hence, the correction factors should be converted to quanta per unit frequency interval (K_ν^Q), which is given by the equation[30] :

$$K_\nu^Q = K_\lambda^Q \cdot \lambda^2 = K_\lambda^E \cdot \lambda^3 \qquad (2.5)$$

FIGURE 2.4

Correction factors for (A) Excitation monochromator – Xenon arc lamp, (B) photomultiplier tube – emission monochromator, as determined by quantum counter (----) and standard lamp (——).



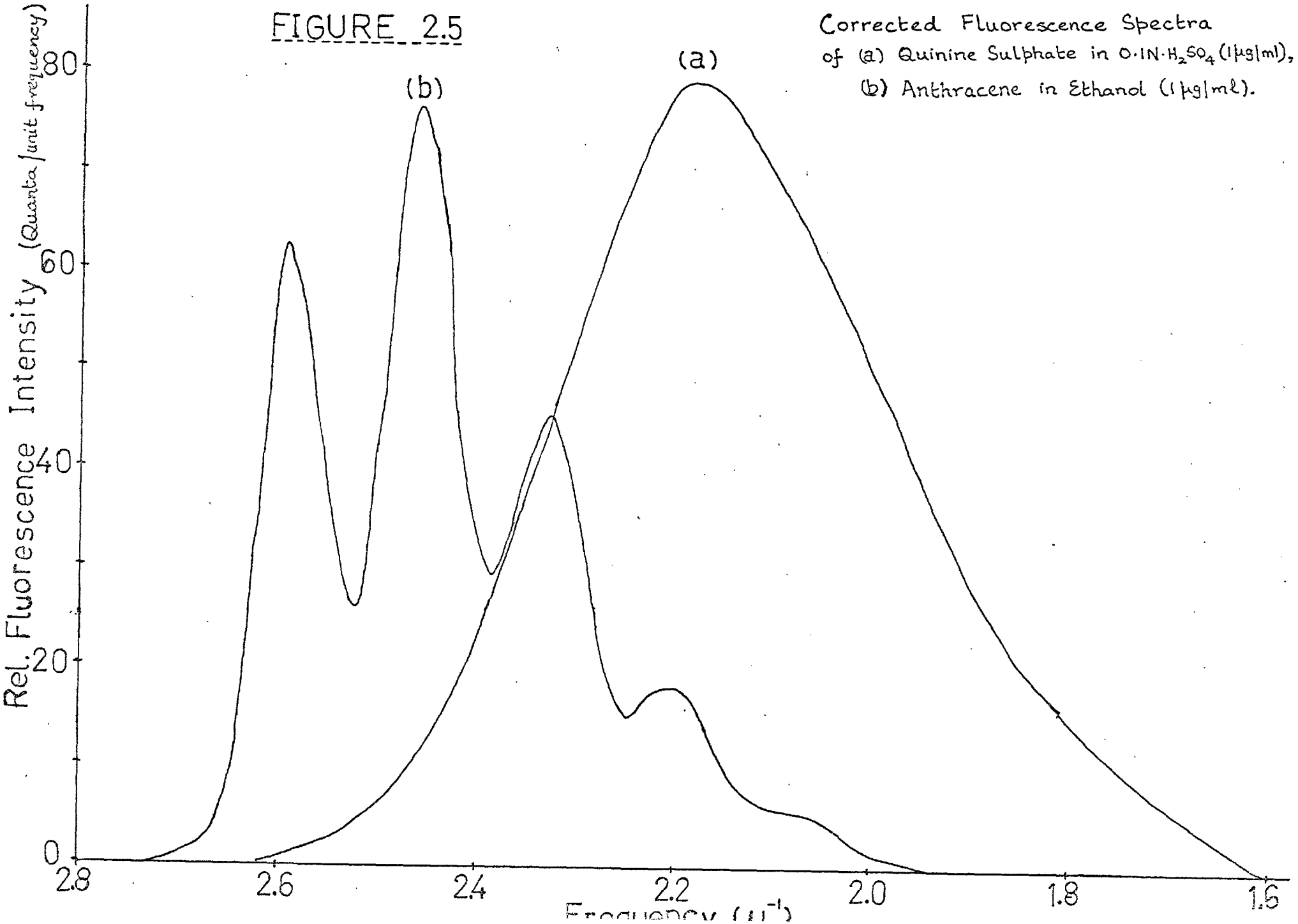
Using the emission monochromator calibrated using the standard lamp as described above, the xenon arc lamp-excitation monochromator combination was calibrated, by passing the radiation from the xenon lamp and excitation monochromator into the emission monochromator by placing the scattering plate at 45^{o} to the incident beam in the standard cuvette holder. The procedure for measurements was as described in section 2.2.3. The distribution of the radiation from the source and monochromator in terms of quanta per unit wavelength interval (E^{Q}_{λ}) was obtained by multiplying the recorded intensity obtained by scattering of light, by the correction factors K^{Q}_{λ}. The data obtained by this procedure were used without correction for the change in coefficient of reflection with wavelength, which varies by only a few per cent over the range 250 - 500 nm[82, 83]. The correction factors in quanta per unit wavelength, obtained by using the standard lamp method are shown by continuous line in Figure 2.4. The figure 2.4 shows the plot of correction factors (K^{Q}_{λ}) with wavelength obtained by the quantum counter method and those obtained by the standard lamp method. These values are normalised at 450 nm. In order to correct the excitation spectrum, the apparent intensity recorded is divided by the correction factors shown in Figure 2.4, and to obtain corrected emission spectrum the apparent intensity is multiplied by the corresponding correction factors at a given wavelength.

In order to test the experimental technique and calculation, the fluorescence spectra of Anthracene in ethanol (1 μg/ml) and Quinine sulphate in 0.1 N sulphuric acid (1 μg/ml) were corrected. These corrected spectra, shown in Figure 2.5, agree with those reported by other workers[30, 62, 69, 70].

2.3 Discussion

The correction factors obtained by the quantum counter method are restricted to the wavelength region 220 to 400 nm and those obtained by using the standard lamp to the region of wavelength 380 to 650 nm. Combination of the correction

FIGURE 2.5



Corrected Fluorescence Spectra of (a) Quinine Sulphate in $0{\cdot}1N{\cdot}H_2SO_4$ (1μg/ml), (b) Anthracene in Ethanol (1μg/mℓ).

factors determined by the two methods can be used for calibration in the range of wavelength 220 - 650 nm (Figure 2.4).

Several other materials, such as calcium carbonate, aluminium, quartz wool, etc. behaved very much like magnesium oxide as scattering surfaces, but magnesium oxide represents the best neutral diffuse reflector which is readily available[69].

A knowledge of both corrected emission spectrum and the wavelength sensitivity curve of the detector is required for the determination of any quantum-yield. An additional check on the experimental technique and calculation would be to determine the fluorescence quantum efficiencies of a few standard compounds. These results are reported in Chapter 6.

CHAPTER 3

THE FLUORESCENCE AND PHOSPHORESCENCE CHARACTERISTICS OF SOME ANTIOXIDANTS AND ULTRAVIOLET ABSORBERS.

3.1 Introduction

Antioxidants and ultraviolet absorbers are often added to polymers to hinder their degradation during manufacture or in sunlight and artificial light. The qualitative identification of these compounds and the determination of the additive concentration in polymers is necessary to correlate moulding or weathering properties with additive content. The antioxidants most commonly used are alkyl substituted phenols, but substituted aromatic amines, phosphites and thio-esters are also used. Ultraviolet absorbers are most commonly hydroxy benzophenones, hydroxy benztriazoles and salicylate esters.

The determination of additives in polymers presents the analyst with some difficult problems, such as the additives being present only in small amounts, the presence of complex mixtures and sometimes mixtures of compounds of completely unknown type. The difficulties involved in the extraction of additives have lead to a search for analytical techniques which do not involve a prior separation of additives from polymers. Direct ultraviolet absorption measurements on thin, hot pressed polymer films have been employed[84] to determine the additive content, with air in the reference beam. By this method, it was possible to estimate antioxidant levels ranging from 0.002 to 1.0% in polyethylene. Infra-red spectra of antioxidants from polymer films have also been obtained[85] using additive-free polymer in the reference beam. Analysis by infra-red is more specific than ultraviolet, but if the additive content is too low, it is difficult to obtain suitable spectra[86]. Drushel and Sommers[87] have reported that certain additives may be determined similarly by direct phosphorescence measurements on polymer films and the samples

examined were all synthesised in the laboratory under identical conditions.

Several methods for the quantitative extraction of additives from polymers prior to analysis by various techniques, have been reviewed by Wheeler[88] and Crompton[89]. When the particular additive which is present in a polymer sample is known, it can be extracted with a suitable organic solvent and determined by ultraviolet or infra-red absorption spectrophotometry[90]. The required extraction time at room temperature has been found to vary linearly with density of the polymer and particle size, and also with the nature of the extraction solvent. Some correlation has also been found between the density of the polyethylene and its permeability to solvents[91, 92]. When the particular additives present in a polymer sample are unknown, the task of positive identification and determination of the compounds present is more difficult. The additives must usually be extracted from the polymer samples, separated by column[89, 93-95] or thin-layer[89, 96-98] chromatography, and determined by spectroscopic methods. Application of gas chromatography has also been studied to simultaneously separate, identify and estimate the additives in complex mixtures[88, 89, 99, 100] and this technique is sometimes combined with infra-red spectroscopy[101, 102]. For some compounds, the ultraviolet absorption spectra may suffice for their identification without prior chromatographic separation. Many of the substituted phenols used as antioxidants, however, cannot be determined in the presence of one another without prior separation, owing to the similarity of their ultraviolet spectra. Topanol OC, Ionox 330 and Binox M, for example, all have a wavelength of maximum absorbance at 277 nm and a shoulder at 282 nm near this peak absorbance. In order to extend the utility of ultraviolet absorption spectophotometry for identification of phenolic additives after extraction, the measurement of the spectra of both neutral and alkaline solutions has been suggested[103]. A more extensive identification scheme has been reported[104] based on the four

ultraviolet absorption spectra obtained for (i) the extract alone, (ii) after making it alkaline with ethanolic potassium hydroxide, (iii) after oxidation by shaking with nickel peroxide, and (iv) after making this oxidised fraction alkaline.

A general colorimetric procedure has been described for the determination of various phenolic and amine type antioxidants in polyethylene[105], viz. Sucronox 18, Topanol OC and Nonox CI. Fluorimetric techniques have been used for analysis of additives in extracts from rubbers[106], and the possibility of using phosphorescence techniques for determining these compounds has been investigated[107]. By virtue of their nature, many ultraviolet absorbers are amenable to analysis by fluorimetric methods[89]. Techniques of fluorimetry and phosphorimetry are well known to provide sensitive and selective methods of analysis for many organic compounds[6]. In order to assess the general feasibility of the fluorimetric and phosphorimetric determination of additives after their extraction from polymers with organic solvents, the fluorescence and phosphorescence characteristics of twenty nine common antioxidants and ultraviolet absorbers in an organic solvent were examined at room temperature and 77°K respectively. The fluorescence and phosphorescence spectral characteristics in EPA-chloroform, calibration data, phosphorescence detection limits and phosphorescence lifetimes are presented in this chapter.

3.2 Experimental

3.2.1 Apparatus

The excitation and emission spectra were obtained using the Aminco-Bowman spectrophotofluorometer, described in section 1.2.1. Fluorescence measurements at room temperature were made with the rotating can phosphoroscope attachment removed. Luminescence studies at liquid nitrogen temperature were conducted with the quartz Dewar flasks of the phosphoroscope attachment

in position, but with the rotating can of the attachment removed. Phosphorescence lifetime measurements were made by observation of the decay curve of phosphorescence. For long-life measurements, this was accomplished by manually closing the shutter on the excitation monochromator and simultaneously observing the decay on the X-Y recorder. For millisecond lifetime measurements the decay curve obtained between pulses with the rotating can phosphoroscope in operation was observed at a Telequipment oscilloscope with built-in time base. Sample tubes used for low temperature luminescence measurements were as described in section 1.2.1.

3.2.2 Materials

The solvent used for nearly all measurements was a mixture of diethyl ether, isopentane, ethanol and chloroform prepared from analytical reagent-grade solvents in the volume ratio 75:75:30:20. The antioxidants and ultraviolet absorbers examined were donated by courtesy of the Plastics Division, ICI Ltd., Welwyn Garden City, Herts. Stock 1000 µg per ml solutions of the additive compounds were prepared by direct dissolution in the solvent mixture, or by dissolution in chloroform followed by dilution with EPA (5:5:2 v/v mixture of diethyl ether, isopentane and ethanol). Nonox CI was dissolved in diethylamine to prepare a 1000 µg per ml solution; 1 ml was then diluted to 10 ml with EPA.

3.2.3 Procedure

The room temperature fluorescence excitation and emission spectra were recorded for the 1000 µg/ml solutions of the compounds. The same solutions were then transferred to the thick-walled sample tubes aligned in the quartz Dewar flask containing liquid nitrogen. The low-temperature luminescence excitation and emission spectra were then recorded. The phosphorescence

spectra at 77°K were then obtained after the phosphoroscope rotating can had been fitted. The phosphorescence lifetime was determined by observation of the decay curve of the phosphorescence at the X-Y recorder or with the oscilloscope.

Working curves for both low temperature luminescence intensity and phosphorescence intensity and for room temperature fluorescence intensity vs. concentration in parts per million (μg/ml) of the compound in the solvent mixture were obtained for each of the stabiliser compounds which exhibited appreciable luminescence. The wavelengths of maximum excitation and emission were set on the spectrofluorimeter, and the relative intensity readings from the photomultiplier microphotometer were recorded for a series of standard solutions containing 0.01 - 1000 μg per ml of each compound. In the preparation of dilute solutions, all glassware was cleaned according to the manufacturer's directions[108] in order to minimise pickup of phosphorescent impurities. After each intensity measurement had been made at liquid nitrogen temperature, the sample tube was removed from the Dewar flask and the sample glass was examined visually for freedom from cracking. When cracked glasses were obtained, the sample was re-measured after the glass had been allowed to melt and then refrozen.

The additive compound Santonox R was extracted from polythene samples (pellets, films or powder) by shaking a known weighed amount with 10 ml of the solvent in a 50 ml stopped flask by a wrist-action shaker, for a given time and temperature. A blank was also prepared simultaneously.

3.3 Results and Discussions

Table 3.1 lists the trade name, chemical composition and wavelengths of excitation and emission for room-temperature fluorescence, total luminescence at 77°K and phosphorescence at 77°K for each of the compounds examined. The phosphorescence lifetimes are also listed where applicable.

TABLE 3.I

LUMINESCENCE CHARACTERISTICS OF COMPOUNDS EXAMINED

No.	*Trade name*	*Chemical composition*	*Room temp. fluorescence*		*Low temp. luminescence*		*Phosphorescence*		*Phosphorescence life-time (sec)*	*Phosphorescence detection limit (p.p.m.)*
			ex λ_{max}	*em* λ_{max}	*ex* λ_{max}	*em* λ_{max}	*ex* λ_{max}	*em* λ_{max}		
1.	Topanol A	2,4-Dimethyl-6-*t*-butylphenol	–	–	289	425	285	420	0.5	1.0
2.	Topanol OC	2,6-Di-*t*-butyl-4-methylphenol	255	318[a]	–	–	–	–	–	–
3.	Tenox BHA	Mixture of 2- and 3-*t*-butyl-4-hydroxyanisole	312,255	380,335	299	420	295	420	1.80	0.06
4.	Binox M	Bis(3,5-di-*t*-butyl-4-hydroxyphenyl)methane	–	–	–	–	–	–	–	–
5.	Ionox 330	1,3,5-Trimethyl-2,4,6-tris-(3,5-di-*t*-butyl-4-hydroxybenzyl)benzene	295	335[a]	–	–	–	–	–	–
6.	Nonox WSP	Bis(2-hydroxy-3-α-methylcyclohexyl-5-methylphenyl)-methane	372	440[a]	290	415	290	412	1.53	0.1
7.	Nonox WSL	2,4-Dimethyl-6-α-methylcyclohexylphenol	372	464[a]	296	430	282	415	0.5	1.0
8.	Nonox DCP	2,2-Bis(3-methyl-4-hydroxyphenyl)propane	310,370	390,460[a]	290	410	285	405	1.9	0.05
9.	Calco 2246	Bis(2-hydroxy-3-*t*-butyl-5-methylphenyl)methane	325	375[a]	282	410	285	410	1.56	0.2
10.	Topanol CA	1,1,3-Tris(2-methyl-4-hydroxy-5-*t*-butylphenyl)-methane	255,318	318,408[a]	280	405	285	405	0.70	0.07
11.	Santonox R	Bis(2-methyl-4-hydroxy 5-*t*-butylphenyl)sulphide	360	410[a]	300	430	305	430	0.035[c]	0.07
12.	Topanol TP	Bis(2-hydroxy-3,5-di-*t*-butyl-6-methylphenyl)-sulphide	–	–	295	428	300	426	0.033[c]	0.1
13.	Suconox 18	N-Stearoyl-*p*-aminophenol	318,345	386[a]	300	415	300	415	1.0	0.12

[a] Denotes weak room temperature fluorescence.
[b] Denotes weak phosphorescence at 77°K, only approximate estimates of life-time made.
[c] Life-time measurement reproducible within ± 10%.

TABLE 3.1 (continued).

LUMINESCENCE CHARACTERISTICS OF COMPOUNDS EXAMINED

No.	*Trade name*	*Chemical composition*	*Room temp. fluorescence*		*Low temp. luminescence*		*Phosphorescence*		*Phosphorescence life-time (sec)*	*Phosphorescence detection limit (p.p.m.)*
			ex λ_{max}	*em* λ_{max}	*ex* λ_{max}	*em* λ_{max}	*ex* λ_{max}	*em* λ_{max}		
14.	Naugawhite	Bis(2-hydroxy-3-nonyl-5-methylphenyl)methane	366,312	446,390[a]	285	408	285	410	1.60	0.08
15.	Agerite Superlite	–	345	405	290	410	290	420	1.85	0.06
16.	Voidox 100%	2,6-Di-*t*-butyl-4-methyl phenol + sorbitan/fatty acid compound	266	318[a]	295	420	292	435[b]	2.5	>10
17.	Irganox 1010	Pentaerythritol-tetra-β-(3,5-di-*t*-butyl-4-hydroxy-phenyl)propionate	300	350[a]	328	380	–	–	–	–
18.	Irganox 1076	n-Octadecyl-β-(3,5-di-*t*-butyl-4-hydroxyphenyl)-propionate	376	430[a]	–	–	–	–	–	–
19.	Irganox 1093	Di-n-octadecyl-3,5-di-*t*-butyl-4-hydroxybenzyl phosphonate	315	375[a]	–	–	–	–	–	–
20.	Polygard	Tris (nonylphenyl)-phosphite	356	422[a]	280	400	282	400	1.75	0.1
21.	Nonox CI	N,N'-Di-β-naphthyl-*p*-phenylenediamine	392	490	386,434	430,455	382	516	0.90	0.02
22.	DLTDP	Dilaurylthiodipropionate	–	–	–	–	–	–	–	–
23.	Salol	Phenylsalicylate	340	464[a]	320	452	302	415[b]	0.45	0.1
24.	Cyasorb UV9	2-Hydroxy-4-methoxy-benzophenone	–	–	300	450	300	450[b]	0.02	>10
25.	Cyasorb UV531	2-Hydroxy-4-n-octoxybenzophenone	–	–	305	450	300	450[b]	0.03	>10
26.	Uvinol 400	2,4-Dihydroxybenzophenone	–	–	–	–	–	–	–	–
27.	Cyasorb UV24	2,2'-Dihydroxy-4-methoxybenzophenone	–	–	305	430	308/360	430/455[b]	0.1	>10
28.	Tinuvin P	2-(2'-Hydroxy-5'-methylphenyl)-benzotriazole	–	–	315	396,480	315	480.515	0.85	0.03
29.	Tinuvin 326	2-(2'-Hydroxy-5'-*t*-butylphenyl)-3-chlorobenzotriazole	–	–	–	–	–	–	–	–

[a] Denotes weak room temperature fluorescence.
[b] Denotes weak phosphorescence at 77°K, only approximate estimates of life-time made.
[c] Life-time measurement reproducible within ± 10%.

Figures 3.1 (A to D) shows the room temperature fluorescence excitation and emission spectra of the compounds whose fluorescence signals were relatively strong. The scale settings at which the spectra were recorded are indicated within brackets, along with the trade names of each of the compounds. Figures 3.2 (A to F) shows the luminescence excitation and emission spectra of the compounds at 77°K and Figures 3.3 (A to D), the phosphorescence excitation and emission spectra at 77°K. The excitation and emission spectra obtained for Nonox CI, is shown in Figure 3.4. The shapes of the spectra are influenced by the particular instrument employed, because they are uncorrected for variations in detector sensitivity, xenon arc lamp emission or grating emission with wavelength. The relevant correction curves are described in the previous chapter. For similar experimental conditions in other laboratories using the same type of instrumentation (source and detector), the optimum peak wavelengths for luminescence measurements should not, however, differ by more than ca 5 nm from the values given in Table 3.1. The phosphorescence lifetimes were measured as the time taken for the phosphorescence intensity to decrease from a certain value to 1/e of that value, and were measured as precisely as possible with the 1000 μg/ml stock solutions of the compounds. Phosphorescence lifetimes greater than 0.5 sec could be measured to within ± 5%. The phosphorescence lifetime measurements of the compounds Santonox R and Topanol TP were reproducible within ± 10% and those of the compounds Voidox 100%, Salol, Cyasorb UV9, Cyasorb UV 531 and Cyasorb UV24, which showed weak phosphorescence, were estimated only approximately. The phosphorescence detection limits for each compound are also listed in Table 3.1. The background noise from the source, photomultiplier and amplifier, in relation to which the detection limit is normally defined in trace analysis, is a less significant source of uncertainty in the technique described here than the magnitude of the variation in the background signal from

FIGURE 3.1A

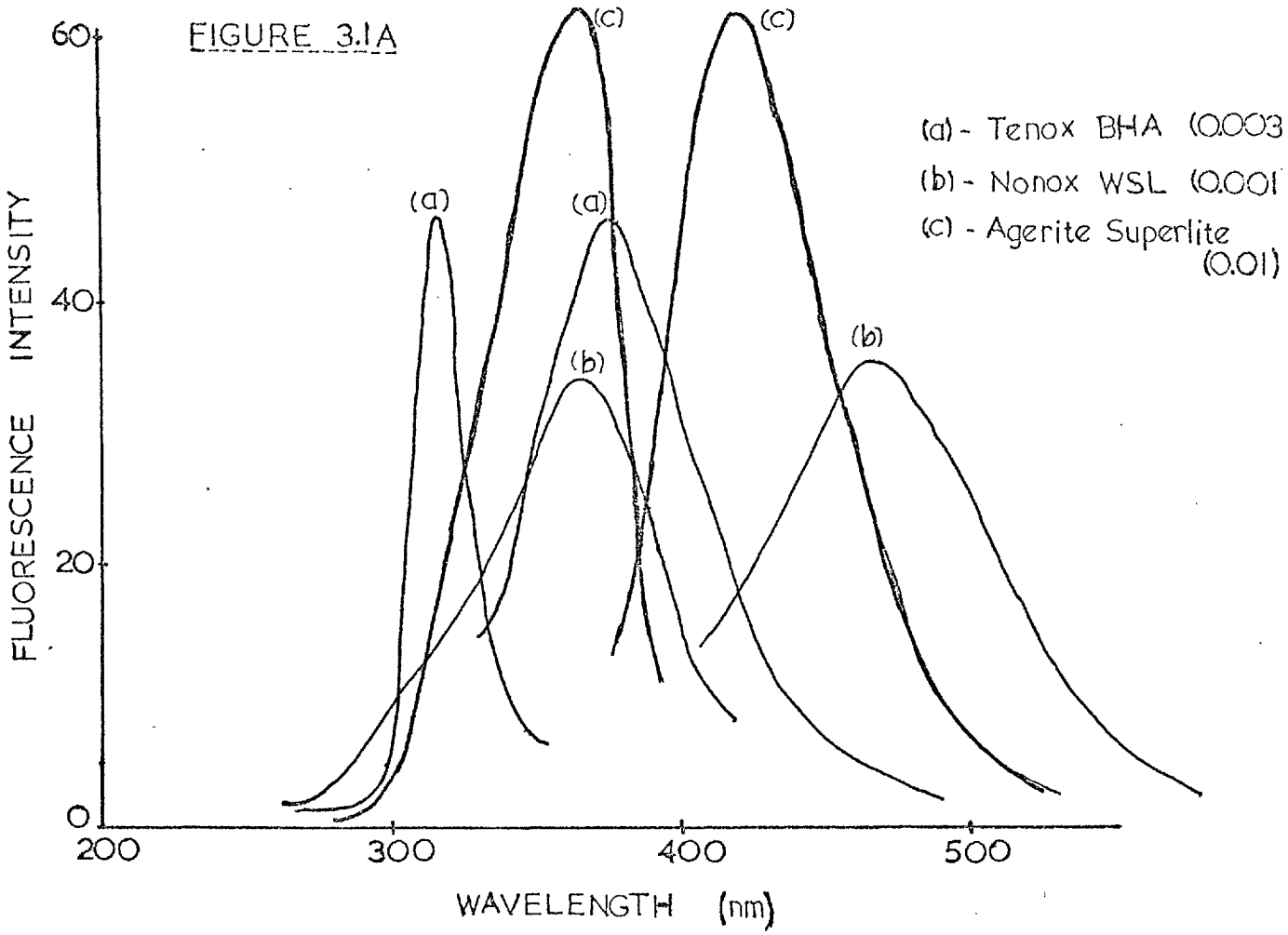


FIGURE 3.1B

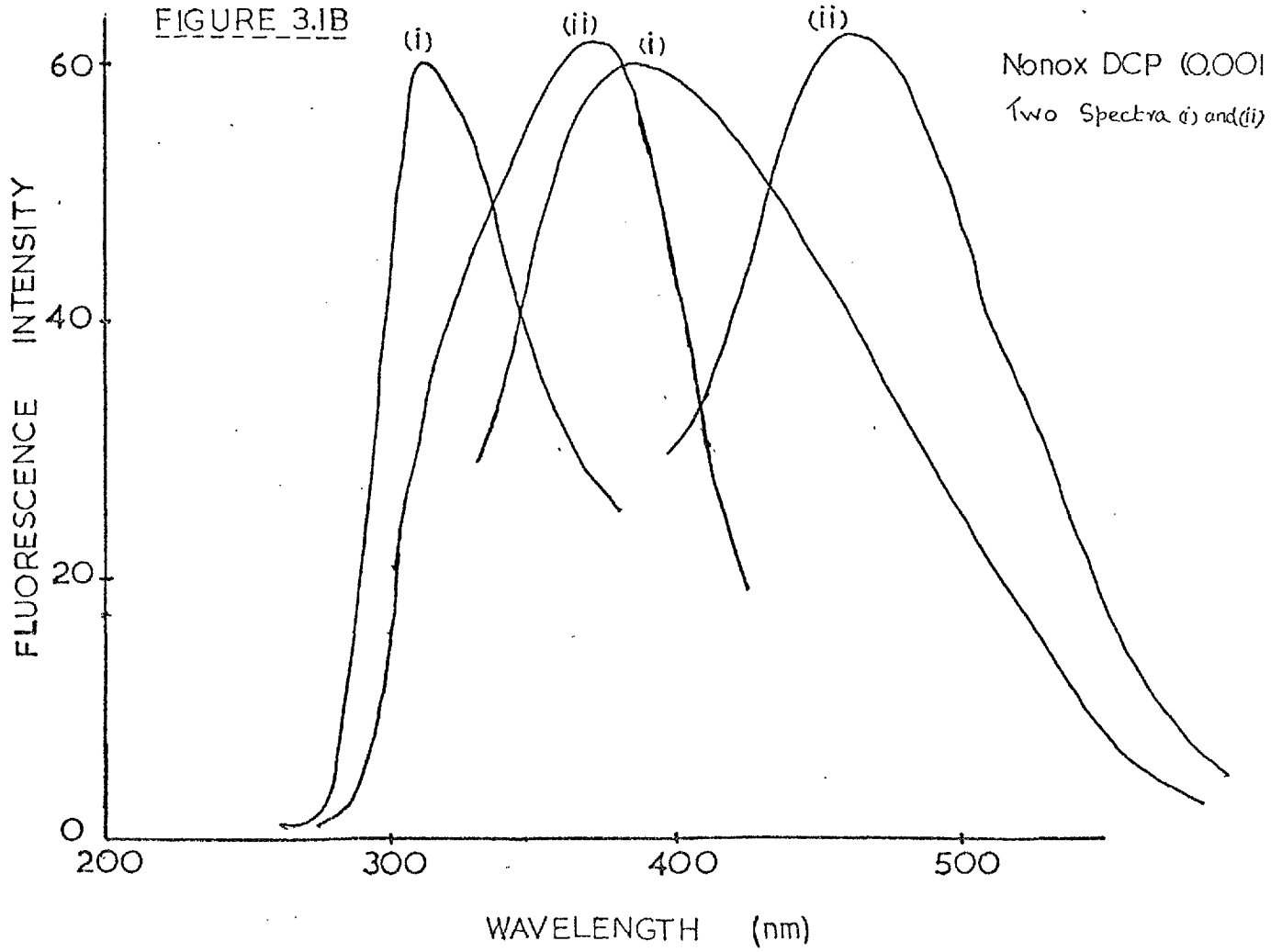


FIGURE 3.1C

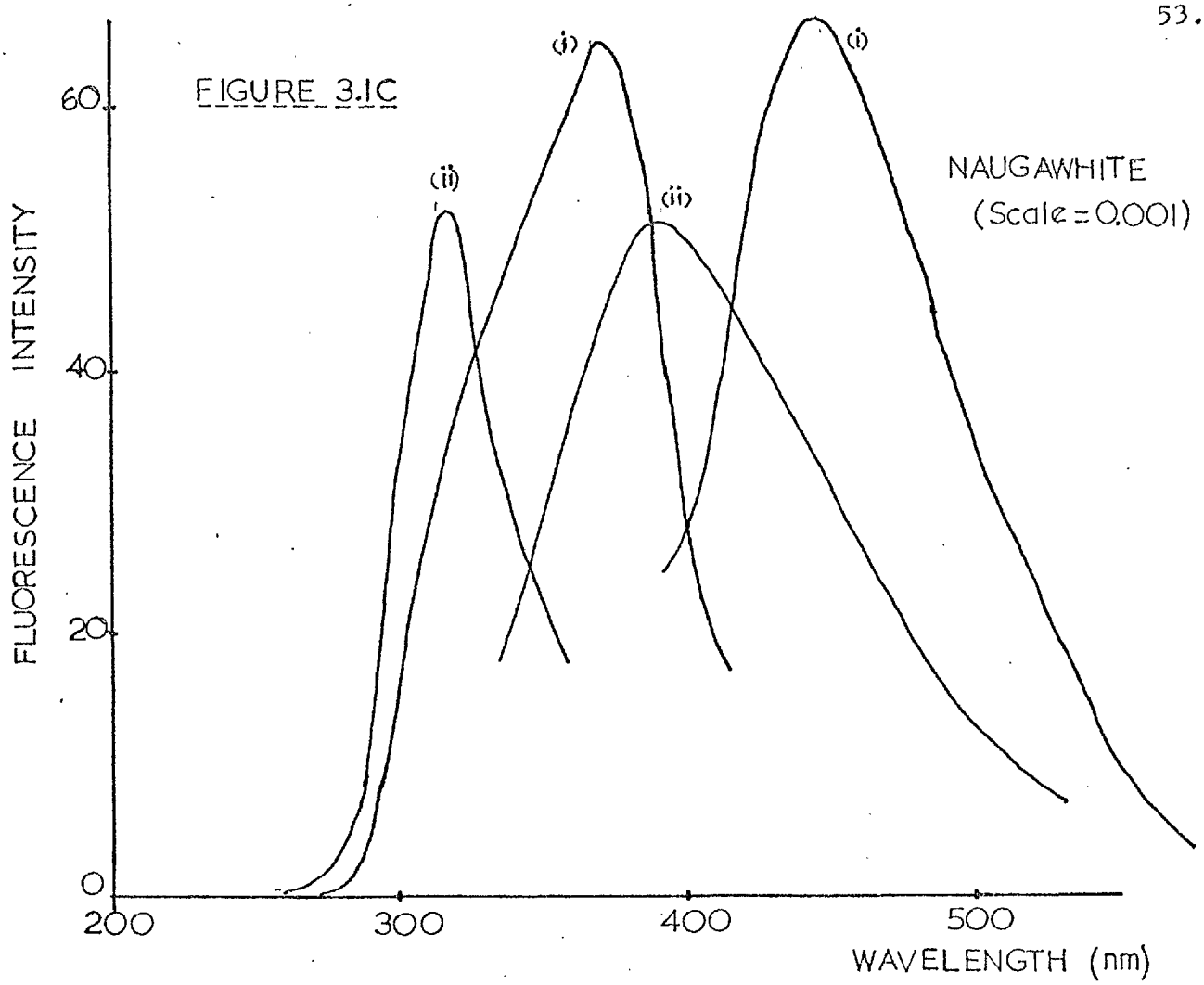


FIGURE 3.1D

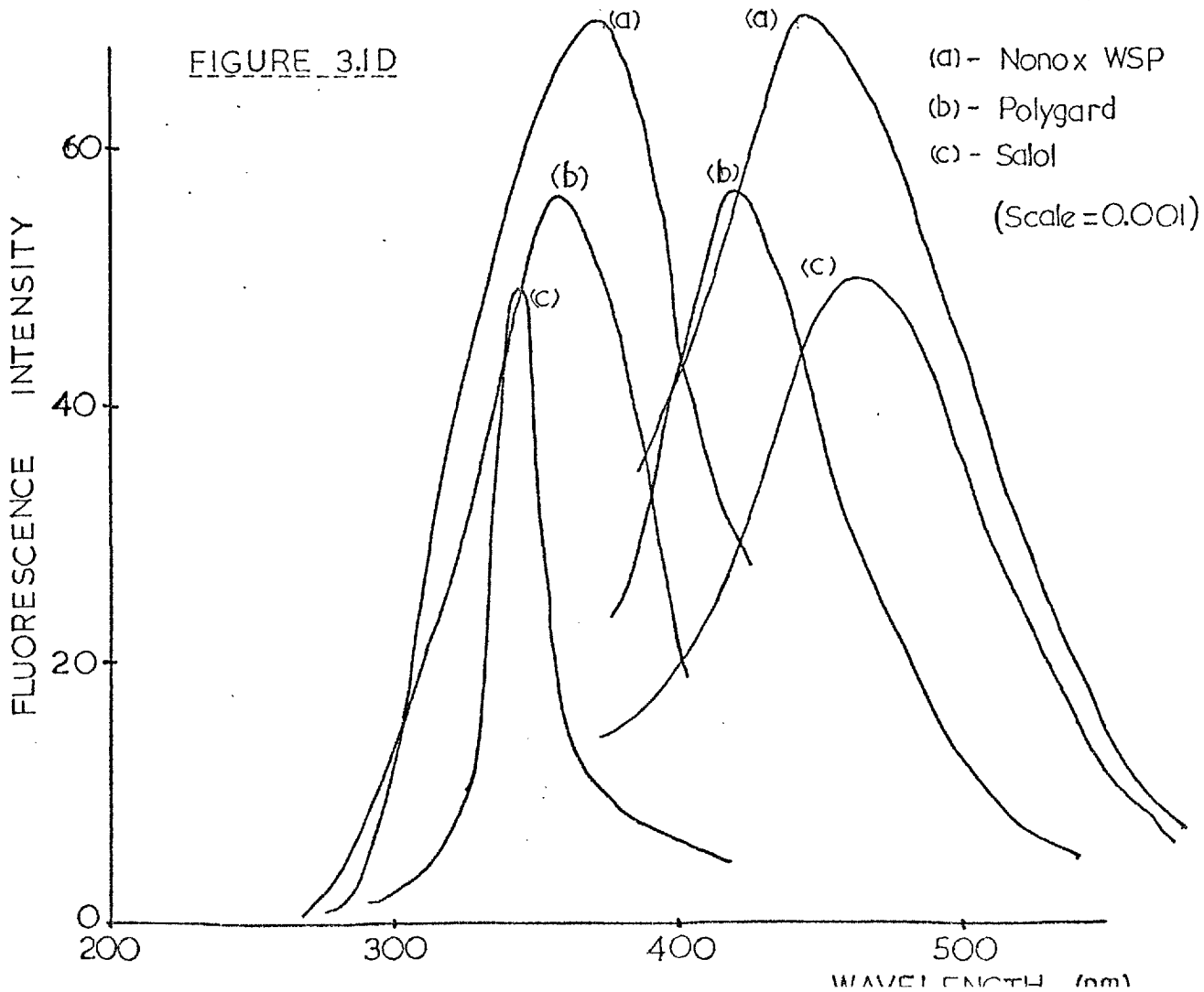
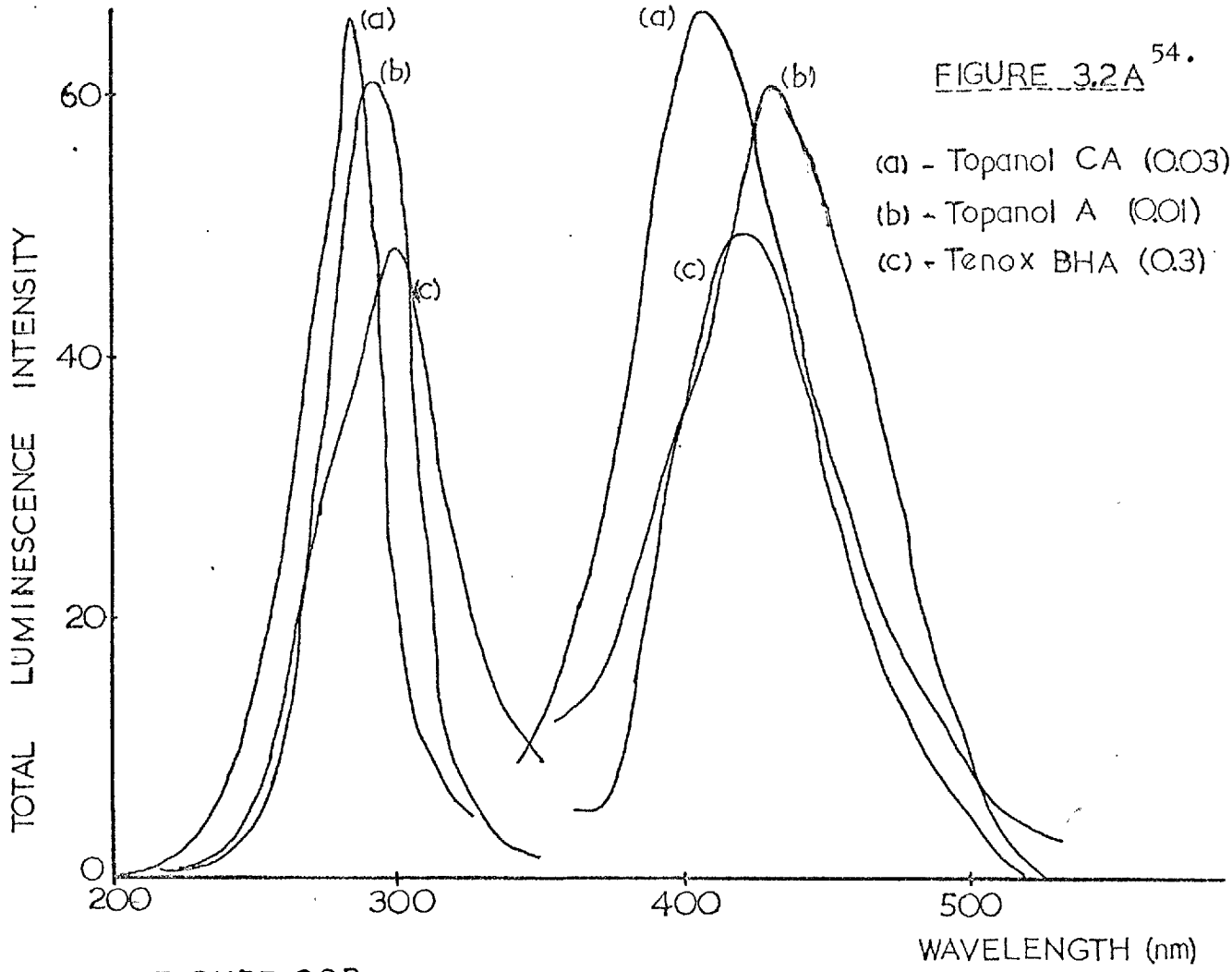
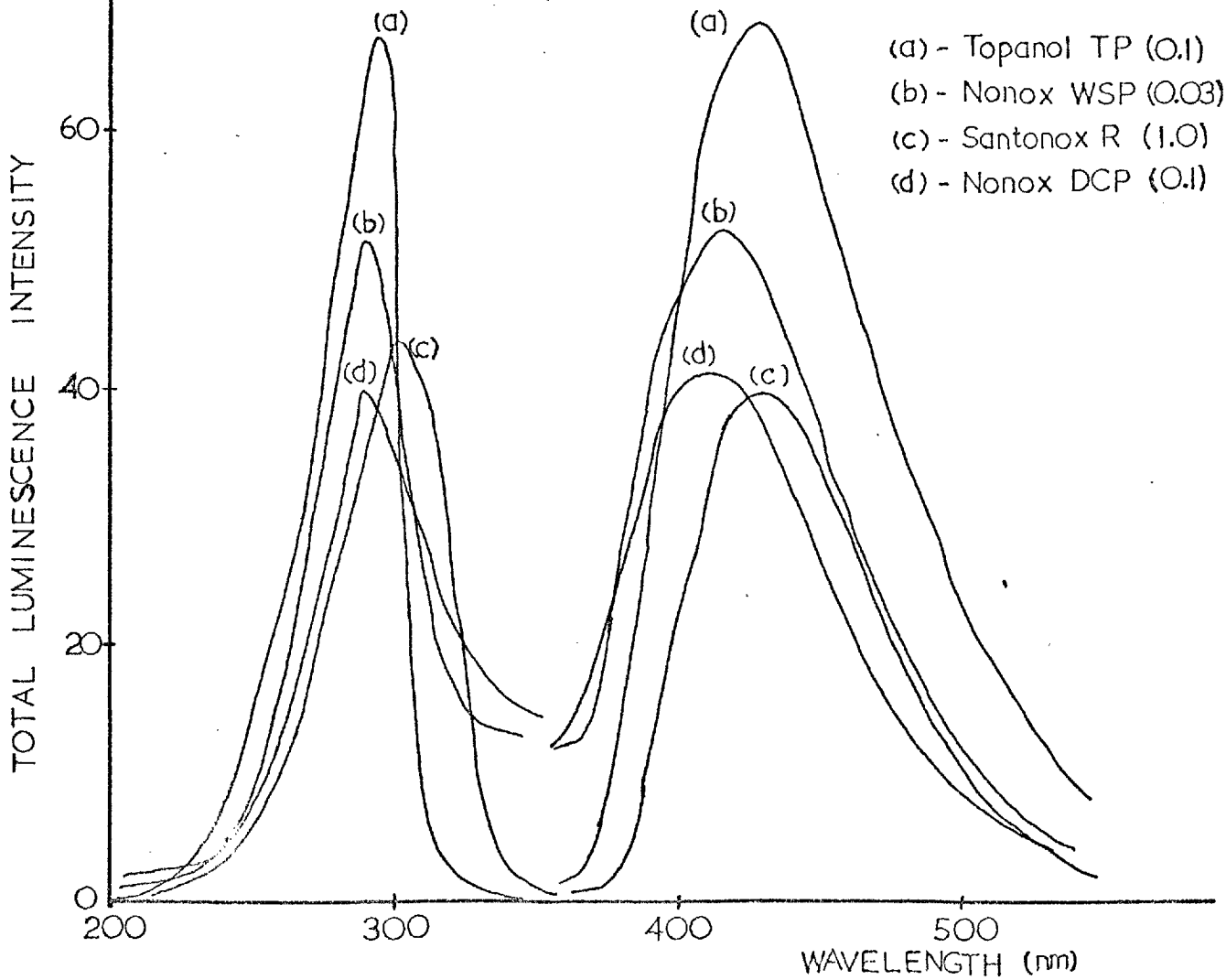


FIGURE 3.2A



(a) - Topanol CA (0.03)
(b) - Topanol A (0.01)
(c) - Tenox BHA (0.3)

FIGURE 3.2B



(a) - Topanol TP (0.1)
(b) - Nonox WSP (0.03)
(c) - Santonox R (1.0)
(d) - Nonox DCP (0.1)

FIGURE 3.2C

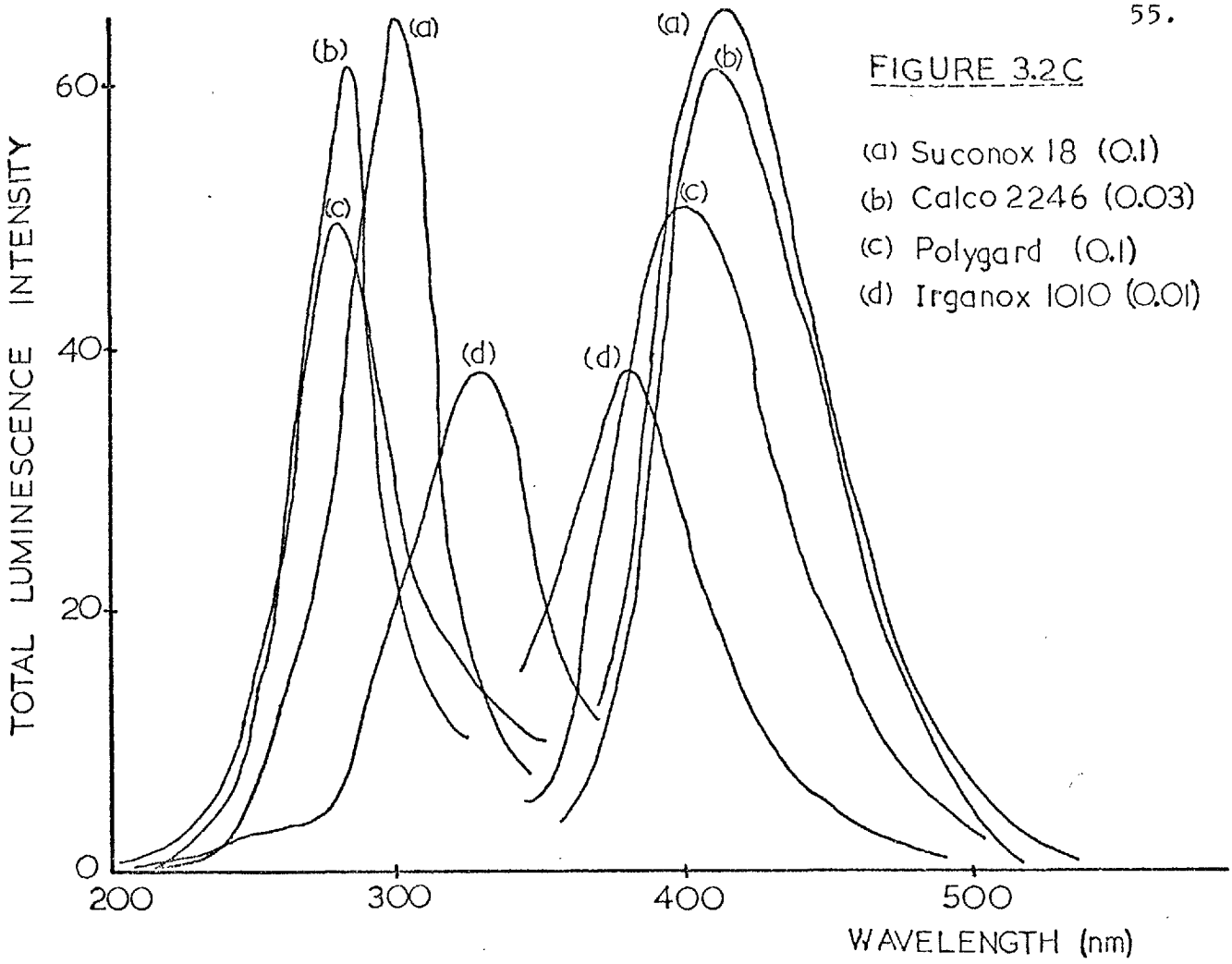


FIGURE 3.2D

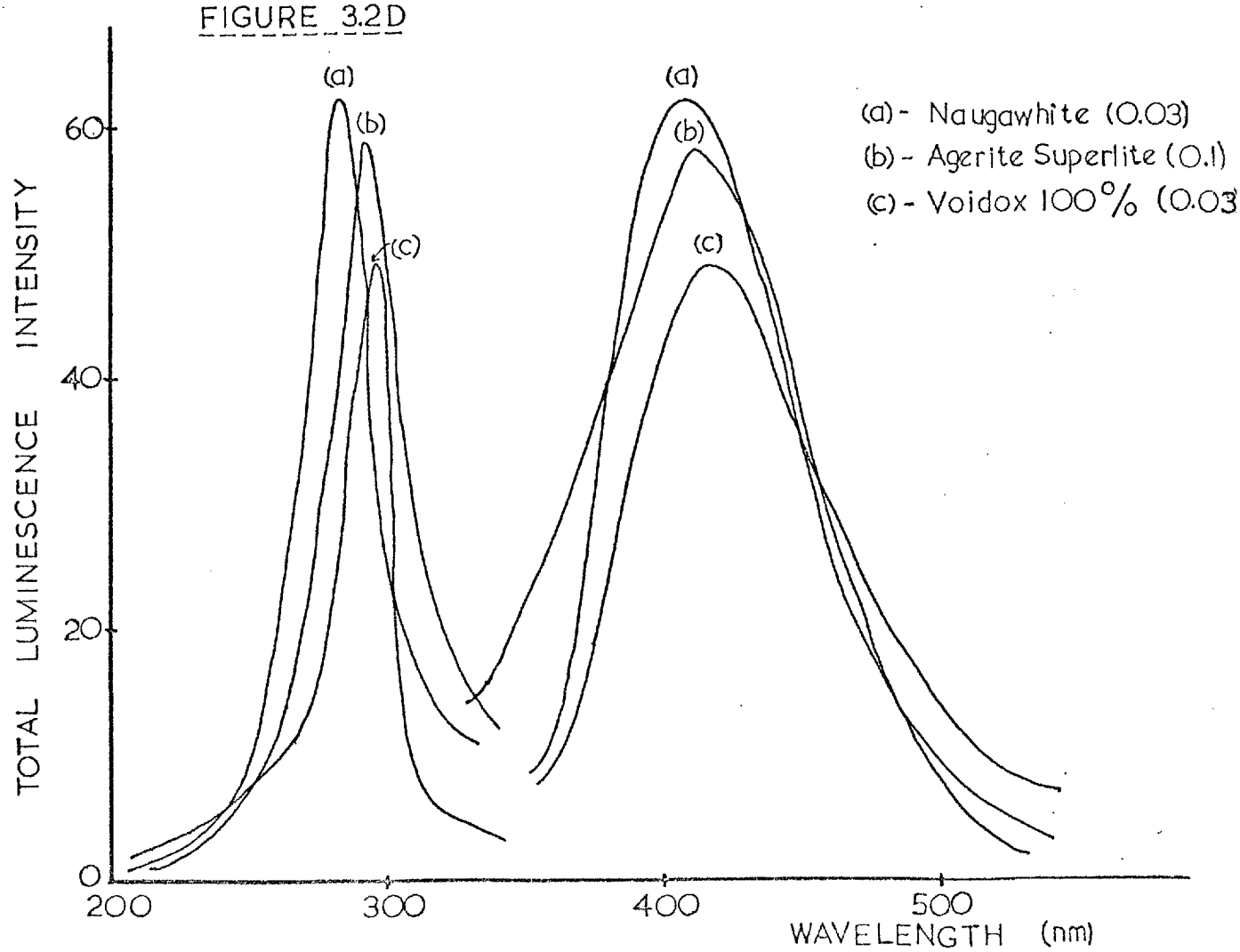


FIGURE 3.2E

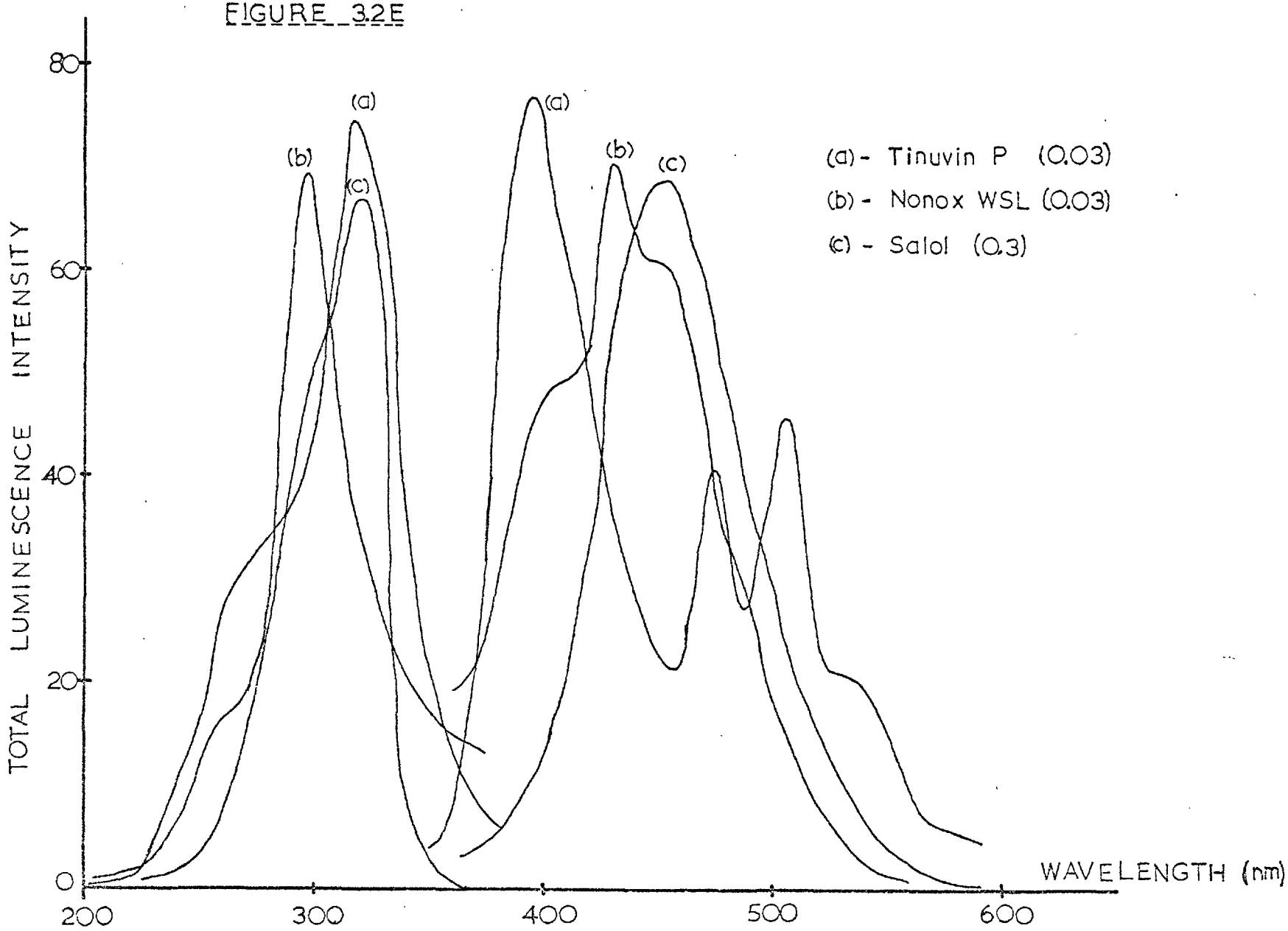

TOTAL LUMINESCENCE INTENSITY
80
60
40
20
0
200
300
400
500
600
WAVELENGTH (nm)
(a)
(b)
(c)
(a)
(b)
(c)
(a) - Tinuvin P (0.03)
(b) - Nonox WSL (0.03)
(c) - Salol (0.3)

FIGURE 3.2 F

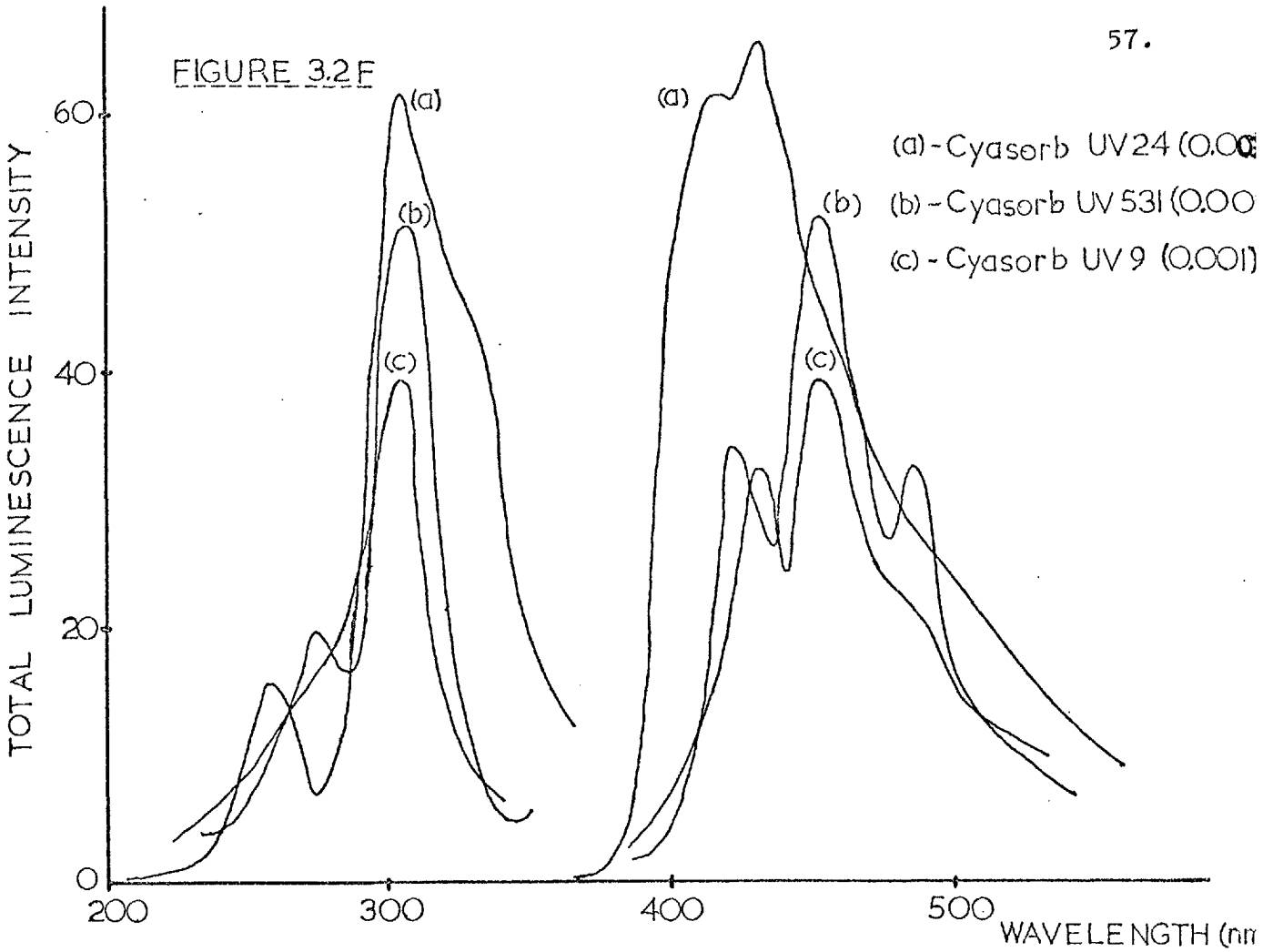


FIGURE 3.3A

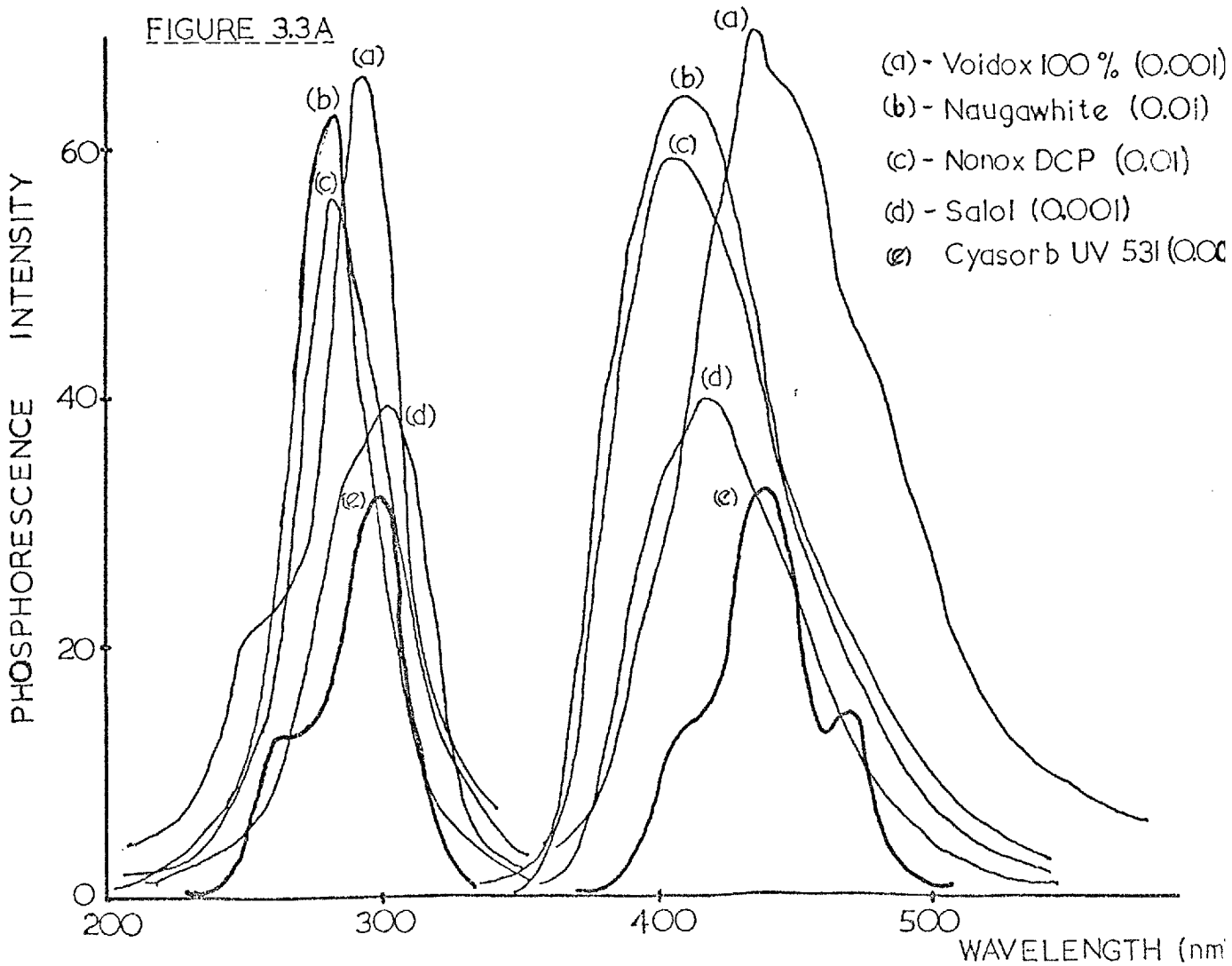
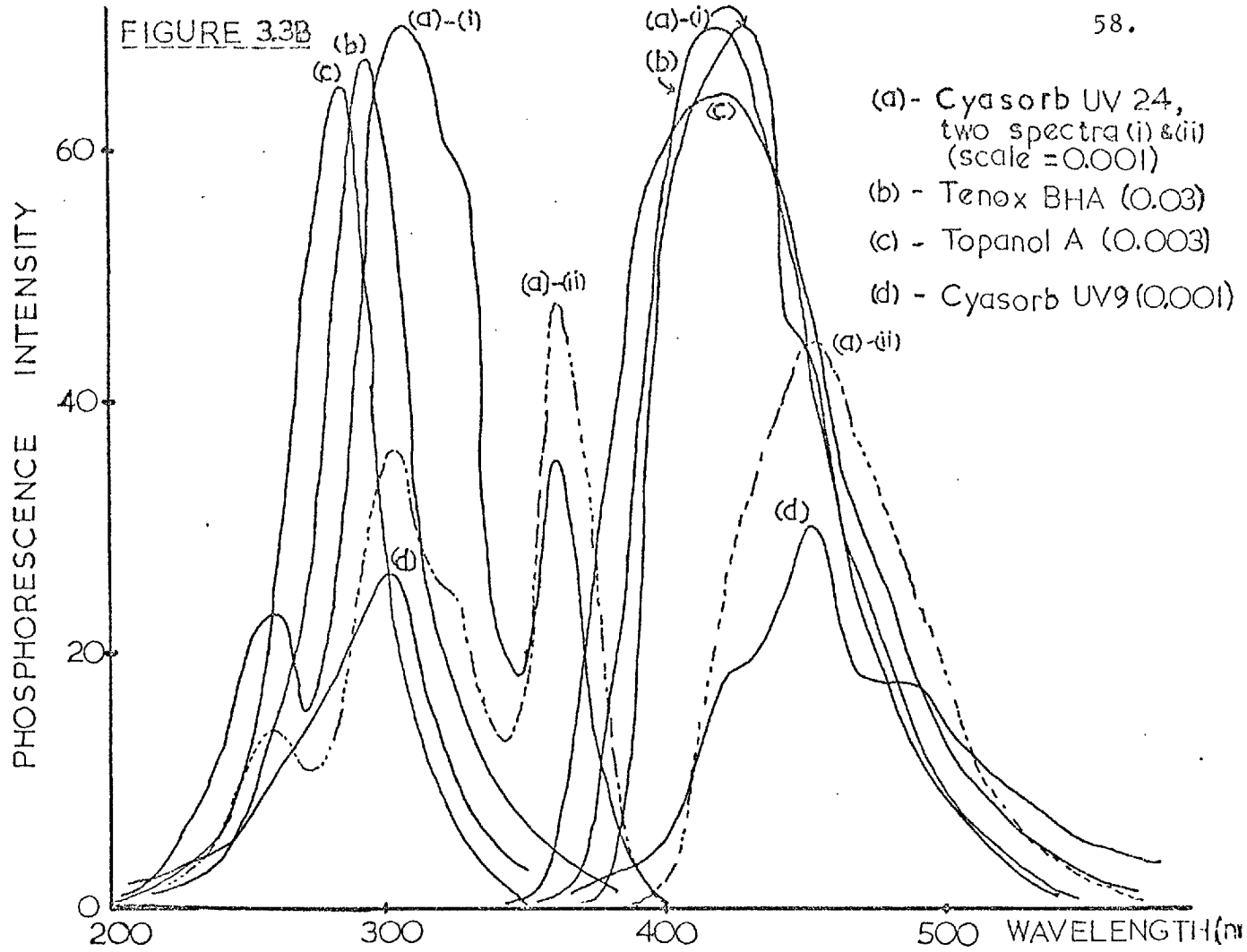


FIGURE 3.3B



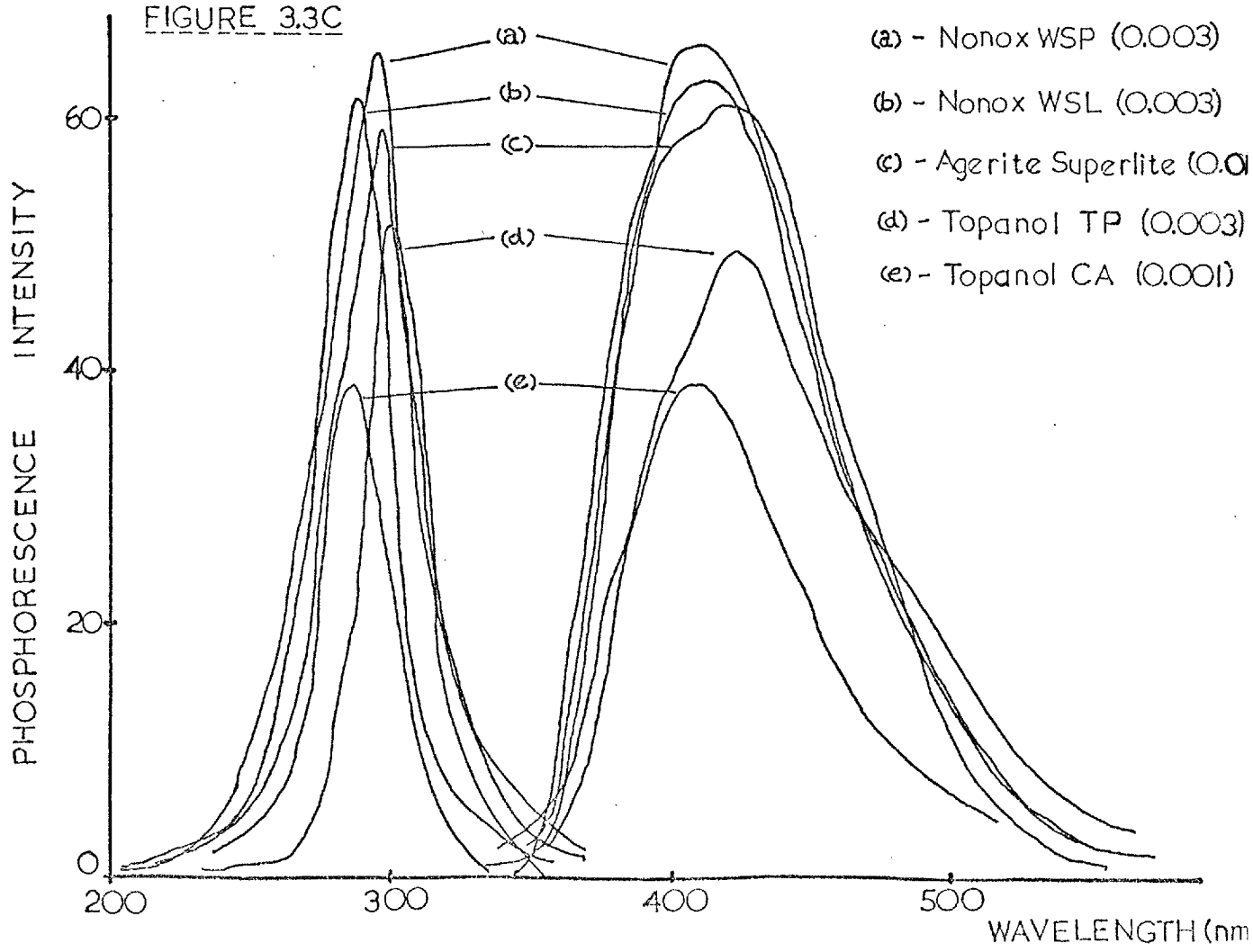
(a) - Cyasorb UV 24, two spectra (i) & (ii) (scale = 0.001)

(b) - Tenox BHA (0.03)

(c) - Topanol A (0.003)

(d) - Cyasorb UV9 (0.001)

FIGURE 3.3C



(a) - Nonox WSP (0.003)

(b) - Nonox WSL (0.003)

(c) - Agerite Superlite (0.0

(d) - Topanol TP (0.003)

(e) - Topanol CA (0.001)

FIGURE 3.3 D

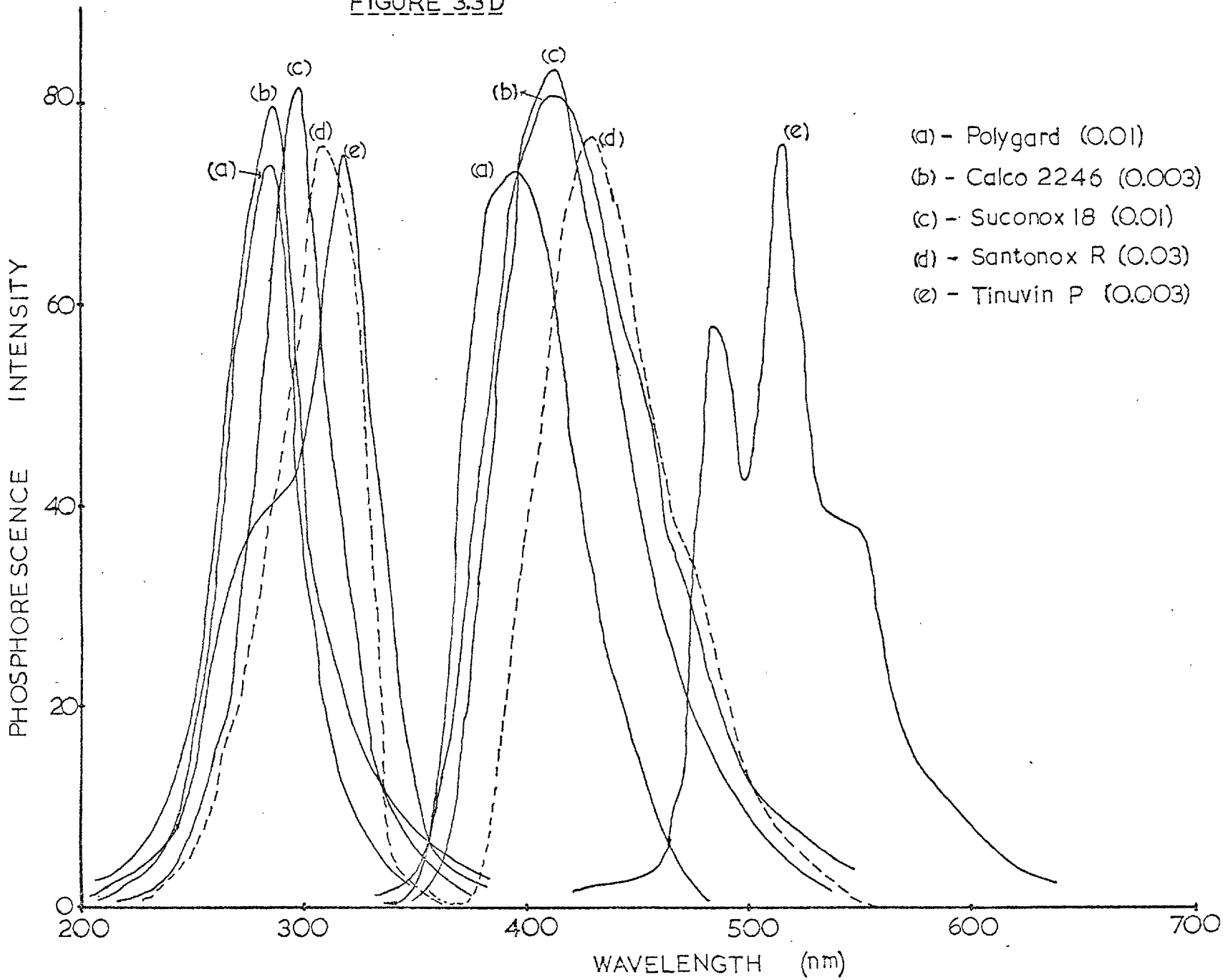
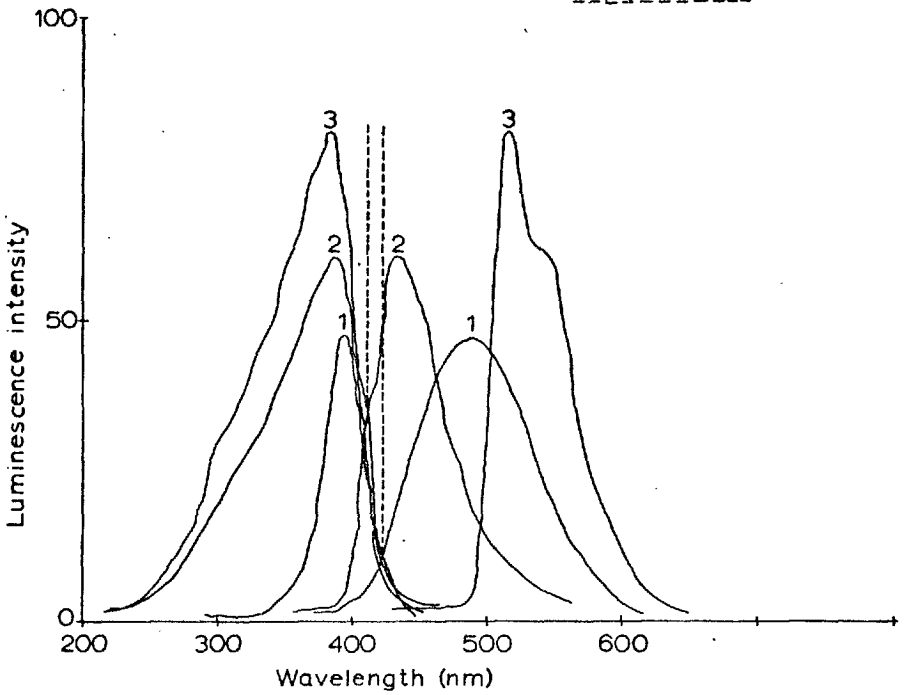
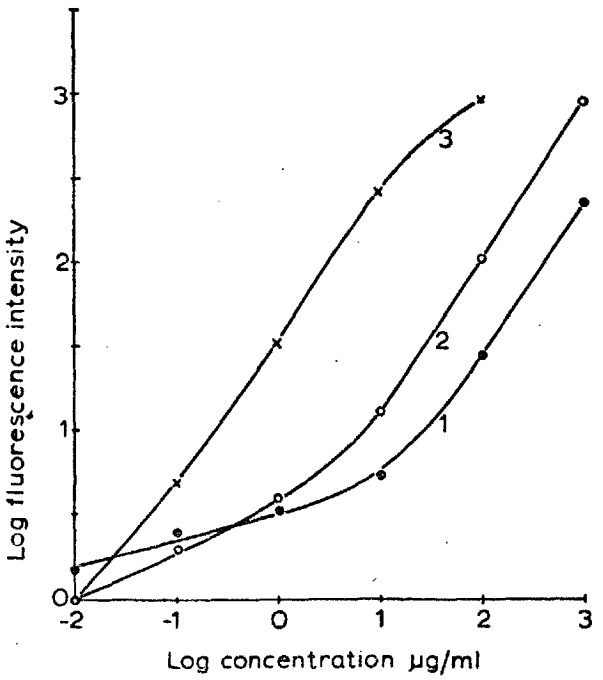

PHOSPHORESCENCE INTENSITY
0
20
40
60
80
200
300
400
500
600
700
WAVELENGTH (nm)
(a)
(b)
(c)
(d)
(e)
(a) - Polygard (0.01)
(b) - Calco 2246 (0.003)
(c) - Suconox 18 (0.01)
(d) - Santonox R (0.03)
(e) - Tinuvin P (0.003)

FIGURE 3.4



Spectral characteristics of luminescence observed for Nonox CI (Sample 21). (1) Excitation and emission spectra at room temperature, sensitivity scale 0.01; (2) excitation and emission spectra at 77°K for total luminescence, sensitivity scale 1.0; (3) excitation and emission spectra at 77°K for phosphorescence, sensitivity scale 0.01.

FIGURE 3.5



Working curves for room temperature fluorescence: (1) Tenox BHA (●), (2) Agerlite Superlite (○), (3) Nonox CI (×).

sample-to-sample caused by absorption of organic material by the solvent, glassware and sample tubes employed. An uncertainty factor of ca 1.5 was estimated for the phosphorescence background. The limit of detection was, therefore, taken as that concentration of the compound in the solvent mixture which produced a detector signal equal to twice the signal caused by variation in the background phosphorescence from the solvent and sample tube at that wavelength. The detection limits can be improved to a certain extent by purification of the solvent to minimise its phosphorescence background but a substantial improvement would result even in relatively impure solvent if the background could merely be maintained constant to permit its subtraction from the signal with confidence. It is, therefore, of great importance that a careful and systematic handling technique and sample tube cleaning procedure should be adopted, in order to minimise the sources of random variation in the values of phosphorescence blank.

The fluorescence signals recorded at room temperature for most of the compounds examined were of low intensity. Thus, while it was detectable at the high concentration (1000 μg/ml) used for study of the spectral characteristics, the room-temperature fluorescence of most of the compounds was of little practical value in trace analysis. Three compounds did, however, show quite intense room-temperature fluorescence; these were Tenox BHA, Agerlite Superlite and Nonox CI. Room-temperature fluorescence calibration graphs for these compounds at their wavelengths of maximum excitation and emission are shown in Figure 3.5. The low-temperature luminescence observed for most of the compounds examined was usually much more intense than the room-temperature fluorescence emission, but largely consisted of phosphorescence. This observation was reflected by the almost exactly similar spectral characteristics of the low-temperature luminescence and phosphorescence and similarly of the respective calibration graphs. Only

two compounds (Nonox CI, Tinuvin P) showed low temperature luminescence characteristics different from their phosphorescence. The compound Irganox 1010 exhibited an apparent low temperature luminescence of different spectral characteristics to the weak room temperature emission, and did not show detectable phosphorescence. The low temperature luminescence observed for Irganox 1010 may be short lifetime phosphorescence which was undetectable with the rotating can phosphoroscope. Four compounds (Topanol OC, Ionox 330, Irganox 1076 and Irganox 1093) showed relatively weak room-temperature fluorescence in EPA but no low-temperature emission was detectable. Four compounds (Binox M, DLTDP, Uvinol 400 and Tinuvin 326) exhibited no luminescence under any conditions, while the phosphorescence of Voidox 100%, Salol, Cyasorb UV9, Cyasorb UV531 and Cyasorb UV24 were too weak to permit its examination in dilute solutions or accurate measurement of the phosphorescence lifetimes.

The working curves between 0.01 and 1000 $\mu g/ml$ for low-temperature luminescence and phosphorescence in the EPA-chloroform solvent mixture for those compounds whose emission at 77^{o}K permitted their sensitive determination, are shown in Figures 3.6 and 3.7. In most cases the log/log plots show linearity over a 100 to 1000-fold concentration range. Self-absorption at high concentrations limits the useful upper working range for several compounds.

Measurement of phosphorescence characteristics of samples obtained after extraction of polymers with organic solvents may yield useful information regarding the nature and concentration of additive present. An attempt was made to investigate the possibility of using phosphorescence measurements in the chemical analysis of an additive. The compound Santonox R was chosen for this study. The additive was extracted from a polythene sample by shaking at room temperature or by refluxing with a solvent, and the concentration was

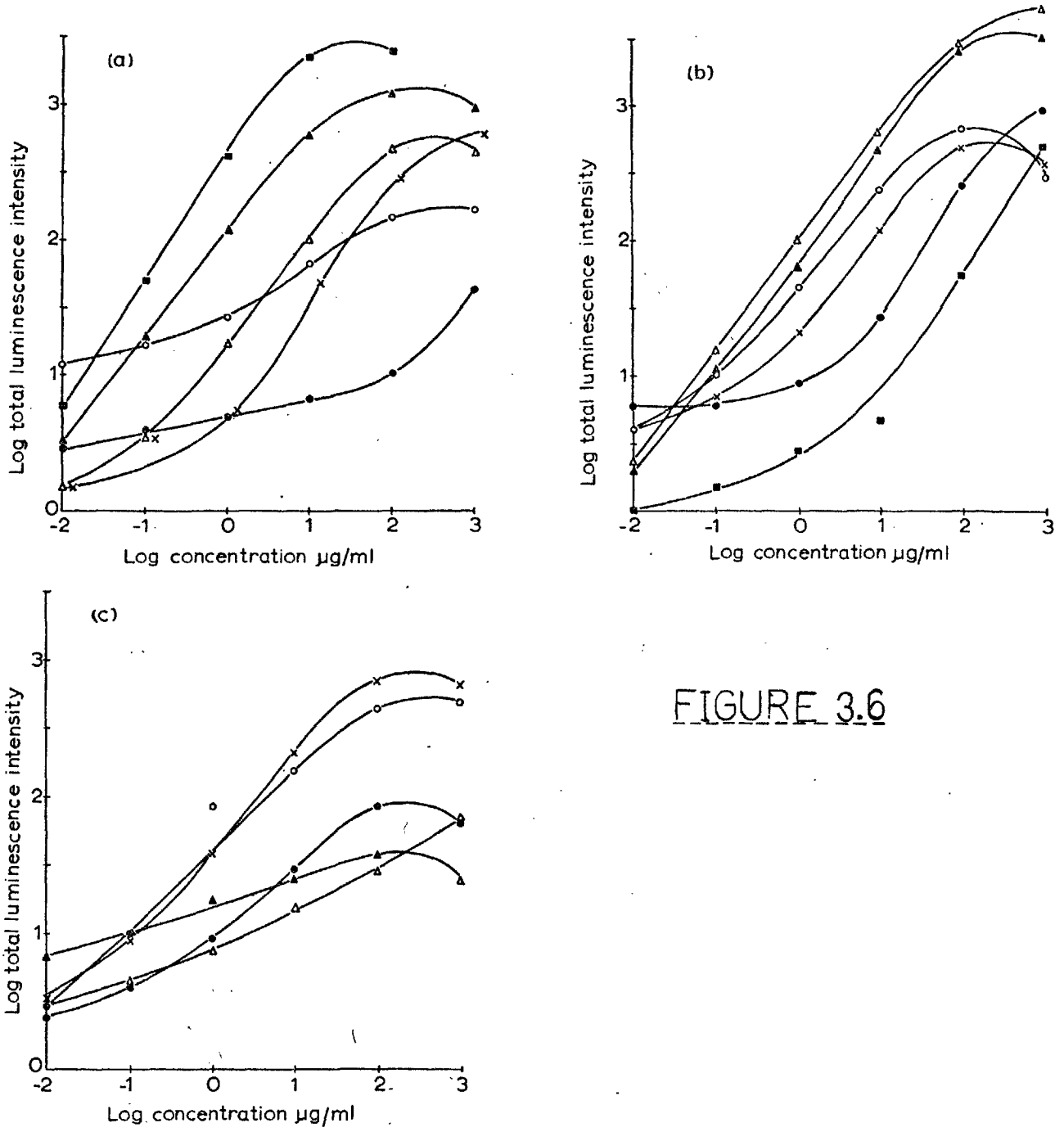
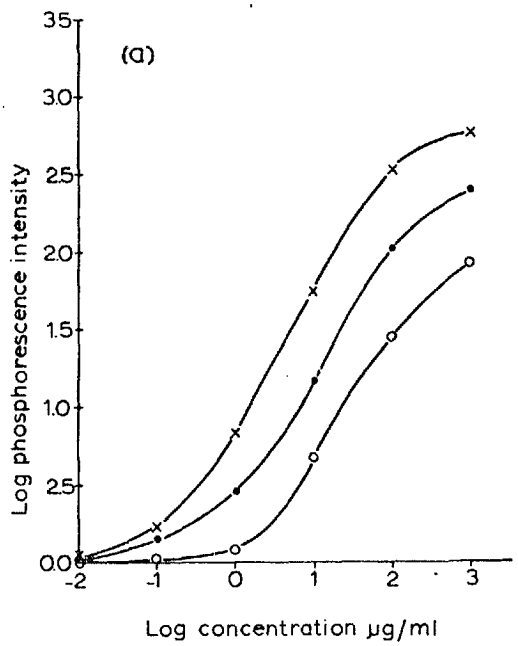


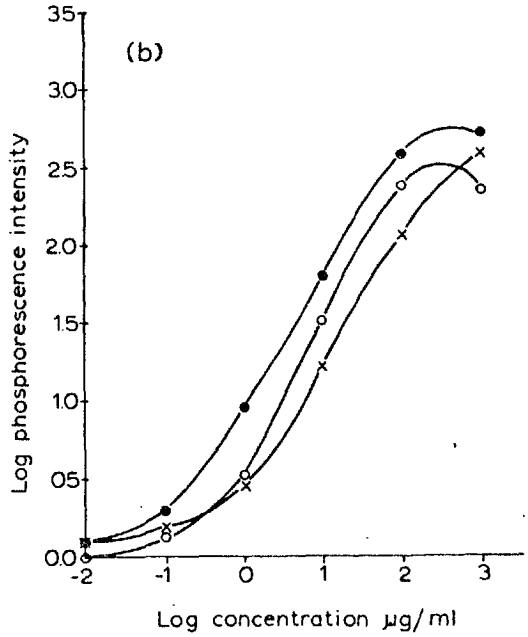
FIGURE 3.6

(a) -. Working curves between 0.01 p.p.m. and 1000 p.p.m. for low-temperature luminescence of the compounds: (■) Nonox CI, (▲) Tinuvin P, (×) Polygard, (△) Calco 2246, (○) Topanol CA, (●) Nonox WSL.

(b)- Working curves between 0.01 p.p.m. and 1000 p.p.m. for low-temperature luminescence of the compounds: (△) Salol, (▲) Santonox R, (●) Agerite Superlite, (■) Irganox 1010, (×) Suconox 18, (○) Tenox BHA.

(c) - Working curves between 0.01 p.p.m. and 1000 p.p.m. for low-temperature luminescence of the compounds: (×) Topanol TP, (○) Naugawhite, (△) Topanol A, (●) Nonox DCP, (▲) Nonox WSP.

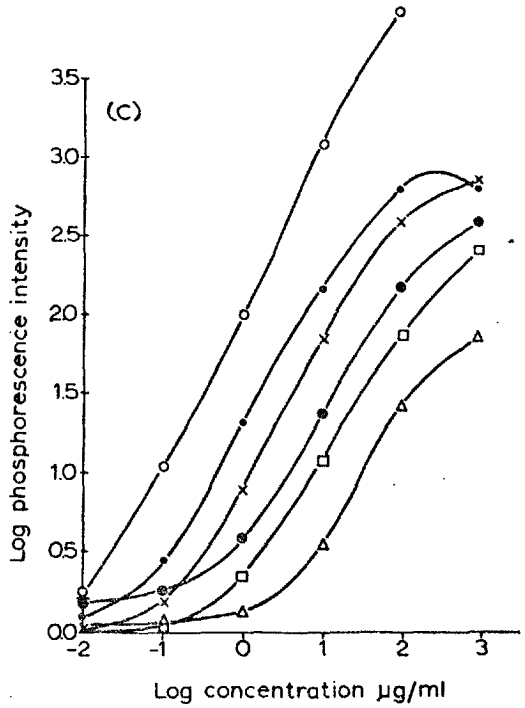


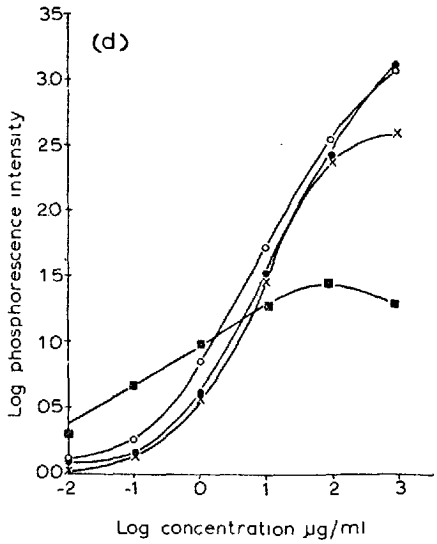


(a) - Working curves between 0.01 p.p.m. and 1000 p.p.m. for phosphorescence of the compounds: (×) Tenox BHA, (○) Nonox WSL, (●) Naugawhite.

(b) - Working curves between 0.01 p.p.m. and 1000 p.p.m. for phosphorescence of the compounds: (×) Suconox 18, (○) Topanol TP, (●) Agerite Superlite.

FIGURE 3.7





(c) - Working curves between 0.01 p.p.m. and 1000 p.p.m. for phosphorescence of the compounds: (×) Nonox DCP, (○) Nonox CI, (●) Tinuvin P, (△) Topanol A, (◍) Nonox WSP, (□) Calco 2246.

(d) - Working curves between 0.01 p.p.m. and 1000 p.p.m. for phosphorescence of the compounds: (×) Topanol CA, (○) Santonox R, (◍) Polygard, (■) Salol.

determined by measurement of its phosphorescence at 430 nm at 77°K with an excitation wavelength of 305 nm and using a standard calibration graph for phosphorescence at 77°K of the additive in the same solvent. The polythene samples used were pellets, thin film (~ 0.06 mm thickness) and powder (2 mm mesh) and the solvents used were chloroform and ethanol. The chloroform extract was diluted with EPA (1:9 v/v) before measurement of its phosphorescence at 77°K while the ethanol extract was directly frozen in liquid nitrogen. Table 3.2 shows the results obtained, from at least two determinations, compared with those given by the manufacturer of the polythene sample.

TABLE 3.2 : DETERMINATION OF SANTONOX R IN POLYTHENE (Phosphorescence at 305/430 at 77°K)

Nature of Polythene Sample	Method of Extraction	Solvent	Amount of Santonox R (%)	
			Found [b]	Present [c]
Pellets	Shaking at room temp. for ~ 3 hours	Chloroform [a]	0.05	0.7 - 1.0
Pellets	-do-	Ethanol	0.05	0.7 - 1.0
Pellets	Repeated extraction by shaking from the same sample	Ethanol	0.052	0.7 - 1.0
Thin Film (~ 0.06 mm thick)	Shaking at room temp. for ~ 3 hours	Ethanol	0.08	0.11
Powder (2 mm mesh)	-do-	Ethanol	0.095	0.11
Powder	Refluxing for ~ 20 hours	Ethanol	0.11	0.11

a - solution diluted with EPA (1:9 v/v)

b - average of at least two determinations

c - values obtained from ICI Plastics Ltd.

The amount of Santonox R extracted with the same solvent, viz ethanol, at room temperature was found to vary with the nature of the polythene sample. The recovery of the additive from the polythene sample increased in the order pellet < thin film < powder. Low results are obtained for extraction at room temperature which most probably correspond to inefficient extraction of the additive from the polymer matrix ; longer extraction times may improve the efficiency. Although the extraction procedure is time consuming, the most efficient extraction appears to be that of refluxing for about 20 hours.

The experiments described above were not subjected to further detailed investigation, yet they illustrate few operations which may be useful in the extraction and determination of the additive. Finally, it should also be possible to determine two or more additive compounds simultaneously by correct choice of excitation and emission wavelengths and phosphoroscope speeds.

CHAPTER 4

THE SPECTROFLUORIMETRIC DETERMINATION OF ORTHOPHOSPHATE AS RHODAMINE B MOLYBDOPHOSPHATE

4.1 Introduction

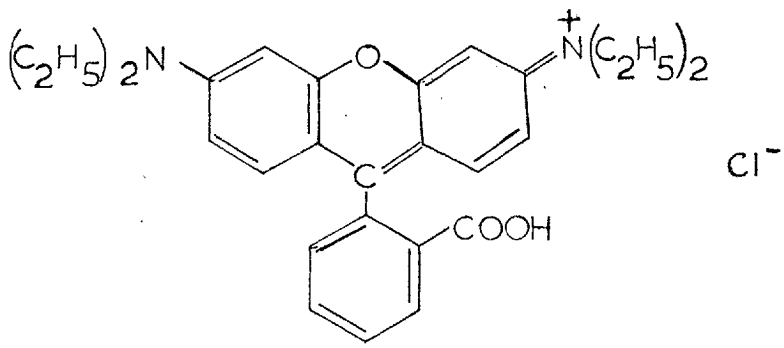
Most of the methods commonly used for the determination of orthophosphate are based on the absorption spectrophotometry in solution of molybdophosphoric[109-118] or molybdovanado phosphoric acids[119-122], or of their reduction products[118,123-131] in aqueous or organic media. These methods are neither highly sensitive nor selective, although selectivity can be obtained by solvent extraction of the heteropoly acid or by the use of masking agents. A variety of reagents has been used for the reduction of 12-molybdophosphoric acid after it had been extracted into an oxygen-containing organic solvent or into mixed solvents consisting of two oxygenated solvents or an oxygenated and non-oxygenated solvents. These include tin(II) chloride[118,123,124], ascorbic acid[126,128], 1-amino-2-naphthol-4-sulphonic acid[110,111,125,126], tin(II) oxalate[127], potassium antimonyl tartrate[128], titanium(III) chloride[129], iron(II)[130,131], and ferrocene[132]. Orthophosphate has also been determined by the determination of the molybdenum of the molybdophosphoric acid as peroxymolybdate by a spectrophotometric method[133]. The catalytic effect of orthophosphate on the reduction of molybdate has been studied for the determination of small amounts of phosphate[134-137]. Titrimetric methods have been used for the determination of phosphorus in which the yellow molybdophosphate is reduced to molybdenum blue and then titrated with potassium permanganate[138]. The phosphorus can also be extracted as molybdophosphoric acid into isobutyl acetate and, after back extraction, is passed through a silver reductor. The molybdenum(V) is then titrated with cerium(IV) sulphate[139]. In a further method for the determination of traces of phosphorus a double amplification

reaction is used, where the molybdophosphoric acid is extracted into isobutyl acetate and back extracted into ammonia solution, and molybdate is precipitated as 8-hydroxyquinoline molybdate. The precipitate is filtered off, dissolved in phosphoric acid and titrated with potassium bromate[140]. Gravimetric amplification methods have been described for the determination of phosphorus, by precipitation of the complex formed between molybdophosphoric acid and 8-hydroxyquinoline[141], quinoline[142-145], diantipyrylmethane[146], and N,N,N',N',-tetrakis-(2 hydroxybutyl)-ethylene diammonium diperchlorate[147]. Sensitive and selective procedures have been described for the determination of orthophosphate by indirect amplification, in which the twelve molybdate ions associated with each phosphate ion in the heteropoly-acid are determined by molecular[148-151] or atomic[152-156] absorption spectrophotometry after its extraction from excess molybdate reagent. The reagents used for the determination of molybdate by molecular absorption spectrophotometry include thiocyanate[148], 2-amino-4-chlorobenzene thiol[149], the azo dyes Acid Chrome Violet and Acid Chrome Black Special[150], and phenyl fluorone[151]. The application of the ion-association complexes formed between molybdophosphoric acid and various organic basic dyestuffs to the molecular spectrophotometric determination of orthophosphate has been studied by several workers. Babko and co-workers[157-159], from a study of the use of a range of dyestuffs in this way, recommended the use of crystal violet[157,159] and iodine green[157] for the extraction-spectrophotometric determination of phosphate. Spectrophotometric methods based on the molybdophosphate complexes of safranine[160,161], 8-quinolinol[162], brilliant green[163], malachite green[164,165], methyl green[166], and strychnine[167] have also been reported. The organic base quinine[168] has also been used for the determination of orthophosphates by a method in which the insoluble quinine molybdophosphate is precipitated and the volume of the precipitate is then measured. Investigations of the reaction product of

molybdophosphoric acid and strychnine[169], quinine[133], crystal violet[133] and rhodamine B[133] have been made by nephelometry with a view of determining the phosphorus content.

Several types of method for the determination of orthophosphate exploit the high sensitivity of fluorimetric techniques. The quenching by orthophosphate of the fluorescence of the complex formed between aluminium and morin has been used for the determination of orthophosphate[170]. The measurement of the fluorescence of the remaining excess of quinine and rhodamine B[133] after their reaction with molybdophosphoric acid has been used as a method of monitoring the phosphorus content of the solution. Sensitive spectrofluorimetric methods for phosphate based on the conversion of glycogen to glucose-6-phosphate in the presence of inorganic phosphate, activated phosphorylase and phosphoglucomutase have been described. The glucose-6-phosphate then reacts with the oxidised form of triphosphopyridine nucleotide[171] and nicotinamideadenosinediphosphate[172] to yield their reduced forms whose fluorescence is measured. In an attempt to exploit the potentially high sensitivity of solution spectrofluorimetry, while retaining the selectivity for the determination of phosphorus available from earlier work with its determination by indirect amplification methods, we have investigated the reaction of molybdophosphoric acid with the fluorescent dyestuff rhodamine B . The structure of rhodamine B is shown in Figure 4.1

Figure 4.1 : STRUCTURE OF RHODAMINE B



In the method for the determination of phosphorus described in this chapter, the fluorescence of the ion-association complex formed between molybdophosphate and rhodamine B is measured at 575 nm with an excitation wavelength of 350 nm, after its extraction into chloroform-butanol. When optimum conditions for the formation and extraction of molybdophosphate are employed, a selective and sensitive method for the determination of phosphate is obtained. A similar method for the determination of phosphate has been described[133] where the complex rhodamine B molybdophosphate is isolated, dissolved in ethanol and the fluorescence of rhodamine B associated with the molybdophosphate is measured.

4.2 Experimental

4.2.1 Apparatus

An Aminco-Bowman spectrophotofluorometer, as described in section 1.2.1, was used for the fluorescence measurements. Fused silica cells (10 x 10 x 48 mm) were employed. In order to obtain maximum sensitivity 5 mm slits, corresponding to ca 50 mm band pass, were used (Aminco slit arrangement No.5) in both the excitation and analysing monochromators.

One hundred millilitre separating funnels fitted with ground glass stoppers were used throughout. These were cleaned thoroughly in chromic-nitric acid mixture after each use.

4.2.2 Reagents

Standard Phosphorus solution : 0.1258 g of sodium dihydrogen orthophosphate dihydrate, $NaH_2PO_4.2H_2O$, (AnalaR grade) was dissolved in distilled water and diluted to one litre. This solution contains 25 $\mu g\ ml^{-1}$ of phosphorus and was diluted when necessary to 0.1 or 1.0 μg P per ml with distilled water.

Molybdate Solution : 30 g of sodium molybdate dihydrate, $Na_2MoO_4.2H_2O$, (AnalaR grade) was dissolved in distilled water and diluted to one litre. From this solution, a reagent solution was prepared for use, by the following procedure : To 250 ml of the molybdate solution an equal volume of distilled water and sufficient concentrated hydrochloric acid (AnalaR grade) was added to make the solution approximately 1 M with respect to acid. The solution was allowed to stand for 10 minutes and then shaken twice with 100 ml aliquots of chloroform-butanol extractant reagent. The phases were allowed to separate and the organic phase was discarded each time. Finally, the aqueous solution was shaken four times with 100 ml aliquots of chloroform to remove dissolved butanol, and the chloroform phases were discarded. The aqueous phase was stored in a polythene container for use as the molybdate reagent.

Rhodamine B Solution : 0.115 g of rhodamine B (CI 45170) (Hopkin and Williams Ltd., Chadwell Heath) was dissolved in distilled water and diluted to one litre.

Extractant : A 4:1 v/v mixture of analytical reagent grade chloroform and 1-butanol was used.

Hydrochloric Acid : AnalaR reagent (Hopkin and Williams Ltd.) was used.

Diverse Ions : Stock 1000 $\mu g\ ml^{-1}$ solutions of foreign ions were prepared by dissolution of their analytical reagent grade salts in distilled water or dilute hydrochloric acid.

4.3 Results and Discussions

Rhodamine B is extracted readily into most organic solvents from acid solutions. The fluorescence characteristics of the ion-association complex formed between molybdophosphate (MPC) and Rhodamine B (RhB) are similar to those of RhB reagent itself. When the rhodamine B molybdophosphate

(RhBMPC) is to be extracted prior to measurement of its fluorescence, therefore, the excess RhB reagent must be first selectively extracted from the solution. For this purpose, a brief study was made on the use of several organic solvents including chloroform, carbontetrachloride, diethyl ether, ethyl acetate , ethyl acetoacetate, n-butanol, iso-butyl acetate, dioxan, cyclohexanol, benzene, toluene, acetophenone and o-dichlorobenzene. Chloroform was found to be an effective solvent for the extraction of excess RhB reagent. Although the distribution coefficient for RhB between chloroform and water is not very high at the acidity employed, so that several extractions are required, chloroform does not extract the RhBMPC complex. In preliminary experiments two extractions with 10 ml aliquots of chloroform were found to reduce the reagent blank to a constant low value. In all experiments concerned with the establishment of optimum conditions for the phosphate determination, therefore, after formation of RhBMPC in the presence of excess molybdate and RhB in acid medium, the excess RhB was removed by extraction with two 10 ml aliquots of chloroform before extraction of RhBMPC into chloroform-butanol and measurement of its fluorescence.

4.3.1 Spectral Characteristics

The fluorescence excitation and emission spectrum for a 2.4×10^{-6}M RhB solution in chloroform-butanol (4:1 v/v) after extraction from 1 M.HCl is shown in Figure 4.2a. The absorption band at 350 nm is used to stimulate fluorescence at 565 nm ; when the higher wavelength band at 550 nm is employed for excitation, high blank readings due to scattered light are obtained when the fluorescence emission at 565 nm is measured with wide slit widths. In addition, the wavelength of the 550 nm excitation maximum varies with the RhB concentration. Figure 4.2b shows the fluorescence excitation and emission spectrum of the RhB.MPC extracted into chloroform-butanol after formation in a 1M.HCl solution containing 0.5 µg P.

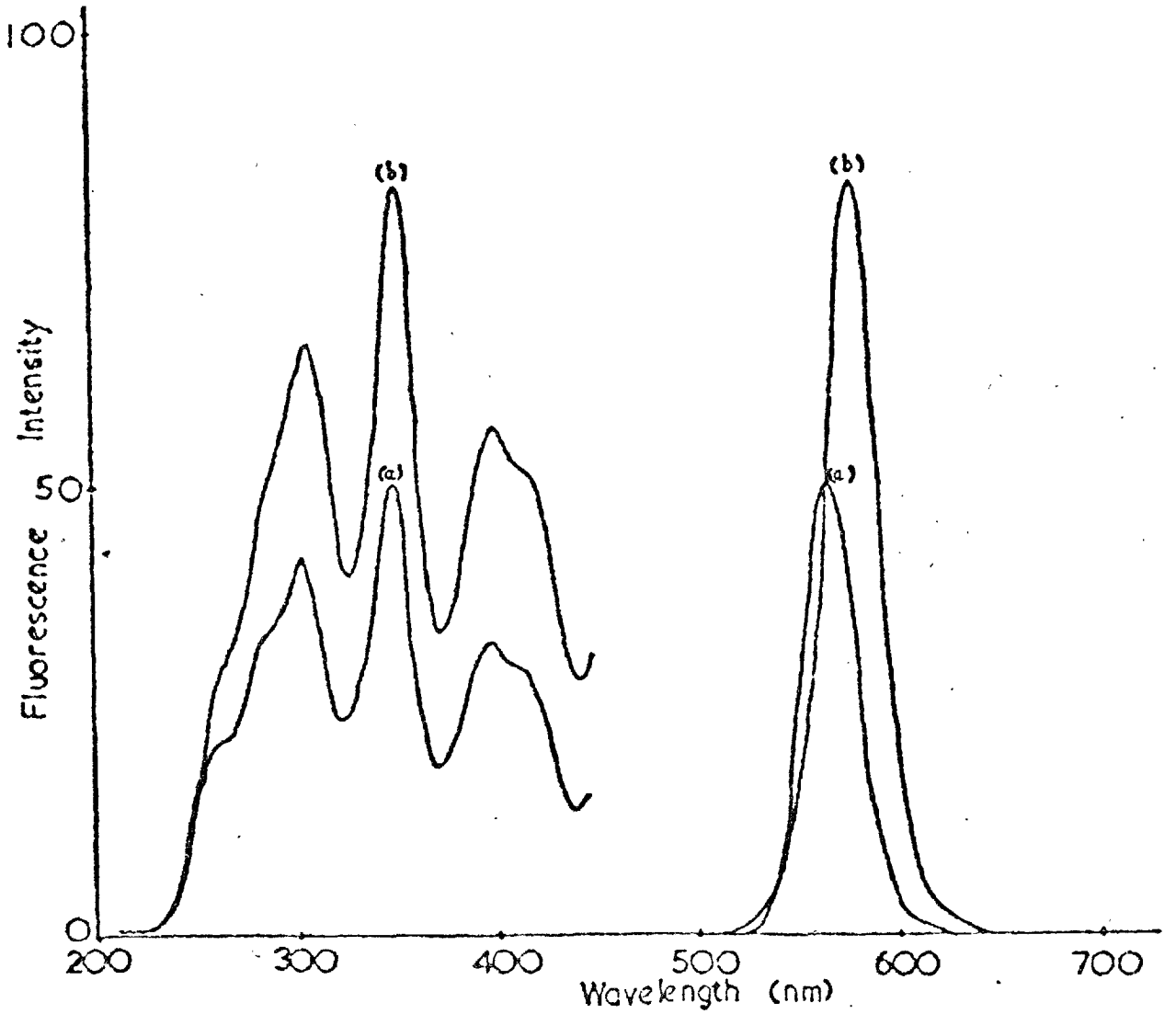


Figure 4.2 Uncorrected excitation and emission spectra in butanol/$CHCl_3$ of:

(*a*) Reagent (RhB) alone (2.4×10^{-6} *M*), and

(*b*) Molybdophosphate RhB complex (equivalent to 0.5 μg of P)

The appearance of the spectrum is similar to that of RhB itself, there being only a slight shift in the fluorescence emission maximum to 575 nm. This might suggest that the fluorescence measured for analytical purposes is that of free RhB itself, after its liberation from the RhB.MPC ion-association complex on extraction into chloroform-butanol. Alternatively, if the RhB.MPC complex is stable in chloroform-butanol, the inference is that the ion-association between RhB and the molybdophosphate anion does not alter the spectral absorption or emission characteristics of the RhB significantly. In practice, the similarity of spectral characteristics of the RhB and its molybdophosphate does not constitute a disadvantage by the existence of a high blank fluorescence, as the excess RhB is extracted into chloroform before extraction of the RhB.MPC into chloroform-butanol. The excitation and emission spectra of RhB and RhB.MPC in chloroform-butanol shown in Figure 4.1, are uncorrected for variation in detector sensitivity, xenon arc lamp emission or grating emission with wavelength. The spectra were plotted on the same scale of the photomultiplier microphotometer.

4.3.2 Effects of Hydrochloric Acid, Rhodamine B and Molybdate Concentrations

The conditions employed initially for the formation of MPC were similar to those used by Wadelin and Mellon[109]. The experimental study of the effect of hydrochloric acid concentration in the aqueous phase on the formation of RhB.MPC was conducted using 7.5 μg of P in 0.3 ml of aqueous solution to which 10 ml of 0.24 mM RhB solution and 20 ml of diluted molybdate reagent solution (1:1) were added in the presence of varying molarities of hydrochloric acid. The excess RhB reagent was removed by three extractions with 10 ml aliquots of chloroform before final extraction of RhB.MPC with 25 ml of chloroform-butanol. The effect of the hydrochloric acid molarity in the original solution on the fluorescence intensity measured in the final chloroform-butanol extract is shown in Figure 4.3. A concentration of 1 M HCl was

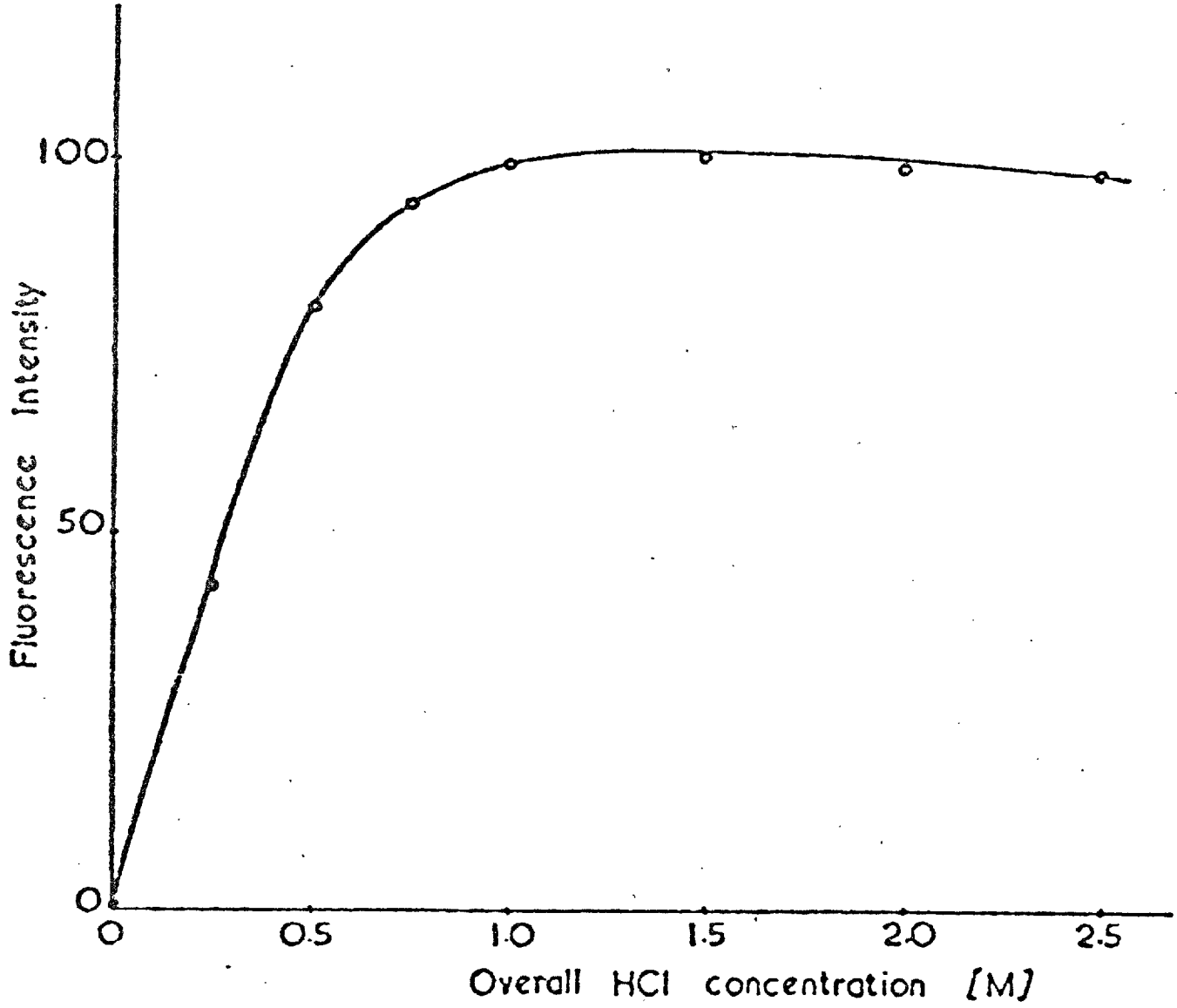


Figure 4.3 Effect of hydrochloric acid concentration on fluorescence in chloroform–butanol

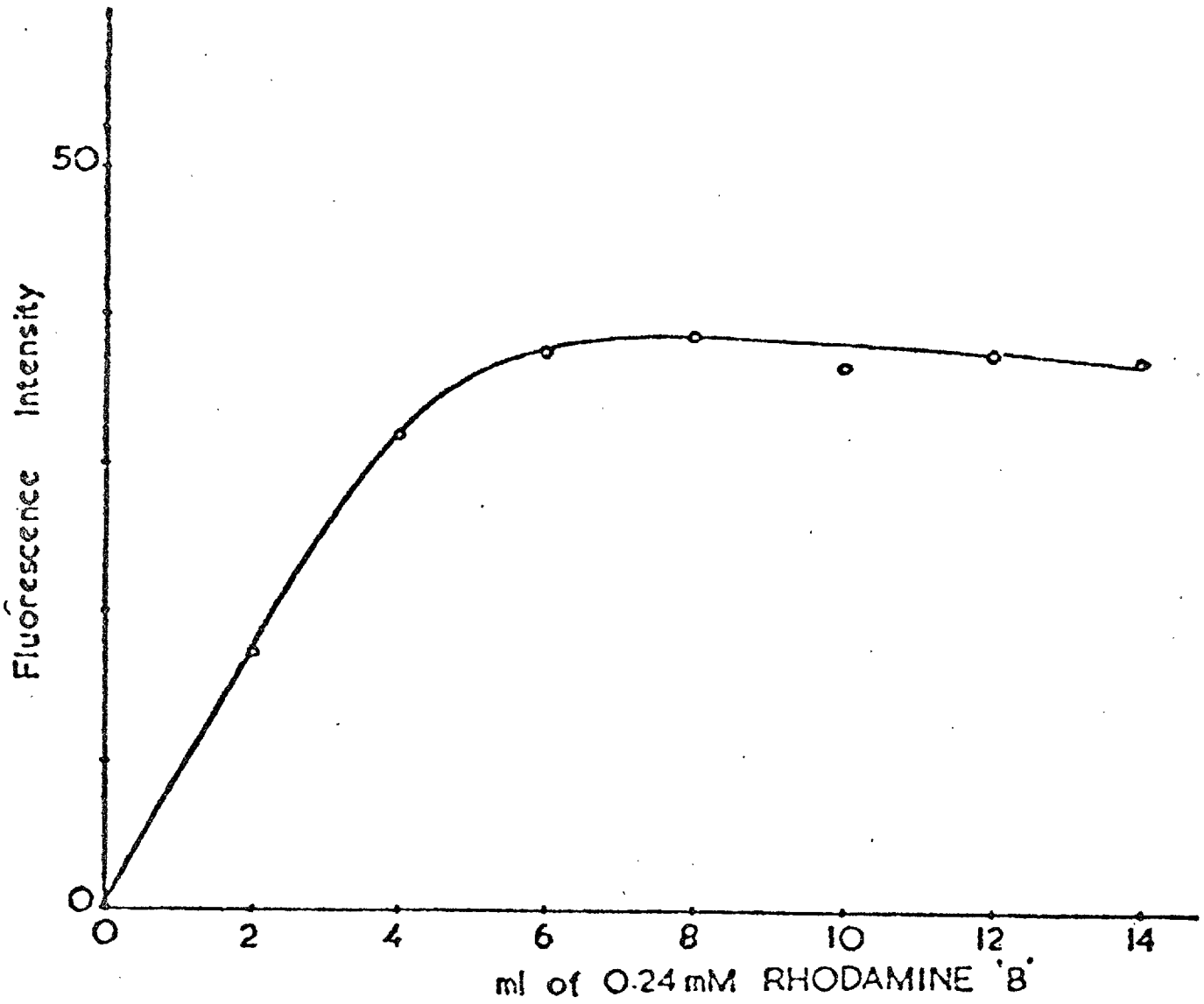


Figure 4.4 Effect of Rhodamine B reagent concentration on fluorescence in chloroform–butanol

chosen as the optimum in the initial solution and was used in all further work.

The optimum RhB concentration was determined similarly using 12.5 µg of P in 0.5 ml of aqueous solution, 20 ml of the diluted molybdate reagent (1:1) and an overall acidity of 1 M.HCl. The excess RhB, if any, was removed as described above by three extractions with 10 ml aliquots of chloroform and the RhB.MPC complex was extracted with 25 ml of chloroform-butanol. As shown in Figure 4.4 the fluorescence increases up to 8 ml of 0.24 mM RhB and this remains constant when the reagent concentration is increased further. Ten millilitres of 0.24 mM RhB solution was therefore chosen for use ; this represents an approximately 90-fold weight excess over 1 µg of P. This rhodamine B concentration was used for further work, although to permit the determination of small weights of phosphorus the volume of all the reagents was halved compared to the above experiments.

A significantly lower reagent blank was obtained in the procedure if the molybdate reagent was prestripped of phosphate impurities before use. This procedure, which involves the formation of molybdophosphoric acid and its extraction from the molybdate solution, is described in the experimental section. The molybdate reagent concentration employed was that shown in an earlier work[153] to result in quantitative formation of molybdophosphoric acid with microgram amounts of phosphorus. Thus 5 ml of 56 mM molybdate reagent solution was used in all experiments.

4.3.3 Extraction of Excess Rhodamine B Reagent and Rhodamine B Molybdophosphate

In order to obtain low blank fluorescence in the determination of phosphorus it was necessary to remove the excess RhB reagent before extraction of RhB.MPC into chloroform-butanol. The number of chloroform extractions required to reduce the reagent blank to a low value was determined. With the optimum conditions of acidity and RhB and molybdate concentrations, and in the absence of phosphorus, the fluorescence of the RhB reagent was measured in the organic

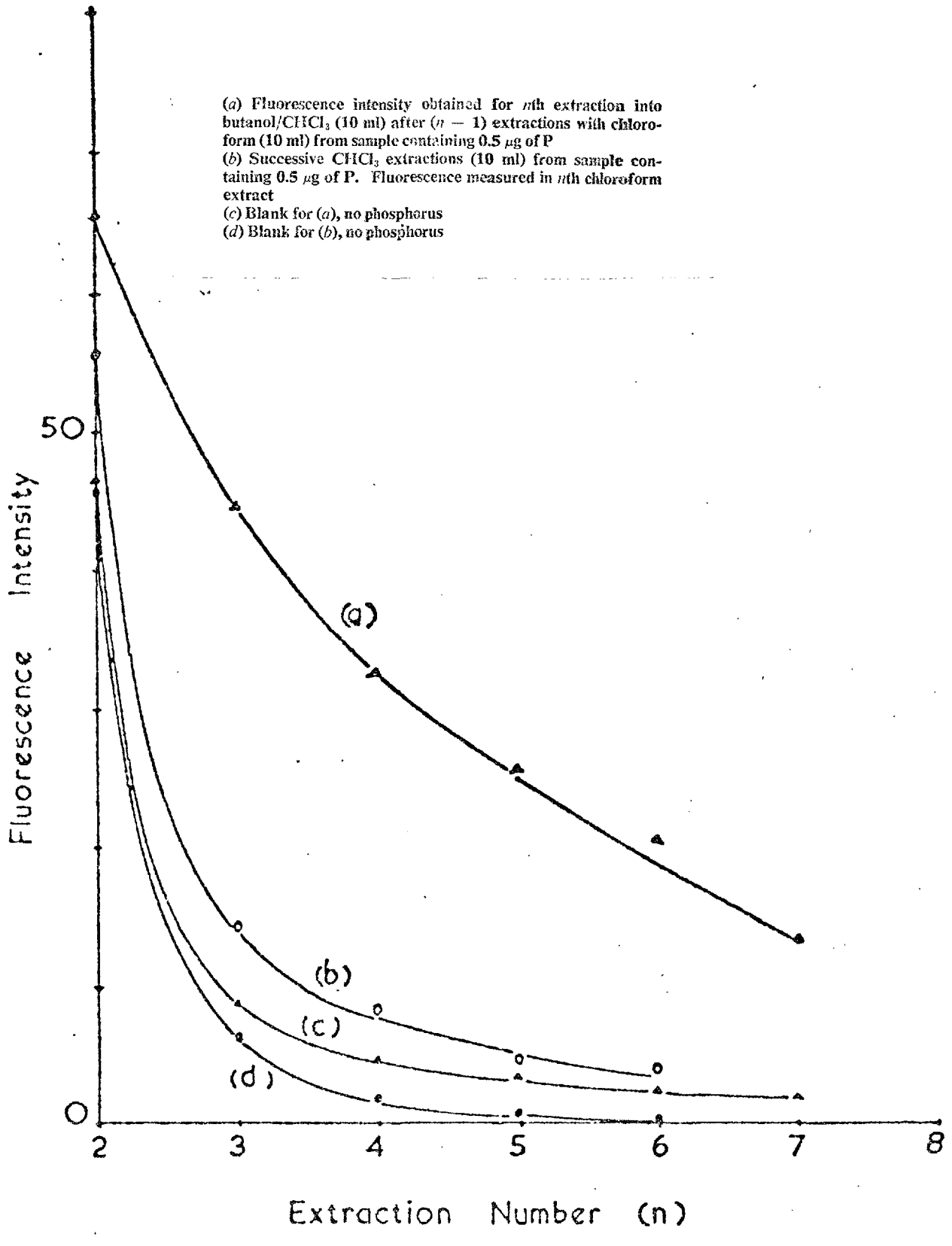


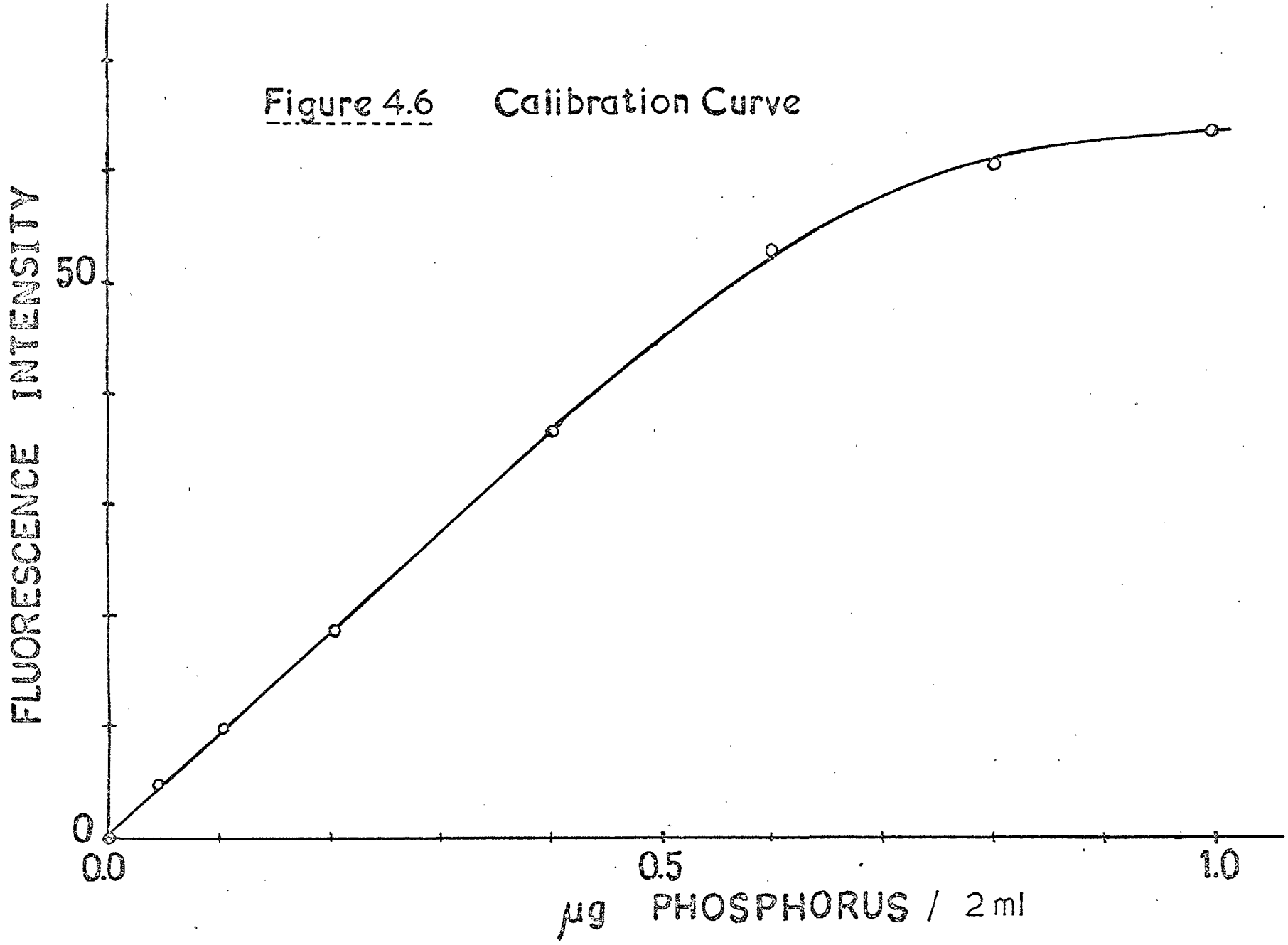
Figure 4.5 Variation in fluorescence intensity with number of extractions with chloroform

phases obtained on repetitive extraction with 10 ml aliquots of chloroform. As shown in Figure 4.5, the excess RhB is extracted from the blank solution effectively with two 10 ml aliquots of chloroform. It is also evident from Figure 4.5, that when the experiment is repeated in the presence of 0.5 µg of phosphorus, the fluorescence in the chloroform phase is still appreciable after two extractions and then decreases slowly with further extractions. It appears that after extraction of the excess free RhB by two extractions with two 10 ml aliquots of chloroform, RhB formed by progressive dissociation of RhB.MPC is removed when further chloroform extractions are made. The ion-association complex itself is not extracted into chloroform; no molybdenum was detectable in the chloroform phase after extraction. The highest net fluorescence signal intensity ratio was observed in the final chloroform-butanol extract after two extractions with 10 ml aliquots of chloroform. It was also observed that a single extraction with a 10 ml aliquot of chloroform-butanol extractant solvent was sufficient to extract the RhB and its associated molybdophosphoric acid quantitatively from the residual aqueous phase after the chloroform extractions.

4.3.4 Calibration Graph for Phosphorus

To 100-ml separating funnels, 5 ml of prepared molybdate reagent solution, 0.5 ml of concentrated hydrochloric acid and 5 ml of rhodamine B solution were added. 0, 0.04, 0.1, 0.2, 0.4 and 0.6 µg of phosphorus (each in 2 ml of distilled water) was added to the funnels, and the solutions were mixed and allowed to stand for 10 minutes. 10 ml of chloroform was then added to each funnel and the excess RhB reagent was extracted. After allowing the phases to separate, the chloroform phase was carefully separated and discarded. The chloroform extraction was repeated, and the rhodamine B molybdophosphate was extracted using 10 ml of chloroform-butanol extractant from the aqueous

Figure 4.6 Calibration Curve



phase. The phases were separated and an aliquot of the organic phase was transferred to a clean spectrofluorimeter cell. The fluorescence intensity of the solution was measured at 575 nm with an excitation wavelength of 350 nm. Figure 4.6 shows the calibration graph obtained after subtraction of the reagent blank prepared simultaneously.

4.3.5 Sensitivity and Precision

With the optimum conditions used in the procedure described, the calibration graph is linear in the range 0.04, to 0.6 µg of phosphorus in the original 2 ml sample solutions, i.e. 0.02 to 0.3 µg per ml. The blank fluorescence is low and reproducible; the use of prestripped molybdate reagent, from which phosphate impurity has been removed, results in a considerably lower blank value than when untreated reagent is employed. Appreciable curvature of the calibration graph towards the concentration axis occurs when more than 0.6 µg of P is determined under the recommended conditions. The use of higher molybdate and rhodamine B concentrations does not extend this upper limit; concentration quenching of the rhodamine B fluorescence appears to be responsible for this effect. This was demonstrated by the construction of a calibration graph for rhodamine B reagent alone in chloroform-butanol extractant reagent; the graph of fluorescence intensity against rhodamine B concentration became curved towards the concentration axis at concentrations similar to those produced when more than 0.6 µg of P was determined.

When 0.5 µg of phosphorus in 2 ml of sample solution was determined ten times by the recommended procedure, a coefficient of variation of $\pm$ 3.9% was obtained. The results are shown in Table 4.1.

Table 4.1 : DATA FOR PRECISION STUDY OF 0.5 μg OF P

Sample	Fluorescence Intensity (Scale x 0.03)	Net Fluorescence Intensity (x)	Deviation ($x - \bar{x}$)	Variance ($x - \bar{x}$)^2
1	37.0	31.0	-1.1	1.21
2	38.0	32.0	-0.1	0.01
3	38.0	32.0	-0.1	0.01
4	40.0	34.0	+1.9	3.61
5	37.0	31.0	-1.1	1.21
6	36.0	30.0	-2.1	4.41
7	40.0	34.0	+1.9	3.61
8	38.0	32.0	-0.1	0.01
9	38.0	32.0	-0.1	0.01
10	39.0	33.0	+0.9	0.81
Blank	6.0	-		
		Mean ($\bar{x}$) = 32.10		Total = 14.90

Standard Deviation = $\sqrt{\frac{(x-\bar{x})^2}{n-1}} = \sqrt{\frac{14.9}{9}} = 1.28$; Coefficient of Variation = $\frac{1.28}{32.1} \times 100 = 3.9\%$.

4.3.6 Order of Addition of Reagents and Time of Complex Formation

The order of addition of reagents which was found to enable rapid complex formation is as described in the procedure given above (section 4.3.4). In this method, the phosphorus sample solution is added after the acid, molybdate and RhB solutions. Under these conditions, complex formation is complete in the initial aqueous solution after 5 minutes, so that the excess RhB may be extracted at any time after this. In practice, when several sample and standard solutions are to be treated, it is convenient to allow 10 minutes after mixing the reagents before the excess reagent is extracted.

4.3.7 Effect of Time on Fluorescence Intensity

The variation of fluorescence intensity of RhB.MPC in chloroform-butanol, formed from 0.5 µg of phosphorus by the recommended procedure, with time was investigated. No change in fluorescence intensity was observed when the solution was allowed to stand for two hours in darkness or in normal laboratory conditions, i.e. under fluorescent tube lighting. Continuous irradiation of the solution at 350 nm by xenon arc in the spectrofluorimeter for a period of one hour caused a decrease in fluorescence intensity at 575 nm of 4.5%; correspondingly an increase in temperature of 3°C was measured in the solution. When cooled to room temperature (~ 30 minutes), the above solution retained its original fluorescence intensity. This reduction in fluorescence intensity due to irradiation, therefore, was due to increase in temperature; the temperature coefficient was -1.5% per °C. A change in fluorescence intensity of +3.3% was observed when the solution was allowed to stand for 24 hours in normal laboratory conditions. These results indicate that the fluorescence of the solution of RhB.MPC in chloroform-butanol is quite stable, certainly over the time required for measurement of the fluorescence emission intensity of sample solutions.

4.3.8 Effects of Foreign Ions

The effect of 100-fold weight excesses of 37 foreign ions on the fluorescence intensity obtained in the final chloroform-butanol extract for 1 μg of phosphorus determined by the recommended procedure was investigated. A foreign ion was considered to interfere when it produced an error in fluorescence intensity compared to that for 1 μg of P alone of greater than twice the coefficient of variation (i.e. 8%). The following ions did not interfere at the 100-fold weight excess level under these conditions : aluminium, antimony(III), bismuth, cadmium, calcium, cerium(IV), cobalt(II), copper(II), germanium, gold, iron(III), lead, magnesium, manganese, mercury(II), nickel, selenium(IV), silicon, silver, tellurium(IV), thallium(III), thorium, tin(IV), titanium(IV), tungsten(VI), zinc, zirconium, fluoride, nitrate, perchlorate, sulphate and EDTA. The presence of a 100-fold excess of the following ions caused the error given in parenthesis : arsenic(V), (+ 150%); arsenic(III), (+ 28%); vanadium(V), (+ 20%); chromium(VI), (+ 16%); chromium(III), (- 12%). The presence of arsenic(III) and vanadium(V) was tolerable at 25-fold and 50-fold weight excesses, respectively, whereas arsenic(V) interferes seriously even when present only at equal weight with phosphorus, i.e . 1 μg. Chromium(III) may be present at 50-fold excess without interference. Silicon as silicate does not interfere; the acidity employed(1 M) is unfavourable for the formation of molybdosilicate, and chloroform-butanol (4:1) is known not to extract this heteropoly acid even if it is formed[109].

4.3.9 Accuracy

A measure of the accuracy of the method was obtained by the determination of phosphorus in synthetic solutions containing foreign ions which were treated as unknown samples. Two standards and a blank were prepared and measured with each group of samples. The results of these analyses are shown in Table 4.2.

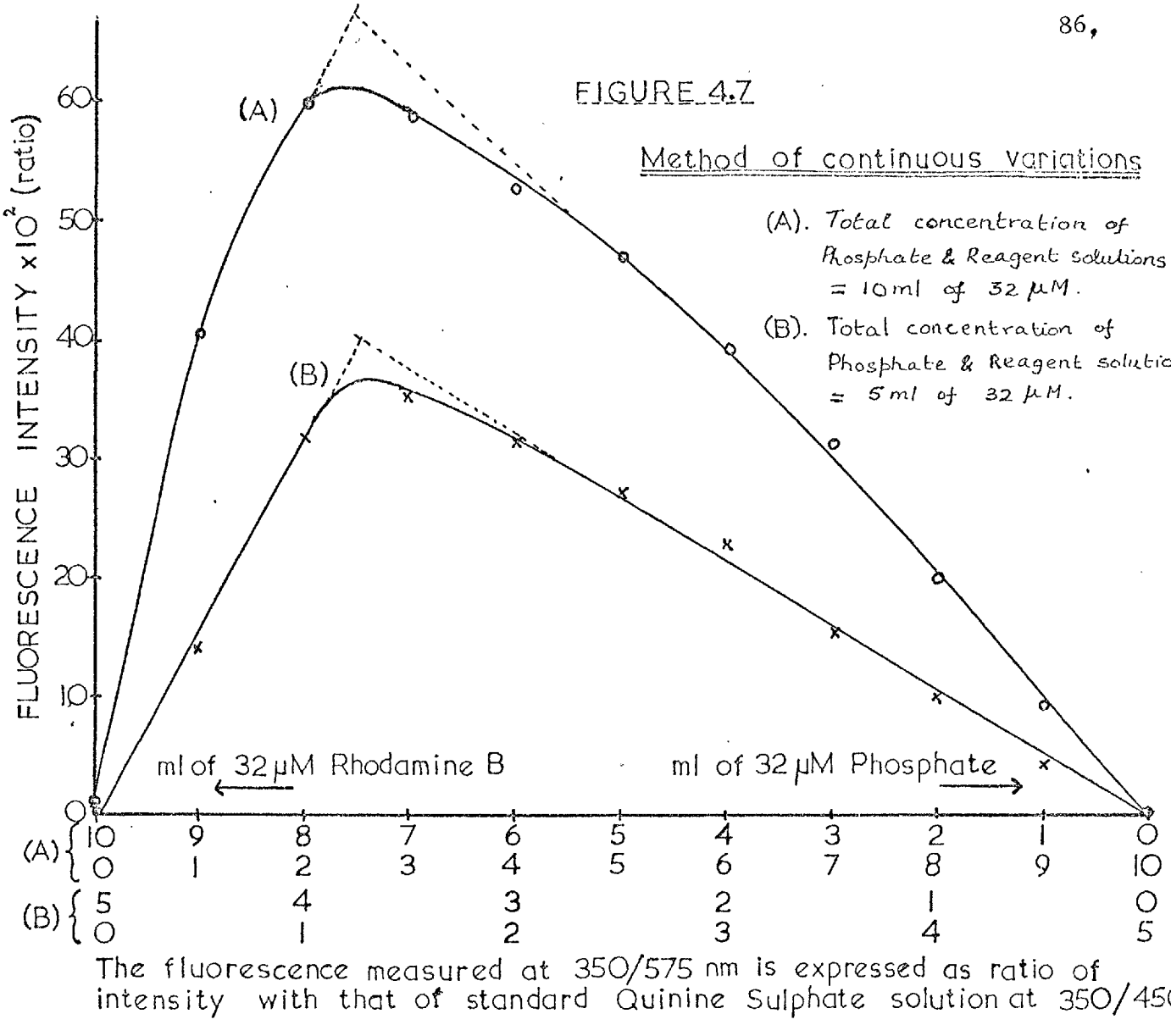
Table 4.2 Determination of Phosphorus in Synthetic Mixtures Treated as Unknowns

Phosphorus taken, μg	Phosphorus found, μg[a]	Error, %	Foreign ions present (weight excess over P)
0.150	0.152	+1.33	Ca(20), Fe(10), Cu(15)
0.300	0.295	−1.67	Al(30), Si(30)
0.300	0.296	−1.33	Zn(30), Pb(20)
0.400	0.420	+5.00	As^{III}(10), Cd(20)
0.250	0.260	+4.00	Se(20), Te(20)
0.200	0.200	0.0	Bi(15), Cu(20)
0.350	0.335	−4.30	W(20), Sb(25), Ge(25)
0.080	0.079	−0.62	Si(15), Ni(10)

[a] Each result is the average of two determinations in good agreement.

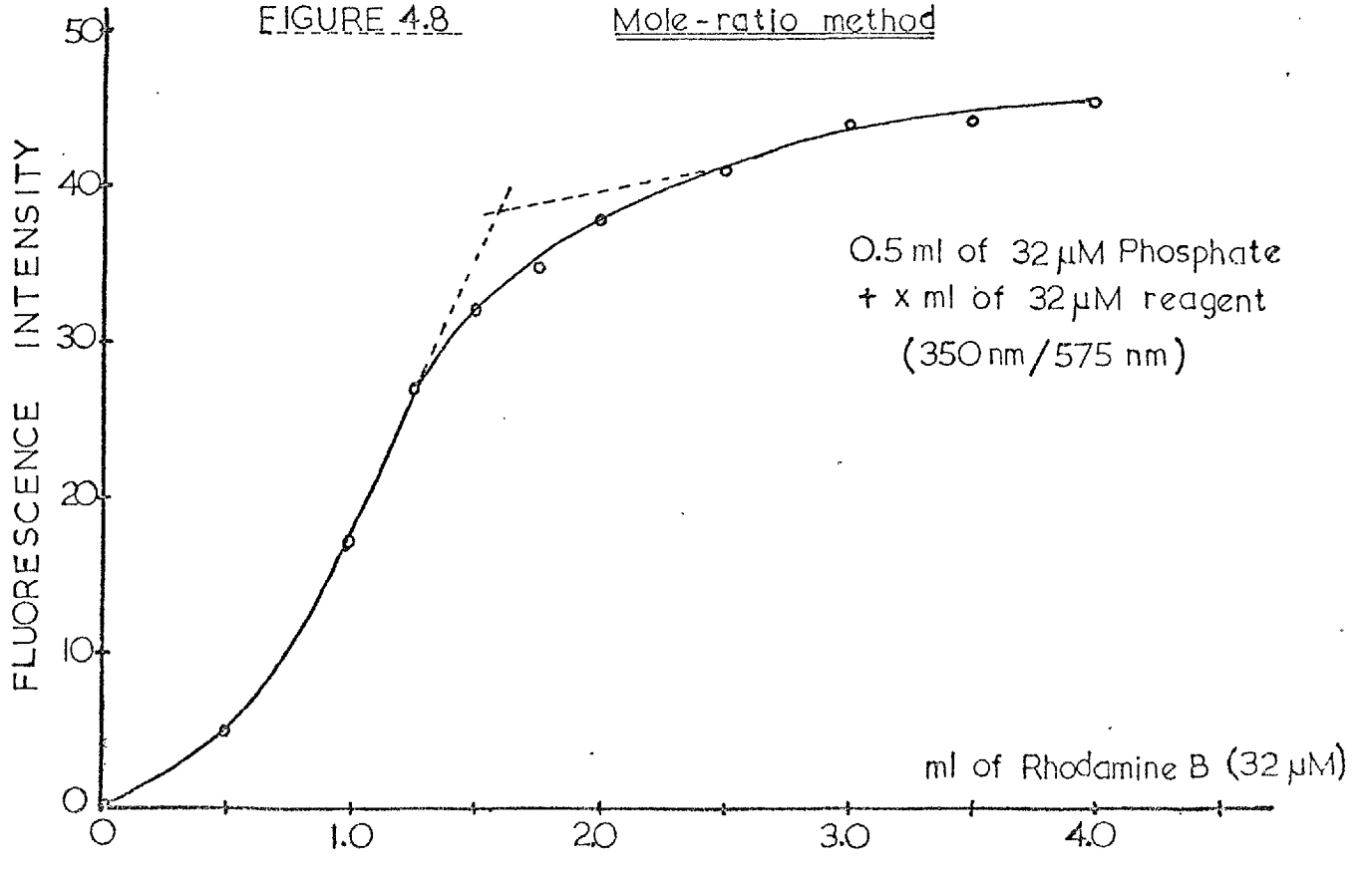
FIGURE 4.7

Method of continuous variations



The fluorescence measured at 350/575 nm is expressed as ratio of intensity with that of standard Quinine Sulphate solution at 350/450 nm

FIGURE 4.8 Mole-ratio method



4.3.10 Nature of Ion-Association Complex

The nature of the rhodamine B molybdophosphate complex was investigated by the continuous variations[173] and mole-ratio[174] procedures with the established optimum conditions of acid concentration, etc., and using the recommended extraction procedure. The experiments were conducted using 20 ml of diluted molybdate reagent (1:1) and 32 μM solutions of orthophosphate and rhodamine B. Figure 4.7 shows the curves obtained by the method of continuous variations and Figure 4.8 that obtained by the mole-ratio method. The results indicate a combining ratio of 3 moles of rhodamine B to 1 mole of molybdophosphate in the ion-association complex. This is in accordance with the formation of an unchanged complex of the type $[RhB^+]_3 [PMo^{3-}]$. An estimate of the apparent overall formation constant of the complex was obtained via experiments in which the rhodamine B recovered in the final butanol-chloroform phase from the complex after its formation from known amounts of phosphorus was determined fluorimetrically. Allowance for depletion of the rhodamine B concentration in the final butanol-chloroform phase due to the chloroform extractions, which promote the dissociation of the complex by removal of the excess reagent, was made. The overall cumulative formation constant, K, defined as

$$K = \frac{[RhB.MPC]}{[MPC] \cdot [RhB]^3} \qquad (4.1)$$

under these conditions was estimated to be $(1.6 \pm 0.3) \times 10^{11}$ litre^3 mole^{-3}.

4.4 Conclusions

High sensitivity is obtained for the determination of phosphorus by the spectrofluorimetric method developed. The sensitivity achieved is higher than that for most other methods based on the formation of molybodophosphoric acid, and compares favourably with that of more complex enzymatic methods.

High selectivity is achieved with the optimum conditions established. Large amounts of silicate do not interfere with the determination of phosphate. Of the ions investigated, only arsenic(V) interferes seriously, by formation and extraction of the corresponding rhodamine B molybdoarsenate; arsenic(III) and vanadium(V) may be tolerated when present in moderate excess with respect to phosphorus.

CHAPTER 5

THE SPECTROFLUORIMETRIC DETERMINATION OF ORTHOPHOSPHATE AS QUININEMOLYBOPHOSPHATE

5.1 Introduction

Most procedures for the determination of orthophosphate employ spectrophotometric determination of molybdophosphoric acid, reduced molybdophosphoric acid (molybdenum blue), and molybdophosphate complexes of aromatic amines and basic dyestuffs in a suitable, often organic, medium[109-168]. Several types of methods for the determination of phosphate using spectrofluorimetric techniques involve the use of quenching of fluorescence by phosphate of the fluorescing system[133,170] or of enzymic reactions[171,172]. These methods have been mentioned in the previous chapter. In an attempt to combine in one method the advantages of the methods based on the formation of ion-association complexes between organic bases and molybdophosphoric acid with those of a spectrofluorimetric finish to the determination, a sensitive and selective procedure for the determination of orthophosphate based on the formation of the complex between rhodamine B and molybdophosphate has been discussed in Chapter 4. In this chapter, a similar method is described that has the additional advantage that the final fluorescence measured is that of the organic base quinine; by virtue of its high fluorescence intensity and stability in solutions of sulphuric acid, quinine is frequently used as a standard for measurements of luminescence quantum efficiency and for the calibration of spectrofluorimeters. The structure of the organic base quinine is shown in Figure 5.1 Quinine has been used previously for the determination of orthophosphate by precipitation of quinine molybdophosphate and measurements of the volume of the precipitate[168] or by a nephelometric method[133]. In the procedure described in this chapter, quinine molybdophosphate is precipitated

in an acidic medium, washed free from excess quinine and dissolved in acidified acetone. The fluorescence of the solution is then measured at 445 nm with an excitation wavelength of 352 nm to provide a sensitive and selective method for the determination of orthophosphate.

5.2 Experimental

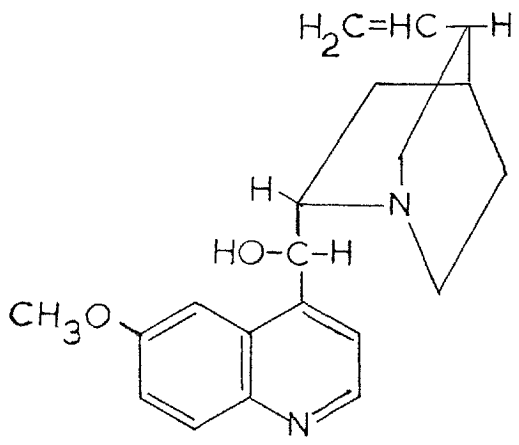
5.2.1. Apparatus

The Aminco-Bowman Spectrophotofluorometer described in section 1.2.1 was employed. Fused silica cells (10 x 10 x 50 mm) were used. Slits of width 3 mm (Aminco slit arrangement No.3, spectra band width 30 nm) were used in both the excitation and analysing monochromators.

Pyrex glass centrifuge tubes of 10 ml capacity were used throughout. These were cleaned thoroughly in a 4% solution of Decon (Decon Laboratories Ltd., Brighton) after each use.

Atomic absorption measurements were carried out using nitrous oxide-acetylene flame on a Techtron AA4 flame spectrophotometer, fitted with a Techtron high temperature burner and molybdenum high intensity hollow-cathode lamp. The instrument settings used for the determination of molybdenum were : lamp current, 10 mA; slit width, 100 μm; wavelength, 313.2 nm.

FIGURE 5.1 : Structure of Quinine



The pH measurements were made using a Model 23A pH meter (Electronic Instruments Ltd) using a glass electrode with a calomel electrode as the reference electrode. The solution was contained in a closed cell assembly fitted with a stirrer.

5.2.2 Reagents

Phosphate Solution : 0.1258 g of sodium dihydrogen orthophosphate dihydrate, $NaH_2PO_4.2H_2O$ (AnalaR grade) was dissolved in distilled water and diluted to 1 litre. This solution contains 25 $\mu g\ ml^{-1}$ of phosphorus and was diluted when necessary to 0.1 or 1.0 $\mu g\ ml^{-1}$ with distilled water.

Molybdate Solution : 30 g of sodium molybdate dihydrate, $Na_2MoO_4.2H_2O$, (AnalaR grade) was dissolved in distilled water and diluted to 1 litre. From this solution a phosphate-free molybdate reagent solution was prepared by the procedure described in section 4.2.2, using concentrated sulphuric acid (AnalaR grade) instead of concentrated hydrochloric acid making the solution approximately 0.5M with respect to the acid. The aqueous molybdate reagent was stored in a polythene container.

Quinine Sulphate Solution : 7.83 g of quinine sulphate, $(C_{20}H_{24}N_2O_2)_2.H_2SO_4.2H_2O$, (BDH Chemicals Ltd., Poole, Dorset) was dissolved in a few drops of concentrated sulphuric acid and diluted to 1 litre with distilled water. The overall acid concentration was approximately 0.05 M with respect to sulphuric acid.

Sulphuric Acid : AnalaR reagent (Hopkin and Williams Ltd) was used.

Acetone - 0.5 M sulphuric Acid Solvent (9:1 v/v) AnalaR grade acetone and sulphuric acid were used.

Foreign Ions : Stock 1000 $\mu g\ ml^{-1}$ solutions of the foreign ions were prepared by dissolution of their salts (analytical-reagent grade) in distilled water or dilute hydrochloric acid.

Wash Solution : A 0.5 M solution of sulphuric acid in distilled water was used.

5.3 Results and Discussions

During the initial qualitative examination of the procedure for the detection of orthophosphate as quinine molybdophosphate, various organic solvents were tested for their suitability for the dissolution of the quinine molybdophosphate precipitate and as solvents for the fluorescence measurement. It was observed that while several solvents were suitable for the dissolution step, including acetone and ethanol, the fluorescence intensity observed after dissolution was low. The addition of dilute sulphuric acid to these solutions, however, resulted in considerable enhancement of the fluorescence of the dissolved material in the organic solvent. Acetone, to which the dilute sulphuric acid had been added initially, was chosen as solvent for the precipitate, and this mixture was used in all the experiments described below. During the initial qualitative studies, it was also realised that in order to achieve low blank fluorescence signals when small volumes of precipitate were to he handled, it would be necessary to wash the precipitate free from excess of reagent before its dissolution. This was undertaken in all subsequent quantitative work by washing the precipitate with 0.5 M sulphuric acid before its dissolution.

5.3.1 Spectral Characteristics

Curves (b) in Figure 5.2 show the fluorescence excitation and emission spectra obtained after formation of the quinine molybdophosphate precipitate from 0.5 µg of phosphorus and dissolution of the precipitate in the acetone-sulphuric acid solvent. The wavelengths of maximum excitation and emission

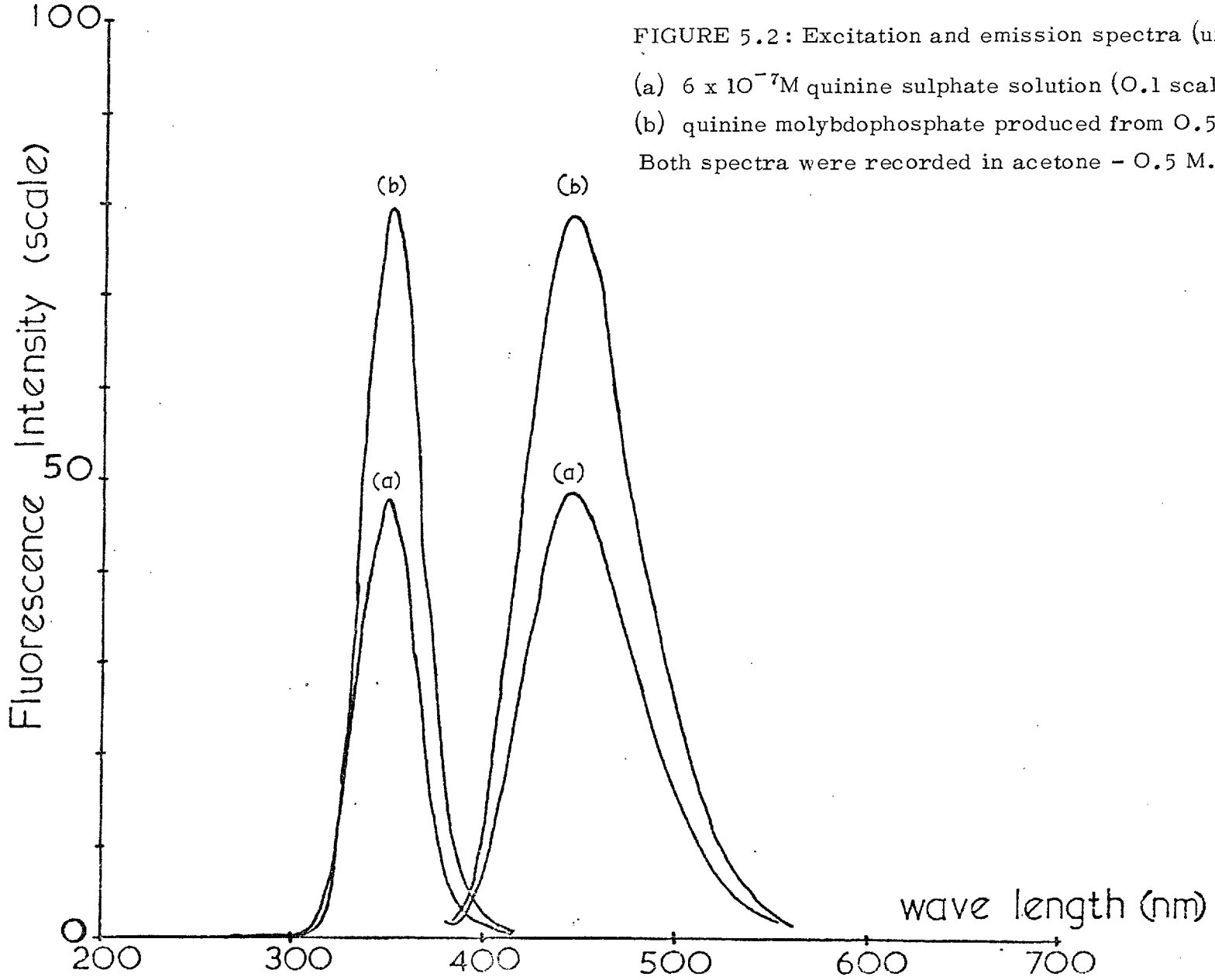


FIGURE 5.2 : Excitation and emission spectra (uncorrected) for :

(a) 6×10^{-7}M quinine sulphate solution (0.1 scale); and

(b) quinine molybdophosphate produced from 0.5 µg of P (0.3 scale).

Both spectra were recorded in acetone - 0.5 M.H_2SO_4 (9 + 1 v/v)

observed in this solvent were 352 nm and 445 nm, respectively. It is apparent that the spectral characteristics are identical to those exhibited by quinine sulphate itself in this solvent; Curves (a) show the excitation and emission spectra for a 6×10^{-7} M solution of quinine sulphate in the same solvent. This suggests that the fluorescence measured is that of free quinine sulphate itself, and that the acetone-sulphuric acid mixture serves to decompose the quinine molybdophosphate. There is no evidence to suggest that the quinine forms a stable complex with molybdophosphate rather than a simple insoluble salt in aqueous medium. The similarity between the spectral excitation and emission characteristics of the quinine sulphate and the sample fluorescence does not constitute a disadvantage provided that the excess of reagent used to precipitate the quinine molybdophosphate is removed by washing before dissolution of the precipitate.

5.3.2 Effects of the Concentrations of Sulphuric Acid, Quinine Sulphate and Molybdate reagent

The conditions initially used for the formation of molybdophosphoric acid were similar to those used by Wadelin and Mellon[109]. The experiments for the study of the effect of the concentration of the sulphuric acid in the aqueous phase on the formation of quinine molybdophosphate were carried out by using 2 µg of phosphorus in 2 ml of aqueous solution to which 2 ml of molybdate reagent and 1 ml of sulphuric acid of various molarities were added. The quinine molybdophosphate was precipitated by adding 1 ml of quinine sulphate reagent, washing with 5 ml of 0.5 M sulphuric acid and dissolved in 5 ml of acetone-sulphuric acid solvent. The effect of the sulphuric acid concentration, in the initial solution on the fluorescence intensity at 445 nm measured in the final acetone-sulphuric acid solvent is shown in Figure 5.3. One ml of 2.4 M sulphuric acid was chosen as optimum for the initial solution and was used in all further work.

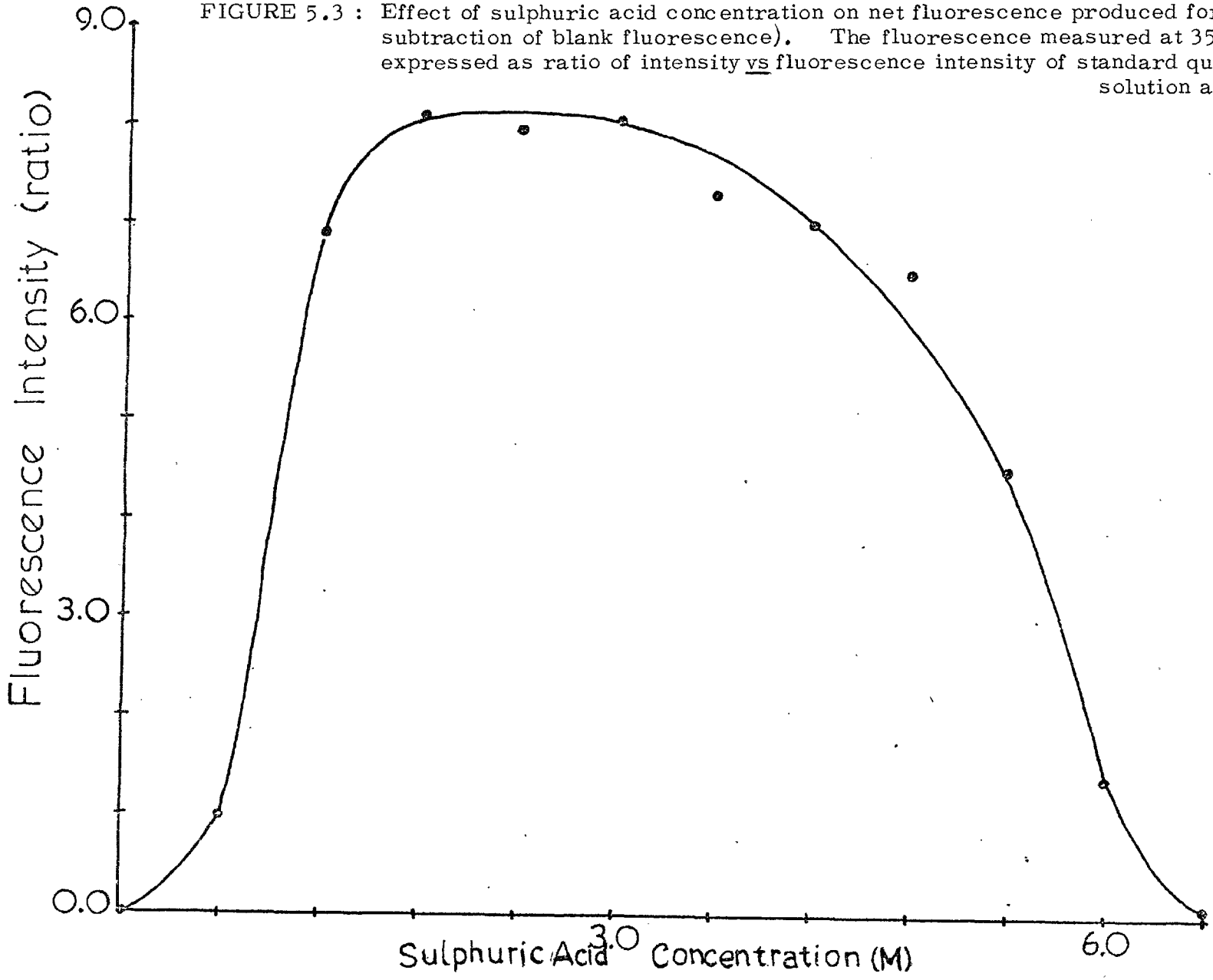
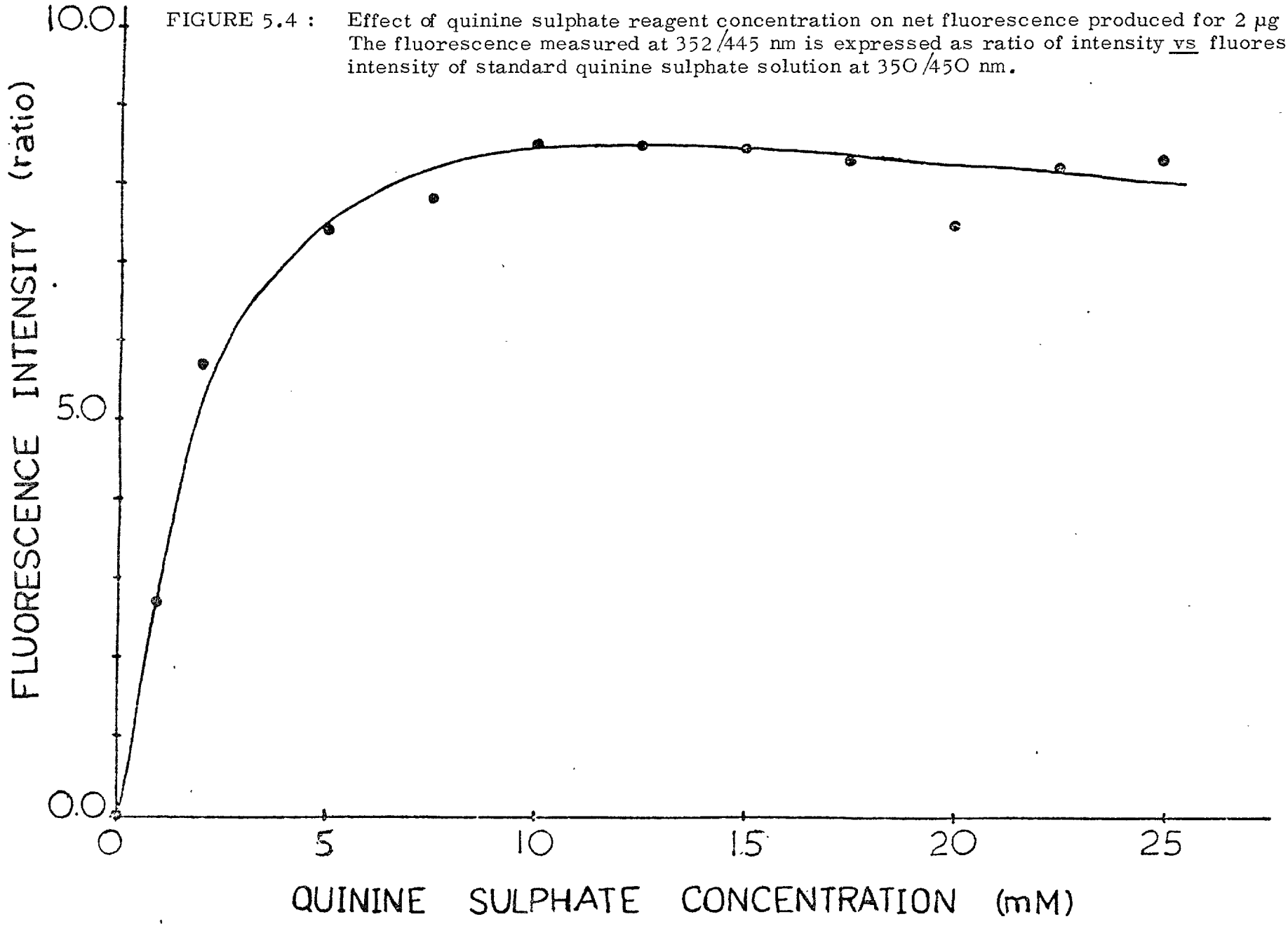


FIGURE 5.3 : Effect of sulphuric acid concentration on net fluorescence produced for 2 µg of P (after subtraction of blank fluorescence). The fluorescence measured at 352/445 nm is expressed as ratio of intensity vs fluorescence intensity of standard quinine sulphate solution at 350/450 nm.

The optimum quinine sulphate concentration was determined in a similar way by using 2 μg of phosphorus in 2 ml of aqueous solution, 2 ml of molybdate reagent and 1 ml of 2.4 M sulphuric acid, to which 1 ml of various molarities of quinine sulphate reagent were added. The precipitate was washed with 5 ml of 0.5 M sulphuric acid before dissolving it in 5 ml of acetone-sulphuric acid solvent for measurement of the fluorescence. As shown in Figure 5.4, the fluorescence intensity increased up to a concentration of 10 mM. quinine sulphate reagent and then remained constant as the reagent concentration was increased further. This concentration, 10 mM, was chosen for use in all further work; the use of a larger amount of quinine sulphate than necessary serves only to increase the magnitude of the reagent blank and to decrease the precision.

In order to ensure a low blank, the molybdate reagent solution was prestripped of any phosphate impurities before use; this procedure, which involves the formation of molybdophosphoric acid and its extraction from any phosphate present in the reagents used, is described in the Experimental section 4.2.2. The optimum concentration of the molybdate reagent was determined by using 2 μg of phosphorus in 2 ml of aqueous solution added to a mixture of 1 ml of 2.4 M sulphuric acid and 2 ml of molybdate reagent solution that had various concentrations. Quinine molybdophosphate was then precipitated by the addition of 1 ml of 10 mM quinine sulphate reagent solution. The effect on the final fluorescence intensity of variations in the concentration of the molybdate reagent solution in this way is shown in Figure 5.5; 2 ml of 60 mM molybdate reagent solution was chosen as the optimum and was used in all further work. This was the concentration corresponding to the prepared molybdate reagent solution.

FIGURE 5.4 : Effect of quinine sulphate reagent concentration on net fluorescence produced for 2 µg of P. The fluorescence measured at 352/445 nm is expressed as ratio of intensity vs fluorescence intensity of standard quinine sulphate solution at 350/450 nm.



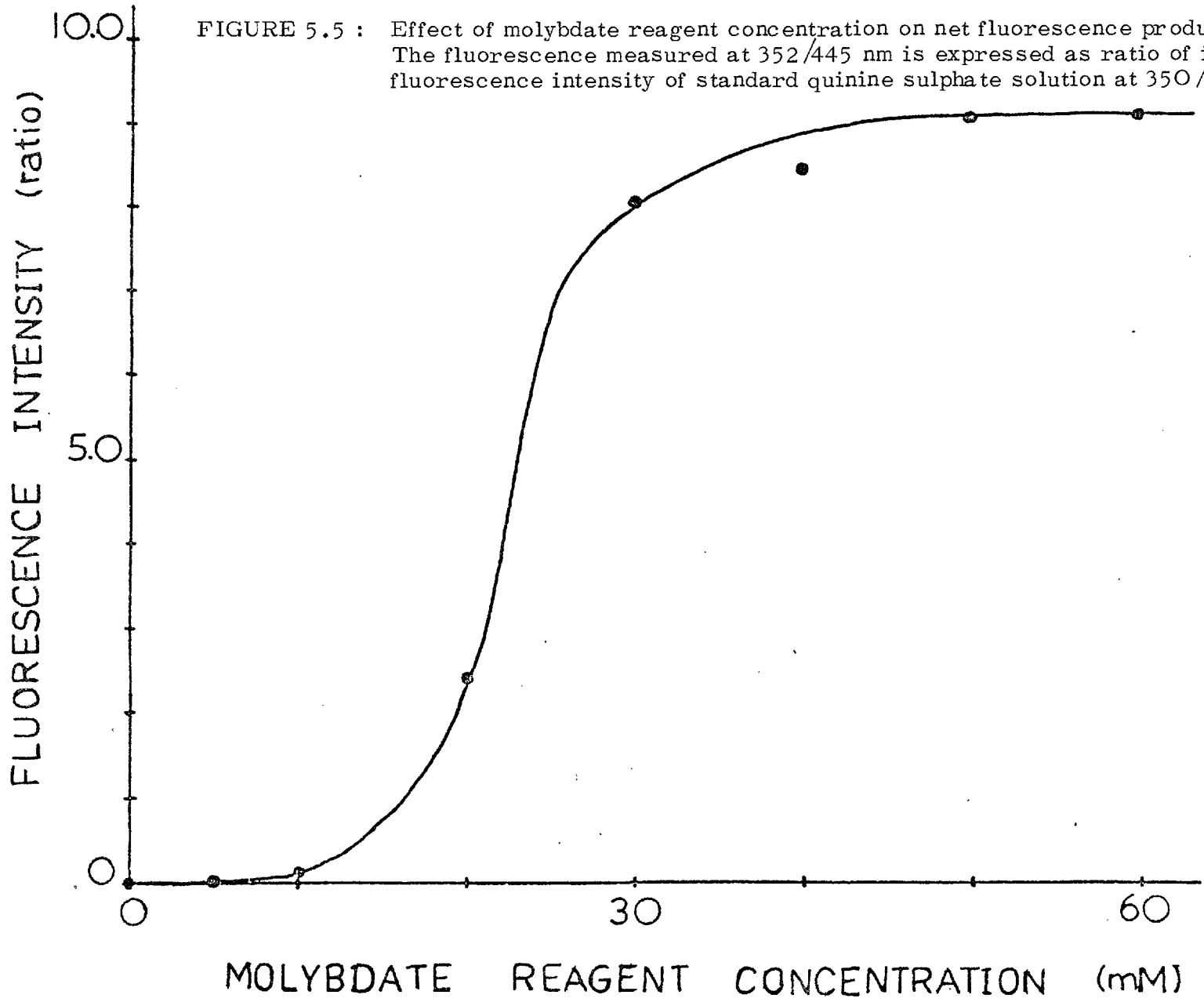


FIGURE 5.5 : Effect of molybdate reagent concentration on net fluorescence produced for 2 µg of P. The fluorescence measured at 352/445 nm is expressed as ratio of intensity vs fluorescence intensity of standard quinine sulphate solution at 350/450 nm.

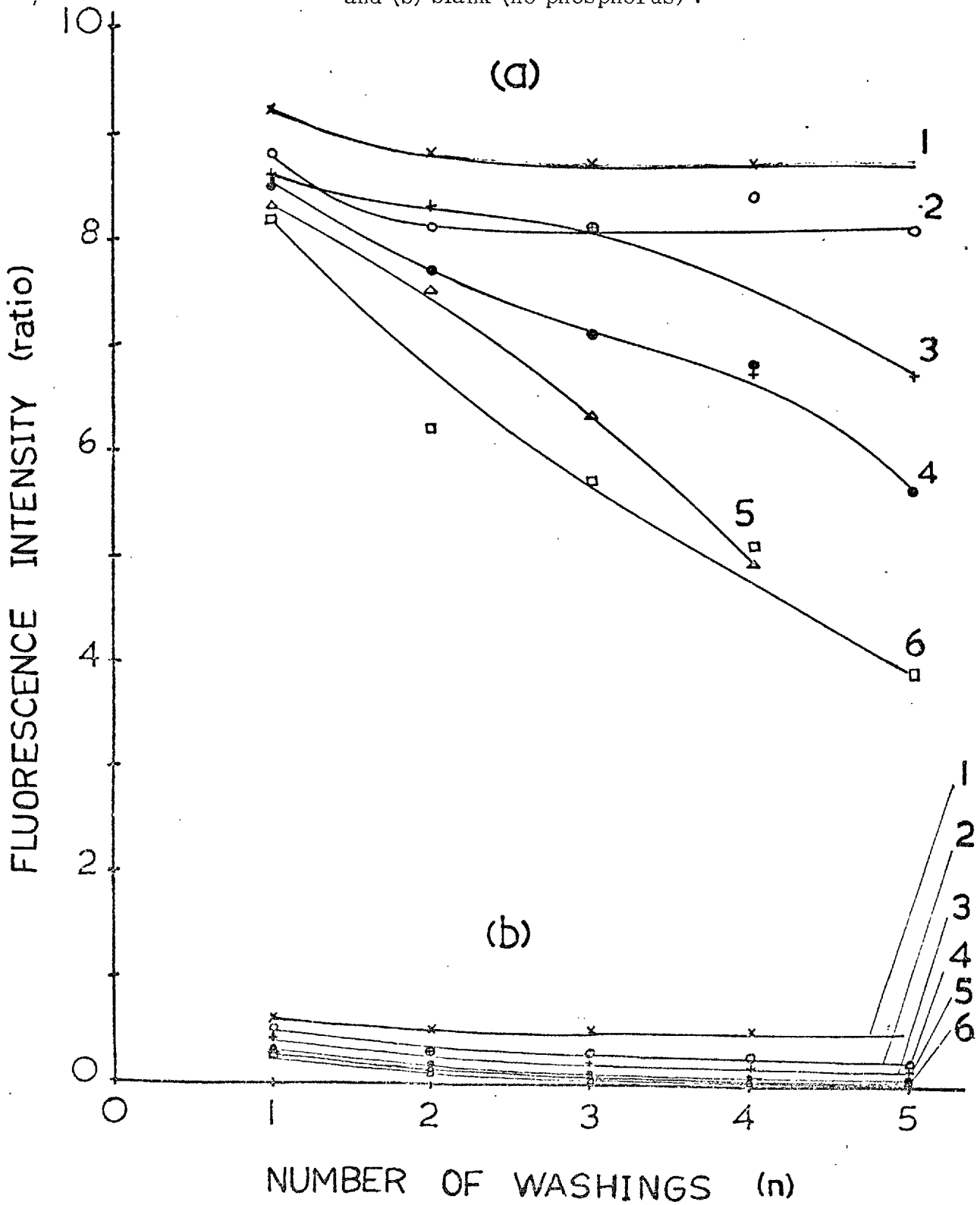
5.3.3 Effect of Washing the Quinine Molybdophosphate Precipitate

The effects on the final fluorescence intensity of the acidity of the solution used to wash the precipitate free from excess of reagent and of the number of washes used are shown in Figure 5.6. The precipitate formed from 2 µg of phosphorus was washed with 5 ml volumes of sulphuric acid solutions having various concentrations between O.O5 and 2M, and also with distilled water. It is evident that a single wash with 5 ml of dilute acid was sufficient to produce a low blank fluorescence after the removal of excess of quinine, and also to retain the quinine molybdophosphate precipitate without significant dissolution. The concentration of the acid used did not significantly affect the net fluorescence signal:blank intensity ratio after only a single washing step, although when the concentration of the acid was greater than O.5 M a significant decrease in the fluorescence signal was produced on further washing of the precipitate.

5.3.4 Calibration Graph for Phosphorus

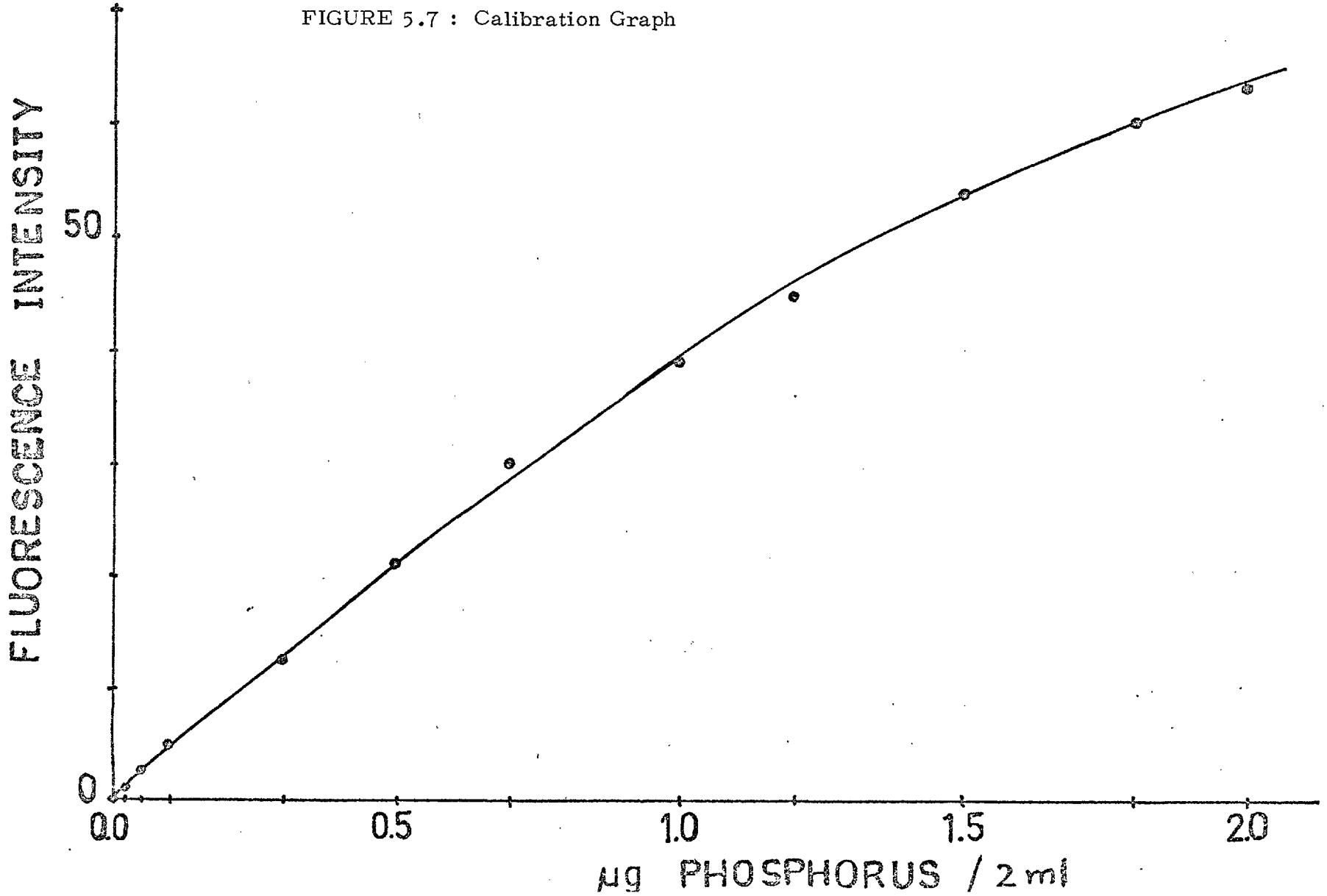
To a series of 10 ml centrifuge tubes, 2 ml of molybdate reagent solution and 1 ml of 2.4 M sulphuric acid were added. To each of the tubes 2 ml of phosphate solution in distilled water was added each containing O, O.O2, O.O5, O.1, O.3, O.5, O.7, 1.O, 1.2 and 1.5 µg of phosphorus. The solutions in each tube were mixed and allowed to stand for 5 minutes. One millilitre of quinine sulphate reagent solution was added to each tube, each solution was mixed again, and the tubes were placed in a beaker of boiling water for 5 minutes. The tubes were then placed in a laboratory centrifuge and the solutions centrifuged for a sufficient time (1 - 2 minutes) to produce a closely packed precipitate at the base of each tube. The clear solution was decanted, the precipitate in each tube washed with 5 ml of O.5 M sulphuric acid and centrifuged again. The wash liquid was decanted. The precipitate was then dissolved by adding 5 ml of acetone-sulphuric acid solvent to each tube. Suitable volumes of

FIGURE 5.6 : Effect of number of washes with 5 ml volumes of wash liquid for (a) quinine molybdophosphate from 2 µg of P, and (b) blank (no phosphorus) :



1 - water; 2 - 0.05 M H_2SO_4; 3 - 0.5 M H_2SO_4; 4 - 1.0 M H_2SO_4; 5 - 1.5 M H_2SO_4; and 6 - 2.0 M H_2SO_4.

FIGURE 5.7 : Calibration Graph



each solution were transferred to the fused silica sample cell and the fluorescence of each solution was measured at 445 nm with an excitation wavelength of 352 nm. After subtraction of the reagent blank fluorescence, prepared simultaneously, the calibration graph obtained for phosphorus was linear and passed through the origin. Figure 5.7 shows the calibration graph obtained by the procedure described above.

5.3.5 Sensitivity and Precision

With the optimum conditions described, the calibration graph for phosphorus was linear in the range O.O2 to 1.2 µg of phosphorus in the original 2 ml of aqueous sample solution taken, i.e O.Ol to O.6 µg per ml in the sample solution. Appreciable curvature of the calibration graph towards the concentration axis occurred when more than 1.2 µg of phosphorus was determined under the recommended conditions. This is not caused by the presence of insufficient quinine or molybdate reagent to allow the quantitative recovery of the larger amounts of quinine molybdophosphate, but is caused by concentration quenching of the quinine fluorescence at higher concentrations. This was demonstrated by the construction of a calibration graph for quinine sulphate alone in acetone-sulphuric acid solvent; the graph of fluorescence intensity against quinine concentration became curved towards the concentration axis at concentrations similar to those produced when more than 1 µg of phosphorus was determined.

The limit of the sensitivity of the procedure is determined by the magnitude and reproducibility of the fluorescence of the procedure blank. By using the procedure described here, the limiting detectable phosphorus concentration was O.Ol µg in 2 ml of aqueous solution.

When 1 µg of phosphorus in 2 ml of sample solution was determined ten times by the recommended procedure, a coefficient of variation of $\pm$ 4.7% was obtained. The results of the precision study are shown in Table 5.1.

Table 5.1 : DATA FOR PRECISION STUDY OF 1.0 µg OF P

Sample	Fluorescence Intensity (Scale = 1.0)	Average net Fluorescence Intensity (x)	Deviation $(x - \bar{x})$	Variance $(x - \bar{x})^2$
1	47, 48	45.0	+3.4	11.56
2	42, 42	39.5	-2.1	4.41
3	44, 45	42.0	+0.4	0.16
4	45, 45	42.5	+0.9	0.81
5	44, 45	42.0	+0.4	0.16
6	45, 45	42.5	+0.9	0.81
7	45, 46	43.0	+1.4	1.96
8	43, 43	40.5	-1.1	1.21
9	40, 41	38.0	-3.6	12.96
10	44, 44	41.5	-0.1	0.01
Blank	2, 3	-		
		Mean $(\bar{x})$ = 41.6		Total = 34.05

Standard Deviation = $\sqrt{\frac{(x - \bar{x})^2}{n - 1}} = \sqrt{\frac{34.05}{9}} = 1.95$; Coefficient of variation = $\frac{1.95}{41.6} \times 100 = 4.7\%$.

5.3.6 Order of Addition of Reagents

The order of addition of the reagents used must ensure that the molybdophosphoric acid is formed in acidic medium before the quinine sulphate reagent is added. Although the rate of formation of molybdophosphoric acid was quite rapid under the conditions employed, it was preferred to allow the solution to stand for 5 minutes before adding the quinine sulphate reagent. The order of addition used was therefore molybdate reagent, acid, sample and quinine sulphate reagent.

5.3.7 Effect of Time on Fluorescence Intensity

The effect of time on the intensity of fluorescence of quinine molybdophosphate in the acetone-sulphuric acid solution, prepared by the recommended procedure from (i) 0.5 µg P, and (ii) 1.0 µg P, was investigated under the following conditions :

(a) when the solutions were allowed to stand under normal laboratory conditions (fluorescent strip lighting) for 2 hours,

(b) when the solutions were stored in darkness for 2 hours, and

(c) on continuous irradiation of the solutions in the spectrofluorimeter for 1 hour at 352 nm by xenon arc light.

The results are shown in Table 5.2.

Table 5.2 : EFFECT OF TIME ON FLUORESCENCE EMISSION INTENSITIES

Amount of P used to prepare the quinine molybdophosphate (µg)	Change in Fluorescence Intensity, %		
	Under Laboratory Conditions (2 hrs)	Standing in Darkness (2 hrs)	Continuous Irradiation (1 hr)
0.5	0.0	+1.7	-0.7
1.0	0.0	+1.2	-0.2

These results, which were obtained with solutions contained in stoppered flasks, indicate that the fluorescence of the quinine molybdophosphate solutions are quite stable over the period of time (2 - 3 minutes) required for measurement of the fluorescence emission intensity, When the above experiments were repeated with the acetone-sulphuric acid solutions exposed to the atmosphere, increase of fluorescence intensity $>$ 5% was observed, which is probably due to evaporation of the acetone solvent. Hence the final acetone-sulphuric acid solutions of quinine molybdophosphate were contained in stoppered flasks before measurement of their fluorescence.

5.3.8. Effects of Foreign Ions

The effects of 100-fold weight excesses of thirty-eight foreign ions on the fluorescence intensity obtained in the final acetone-sulphuric acid solution for 1 µg of phosphorus determined by the recommended procedure were investigated. An ion was considered to interfere when it produced an error in fluorescence intensity compared with that produced by 1 µg of phosphorus alone of greater than the coefficient of variation (i.e. about 5%). The following ions did not interfere under these conditions : aluminium, antimony(III), bismuth, cadmium, calcium, cerium(IV), cobalt(II), chromium(III), copper(II), gold, iron(III), lead, magnesium, manganese(II), mercury(II), nickel, selenium(IV), silicon, silver, tellurium(IV), thallium(III), tin(IV), titanium(IV), vanadium(IV), vanadium(V), zinc, zirconium, fluoride, chloride, nitrate, perchlorate, and ethylenediaminetetraacetate. The presence of a 100-fold weight excess of the following ions produced the errors in fluorescence intensity given in parenthesis : arsenic(III), (+ 28%); chromium(VI), (- 11%); germanium(IV), (+ 155%); thorium, (+ 7%); tungsten(VI), (+ 13%). The presence of a 50-fold weight excess of arsenic(V) caused an error in fluorescence intensity of + 97%. Arsenic(III) and tungsten(VI) can be tolerated in 20 and 50-fold weight excesses,

respectively, whereas germanium(IV) in only a 2-fold weight excess and arsenic(V) present in the same amount as phosphorus interfere seriously. Silicon as silicate does not interfere; under the conditions used for the formation of molybdophosphoric acid, the formation of molybosilicate is inefficient, and an insoluble quinine molybdosilicate does not appear to be formed.

5.3.9 Accuracy

An estimate of the accuracy of the procedure was obtained by the determination of phosphorus in synthetic solutions containing foreign ions that were treated as unknown samples. Analysis of the sample was conducted by the procedure described in section 5.3.4, omitting the addition of phosphate solution but adding 2 ml of sample solution. Two standards containing 0.2 and 0.4 µg of phosphorus in 2 ml of aqueous solution and a blank (no phosphorus) were prepared and measured with each group of samples. The results of these analyses are shown in Table 5.3

5.3.10 Nature of the Precipitate

Several attempts were made to isolate and dry the quinine molybdophosphate precipitate so that complete elemental analysis could be carried out. Each time, however, it was apparent that decomposition of the compound occurred during drying, even at room temperature. The decomposition was evident from the change in colour of the precipitate from very pale green to blue. The precipitate was therefore prepared wet from known amounts of orthophosphate (6 µg of P) centrifuged off and washed with water or dilute sulphuric acid. The molybdenum content of the precipitate was determined by atomic absorption spectroscopy, after dissolution in 3 ml of concentrated sulphuric acid and diluting the resulting solution to 25 ml with distilled water. This determination

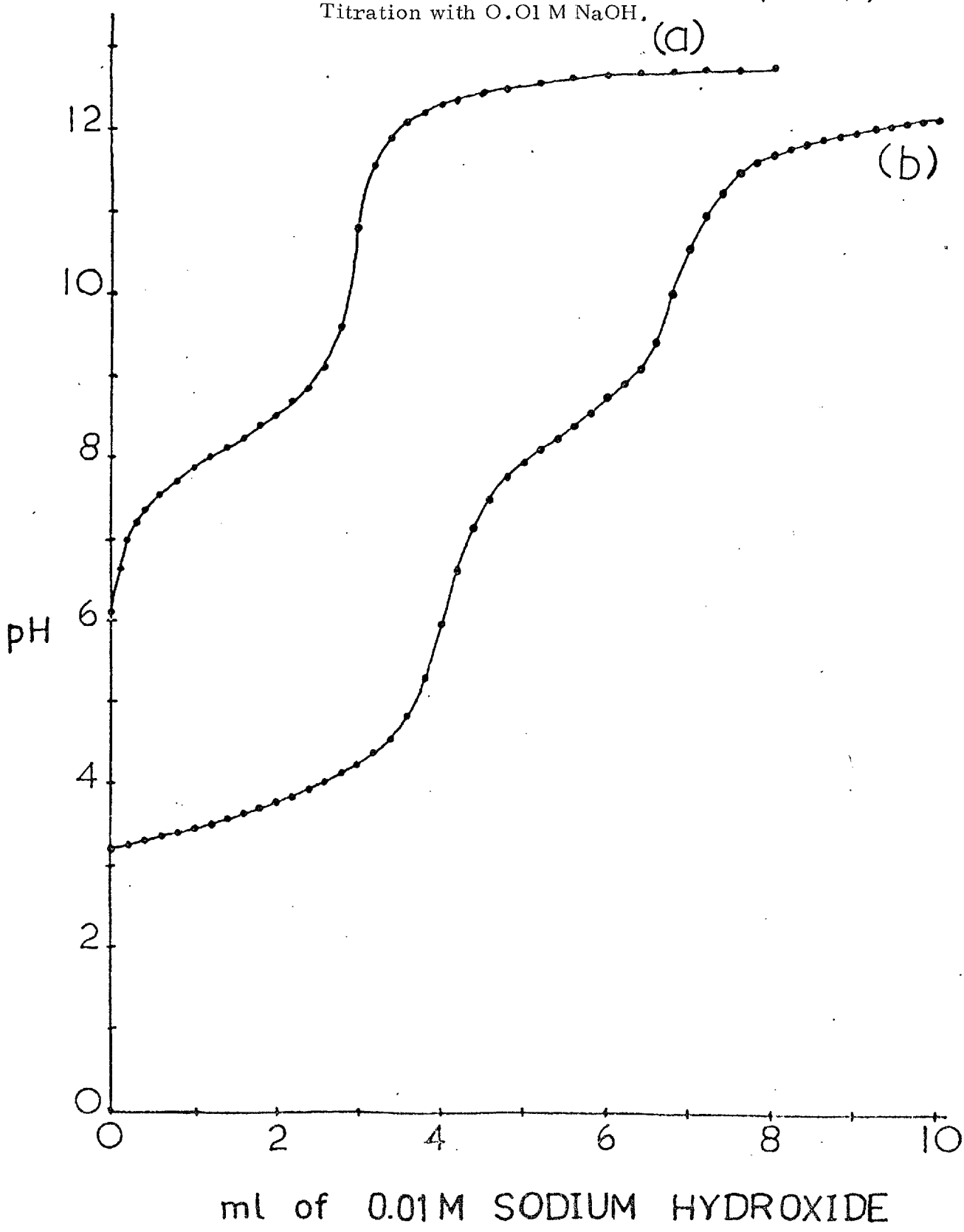
TABLE 5.3

DETERMINATION OF PHOSPHORUS AS ORTHOPHOSPHATE IN SYNTHETIC MIXTURES TREATED AS UNKNOWNS

Phosphorus taken/μg	Phosphorus found*/μg	Error, per cent.	Foreign ions present. Values in parentheses are the weight excess ratios of the ions over phosphorus
0·40	$0·37_5$	−6·25	Zn (30); Pb (20)
0·20	$0·18_5$	−7·50	Cu(II) (15); Ni (15)
0·08	$0·08^1$	+1·25	Al (15); Si (10)
0·25	0·26	+4·0	As(III) (5); Co (10)
0·14	0·13	−5·7	Bi (10); Mg (15)
0·45	0·46	+2·2	Ca (20); Fe(III) (10)
0·30	$0·28_5$	−5·0	F (10); ClO_4 (10)
0·22	0·22	0	Cr(III) (10); Fe(III) (15)

* Each result represents the average of three determinations in good agreement.

FIGURE 5.8 : Potentiometric titration curves for :
(a) quinine sulphate (2.5 ml of 0.005 M solution), and
(b) quinine molybdophosphate (from 150 µg of P).
Solutions were made in acetone/water (3 + 2 v/v)
Titration with 0.01 M NaOH.



showed that the added orthophosphate was completely recovered, as 12 moles of molybdate were recovered for each mole of orthophosphate taken. The quinine content of the precipitate was determined similarly by fluorimetric measurement and comparison with standards prepared from quinine sulphate alone in acetone-sulphuric acid medium. This determination indicated a combining ratio of quinine to molybdophosphate of 1.8:1, a ratio which is probably slightly high owing to the difficulty of removing the last traces of excess of reagent from the precipitate. Potentiometric titration of the precipitate, obtained from 150 μg of phosphorus precipitated by the recommended procedure, and washed with distilled water to free it from acid before dissolving it in 60% acetone in water, against standard 0.01 M sodium hydroxide solution gave the results shown in Figure 5.8. The results indicate that there are two titratable protons present in the compound formed. The pH values at the points corresponding to these two points on the titration curves are similar to the pH values corresponding to the two protonation steps for quinine itself, which suggests that the quinine (Q) is precipitated as QH_2^{2+} to form the insoluble molybdophosphate. The titre from these experiments and the results of the fluorimetric determination of the quinine of the quinine molybdophosphate precipitate suggest that the precipitate corresponds to the composition $(C_{20}H_{24}O_2N_2H_2)_3(PMo_{12}O_{40})_2$, which is an uncharged species. The structure may be similar to that of quinine molybdovanadophosphate proposed by Ripan and Suteu[175].

5.4 Conclusions

High sensitivity is obtained for the determination of phosphorus by the method developed. The sensitivity achieved is slightly higher than that obtained in the determination of orthophosphate as the rhodamine B-molybdophosphate complex described in Chapter 4. Of the foreign ions investigated, only

arsenic(V) and germanium(IV) cause serious interference as a result of the formation and extraction of the corresponding quinine molybdoarsenate and molybdogermanate, respectively. Arsenic(III), however, can be tolerated when it is present in moderate excess over phosphorus. Silicate, the elimination of interference from which can be troublesome in other procedures involving the use of molybdophosphoric acid for the determination of orthophosphate, does not interfere in the present procedure. A particular advantage of the method described here is the use of the fluorescent base quinine, which is often used as a standard for fluorescence intensity measurements as the component whose fluorescence is monitored for analytical purposes.

CHAPTER 6

LUMINESCENCE LIFETIMES AND QUANTUM YIELDS OF SOLUTIONS OF INORGANIC IONS IN HYDROHALIC ACIDS

6.1 Introduction

The luminescence of the aqueous solutions of halide salts containing heavy metal ions have been studied by several workers. The fluorescence observed from solutions of thallium(I)[176] and lead(II)[177] in the presence of excess of chloride have been used for the determination of the ions. The violet fluorescence observed in both cases was attributed to complex anionic $TlCl_3^{2-}$ and $PbCl_4^{2-}$ species[178]. Low temperature luminescence characteristics of several heavy metal ions have been investigated and used for the determination of few of the ions in hydrochloric and hydrobromic acids[179-187].

The luminescence quantum yield of thallium(I) in aqueous solution has been found to decrease slightly with increase in concentration of the chloride ion[188] 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 temperature[189,190]. Similarly the luminescence lifetimes of thallium(I), lead(II) and bismuth(III) ions in lithium chloride solutions have been found to increas with decrease in temperature[191].

6.1.1 Measurement of Luminescence Lifetimes

The experimental procedures for measuring luminescence lifetimes vary according to the order of magnitude of the decay period. Phosphorescence lifetimes are alsmost 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 rotating phosphoroscope can or disks are used[192].

The decay time of the 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 the phase-shift techniques[57,193-200] and the pulse (or flash) techniques[198,201-207]. Commercial flash lamp systems capable of generating nanosecond light pulses are available; one such instrument which was employed by the author (section 1.2.2) has been used previously for determining fluorescence lifetimes[60,191,207-210].

6.1.2 Measurement of Luminescence Quantum Yields

Several types of methods have been described for the determination of quantum yields of solutions. These include the use of standard scatterers, such as magnesium oxide[24,27,29,211] or colloidal solutions[19,212], or relative methods which substitute a compound of known quantum yield in place of a standard scatterer as a reference. The solutions of the standard compounds and that of the sample can be either optically dense[12,211] or optically dilute.[6,20,30,69] Calorimetric methods have also been described as procedures for measuring quantum yields, which are calculated from the energy yields obtained from temperature changes during irradiation[213-215].

In order to ascertain the mechanism of luminescence observed in the hydrochloric acid and hydrobromic acid matrices in the presence of heavy metal ions, the lifetimes of several of the ions in 6M hydrochloric and hydrobromic acids were measured at liquid nitrogen temperature ($77^{\circ}K$) and for cerium(III)

and thallium(I) at room temperature. The quantum yield of luminescence at room temperature for Tl(I), Pb(II) and Ce(III) in 6M HCl and for Tl(I) and Ce(III) in 6M HBr were also measured. The results are presented in this chapter and a mechanism for luminescence is suggested.

6.2 Experimental

6.2.1 Reagents

Metal Salts : Analytical reagent grade tin(II) chloride dihydrate, lead nitrate, arsenic(III) oxide, sodium arsenate heptahydrate, potassium antimonyl tartrate, bismuth nitrate pentahydrate, copper(II) sulphate pentahydrate, and uranyl nitrate hexahydrate were used to prepare 10^{-2}M solutions. Tellurium metal, laboratory reagent grade cerium(III) nitrate hexahydrate, Tin(IV) chloride pentahydrate, antimony pentoxide monohydrate, and freshly prepared cuprous iodide[216] were used for the other ions to prepare 10^{-2}M solutions. The salts were dissolved directly in 6M hydrochloric and hydrobromic acids. An aqueous solution of thallium(I) chloride was used to prepare 10^{-3}M Tl(I) ion solution in the acids.

Acids : Analytical reagent grade concentrated hydrochloric acid and re-distilled, 48%, "AnalaR" hydrobromic acid (both of Hopkin and Williams Ltd.) were used.

Quinine Sulphate Standard : This was prepared from quinine sulphate (BDH Ltd.), by dissolving in O.1 N sulphuric acid to give a 100 $\mu g\ ml^{-1}$ solution. From this solution, 1 $\mu g\ ml^{-1}$ of quinine sulphate solution was prepared by diluting with O.1 N sulphuric acid.

Standard Compounds : 100 $\mu g\ ml^{-1}$ solutions of anthracene (recrystallised from ethanol) and purified rhodamine B (see Chapter 2) were prepared in ethanol,

eosin in O.1 N sodium hydroxide, and acriflavine in distilled water. The solutions were diluted as required, when necessary, with the appropriate solvent.

All solvents used were of analytical reagent grade purity; water was distilled from an all-glass apparatus.

6.2.2 Measurement of Luminescence Lifetimes

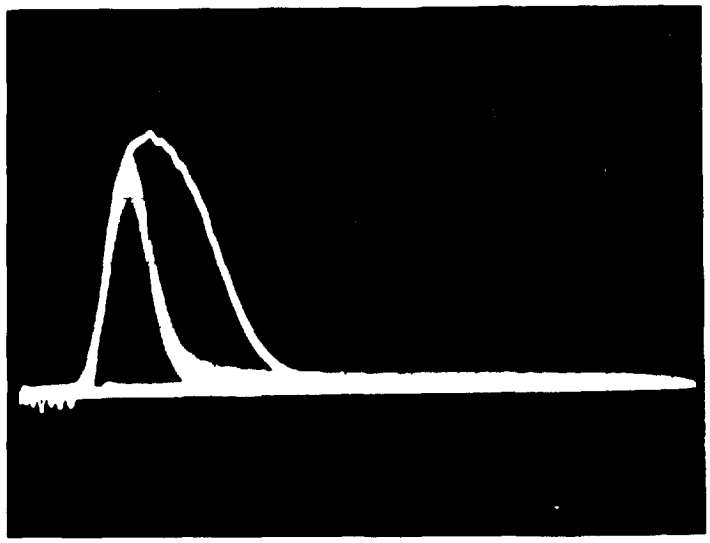
6.2.2.1 Apparatus

The apparatus used for measuring the lifetimes was the TRW Nanosecond Spectral Source System (section 1.2.2). The signal, measured across a suitable load resistor, was preamplified and displayed on the oscilloscope. The decay traces were photographed with the Polaroid Oscilloscope camera from the face of the oscilloscope. Figure 6.1 shows the decay trace obtained for a 10^{-4}M solution of quinine sulphate in O.O5M sulphuric acid. The short decay pulse shown in the figure is that of the source (nitrogen lamp). Figure 6.2 shows a decay trace of Te(IV) (10^{-2}M) in 6 M HCl. This is typical of decay traces that were obtained from other inorganic ions. Determination of the fluorescence lifetimes of quinine sulphate in O.O5 M sulphuric acid (10^{-4}M) and naphthalene in n-hexane (deoxygenated) (O.2 M), gave values of (20.1 $\pm$ O.1) nanoseconds and (98.4 $\pm$ 5.8) nanoseconds respectively. These values agree closely with previous investigations[12,59,60].

6.2.2.2 Filters

Filters with high transmission characteristics were used for selecting luminescence excitation and emission light. They were Corning, Interference (Baird Atomic Co.), or Locarte filters. A solution filter of 1 cm thickness contained in a filter holder (both constructed by the author) was used for excitation light below 3OO nm. The solutions used were either a saturated

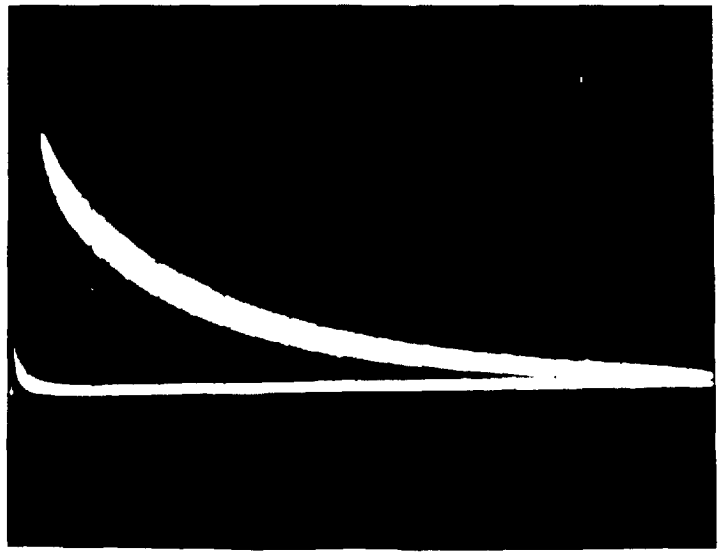
FIGURE 6.1



FLUORESCENCE DECAY OF QUININE SULPHATE AT ROOM TEMPERATURE (294°K).

10^{-4}M solution of quinine sulphate in 0.05M H_2SO_4. Time base: 50 nanoseconds per major division. Termination: 50 ohms.

FIGURE 6.2



LUMINESCENCE DECAY OF TELLURIUM COMPLEX IN 6M.HYDROCHLORIC ACID AT LIQUID NITROGEN TEMPERATURE (77°K).

10^{-2}M Tellurium(IV) solution in 6M.HCl.

Time base: 2 microseconds per major division.

Termination: 1000 ohms.

solution of nickel sulphate and cobalt sulphate or the above saturated solution containing O.1 g per litre of 2,7-dimethyl-3,6-diazacyclohepta-1,6-diene perchlorate[217]. This compound was synthesised by the procedure given by Schwarzenbach and Lutz[218].

6.2.2.3 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 top of the trace, and the other from the base line to the bottom of the trace. Two plots of log intensity vs time were 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 Tables 6.2 and 6.3 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.

6.2.3 Measurement of Quantum Yields

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 solutions 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. Quinine in N/10 sulphuric acid was used as the standard and its quantum yield of fluorescence was taken as O.55. This compound has only a small overlap of its fluorescence excitation and emission spectr

6.2.3.1 Calculation

The total fluorescence emission F from a solution is given by the equation (1.8),

$$F = k I_o (1 - 10^{Al}) \cdot \phi$$

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

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

or

$$\phi_x = \phi_s \cdot \frac{F_x}{F_s} \cdot \frac{I_o^s}{I_o^x} \cdot \frac{(1 - 10^{-A_s l})}{(1 - 10^{-A_x l})} \qquad (6.1)$$

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 of light is absorbed, equation 6.1 reduces to :

$$\phi_x = \phi_s \cdot \frac{F_x}{F_s} \cdot \frac{I_o^s}{I_o^x} \cdot \frac{A_s}{A_x} \qquad (6.2)$$

This equation was used for calculating quantum yields of fluorescence of some standard compounds. In the case of metal ions the absorbance of the solutions used were quite high, although this concentration (10^{-4}M) was essential to obtain a reasonable luminescence emission spectrum from the samples. The solution of the standard (quinine sulphate) was very dilute (1 μg/ml), and therefore the equation used for calculating the quantum yield was :

$$\phi_x = \phi_s \cdot \frac{F_x}{F_s} \cdot \frac{I_o^s}{I_o^x} \cdot \frac{A_s}{(1 - 10^{-A_x l})} \qquad (6.3)$$

The quantum yields (ϕ) were corrected for the different refractive indices of the solvents by multiplying ϕ by the ratio $(n_x/n_s)^2$, where n is the refractive index of the solvent.

6.2.3.2 Apparatus

Luminescence measurements were made with the Aminco-Bowman Spectrophotofluorometer (section 1.2.1). Fused silica cells (50 x 10 x 10 mm) were used. Absorption spectra of the solutions were obtained using a Higher-Watts Ultrascan Spectrophotometer. Two identical fused silica cells were employed. All measurements were made at room temperature.

6.2.3.3 Procedure

The recorded luminescence emission spectra of the solutions of unknown and of the standard quinine sulphate (in 0.1 N sulphuric acid, 1 μg/ml) were corrected using the 'correction factors' obtained by the methods described in Chapter 2. The corrected spectra were plotted on an axis of corrected luminescence intensity vs frequency (in μ^{-1}). The area under the curves were then calculated by using Simpson's Rule[219]. The following compounds were used as standards whose quantum yields were determined using quinine sulphate solution as standard : acriflavine in water, (4 μg/ml); anthracene in ethanol, (1 μg/ml); eosin in N/10 sodium hydroxide, (2 μg/ml); and rhodamine B in ethanol, (1 μg/ml). For metal ion solutions, 10^{-4}M solution of the ions in 6M hydrochloric or hydrobromic acid were used. The quantum yields of the unknowns were calculated using the equation 6.2 (for standard compounds) or 6.3 (for metal ions). To test the experimental technique and calculation the results for the quantum yields of fluorescence of the standard compounds were measured. These results, obtained from single determinations and compared with literature values obtained by both relative and absolute methods, and are given in Table 6.1. The experimental results, corrected

for the different refractive indices of the solvents, appears to be in good agreement.

Table 6.1 : DETERMINATION OF FLUORESCENCE QUANTUM YIELDS USING QUININE SULPHATE IN N/10 SULPHURIC ACID AS A STANDARD (ϕ = 0.55)

Compound	Solvent	Concentration (μg/ml)	ϕ	Literature
Acriflavine	Water	4.0	0.52	0.56[212]; 0.54[19]; 0.57[20];
Anthracene	Ethanol	1.0	0.27	0.27[26,76]; 0.30[20,212]; 0.28[30]; 0.26[27];
Eosin	0.1 N. NaOH	2.0	0.23	0.23[30]; 0.19[19]; 0.15[24]
Rhodamine B	Ethanol	1.0	0.80	0.70[20]; 0.76[69]; 0.97[19]; 0.69[30];

The quantum yields of luminescence for metal ions are given in Table 6.3.

6.2.3.4 Refractive Index Measurements

The refractive indices of the solvents were measured using an Abbe Refractometer (Hilger and Watts Ltd.), and the values obtained are given in parenthesis : Water, (1.333); ethanol, (1.361); 0.1 N sulphuric acid and 0.1 N sodium hydroxide, (1.333); 6 M hydrochloric acid, (1.381); and 6 M hydrobromic acid, (1.408).

All measurements were made at room temperature (20°C).

6.3 Results and Discussions

The measured lifetimes of several inorganic ions in 6 M hydrochloric and hydrobromic acids at liquid nitrogen temperature ($77^{o}K$) are given in Table 6.2 and those at room temperature in Table 6.3. The Stokes Shift values were calculated from the wavelengths of maximum excitation and emission[179,180] as measured by the spectrofluorimeter. Each mean life shown in Tables 6.2 and 6.3, is the average of at least two determinations, and was calculated as described in section 6.2.2.3. The error in the measurements of the lifetimes, in general, range from 5% to 15% and for ions showing weak luminescence the error is greater than 15%. The log intensity vs time plots for all decay traces were found to be linear, which indicates that the luminescence measured from all the ions decay exponentially.

The mean lifetimes of several of the heavy metal ions are of the order of microseconds and widely spread. These mean lifetimes are at least an order of magnitude longer than is normally observed for spin-allowed transitions and at least an order of magnitude shorter than normally expected for spin-forbidden transitions. Bismuth(III) and Uranium(VI), however, have long lifetimes (milliseconds), and the luminescence probably consists of phosphorescence. The luminescence from uranium is attributed to the uranyl ion (UO_2^{++})[220-222], and is different to the other ions listed in the Table. The short lifetimes observed from most of the ions can be explained if the luminescence could be of a low quantum yield, spin-forbidden transition. The radiative lifetime (τ_o) is related to the measured lifetime (τ_m) and the luminescence quantum yield, Q, by the equation $\tau_m = \tau_o . Q$, and the radiative life for a spin-forbidden transition is normally longer than 100 μsec. For the heavy metal ions the radiative lifetimes would be those expected for spin-forbidden transitions, if the quantum yield of luminescence were less than 10% at low temperature, and less than about 0.1% at room temperature. Table 6.3 lists the luminescence

Table 6.2 : MEASURED LUMINESCENCE LIFETIMES OF INORGANIC IONS IN HYDROCHLORIC AND HYDROBROMIC ACIDS AT 77°K (CONCENTRATION OF Tl(I) = 10^{-3} M, AND OF REMAINING IONS = 10^{-2} M).

Ion	Salt used	Stokes' Shift (eV)		Lifetimes (μ-seconds)	
		6 M HCl	6 M HBr	6 M HCl	6 M HBr
Tl(I)	TlCl	1.58	1.57	4.18 ± 0.76	3.51 ± 0.95
Sn(II)	$SnCl_2.2H_2O$	-	1.69	-	4.15 ± 0.25
Sn(IV)	$SnCl_4.5H_2O$	1.38	-	w	-
Pb(II)	$Pb(NO_3)_2$	1.31	1.08	34.00 ± 4.00	52.40 ± 8.30
As(III)	As_2O_3	-	1.36	-	3.77 ± 1.09
As(V)	$Na_2HAsO_4.7H_2O$	-	1.29	-	3.64 ± 1.19
Sb(III)	$KSbO.C_4H_4O_6$	1.92	1.33	6.59 ± 0.82	2.92 ± 0.25
Sb(V)	$Sb_2O_5.H_2O$ *	1.04	1.33	w	3.41 ± 0.25
Bi(III)	$Bi(NO_3)_3.5H_2O$	0.73	0.52	559 ± 44	267 ± 10
Te(IV)	Te-powder	1.55	1.31	7.22 ± 0.77	w
		1.15 **	-	7.11 ± 0.86	-
Cu(I)	Cu I	1.65	1.62	15.30 ± 1.30	11.90 ± 0.70
	$CuSO_4.5H_2O$ reduced with ascorbic acid	1.55	1.48	35.90 ± 4.70	105.6 ± 8.0
Ce(III)	$Ce(NO_3)_3.6H_2O$	1.36	1.42	0.040 ± 0.005	0.034 ± 0.004
U(VI)	$UO_2(NO_3)_2.6H_2O$	1.60 1.70	1.28)*** 1.39)	590 ± 30	290 ± 10

* Salt with approximate composition; ** 10 M HCl; *** Two strong emission peaks; w Denotes weak luminescence signal that decay trace could not be obtained

Table 6.3 : LUMINESCENCE QUANTUM YIELDS AND LIFETIMES AT ROOM TEMPERATURE (293°K) CONCENTRATION OF THE IONS = 10^{-4}M (QUANTUM YIELD OF QUININE SULPHATE STANDARD = 0.55)

Ion	6 M HCl				6 M HBr			
	Stokes' Shift	Quantum Yields (a) Q, ± std. dev.	Lifetime (b) measured (τ_m) (μ.sec)	Radiative (c) Lifetime (τ_o) (μ sec)	Stokes' Shift	Quantum Yields (a) Q, ± std. dev.	Lifetime (b) measured (τ_m) (μ.sec)	Radiative (c) Lifetime (τ_o) (μ.sec)
Tl(I)	1.95	$(5.9 \pm 0.3) \times 10^{-2}$	0.102 ± 0.022	1.73	1.85	$(5.66 \pm 0.53) \times 10^{-3}$	0.094 ± 0.014	16.60
Pb(II)	1.98	$(9.41 \pm 0.64) \times 10^{-4}$	-	-	-	-	-	-
Ce(III)	0.58	(0.31 ± 0.01)	0.021 ± 0.002	0.07	0.62	(0.153 ± 0.004)	0.019 ± 0.002	0.12

(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 $\tau_o . Q = \tau_m$.

quantum yields for Tl(I) and Ce(III) in 6 M HCl and 6 M HBr, and for Pb(II) in 6 M HCl measured at room temperature. The results obtained are the average of at least three determinations. No luminescence was observed from Pb(II) in 6 M HBr at room temperature. The quantum yields were calculated using equation 6.3, and were corrected for high absorbance of the solutions. The values of τ_o (Table 6.3) for Tl(I) in 6 M HCl and 6 M HBr, are of the order of microseconds which is again intermediate between the expected lifetimes for spin-allowed and spin-forbidden transitions. The luminescence from Ce(III) ion appears to be fluorescence as the radiative lives in both hydrohalic acids are several nanoseconds.

Another possible explanation for short lifetimes measured for many of the ions is based upon the observation that the presence of heavy atoms decrease phosphorescence lifetimes due to increased spin-orbit coupling in the molecules[1,9] The metal ions investigated all have high atomic number, and they are possibly directly involved in the electronic transition; the absorption may correspond to $^1S_o \rightarrow {}^3P$ transition and the emission to the reverse transition.

The ions whose lifetimes of luminescence have been measured in HCl and HBr, can be classified into two groups ; (1) ions with outer electron shell $nd^{10}(n+1)s^2$ [Tl(I), Pb(II), Sn(II), Bi(III), Sb(III), As(III), Te(IV)] , and (2) ions with a shell of the type nd^{10} [Sn(IV), Sb(V), As(V), Cu(I)] . The rare earths, Ce(III) and U(VI) do not fall into any of the above groups. The outer electron shells of the ions determine their spectral properties. The luminescence observed from those ions with two s electrons are more intense, while the ions with d^{10} outer shell show weak luminescence. Belyi and co-workers[187] have found that the consideration of s electrons alone does not explain several observations in the spectral characteristics of the ions. As a result of observations of similarity of the absorption spectra of solutions containing ions with outer electronic shell $nd^{10}(n+1)s^2$ and nd^{10}, they formulated a hypothesis

for absorption and emission by the transitions $nd^{10}(n+1)s^2 \rightleftharpoons nd^9(n+1)s^2(n+1)p$ and $nd^{10} \rightleftharpoons nd^9(n+1)p$. The possibility of transfer of an electron within the ion has been used to explain the absorption spectra of thallium activated alkali metal chloride phosphors[223] and the luminescence of several heavy metal haloid salts in solutions[187]. It has been suggested that the absorption peaks of Tl(I), Pb(II) and Bi(III) in the presence of chloride ions are due to $s \rightarrow sp$ transitions of the 6s electrons[224] and that in aqueous solution of thallium(I) chloride, a quasi-crystalline assembly of ions exist with the Tl(I) ions surrounded by chloride ions[225]. It is also possible that luminescence from these compounds originate from activated halide ions, and the lifetime for emission be shortened by the heavy atom effect of the metal ion, but this fails to explain the observation of luminescence from metal ions in the absence of halide ions, for e.g. from aqueous solutions of Tl^+ (e.g. Tl_2SO_4) in the absence of chloride ions[178,188].

Increase in measured luminescence lifetimes are observed for Tl(I) and Ce(III) ion with decrease in temperature, which is due to an effect of reducing the quenching of luminescence which is in turn is due to reduction in the probability of collisions between excited and non-excited species in solution.

The effect of dissolved oxygen on the luminescence lifetimes of Tl(I) (10^{-3}M) in 6 M HCl at 77°K and at room temperature and of Pb(II) (10^{-2}M) in 6 M HCl and 6 M HBr at 77°K, was studied. The solutions were deoxygenated by bubbling nitrogen through the solutions for one hour, by passage through a 'Nilox Scrubber'[226]. No significant changes in the measured lifetimes were observed. However, when a solution of Tl(I) (10^{-3}M) in 6 M HCl was saturated with oxygen a reduction in measured lifetime up to ~50% was observed at room temperature. Similar study with Ce(III) (10^{-2}M) in 6 M HCl did not show any significant change in the measured luminescence lifetime. This study probably suggest that the emission from Tl(I) may originate from a triplet state. Triplet molecules are

are susceptible to quenching by oxygen[6] and therefore a reduction in lifetime is observed for Tl(I).

The effect of concentration of metal ions Tl(I) and Pb(II) in 6 M HCl on their luminescence lifetimes was studied. The solutions used were 10^{-4}, 5×10^{-4}, 10^{-3}M for Tl(I) and 10^{-3}, 5×10^{-3}, 10^{-2}M for Pb(II). The measured lifetimes were found to be independent of concentration of the metal ion at low temperature (77°K). However, at room temperature for Tl(I) in 6 M HCl, the measured lifetimes decreased with increase in concentration of the metal ion. This may be due to quenching due to collisional effects which would be removed when the solutions are frozen to 77°K. With the exception of Pb(II) and Cu(I) (reduced Cu(II)), the observed luminescence lifetimes in HCl for all the remaining ions are greater than those in HBr. This is probably due to the heavy atom effect of the halide (bromide) ion on the luminescence life of the metal ion. In the case of Cu(I) (reduced Cu(II)) and Pb(II) ions, the luminescence lifetimes in HBr are greater than those measured in HCl. The reasons for this anomaly are not clear.

6.4 Conclusions

From the observations made and the results of other workers, it is reasonable to conclude that the absorption and luminescence observed from heavy metal ion solutions in 6 M HCl and 6 M HBr 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 $^3P \rightarrow {}^1S_0$ 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 hydrochloric and hydrobromic acids. In Ce(III) ion, the luminescence is probably caused by the transition of the 4f electron to higher energy levels.

CHAPTER 7

CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

The spectrofluorometric technique may offer greater sensitivity by two or three orders of magnitude than absorption spectrometry as it depends on the amplification of the first faint re-emission of a small percentage of absorbed radiation rather than the discrimination of small differences in intensity between the incident and transmitted beams in absorption work. Fluorometric methods are also more selective, as both excitation and emission spectra can be used in the analysis of mixtures. The versatility of fluorescence and other luminescence techniques is demonstrated by their use in both organic and inorganic trace analysis. The basic reason why luminescence spectrometry is more subject to elemental interferences than absorption spectrophotometry is that the analyte molecule in the excited state is quite different than the ground state. Its reactivity, pK and geometry may be radically changed so that any differences between standards and samples are enhanced as errors. Furthermore photochemical reactions such as dimerisation and energy-transfer can readily take place.

The measurement of prompt fluorescence of solutions at room temperature is still the technique most widely employed, because it is simple, rapid and the instrumentation available at present operates with high sensitivity over a wide range of the spectrum. The most intense source used is a high-pressure xenon arc lamp, and an important improvement in the instrumentation would be the development of a light source giving a continuum with large intensity below 250 nm to permit the measurement of excitation spectra of very dilute solutions in this spectral region. Luminescence measurement at low temperature, with its additional advantages of providing high intensity, are becoming more widely recognised. Systems which are non-fluorescent under normal conditions,

may emit luminescence at low temperature where processes which have an adverse effect on the luminescence emission are reduced to a very great extent. During the author's investigation of low-temperature luminescence, certain practical difficulties have been experienced. One of the difficulties was the cracking of silica sample tubes used for low temperature measurements due to internal pressure set up on the walls of the tubes when a crack in the glassy medium occurs. This difficulty can be overcome if thick-walled tubes are used to hold the sample solution. To obtain reproducible results the positioning of the sample tube in the Dewar flask was a critical operation. To overcome this kind of problem, a rotating sample cell designed by Hollifield and Winefordner[227] for use in phosphorimetry, may be useful to improve the precision in the measurements. The boiling of liquid nitrogen presented another problem, which usually occurred when the outside of the sample tube and inside of the Dewar flask were not thoroughly cleaned. This bubbling of liquid nitrogen tends to vibrate the sample tube and causes decrease in sensitivity and precision. In such events, a Dewar tube assembly constructed by Mayne[191] may be very useful and provide increased sensitivity and precision.

This thesis has described various aspects of the luminescence technique and its applications. Procedures for obtaining factors for correction of luminescence spectra obtained from the Aminco-Bowman Spectrophotofluorometer are described in Chapter 2. For analytical requirements such corrections are not essential as the maximum response in the operator's own instrument would be of a more practical significance. The excitation and emission spectra presented in Chapters 3, 4 and 5 are uncorrected and are given as recorded in the instrument used by the author.

The luminescence characteristics of several additives used in plastics are described in Chapter 3. Lack of sufficiently specific analytical techniques makes the separation of an additive first from the polymer and from other

additives quite important before any comprehensive scheme of analysis could be made. Separation of additives from polymer samples have not been extensively studied due to insufficient knowledge of the component to be separated. Useful indications as to how this can be achieved are given by the in situ techniques of spectroscopy[84,87]. The separations of additives from one another are clearly achieved by chromatographic techniques and simultaneously the additives can be isolated and identified. For the final quantitative determination of the isolated additives several types of techniques are available and this is facilitated by inherent reactivity of the additives.

A number of organic amines and basic dyes have been used for the determination of several anions. The methods pertaining to the determination of phosphorus as orthophosphate are reviewed in Chapters 4 and 5. The anions arsenate, silicate and germanate too have been determined using organic amines and dyes via their heteropolyanions by procedures similar to those of phosphate. Many different elements can function as the central atom in the heterpolyanions, commonly used element being molybdenum. Tungsten can replace some or all of the molybdenum; pentavalent vanadium and niobium can replace some of the molybdenum or tungsten. Many different dyes also find application in the determination of metal-containing and other anions via the formation of ion-association complexes[228]. The main advantage of using basic dyes is that many of them possess high molar absorptivity and spectrometric methods using the dyes provide substantially better sensitivity. Chapters 4 and 5 in this thesis describe sensitive methods for the determination of phosphorus as orthophosphate, which make full use of the maximum fluorescence intensity of rhodamine B and quinine by isolating their reaction product with molybdophosphoric acid. The rhodamine B and quinine reagents recovered from the ion-association complexes can be measured fluorimetrically in the parts per billion range, and have chemical amplification factor for phosphorus of 50:1 and 40:1 respectively.

The measurement of quantum yield and lifetime of luminescence of heavy metal ions in aqueous solutions of hydrochloric acid and hydrobromic acid has been described in Chapter 6. The luminescence of several of these ions has also been observed in strong solutions of other haloid salts (fluoride and iodide) at low temperature. From the data presented in Chapter 6, it is difficult to make any definite conclusions regarding the mechanism of luminescence of the ions in aqueous solutions of hydrohalic acids. The measurement of luminescence lifetimes and quantum yields of the ions in all four halogen acid media would provide more useful information regarding the luminescence processes in these systems. Measurement of quantum yields at low temperature requires the knowledge of the absorbance of the solutions at this temperature. One of the problems in the present work was the measurement of absorbance at 77°K in inorganic glasses. Future work in this field would be to design a low-temperature absorption cell. Several workers, however, have designed low temperature absorption cells for various spectrophotometers[229,230].

Measurement of lifetimes and quantum yields over a range of temperatures (between 77°K and room temperature) would give an indication of the effects of temperature and structure of solvent on the luminescence processes. Crystallisation of hydrohalic acids at some intermediate temperature may present a problem, and to overcome this aqueous solutions of hydrohalic acids could be replaced with aqueous solutions of lithium halides[189-191].

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