THE UNIVERSITY OF HULL

CHEMILUMINESCENCE DETECTION IN FLOW INJECTION ANALYSIS AND LIQUID CHROMATOGRAPHY

being a thesis submitted for the degree of

DOCTOR OF PHILOSOPHY

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by

JAMES STEVEN LANCASTER

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ABSTRACT

This thesis describes the development of chemiluminescence procedures for the detection of some organic compounds of interest to the oil industry.

Water soluble tertiary amines were determined using flow-injection analysis with chemiluminescence detection. The chemiluminescence was generated by reacting the aqueous tertiary amine solution with aqueous sodium hypochlorite at pH 11.0, in the presence of rhodamine B, which sensitizes the emission. The simplex optimized flow-injection manifold was used to determine trimethylamine, triethylamine and tripropylamine in water and sea water. A standard addition calibration procedure was used to determine trimethylamine in an industrial scrubbing medium.

The chemiluminescence of tertiary amines was extended to the detection of the non-ionic surfactant, Nonidet AT 85, which contains a tertiary amine group. The flow-injection manifold was simplex optimised for this analyte in sea water. The surfactant was also determined in aqueous extracts of marine sediment. Calibration was by a standard addition procedure.

Primary amines were determined by flow-injection analysis after fluorimetric derivatization with o-phthalaldehyde and 2-mercaptoethanol in non-aqueous media. The fluorescent derivative was detected by

excitation using the peroxyoxalate chemi-excitation reaction.

A pre-column fluorimetric derivatization procedure was developed for the determination of carboxylic acids in non-aqueous media. Straight chain acids over the range C_2 to C_{20} and benzoic acid were derivatized with 9-anthracene methanol, using dicyclohexylcarbodiimide (DCC) as a coupling reagent to yield fluorescent esters. Separation was carried out by reversed-phase high-performance liquid chromatography and the peroxyoxalate chemi-excitation reaction was used to detect the fluorescent species.

An automated flow-injection manifold was developed, including computerised data acquisition and automated sample uptake and injection. This enabled the system to operate whilst unattended. The precision of the data was higher than that obtained with the manual system.

Finally, a flow-injection manifold was designed which incorporated a monochromator between the flow cell and the detector, thus enabling chemiluminescence spectra to be obtained.

Presentations And Publications

Presentations

1. Flow-injection Determination of Organosulphur Compounds With Chemiluminescence Detection. Poster presentation, Research and Development Topics Meeting, Polytechnic South West, Plymouth, 18/7/88.

2. Flow-injection procedure for the Determination of Tertiary Amines in Water and Sea water Using Chemiluminescence Detection. Lecture presentation, Research and Development Topics Meeting, Dublin City University, Dublin, 21/3/1989.

3. Industrial and Environmental Applications of Chemiluminescence, Update course, Sac 89, Cambridge, 2/8/1989.

4. Determination of Fatty Acids in Non-Aqueous Media by HPLC with Chemiluminescence Detection. Lecture presentation, Euroanalysis VII, Technical University of Vienna, Austria, 31/8/1990.

Publications

1. Flow Injection Determination of Organosulphur Compounds With Chemiluminescence Detection, Anal. Proc. 26 (1989) 19.

2. The Application of Chemiluminescence Reactions to the Analysis of Industrial and Environmental Samples, Anal. Proc. 26 (1989) 362.

3. Flow Injection Procedure for the Determination of Tertiary Amines in Water and Sea Water Using Chemiluminescence Detection, Analyst, 114 (1989) 1659.

4. Determination of a Non-Ionic Surfactant in Aqueous Environmental Samples by Flow-Injection Analysis with Chemiluminescence Detection, Anal. Chim. Acta, 239 (1990) 189.

Contents

Chapter One

Introduction

1.1 Molecular Luminescence

The term luminescence describes a wide range of light emitting processes [1], and was first used in 1888 by Wiedemann [2], to describe the phenomenon of cold light. Many types of luminescence are now known and have been classified according to the source of energy responsible for generating the excited state, or for providing the activation energy required to release the excess energy stored within a molecule by previous irradiation [3]. Examples of luminescence include pyroluminescence, thermoluminescence, electroluminescence, piezoluminescence, crystalloluminescence, lyoluminescence and photoluminescence. Pyroluminescence occurs when gaseous metal atoms are excited by the thermal energy of a flame. Thermoluminescence is the emission of light on heating to temperatures below that needed for incandescence, and is characteristic of certain crystals which are able to store the energy of previous irradiation. This is released as light when heating provides the activation energy for luminescence. An example of this is fluorspar. Electroluminescence is the emission accompanying an electrical discharge in a gas, and is due to bombardment of the gas molecules by electrons. Piezoluminescence or triboluminescence occurs when bonds are ruptured by compression of certain crystals. Frictional forces which occur during formation or dissolution of crystals may give rise to

crystalloluminescence and lyoluminescence respectively. Fluorescence is the re-emission of light from substances during irradiation. If the emission persists after the exciting radiation is removed, the process is known as phosphorescence. Fluorescence and phosphorescence require irradiation by an external energy source, and the terms photoluminescence, cathodoluminescence, anodoluminescence and radioluminescence have been used to indicate the type of exciting radiation.
Chemiluminescence (CL) is the emission of light during certain chemical reactions, and may occur in the solid, liquid and gas phases. Many organic and inorganic compounds exhibit chemiluminescence when oxidized.
Bioluminescence (BL) is the enzyme mediated chemiluminescence which occurs in biological systems. Many organisms exhibit BL and it is particularly common in marine organisms. BL also occurs in certain bacteria, fungi and insects.

1.2. Principles Of Photoluminescence

Photoluminescence occurs when certain molecules, promoted to an electronically excited state by the absorption of ultra-violet or visible radiation decay back to the ground state by emitting the excess energy as electromagnetic radiation in the visible region. Photoluminescence can be further classified according to the type of electronic transition involved in the decay process. Fluorescence occurs when an excited singlet state (the spin of the excited state electron is

unchanged) decays to the ground singlet state. This is known as an allowed transition as no change in electron spin is involved. Consequently fluorescence is very rapid and the excited state has a lifetime of between 10^{-8} s and 10^{-6} s. Phosphorescence occurs when an excited triplet state (the spin of the excited state electron is inverted) relaxes to the ground singlet state. As this transition involves a change in electron spin the transition is much less probable than a singlet to singlet transition. This is known as a forbidden transition. Consequently the lifetime of the excited triplet state is much longer than that of the corresponding singlet state, and phosphorescence has a lifetime between 10^{-4} s and several seconds. Figure 1.1 shows an energy level diagram including first and second excited singlet and triplet states. Phosphorescence usually occurs at longer wavelengths than fluorescence as the first excited triplet state has a lower energy than the corresponding singlet state. Radiative and non-radiative mechanisms for deactivation are shown. The absorption of electromagnetic radiation to give an excited state, may result in the population of any one of the closely spaced vibrational and rotational energy levels. The excess vibrational and rotational energy is lost rapidly ($<10^{-12}$ s) to the environment by vibrational and rotational relaxation. Consequently, luminescence occurs from the lowest vibrational level within the excited state. When two electronic energy levels are sufficiently close to permit overlap of the vibrational

levels, a very efficient mechanism for non-radiative deactivation exists. This is known as internal conversion. Deactivation may also occur by collisional energy transfer from the excited species to the solvent molecules, and this is external conversion. This becomes more efficient with increasing frequency of collisions, therefore reducing temperature or increasing solvent viscosity often results in an increase in fluorescence intensity. The excited species may also be shielded within micelles [4,5] and cyclodextrins [6] or adsorbed onto solid surfaces [7]. Population of the excited triplet state occurs by intersystem crossing from the corresponding singlet state. Direct population from the ground state is rare. As with internal conversion the probability of intersystem crossing is greatly increased if overlap of the vibrational energy levels within the two states occurs. The probability of spin inversion is also increased by the presence of a heavy atom such as bromine [6]. This is due to spin-orbit interactions promoted by the heavy atom making the singlet to triplet transition more probable. Due to the time scale of phosphorescence, non-radiative deactivation pathways are kinetically favourable in solution. Phosphorescence is therefore observed only when the excited species is shielded from solvent interactions, for example by adsorption onto a solid surface. These processes are illustrated in Fig. 1.1. The favoured deactivation

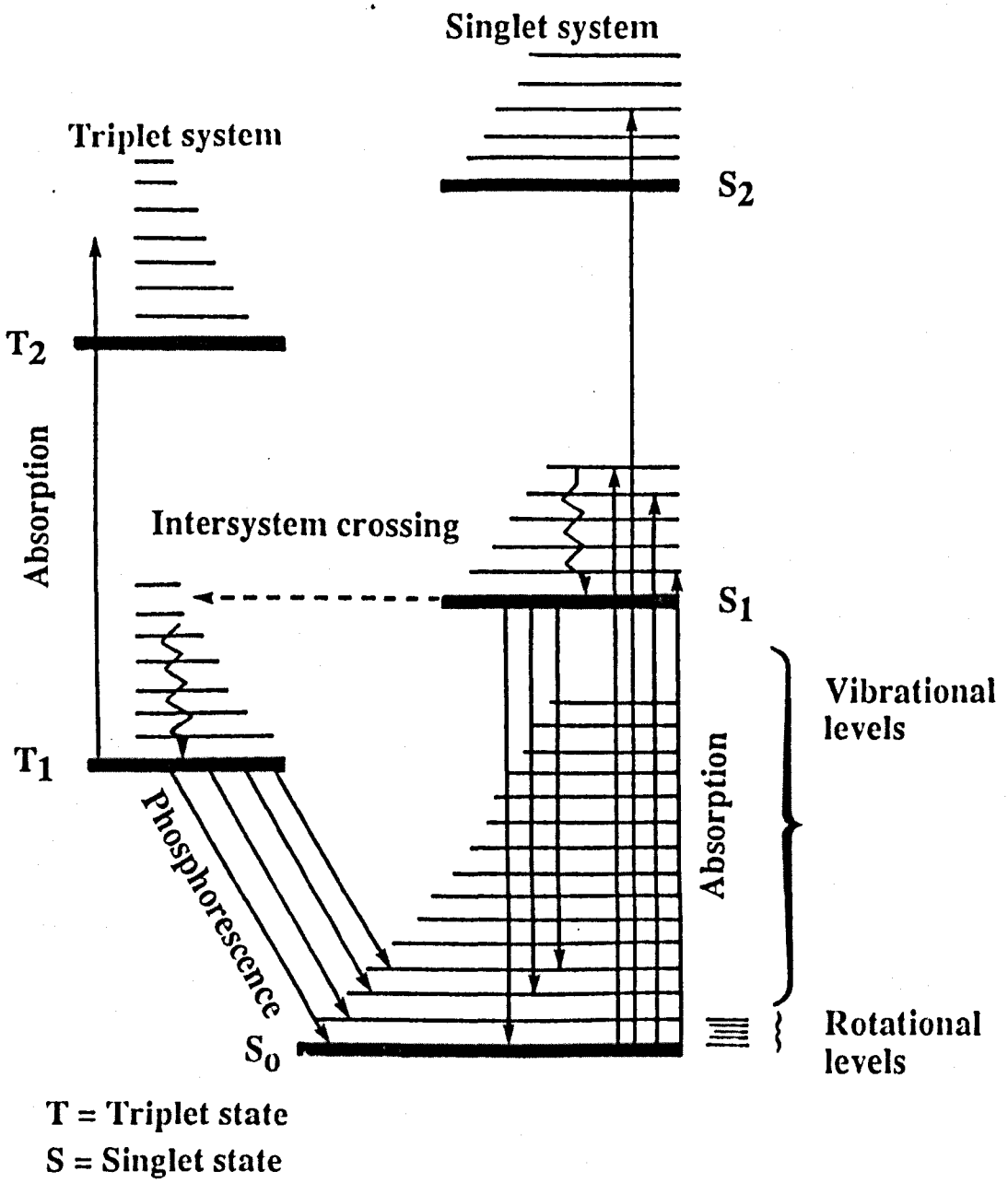
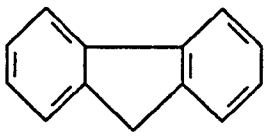
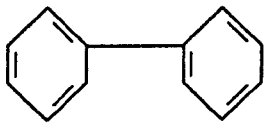


Figure 1.1. Energy level diagram showing absorption and relaxation processes of a molecule.

pathway is the one which minimises the lifetime of the excited state, therefore luminescence processes are in competition with non-radiative deactivation processes. Photoluminescence is limited to a relatively small number of molecules which have structural features causing the non-radiative deactivation processes to be slowed sufficiently to allow deactivation by luminescence to compete kinetically. The most intense fluorescence occurs in compounds containing aromatic groups and other conjugated π systems which enable low energy π bonding to π antibonding transitions ($\pi - \pi^*$) to occur. Compounds containing aliphatic carbonyl groups may exhibit fluorescence due to the possibility of non-bonding to π antibonding transitions ($n - \pi^*$). These transitions have lower molar absorptivities than $\pi - \pi^*$ transitions, and, as emission is the reverse process of absorption the resulting fluorescence is less intense. Another feature of fluorescent molecules is structural rigidity. Lack of rigidity within a molecule is thought to result in the enhancement of the rate of deactivation of the excited state by internal conversion. This is due to the increased probability of the overlap of vibrational energy levels of the ground state and first excited states. The quantum efficiency of fluorescence, ϕ_F, is the fraction of excited molecules which lose their excess energy by radiative decay, for example, the quantum efficiency of biphenyl and fluorene are 0.2 and almost 1 respectively. The difference being due to the increased rigidity of fluorene [8].



FLUORENE



BIPHENYL

1.3. Historical Aspects Of Chemiluminescence

The phenomenon of cold light has been known throughout the ages and has intrigued man since records began. The appearance of light without heat or fire was often thought to be of supernatural or religious significance. The superstitions of every race refer to strange light or flames such as glowing hands and luminous corpses, glowing trees and shining animals [2]. Many of these stories undoubtedly originate from actual observations of natural luminescent phenomena. The first recorded observation of firefly luminescence is probably that found in Chinese literature dating from around 1500 to 1000 B.C. [9]. Aristotle (384 to 322 B.C.) is credited with the first recorded study of cold light [10], and recorded some well known types of luminescence, including the light from dead fish and fungi. He also refers to the luminous "juice" of the cuttlefish, and was probably referring to the secretion from the open glands

of the deep sea squid Heteroteuthis dispar, which contains bioluminescent bacteria.

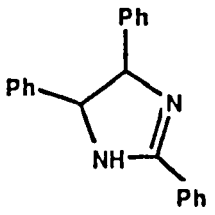
During the dark and middle ages (200 to 1400 AD.) there is little recorded observation of cold light and knowledge of luminescence remained largely unchanged until the renaissance of science in the fifteenth and sixteenth centuries. During this time Gesner published the first book wholly devoted to luminescence [11], in which he described many types of natural luminescence.

In 1669 Robert Boyle established some of the properties of bacterial and fungal luminescence, and demonstrated that the removal of air surrounding "shining flesh" and "shining wood" caused a large reduction in the intensity of the light. In addition, he recognised that the luminescence of minute amounts of material could be detected. In the same year the first example of artificial chemiluminescence was reported by Hennig Brandt, a Hamburg physician intent on making his fortune through the practice of alchemy. In 1669 he isolated a substance by the distillation of urine, and on reduction of the remaining material Brandt found the product glowed with a blue light without previous exposure to light. He called this substance phosphorus mirabilis, or miraculous light [2].

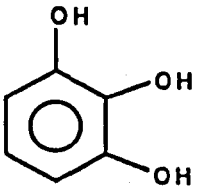
In 1821 Macaire [12] studied certain luminescent organisms and concluded that the luminous material was composed mainly of "albumine", and required oxygen.

Macaire's experiments disproved the idea that the light was caused by phosphorus. Over half a century later Raphael Dubois reported a series of experiments which were to lead to the modern study of bioluminescent reactions. He isolated two extracts from the luminous organ of the beetle Pyrophorus, one was a cold water extract which he called luciferase, the other was a hot water extract, luciferin. He found that mixing the two extracts resulted in restoration of the emission, and concluded that the luminescence was the result of a chemical reaction between the two substances [13].

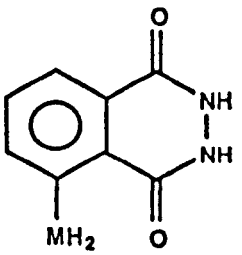
The first synthetic organic compound to exhibit chemiluminescence was lophine (Table 1.1) prepared by Radziszewski [14] in 1877. This was followed by the discovery of the chemiluminescence of pyrogallol (Table 1.1) [15]. Many more CL reactions were discovered during the early 20th century, including two of the more efficient chemiluminescent substances known to date. These being 5-amino-2,3-dihydrophthalazine-1,4-dione (luminol), discovered by Albrecht [16] in 1928, and dimethyldiacridinium nitrate (lucigenin), discovered by Gleu and Petsch [17] in 1935 (Table 1.1). Since then many more chemiluminescent reactions have been discovered, and much work has been done to establish the mechanisms of these reactions, though many of the proposed mechanisms are still the subject of speculation.



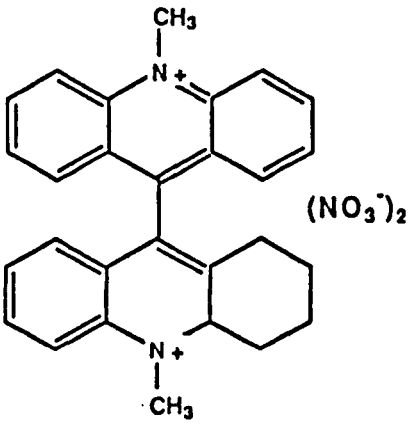
Lophine



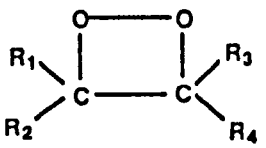
Pyrogallol



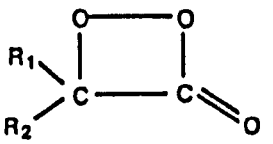
Luminol



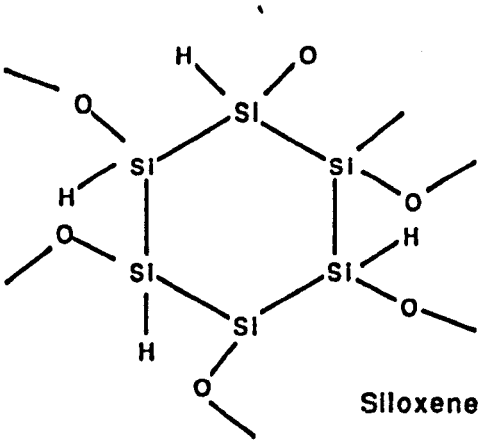
Lucigenin



A dioxetane



A dioxetanone



Siloxene

Table 1.1. Some chemiluminescent compounds.

1.4. General Principles Of Chemiluminescence

Chemiluminescence (CL) is observed when a chemical reaction (usually an oxidation reaction) yields a product in an electronically excited state which relaxes to the ground state by releasing a photon. In its simplest form CL may be represented as:

$$A + B \longrightarrow P^* \longrightarrow P + \text{LIGHT}$$

where A is an oxidizing reagent, B is a chemiluminescent molecule, P^* is an excited product and P is the ground state product.

The first stage of the CL process is the formation of a product or metastable intermediate in an electronically excited state. The second stage is the relaxation of the excited state product by the emission of electromagnetic radiation, which can occur from the near ultra-violet to the infra-red [18]. In solution phase CL the emission process is usually fluorescence. In many cases the excited state product is either weakly or non-fluorescent. The CL efficiency may be enhanced by the addition of an energy acceptor (sensitizer) which accepts the energy of the initial excited product and becomes the emitter. This is known as energy transfer or sensitized CL, and can be represented as:

$$A + B + S \longrightarrow P^* + S \longrightarrow P + S^* \longrightarrow P + S + \text{LIGHT}$$

where S is the energy acceptor.

CL is relatively rare as most reactions yield their excess energy as heat rather than light. For CL to occur three requirements must be satisfied:

1. The reaction must generate sufficient energy to enable the formation of an electronically excited product or intermediate to occur.

2. A suitable reaction pathway must exist to enable this energy to form an electronically excited state.

3. The excited product must decay to the ground state either by emitting a photon (direct CL) or by transferring its energy to a suitably fluorescent energy acceptor (sensitized CL).

The energy of a photon can be related to its wavelength by the equation:

$$E = h\nu = hc/\lambda$$

where E is the energy (J), h is Planck's constant (J s), v is the frequency of the radiation (Hz), c is the velocity of light (m s^{-1}) and λ is the wavelength of the light (m). Therefore the energy which the reaction must provide for emission at 400 nm is:

$$E = hc/4X10^{-7} = 4.97X10^{-19}\ J = 300\ kJ\ mol^{-1}$$

Similarly, it can be shown that 184 kJ mol^{-1} is required for emission at 650 nm. Hence reactions which

provide sufficient energy are usually oxidation reactions involving molecular oxygen, hypochlorite ion, hydrogen peroxide or other strong oxidizing agents. The excitation step frequently involves electron exchange or the cleavage of linear or cyclic peroxides [3].

The efficiency of chemiluminescence can be defined as the ratio of the number of molecules emitting light to the number of molecules reacting:

$$\phi_{CL} = \frac{\text{The number (or rate) of molecules emitting}}{\text{The number (or rate) of molecules reacting}}$$

Alternatively, the CL efficiency can be shown as a function of the efficiency of the excitation stage and the fluorescence stage:

$$\phi_{CL} = \phi_{EX} \times \phi_{FL}$$

Chemiluminescence reactions can often be used for quantitative analysis as the concentration of either a reactant or a catalyst can, by suitable control of the reaction conditions, be made proportional to the rate of the reaction. The CL intensity (I_{CL}) is a function of the CL efficiency and the reaction rate [19]:

$$I_{CL}\ (t) = \phi_{CL}\ dc/dt(t)$$

Where I_{CL} is the CL intensity at time t, ϕ_{CL} is the quantum efficiency of the CL reaction and dc/dt (t) is the rate of reaction at time t.

In many CL reactions there are several possible reaction pathways available to the reaction and the pathway leading to luminescence is often a minor one. This is reflected in the relatively poor efficiency of many CL reactions, even when the fluorescence efficiency of the excited state product is high. For example, the luminol reaction has an overall quantum yield of only 1%, though the fluorescence efficiency of the emitting species (3-aminophthalate) is about 30% [20]. From this it can be deduced that the excitation efficiency of this reaction is 3%. This is in contrast to bioluminescence reactions, which often have much higher efficiencies, e.g., firefly luminescence has a quantum yield of 88% [21]. This is attributable to the use of enzymes which ensure both high excitation and emission efficiencies.

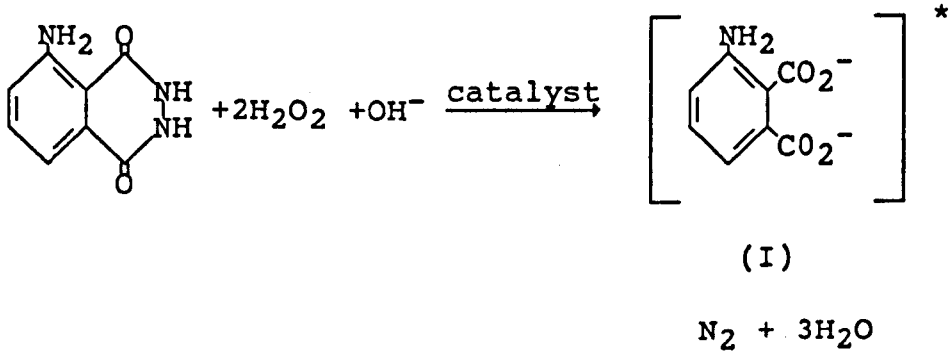
1.5 Solution Phase Chemiluminescence

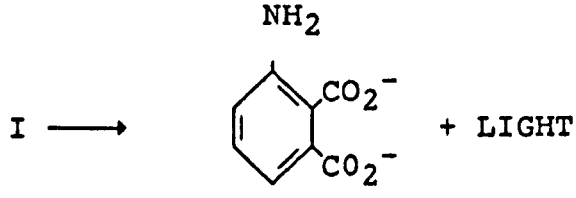
Many solution phase CL reactions are now known, and several of these have considerable analytical potential. Examples of solution phase CL reactions include:

1.5.1. The Luminol Reaction

One of the most well known and widely studied CL reactions is the oxidation of luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) by hydrogen peroxide in the presence of a catalyst in basic aqueous solution. Since its discovery in 1928 [16] the luminol reaction (and those of related hydrazides) has been studied extensively [22,23]. However, the detailed mechanism is still the

subject of speculation. The luminol CL spectrum has a maximum at 425 nm in aqueous media (Fig. 2.7) and this is identical to the fluorescence spectrum of 3-aminophthalate (I), which is known to be the emitting species [24]. Although the detailed mechanism is not known, the overall reaction is:



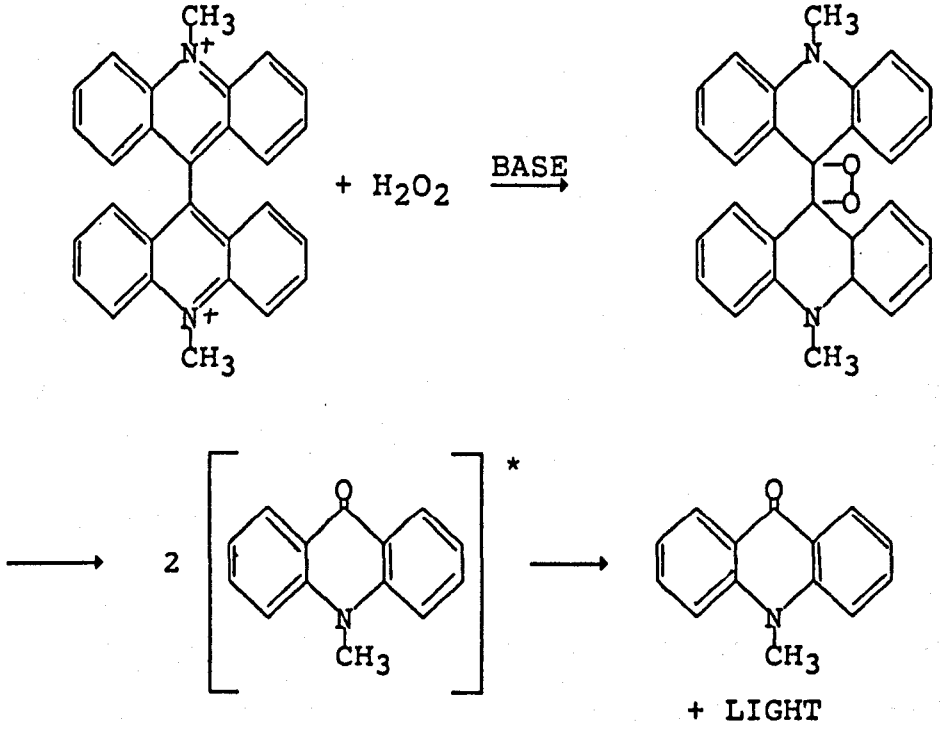


A number of other oxidizing agents including molecular oxygen also result in CL and the reaction is catalysed by at least forty metal ions [25]. One of the first analytical applications of the luminol reaction was reported in 1937 for the quantitation of hydrogen peroxide [26]. Since then many more analytical applications have been reported, including the

determination of cobalt (II) [27], chromium (III) [28] and hydrogen peroxide [29].

1.5.2 The Lucigenin Reaction

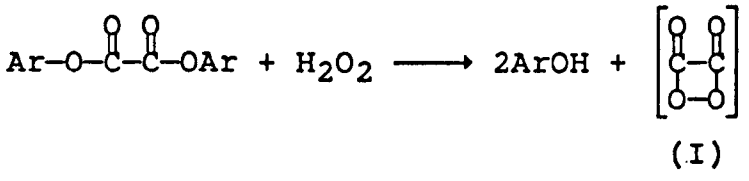
Lucigenin (dimethyldiacridinium nitrate) gives a CL emission when oxidized in a basic aqueous medium and, like luminol, is catalysed by certain metal ions. The emission spectrum has a maximum intensity at 500 nm and the emitting species is N-methyl acridone [18]. The overall mechanism of the CL reaction is shown below and is thought to proceed via a dioxetane intermediate [30].



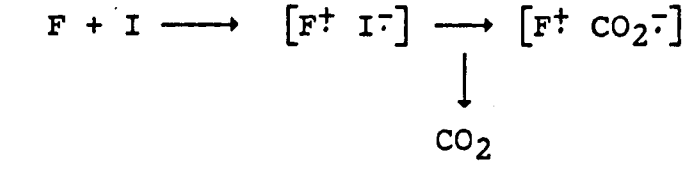
1.5.3. Peroxyoxalate Chemiluminescence

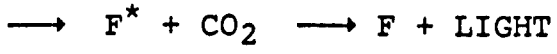
Peroxyoxalate CL was first reported by Rauhut in 1967 [31]. The emission is generated by the reaction of an oxalate ester with hydrogen peroxide in the presence of a suitably fluorescent energy acceptor. The two most widely used oxalates are bis(2,4,6-trichlorophenyl) oxalate (TCPO) and bis(2,4-dinitrophenyl)oxalate (DNPO). Other aryl oxalates have been synthesized and evaluated with respect to their possible analytical applications [32]. Peroxyoxalate CL is an example of indirect or sensitized CL in which the energy from an excited intermediate is transferred to a suitable fluorescent molecule, which relaxes to the ground state by emitting a photon. Rauhut and co-workers have reported that the intermediate responsible for providing the energy of excitation is 1,2-dioxetanedione [31,33]. The peroxyoxalate reaction is able to excite many different compounds, having emissions spanning the visible and infrared regions of the spectrum [33,34], and the reaction can supply up to 440 kJ mol^{-1}, corresponding to excitation at 272 nm [35]. It has been found, however, that the CL intensity corrected for quantum yield decreases as the singlet excitation energy of the fluorescent molecule increases [36]. There is also a linear relationship between the corrected CL intensity and the oxidation potential of the molecule [36]. This suggests the possibility of an electron transfer step in the mechanism, as demonstrated in several other CL systems [37-40]. It has been postulated that a transient

charge transfer complex is formed between the intermediate 1,2-dioxetanedione and the fluorescer [41], and a modified mechanism was proposed involving the transfer of an electron from the fluorescer to the reactive intermediate [42]. The excitation is thought to result from the annihilation of the fluorescer radical cation with the carbon dioxide radical formed when the 1,2-dioxetanedione decomposes [43].



(I)





Recently the role of 1,2-dioxetanedione has been questioned and other intermediates have been proposed [44-47]. Peroxyoxalate CL is an example of chemically initiated electron exchange luminescence (CIEEL).

Electron exchange luminescence may also occur when electrochemically generated radical cations and anions react to give excited state products which emit light on relaxing to the ground state.

1.5.4. Electrogenerated Chemiluminescence

Electrogenerated CL or electrochemiluminescence occurs when radical cations and radical anions, generated at the surface of an anode and a cathode respectively, form a charge transfer complex which breaks down to give an excited state product [38].

$$A\cdot \longrightarrow A^{+\cdot} + e^- \quad \text{Anode reaction}$$
$$C + e^- \longrightarrow C^{-\cdot} \quad \text{Cathode reaction}$$
$$A^{+\cdot} + C^{-\cdot} \longrightarrow [A^{+\cdot}\ C^{-\cdot}] \longrightarrow A^* + C$$
$$A^* \longrightarrow A + \text{LIGHT}$$

where A^+ and A^- are the cation and anion radicals of the donor and acceptor molecules. A^* is the excited state species.

1.5.5. Organometallic Compounds

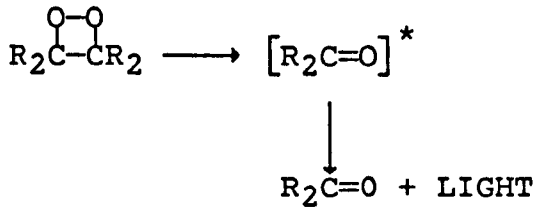
Solution phase CL occurs when certain organometallic compounds are oxidized. This is a well known phenomenon, and the CL of Grignard reagents was first reported in 1906 [48]. Examples include aryl

magnesium halides [49], alkyl magnesium halides and lithium diphenylphosphides and related organophosphides [50]. The most intense CL emission arises from the oxidation of p-chlorophenyl magnesium bromide [51]. The CL emission is thought to be due to the formation of radicals which recombine to give alkylated biphenyls in an excited state [49].

1.5.6. 1,2-Dioxetanes

During the 1960s several workers predicted that 1,2-dioxetanes were the critical intermediates in the chemiluminescence of certain organic compounds including lophine, acridinium salts, indoles, peroxyoxalates and several luciferins.

Simple dioxetanes (Table 1.1) are unstable due to highly strained oxygen bonds and decompose near room temperature to generate excited carbonyl products [52,53]. In 1969 however, trimethyl-1,2-dioxetane was synthesized and was found to be relatively stable, having a half life of 20 minutes at 60°C [54]. Since then many more have been reported and all are chemiluminescent [55].



Both excited singlet and triplet states are formed on thermal decomposition of the dioxetanes, though for simple alkyl carbonyl compounds, radiative efficiencies are very low [56]. The emission intensities can be increased significantly by the addition of a fluorescent energy acceptor [52]. More efficient dioxetane systems based on the highly fluorescent acridan system have been prepared, and quantum yields of up to 30% have been obtained [57].

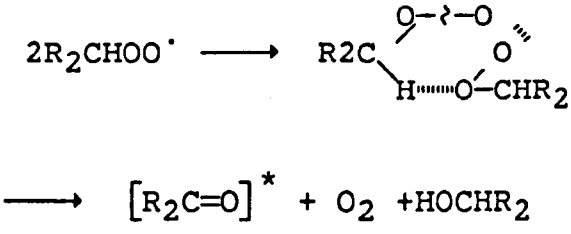
1.5.7. 1,2 - Dioxetanones

1,2-dioxetanones (Table 1.1) are inherently less stable than dioxetanes but are particularly interesting because of their role in several BL reactions [58,59]. These compounds have been synthesized and rapidly decompose at room temperature to give electronically excited aldehydes and ketones [60].

1.5.8. Autoxidation Reactions

Several organic compounds react slowly with molecular oxygen at room temperature to produce a very weak CL emission [61,62]. The emission is thought to be due to the combination of peroxy radicals which are

responsible for the propagation and termination of the autoxidation chain reaction [63]. The reaction is thought to proceed via a six membered transition state as shown below [50].



The emitting species has been found to be the excited carbonyl product [63].

1.5.9. Singlet Oxygen CL

CL may occur in reactions involving electronically excited oxygen. Ground state oxygen exists in the triplet state, and excited state oxygen exists in the singlet state. Excited state oxygen can be produced in a number of ways such as passing molecular oxygen through a microwave discharge [64,65], or by the reaction of hydrogen peroxide with the hypochlorite ion [66]. This results in an emission at 634 nm.

$$OCl^- + H_2O_2 \longrightarrow Cl^- + H_2O + {}^1O_2$$
$${}^1O_2 \longrightarrow {}^3O_2 + \text{LIGHT}$$

The emission maximum occurs at 634 nm.

1.6. Solid Phase Chemiluminescence

Few solid phase CL reactions are known. The best documented example is the oxidation of siloxene. Siloxene is a yellow silicate polymer obtained from the reaction of calcium silicide with hydrochloric acid [67], and has the basic formula $(Si_6H_6O_3)_n$. The silicon atoms are arranged in hexagonal rings joined by oxygen atoms to form a lamina structure [68]. The basic structure (Table 1.1) is randomly substituted by hydroxyl and chlorine groups and this affects both the CL spectrum and the CL efficiency. CL emission in the red region of the spectrum results from the reaction of siloxene with oxidants such as ceric sulphate, chromic acid, potassium permanganate and nitric acid [67]. Lithium organophosphides also exhibit solid phase CL on oxidation with molecular oxygen [69].

1.7. Gas Phase Chemiluminescence

The earliest recorded example of gas phase CL is the oxidation of phosphorus by molecular oxygen, which occurs with phosphorus vapour just above solid phosphorus, to give a green emission. The mechanism of

this reaction is not known [70] though the emitting species have been identified as $(PO)_2$ and HPO [71]. Gas phase CL has recently been used to monitor atmospheric pollutants, including nitrogen oxides [72]. Nitric oxide gives a CL emission when reacted with ozone to yield nitrogen dioxide in an excited state [73]. A linear response from 0 to 1% with a detection limit of 1 ppb was reported.

$$NO + O_3 \longrightarrow O_2 + [NO_2]^* \longrightarrow NO_2 + LIGHT$$

The maximum CL intensity occurs at 600 nm.

Nitrogen dioxide and other nitrogen containing compounds have been determined after thermal catalytic reduction to NO [74]. This technique has also been used to determine volatile nitrosamines following separation by gas chromatography. Catalytic reduction of the nitrosamines takes place within a thermal energy analyser to yield nitrosyl radicals which react with ozone to generate electronically excited nitrogen dioxide [75].

Gas phase CL has been used to determine other atmospheric pollutants, including hydrogen sulphide and dimethyl sulphide. This is based on the CL emission resulting from the oxidation of divalent sulphur compounds with ozone, and a limit of detection of 4 ppb has been reported for hydrogen sulphide [76]. The

reaction mechanism is complex, though excited sulphur dioxide has been identified as the emitting species [77,78], and the final steps in the mechanism are:

$$SO + O_3 \longrightarrow [SO_2]^* + O_2$$

$$[SO_2]^* \longrightarrow SO_2 + \text{LIGHT (400 nm - 330 nm)} \quad (78)$$

Other gaseous pollutants determined by gas phase CL include hydrocarbons [79] and carbon monoxide [80].

CL emission may occur in cool flames at temperatures below that required for thermal excitation [81]. An example of this is the molecular emission of sulphur in cool flames:

$$S + S \longrightarrow [S_2]^* \longrightarrow S_2 + \text{LIGHT}$$

Maximum intensity occurs at 384 nm

This is the basis of the flame photometric detector (FPD) used in gas chromatography [82]. The flame photometric detector can also be used for detecting phosphorus and nitrogen-containing compounds in GC eluents [83,84].

$$H + NO \longrightarrow [HNO]^{*} \longrightarrow HNO + LIGHT$$

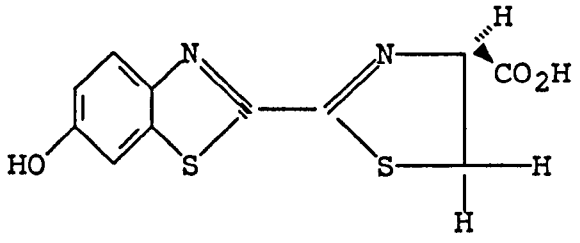
(660 nm - 770 nm)

Molecular Emission Cavity Analysis (MECA) is a sample introduction technique in which samples are introduced into a cool flame within a small cavity at the end of a rod. The resulting CL emission takes place within the cavity [85] and provides a sensitive and reproducible means of analysing small samples. The technique has been applied to a wide range of species, including selenium and tellurium [86], the halogens [87] and arsenic and antimony [88].

1.8. Bioluminescence

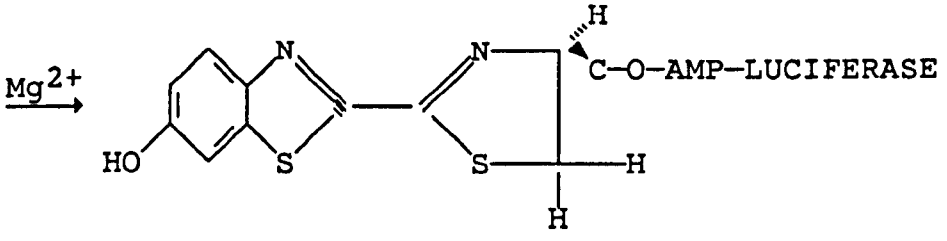
Bioluminescence is an example of chemiluminescence in which an enzyme, known as a luciferase, catalyses the oxidation of a chemiluminescent substrate, known as a luciferin. Molecular oxygen is the oxidant, and the product is formed in an electronically excited state which decays to the ground state by emitting a photon.

Firefly luminescence is the most efficient BL reaction known, and has a quantum efficiency of 88% [3]. In 1947 the reaction was shown to require adenosine triphosphate (ATP) as a cofactor [89]. The reaction mechanism is as shown:

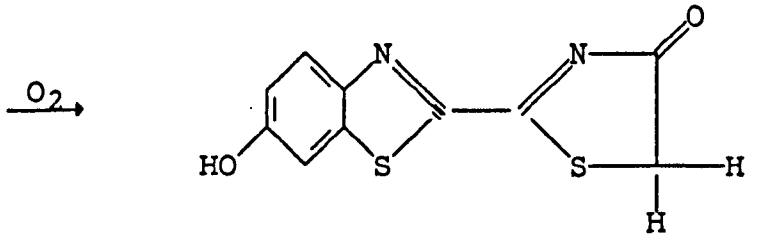


+ ATP + LUCIFERASE

LUCIFERIN



ENZYME SUBSTRATE COMPLEX



+ CO_2 + AMP

EXCITED STATE CARBONYL COMPOUND

The analytical potential of the firefly reaction for sensitive ATP assays and for metabolites and enzymes participating in ATP converting reactions was realised as early as 1952 [90]. The major disadvantage of BL analysis is the expense of the reagents, due to the difficulty of isolating them in sufficient quantity and

purity. The use of immobilized reagents for the firefly BL reaction was reported in 1977 [91]. The benefits of immobilization of the reagents include increased enzyme stability, reduced reagent costs, better sensitivity and compatibility with automated continuous flow analysis. BL reactions exhibit two important characteristics which make them particularly desirable from an analytical viewpoint. They are selective and highly sensitive, and the benefits of this, together with the convenience of immobilized reagents, means that BL assays are becoming increasingly widely employed in clinical and biomass assays.

1.9. Chemiluminescence Detection In Analytical Chemistry

The major advantage that chemiluminescence offers over other related techniques, such as uv-visible and fluorescence detection is the potential for lower detection limits. The development of efficient photomultiplier tubes has enabled the light from CL reactions with very low quantum yields to be detected [92]. The absence of a source eliminates the background due to the native fluorescence of the blank, fluorescent impurities in the reagent and solvent, fluorescence of the cell, light scattering and noise due to source fluctuation. This enables the CL emission to be detected against a much darker background than is possible with fluorescence detection and, depending on the quantum yield of the CL reaction, extremely low limits of detection can be achieved. For example, limits of

detection of $1X10^{-11}$ M hydrogen peroxide [25] and $1X10^{-10}$ M morphine [93] have been reported. Potentially lower limits of detection with BL techniques can be achieved due to the higher quantum yields of these reactions, and attomole detection limits are possible [94].

Chemiluminescence reactions are rapid, and usually reach maximum intensity in less than one or two seconds. Because of this, large numbers of samples can be analysed in a short period of time, particularly when combined with flow injection analysis. CL detection is therefore appropriate for rapid on-site analysis, and assays which require a large sample throughput.

The major disadvantage with many CL reactions is that other species can often affect the CL reaction and either enhance or suppress the emission. For example, many metal ions and organic compounds will affect the light emission from the luminol and lucigenin reactions [95], and a range of fluorescent compounds can be excited by the peroxyoxalate reaction [96]. Because of this, and despite the growing list of publications dealing with CL analysis, the analytical applications of chemiluminescence have remained confined largely to clinical analysis, utilizing BL reactions, for example for ATP assays, or CL immunoassay techniques. The specificity being due to the enzyme substrate reaction in the case of BL reactions, or the immunogenic response in the case of CL immunoassays. A major area of clinical analysis using CL techniques is the measurement of enzyme

generated hydrogen peroxide. Examples of substrates which can be monitored by the generation of hydrogen peroxide during the reaction with their oxidase enzymes include glucose, xanthene, cholesterol, and choline [3]. The firefly luciferin - luciferase assay is now the method of choice for measuring ATP, and is the most sensitive method currently available, having a limit of detection of $1X10^{-17}$ mol. and a linear range from 1 pM to 1 µM [3]. The procedure is also very rapid, enabling many hundreds of samples per day to be analysed, and the generation or degradation of ATP by bacteria, cells and enzymes can be continuously monitored. ATP assays can be used as a measure of biomass in the brewing, food and water treatment industries, and have even been used in the search for life on Mars [3].

Other examples of BL assays include the determination of the coenzyme nicotinamide adenine dinucleotide phosphate (NADP), using bacterial luminescence [97]. Bacterial luciferase catalyses the oxidation of the reduced flavin mononucleotide ($FMNH_2$) and a long chain aliphatic aldehyde (decanal) by molecular oxygen, resulting in the emission of blue light [97].

$$FMNH_2 + RCHO + O_2 \xrightarrow{\text{LUCIFERASE}} FMN + RCOOH + H_2O + \text{LIGHT}$$

In the presence of the reduced form of nicotinamide adenine dinucleotide phosphate, NADPH, bacterial oxidoreductase converts FMN back to the reduced form, and under suitable conditions the emission is directly related to the concentration of NADPH [98,99].

$$NADPH + FMN + H^+ \xrightarrow{OXIDOREDUCTASE} NADP^+ + FMNH_2$$

The luciferase-oxidoreductase system can be coupled with reactions that produce or consume NADPH and can therefore determine dehydrogenase enzymes and their metabolites [100].

Immunoassay techniques rely upon the binding of the analyte (antigen) with an appropriate antibody to give an immune complex. The specificity of the technique is due to the antibody-antigen binding reaction. The advent of monoclonal antibody technology has enabled the development of highly specific assays for hormones, therapeutic drugs, drugs of abuse and pathogens such as salmonella and HIV. Radioimmunoassay (RIA) is widely used for quantitative analysis. In this technique the analyte binds to a limited amount of antibody and competes with a radio-labelled analyte. The amount of bound activity is thus inversely proportional to the original analyte concentration. The limitations of

radioactive labels include handling and disposal problems, short half life of the label and radio-induced decomposition of the labelled molecule. The use of luminescent labels eliminates these problems, and the earliest CL immunoassay involved antigens or antibodies labelled with luminol derivatives [101]. Many assays were based on aminobutylethyl isoluminol (ABEI) [102], though the modification of the luminol molecule resulted in a reduction in quantum efficiency [103]. More sensitive assays are now based on aryl acridinium esters [104], and analytes can be detected down to $1x10^{-19}$ mol, using commercially available luminometers [105].

Enzyme immunoassays may be coupled to CL reactions using labels such as peroxidase (which catalyses the luminol reaction), bacterial luciferase, firefly luciferase and glucose oxidase [106].

1.10 Instrumentation

BL reactions are often much slower than CL reactions and can last for several minutes. This has enabled very simple instrumentation to be used, and commercially available luminometers are based on direct injection of the oxidant into a cell containing the sample. This is shown in Fig. 2.2. The major problem associated with this procedure for mixing the sample and reagent, particularly when the emission is weak and the CL lifetime relatively short, is that of poor repeatability. The two contributing factors to poor

precision are the dark current from the photomultiplier tube and the lack of efficient mixing between the sample and oxidant [107]. Various mechanical devices for promoting more efficient mixing have been reported [108,109], although the problem of low sample throughput and difficulty of automating the batch procedure remain.

1.10.1. Flow-Injection Analysis

The development of flow injection analysis (FIA) in 1975 [110] provided a successful means of monitoring CL reactions. FIA is based on the reproducible injection of a definite sample volume into an unsegmented carrier stream, pumped at a constant rate through narrow bore tubing [111]. The dispersion of the sample slug and the timing between sample injection and detection are highly reproducible. As a result complete mixing is not required. Therefore FIA provides a highly reproducible means of mixing the sample with the reagent, combined with the potential for rapid sample throughput (30 to 200 samples per hour) and ease of automation. A flow injection manifold suitable for monitoring CL reactions is shown in Fig. 2.3. The sample (typically 20 µl to 100 µl) is injected into a flowing carrier stream via a rotary valve. The oxidant stream is merged with the carrier stream at a PTFE T-piece inside a light tight housing. The merged streams then travel through a short length of tubing into a flow cell immediately in front of a photomultiplier tube (PMT). The streams are pumped via a peristaltic pump through PTFE tubing, typically 0.5 mm

internal diameter. The detector response may be recorded on a chart recorder, or handled by a microcomputer via an analogue-to-digital converter for data collection, storage and manipulation. FIA is therefore a useful technique for CL method development. The copper (II) catalysed oxidation of luminol for the quantitative determination of hydrogen peroxide was the first CL reaction to be adapted to a flow injection procedure [112]. The complex matrices of many industrial, environmental and clinical samples however, can subject FIA procedures to matrix interferences. These may be overcome in certain cases by using highly specific CL reactions, such as the firefly BL reaction for the determination of ATP [113,114], accurate matrix matching as for the flow-injection determination of tertiary amines in sea water with CL detection [107], or standard addition calibration procedures [115]. Alternatively, masking reagents may be used to suppress the effect of interfering species. This technique was used for the CL detection of chromium (III) in natural water samples. The interfering cations were masked with EDTA, and as chromium (III) is kinetically slow to form EDTA complexes, it remains available to catalyse the luminol reaction [108].

1.10.2. High-Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) provides a means of separating the components of the mixture prior to detection, and flow-injection procedures

can be readily adapted to HPLC, the CL flow cell serving as the HPLC detector. Examples of HPLC methods with CL detection include the determination of morphine in body fluids [116], the determination of cobalt in rice flour [117] and boiler waters [118], the determination of trace levels of steroids in blood plasma [119] and the determination of polycyclic aromatic amines in oil samples [43].

Due to the need for sensitive and selective HPLC detectors and the relative scarcity of fluorescent molecules, it is often necessary to derivatize the molecule to make it fluorescent. Many examples of fluorimetric derivatization have been reported, including the fluorimetric detection of carboxylic acids [120], tertiary amines [121] and thiols and disulphides [122]. Recently, the peroxyoxalate chemi-excitation reaction has been applied to the HPLC determination of fluorescent species [43,96,123].

In post column derivatization the chromatographic column separates the underivatized compounds of interest. The derivatization is carried out by adding the reagents continuously to the column eluent prior to detection. Post column derivatization is useful for many applications as the sample preparation is minimal, and since the derivatization stage is on-line the precision is high. It does however impose limitations on the chromatographic mobile phase, which must be compatible with the derivatization reaction. As a result of post-

column mixing, the chromatographic resolution may be affected. The technique is also limited to rapid derivatization reactions. A further consideration is detector selectivity which should be very high for the analyte in the presence of the reagent, and ideally the reagent should not be detected.

Pre-column derivatization has the advantage that the reagent is separated from the analyte prior to detection and providing that the chromatographic selectivity is sufficient, the detector need not be selective. This enables pre-column derivatization procedures to be used with the peroxyoxalate chemi-excitation reaction. Furthermore, the derivatization reaction need not be compatible with the column eluent, and therefore reaction conditions are unrestricted. The major disadvantages of pre-column procedures include the more difficult chromatography due to by-products, and the difficulty of automating the derivatization reaction, resulting in lower sample throughput and sample losses due to manual manipulation. This leads to poor repeatability and results in the necessity for an internal standard. The derivatization reaction should be repeatable and give a high yield of derivative in as short a time as possible.

1.11. Research Objectives

The general aim of this project was to increase the scope of the CL detection of industrial and environmental

samples using FIA and HPLC. The initial aim was to screen compounds containing nitrogen, oxygen and sulphur functionalities for the possible generation of CL emission in both aqueous and non-aqueous media, with the intention of developing new CL reactions with analytical applicability to the oil industry.

The first specific aim was to investigate the possible generation of a CL emission from the oxidation of tertiary amines with aqueous reagents. The objective was to develop a sensitive flow-injection procedure to monitor surfactants containing tertiary amine groups in the aquatic environment.

The second objective was to develop an HPLC procedure for the determination of carboxylic acids in lubricating oils in order to asses their oxidative degradation.

The third objective was to improve the performance of the flow-injection manifold by automating the sample introduction, injection, and data collection and manipulation.

The final objective was to design and build a flow-injection system to monitor the emission spectra of CL reactions.

Chapter Two

Instrumentation

2.1. Introduction

Until recently, the major application of CL and BL techniques has been the determination of ATP in clinical analysis and the use of chemiluminescent labels in immunoassays. Many BL reactions have relatively long-lived emissions, in contrast to CL reactions, which often reach maximum intensity in less than a second, and decay rapidly. Fig. 2.1 shows the emission-time profiles for the determination of ATP using the firefly BL reaction and for the oxidation of isoluminol respectively [124]. Thus, for the firefly reaction the maximum light output is attained after about 1 s, and remains fairly steady, falling by a few percent per minute, whereas the isoluminol reaction gives out most of its light in less than 1 s. The slow rate of reaction and high quantum efficiency of BL reactions means that very simple instrumentation can be used, and most commercial luminometers are based on a direct injection batch procedure. This technique relies on the rapid injection of the reagent for mixing. A typical batch luminometer is shown in Fig. 2.2. The sample is transferred to a cuvette which is placed in a light-tight housing, and the CL or BL reagent is injected. More recent instruments rely on automatic reagent injection. The emission is monitored by a photomultiplier tube (PMT) which is protected from outside light by a shutter which closes

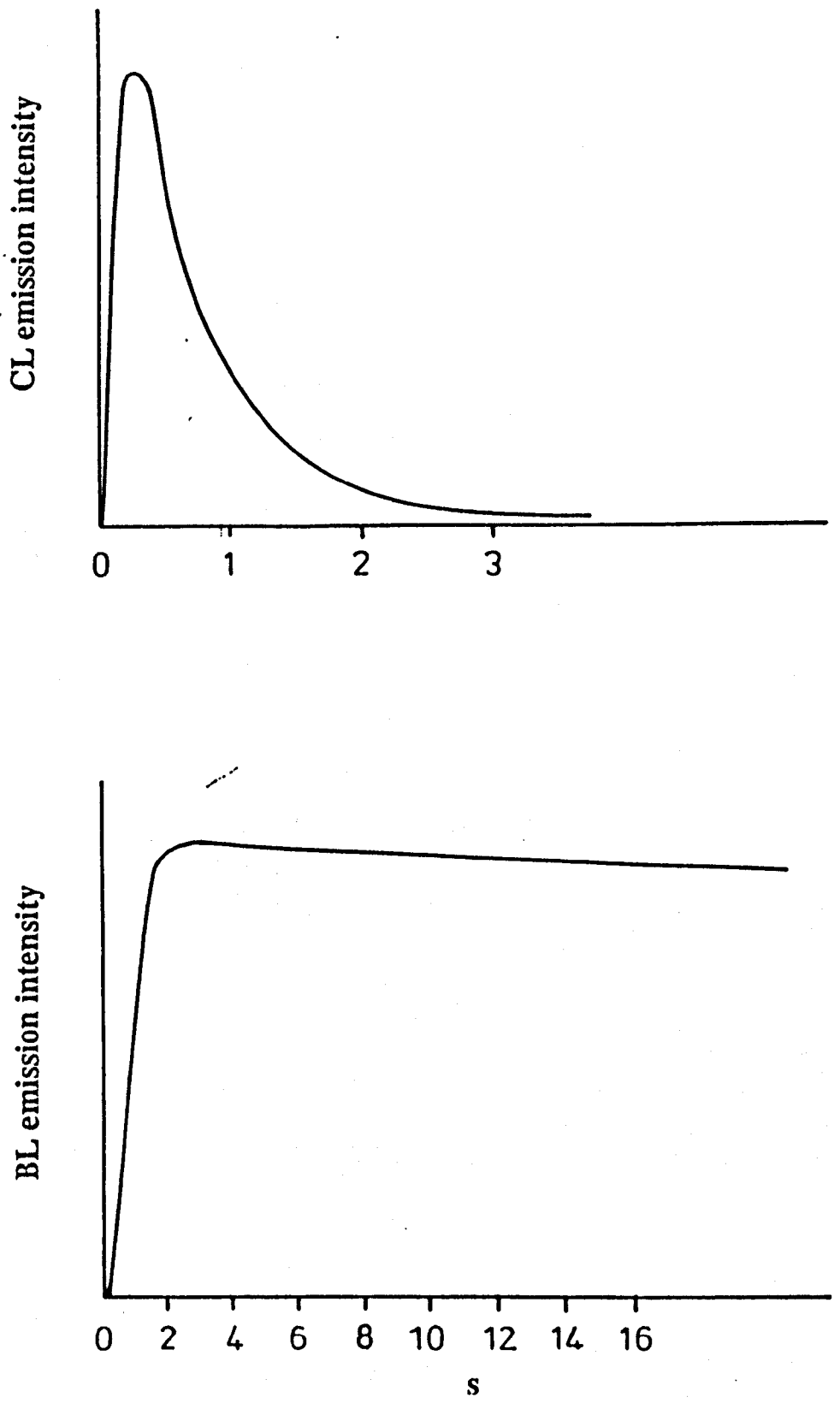


Figure 2.1 **Typical emission profiles for CL and BL reactions.**

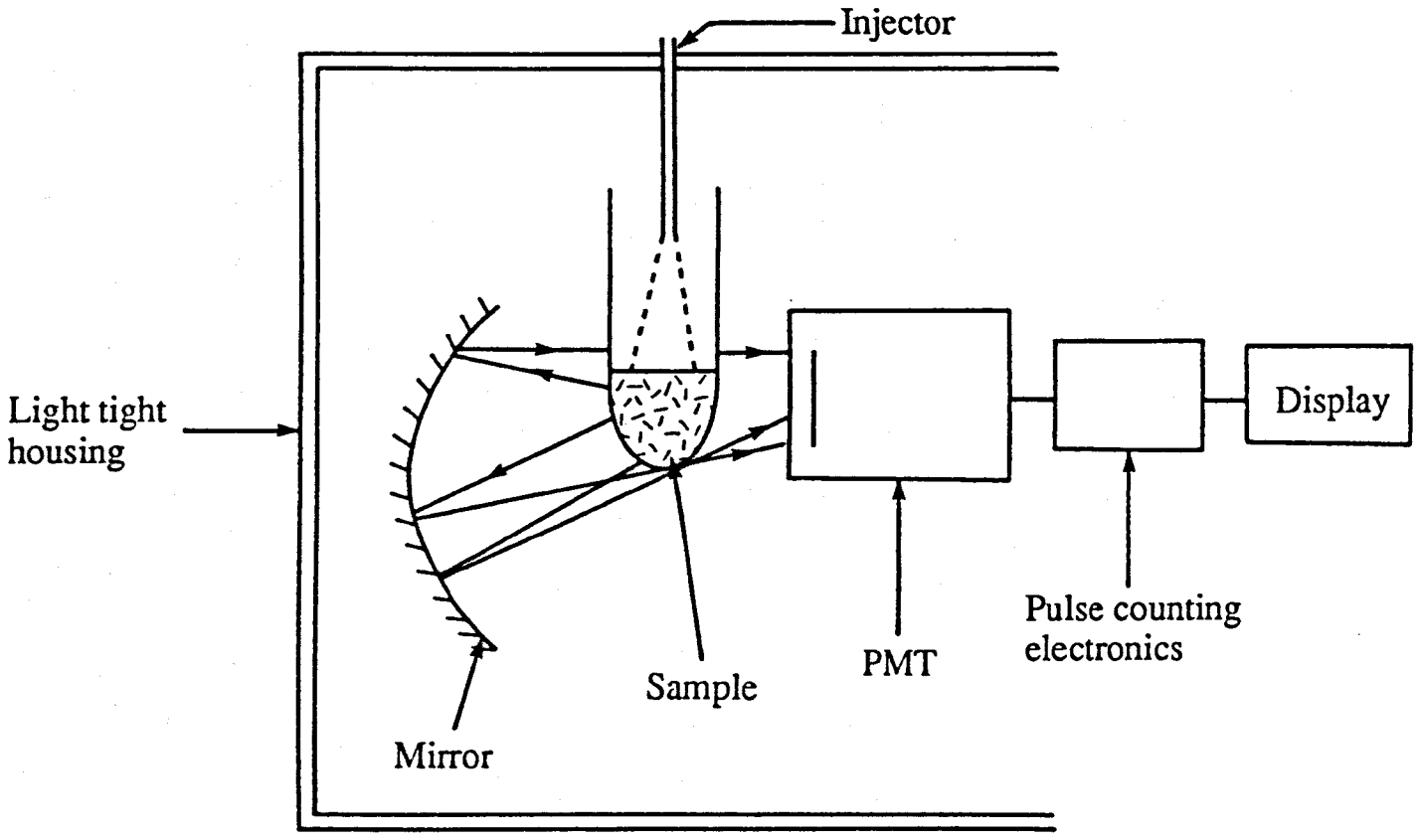


Figure 2.2 Batch luminometer.

automatically when the lid is opened to add the sample. The major problems associated with the batch procedure for monitoring light producing reactions is the difficulty of automation, poor mixing of the sample and reagents and low sample throughput, though recently multi-channel luminometers have become available [125]. Centrifugal analysers have also been adapted to monitor CL reactions [126,127] because of their multi-sample capabilities, though their success was limited because of lower sensitivity due to intermittent light collection.

The rapid nature of many CL reactions makes their reproducible monitoring with simple batch luminometers problematic, as the reactions require rapid and reproducible mixing of the sample with the reagents, and syringe injection is not able to achieve this. The problems of inefficient mixing and low sample throughput have been largely overcome by the use of flow injection analysis (FIA) [128-130]. A simple flow-injection manifold is shown in Fig. 2.3. FIA is based on the reproducible injection of a sample into an unsegmented carrier stream pumped at a constant flow rate (typically 0.2 to 3.0 ml min^{-1}) through narrow bore tubing (typically 0.2 to 2.0 mm i.d.). The carrier and reagent streams are propelled by a pump which is usually a peristaltic pump. The sample is introduced into the carrier stream by a rotary injection valve. The sample slug then disperses within the carrier stream to form a zone which is transported to a detector. The

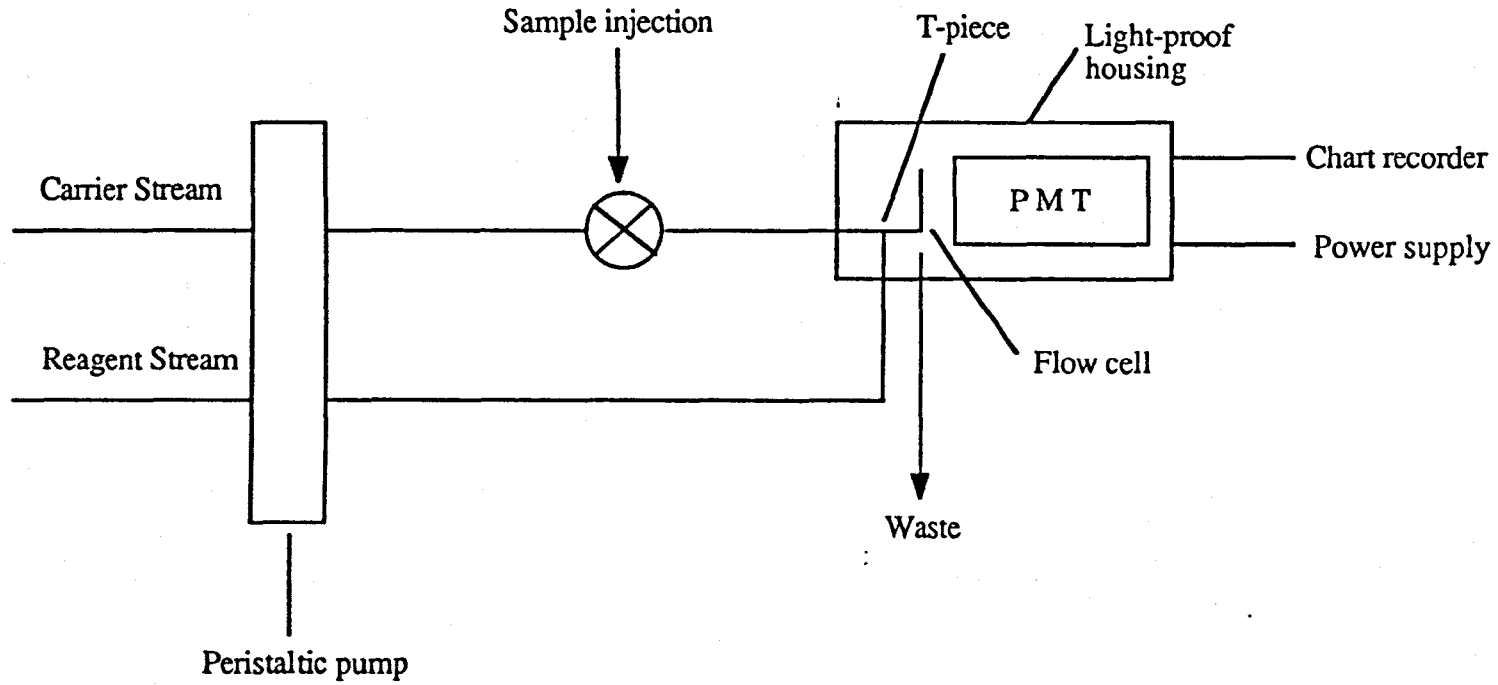


Figure 2.3. Simple flow-injection manifold to monitor CL reactions.

highly reproducible dispersion of the sample slug within the carrier stream, and the reproducible timing between injection and detection means that mixing does not have to be complete, hence rapid sample throughput is possible, together with good repeatability. This is illustrated by a comparison of the CL determination of triethylamine in sea water using a batch procedure and a flow-injection procedure. The flow-injection procedure gave relative standard deviation values (rsd) of 0.6% (n=5) compared with 27% with the batch procedure for a $1X10^{-3}$ M triethylamine standard [107]. Further details are given in chapter three.

2.2. Light Detectors

Chemiluminescence analysis relies on the measurement of very low levels of light. Consequently, the detector must be sufficiently sensitive, have a high signal to noise ratio and have a constant response over a large spectral range. In addition, it should have a sufficiently fast response time and the signal should be directly proportional to the intensity of the radiation. A number of light measuring devices have been used for detecting chemiluminescence.

2.2.1. Photographic Film

Photographic detection is one of the original methods for detecting and recording luminescence. It has, however been of limited value for quantitation because of the insensitivity and non-linearity of

photographic emulsions [131]. An example of the use of photographic detection for CL reactions is the determination of glucose using solid phase luminol and glucose oxidase. The oxidation of glucose produced hydrogen peroxide resulting in a CL emission which was recorded on a photographic film [132]. This procedure proved to be a sensitive threshold test for the presence or absence of glucose.

2.2.2. Photomultiplier Tubes

Photomultiplier tubes are highly sensitive light detectors which provide a current output proportional to the radiation intensity. The PMT consists of a photosensitive cathode and a series of up to 13 dynodes, each at a higher positive potential than the last. On exposure to light the photocathode emits electrons by the photoelectric effect. The electrons are accelerated and focused onto the first dynode where they each liberate several more electrons. The electrons emitted from the final dynode are collected at the anode and the resulting current is proportional to the radiation intensity. Each photon results in up to 10^7 electrons at the anode. Consequently, the PMT is a uniquely sensitive light detector. Other advantages include fast response times (typically nanoseconds), good linearity and low noise (particularly if cooled). The photocathode can be fabricated from a range of compounds to give maximum spectral response over the wavelength range of interest [133].

2.2.3. Photodiode Arrays

Photodiodes consist of a reverse-biased pn junction on a silicon chip. The reverse bias creates a depletion layer which reduces the conductance of the junction to almost zero. When radiation impinges on the junction free electrons are formed and, under the influence of an external potential, a current proportional to the intensity of the radiation is produced. Individual photodiodes are inexpensive, small and can be battery powered, and can therefore be used in portable chemiluminometers in the field [134].

Silicon diode array detectors are made up of many such photodiodes, each paired with a capacitor on a silicon chip. The number of diode-capacitor pairs commonly varies from 211 to 4096. Radiation impinging on a diode enables the corresponding capacitor to discharge. The amount of lost charge is replaced periodically and the charging current is integrated giving a signal proportional to the radiation intensity. Diode array detectors are not as sensitive as photomultiplier tubes, but have the ability to rapidly acquire spectra when placed at the focal plane of a monochromator. Diode array detectors have been used for the acquisition of CL spectra [135-137].

Other light detectors include photovoltaic cells, in which the radiation generates a current at the interface of a semiconductor layer and a metal; phototubes, in which the radiation causes the emission of

electrons from a photocathode (in this case, without the amplification due to the dynodes of a photomultiplier tube) and photoconductivity detectors, in which the absorption of radiation enhances the conductivity.

2.3. Flow Cell Design

Historically, coiled tubes were the preferred flow cell design for monitoring CL emission in flow-injection experiments. These were made of either transparent plastic or glass, the latter having superior optical properties and chemical resistance. Glass coils facilitate the incorporation of immobilized reagents, e.g., bioluminescent enzymes within the flow cell [114], and do not significantly increase the sample dispersion within the manifold. The major limitation of glass coils is their fragility during routine use [115] and the requirement for on site glass blowing facilities. This makes their use in an industrial environment or in the field undesirable. Because of the disadvantages of glass coil flow cells, the investigation of a more robust design was undertaken. Recently, the use of a lamina flow cell for monitoring the CL emission from the luminol reaction has been reported [138]. A flow cell similar to this was designed and built to overcome the problems associated with the routine use of the glass coil flow cell [139]. A schematic diagram of the lamina flow cell is shown in Fig. 2.4. The lamina flow cell has a stainless steel back plate with inlet and outlet ports. A PTFE spacer with an elliptical orifice is sandwiched

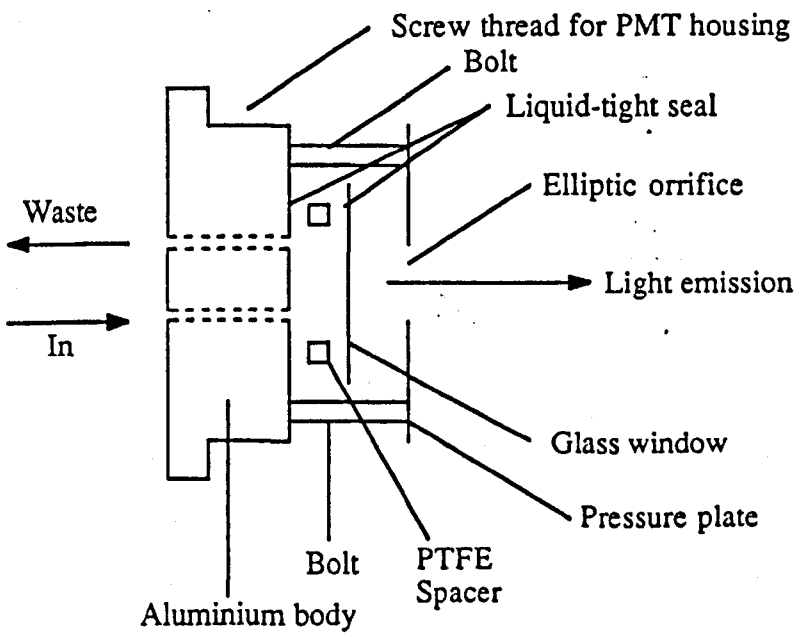


Figure 2.4. Lamina Flow Cell.

between the back plate and a glass front. The assembly is held together by a stainless steel front plate with an elliptical orifice through which the light is transmitted to the PMT. The volume of the cell is easily varied by using different thicknesses of PTFE spacer. The lamina flow cell was compared with the glass coil flow cell. The results are given in section 4.3.3.

2.4. Measurement Of Chemiluminescence Spectra

2.4.1. Introduction

Although not essential for analytical purposes, the CL spectrum can provide useful additional information on the CL reaction mechanism. The comparison of the CL spectrum with fluorescence spectra of the postulated reaction products or intermediates plays a major role in elucidating the CL reaction mechanism and identifying the emitting species. It is therefore useful to be able to measure the spectra of CL reactions. CL spectra, however, are often difficult to measure due to the very weak emission and transient nature of many CL reactions. CL spectra have been obtained in several ways including the use of fluorimeters with the source removed [140,141]. The short duration of the emission, however, means that such procedures are tedious, requiring many sample aliquots and reagent injections. Intensified diode array detectors have been used for the fast acquisition of CL spectra [135-137]. Such detectors have sensitivities approaching that of photomultiplier tubes

[142], though their high cost means that they are not widely available. Because of the requirement for a low cost means of obtaining spectral information on CL reactions, a flow-injection system was designed comprising a glass coil flow cell positioned in front of the entrance slit of a high efficiency grating monochromator. A PMT in a light-tight housing was placed at the exit slit of the monochromator. The assembly is shown in Fig. 2.5. Initial experiments were carried out using the luminol reaction.

2.4.2. Preliminary Studies

2.4.3. Reagents

All solutions were prepared with distilled-deionised water. Borate buffer (0.1 M) was prepared from boric acid (AnalaR; BDH) and the pH adjusted to 12.5 with 10 M sodium hydroxide solution. Luminol solution ($1.13X10^{-3}$ M) was prepared by dissolving luminol (Sigma) in buffer solution. Hydrogen peroxide solution (0.089 M) was prepared by diluting 100 volume hydrogen peroxide (AnalaR; BDH) to the appropriate volume with buffer solution. Cobalt (II) solution ($1X10^{-4}$ M) was prepared in water from cobalt (II) nitrate (AnalaR; BDH).

2.4.4. Instrumentation And Procedures

Spectra were obtained using an f 3.0 Minichrom monochromator model MC1-02 (Macam Photometrics), with a holographic diffraction grating with 1800 lines per mm

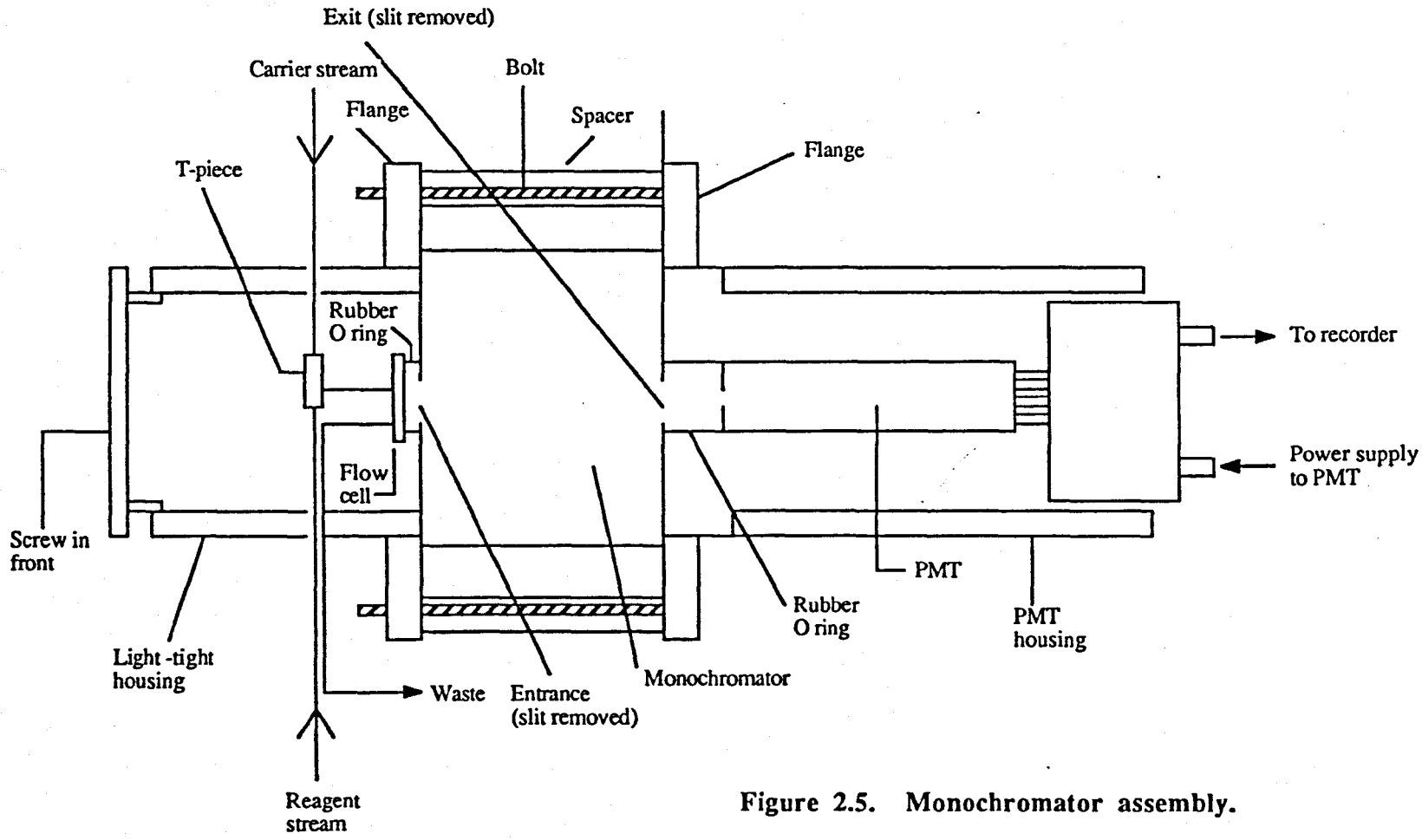


Figure 2.5. Monochromator assembly.

blazed at 250 nm. The linear dispersion was 6 nm mm^{-1} and the working range was 250 nm to 800 nm.

The flow-injection manifold used for presenting the CL emission of the cobalt (II) catalysed oxidation of luminol to the monochromator-detector assembly is shown in Fig. 2.6. Aqueous solutions (100 µl) of the standard cobalt (II) solution were injected into the buffer carrier stream which merged 10 cm down stream with the luminol stream at a PTFE T-piece, and again 15 cm downstream with the hydrogen peroxide stream at a second PTFE T-piece. The three streams were pumped at equal flow rates via a peristaltic pump (Gilson Minipuls 2) with polyvinyl chloride pump tubing (Lab Systems UK). The total flow rate was 2 ml $min.^{-1}$. PTFE tubing (0.5 mm i.d.) was used throughout the remainder of the manifold. The merged zones then travelled through a short length of PTFE tubing (5 cm) before passing into the flow cell. To achieve maximum light throughput to the detector (an end window PMT; Thorn EMI 9789QA operated at 1180 V) the entrance and exit slits were removed. Five replicate injections were made for measurement at each wavelength over the range 325 nm to 550 nm. The monochromator was factory calibrated and was accurate to ± 0.6 nm over the working range. The CL intensities were corrected for PMT spectral response, using data supplied by Thorn EMI [133]. The corrected spectrum for luminol is shown in Fig. 2.7. The CL spectrum of luminol obtained using this equipment is in close agreement with that reported in the

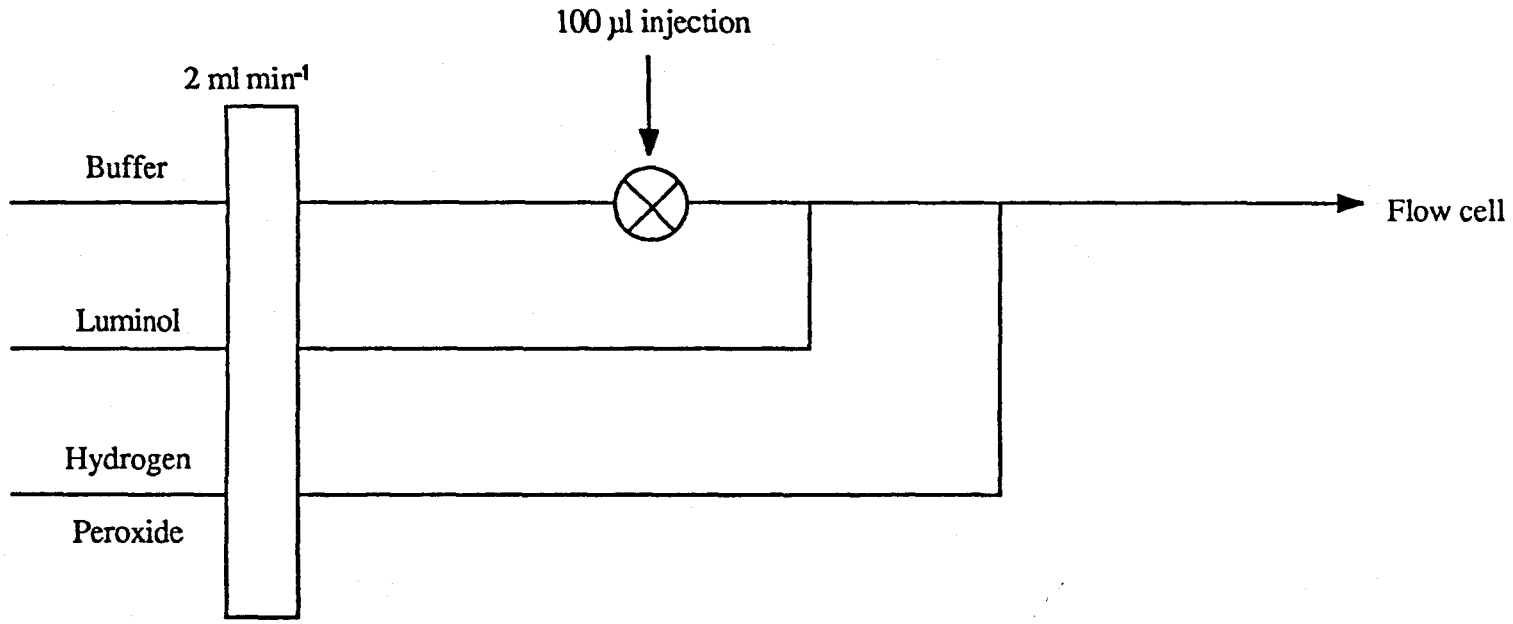


Figure 2.6. Flow injection manifold to monitor the Luminol spectrum.

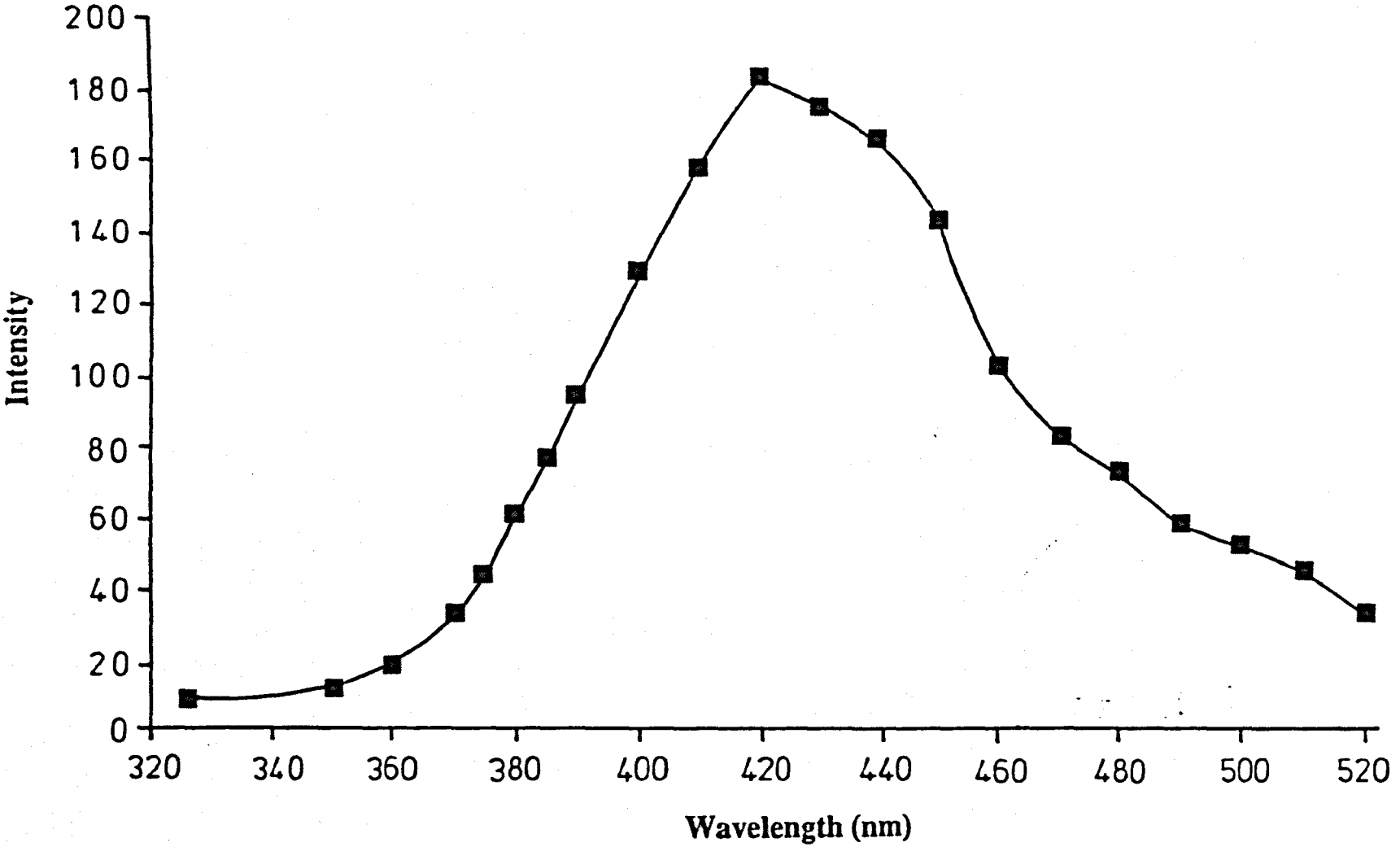


Figure 2.7. **Luminol Spectrum.**

literature. The emitting species being the 2-aminophthalate dianion [24]. The CL spectrum of 9-anthracene methanol is shown in Fig. 6.7.

2.5. Automation Of The Flow-Injection Manifold

2.5.1. Introduction

The driving force for research into the automation of chemical analysis occurred in the late 1950's when clinical laboratories in the United States came under increased pressure due to the availability of medical insurance from both the federal and private sectors of the economy [143]. In addition to this, specialised tests involving the quantitation of serum enzymes, electrolytes, proteins and hormones became routine. Consequently automation first appeared in the clinical laboratory and was gradually adapted for use in other industries.

The automated analysis currently in use utilizes either batch analysers or continuous flow analysers. In a sequential batch instrument, discrete samples are held in individual containers, and the appropriate reagent is automatically transferred to each container. Other procedures such as diluting, mixing, heating and finally measurement are carried out sequentially as the sample containers are transported through a series of stations.

Another well documented approach to batch analysis is the parallel centrifugal analyser [144,145] designed

to perform several spectrophotometric assays essentially simultaneously. It consists of a rotor with up to 17 radially arranged cavities, each connected by a channel to an individual spectrophotometric cell. Each of the cavities is separated into two compartments and the samples are automatically pipetted into one of the dual compartments within each cavity. The reagent is pipetted into the remaining compartment. Mixing of the sample and reagent occurs when the rotor reaches about 350 rpm. The mixture is carried into the cells where monochromatic light passes through the cells, the transmitted light is detected by a PMT. For each revolution of the rotor a series of signals is produced, each corresponding to a sample. The signals are captured and stored by a microcomputer and data manipulation such as signal averaging and statistical calculations are carried out.

Another approach to automatic analysis is that of air-segmented continuous flow analysis. The analyte is carried in a flowing stream to a detector. Reagents can be added at points along the stream and the reaction takes place whilst the sample is flowing towards the detector. Carry over from one sample to the next is minimized by air-segmentation [146]. The Technicon AutoAnalyzer, based on this principle was designed over 30 years ago to monitor urea and glucose in blood samples, and is now widely used in clinical laboratories.

The advantages of FIA, i.e., high sample throughput, highly repeatable signals, simplicity of

instrumentation and the wide range of procedures which can be adapted to FIA has meant that many FIA procedures have been automated. The water industry is one area in which continuous or frequent monitoring is becoming increasingly important. Consequently, automated FIA is now a major technique for the continuous analysis of water quality parameters [147-151], process monitoring and nutrient budget studies [152] and aquaculture monitoring [153].

Many flow injection procedures with CL detection have been reported, though, as yet, no fully automated FIA-CL systems have been described. The advantages of automation include higher sample throughput, improved precision of the sample introduction and the capability of unattended operation. The advantages associated with automated signal capture and data-handling include greater accuracy and precision of measurement and higher rate of sample turnover.

Recently batch CL procedures have been developed which utilize microcomputers to trigger automatic sample injection devices, and store and manipulate the signals from the detector. Hayashi et al. [154] have developed such a system to monitor the heamin catalysed oxidation of luminol. Taniguchi et al. [155] developed a batch procedure, also with automatic injection and computerized data collection, for the determination of cholesterol using the oxidation of luminol by cholesterol oxidase generated hydrogen peroxide. A batch enzymatic assay

using bacterial BL for the determination of formic acid in water samples using computerized data collection has also been reported [156]. A flow-injection system with CL detection using automated sample uptake and computer controlled injection has been described [157]. Though data collection was not computerized and the signals were recorded as peaks on a strip chart recorder. For rapid and accurate processing of the data there is a need for data collection and manipulation to be automated. This section describes the development of an automated flow-injection system, including the use of a microcomputer for controlling sample uptake, valve switching, data capture and data manipulation. The automated system is compared with the equivalent manual system using the peroxyoxalate reaction for the determination of perylene. The flow injection manifold is shown in Fig. 2.8.

2.5.2. Hardware

All the hardware was controlled by a microcomputer (BBC B+), and was interfaced to the user port (set for output) via a buffer (LS742507; Fig. 2.9) to protect the computer from equipment feedback. The buffer mirrors all the outputs from the user port.

The autosampler (Technicon) was connected to the buffer via a transistor (BC183L), with a resistor (330Ω) between this and the buffer to reduce the current supplied to the transistor. The transistor was switched on by setting the user port (channel PB4) high (+5 V), thus allowing a current to flow through the transistor

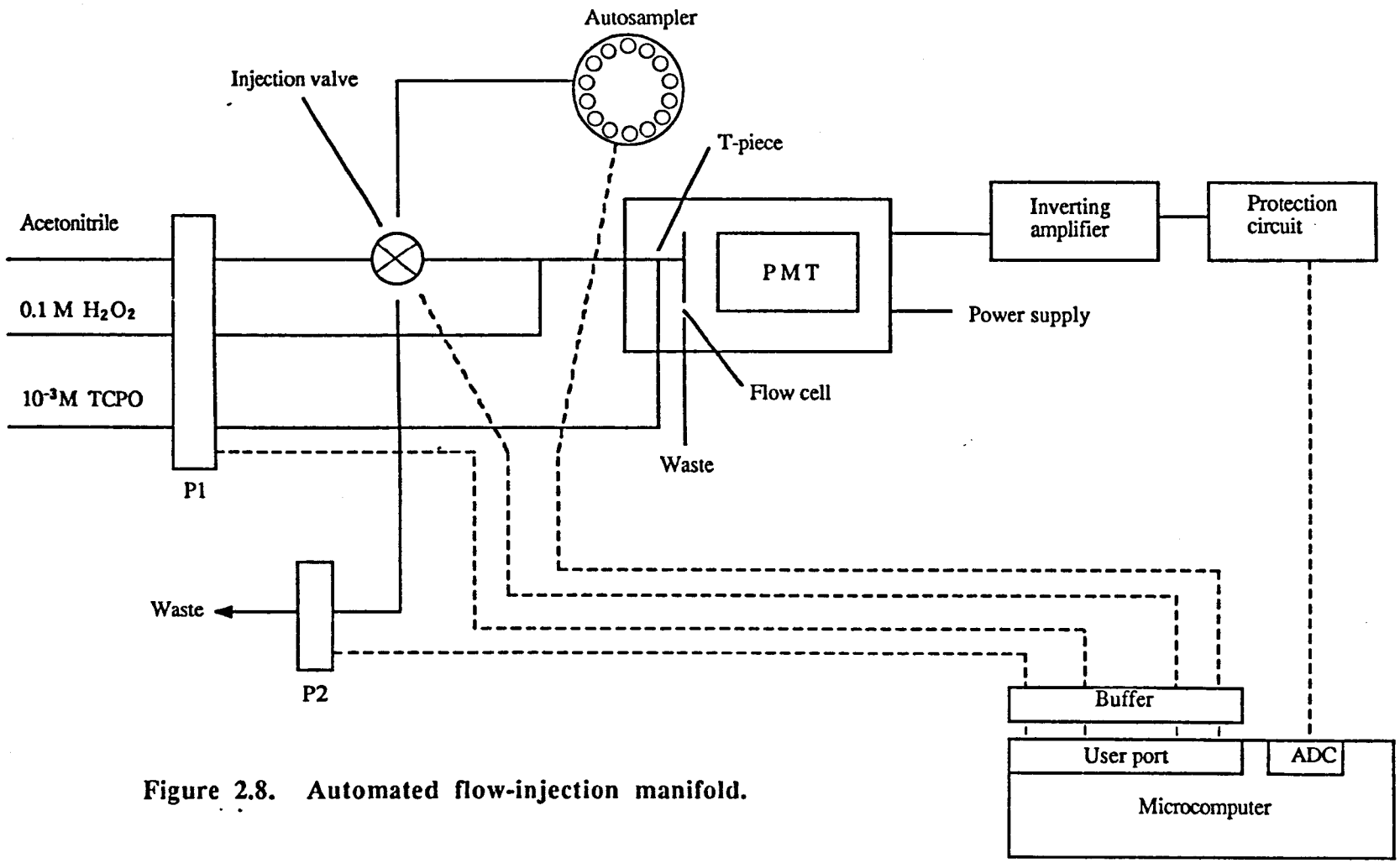


Figure 2.8. Automated flow-injection manifold.

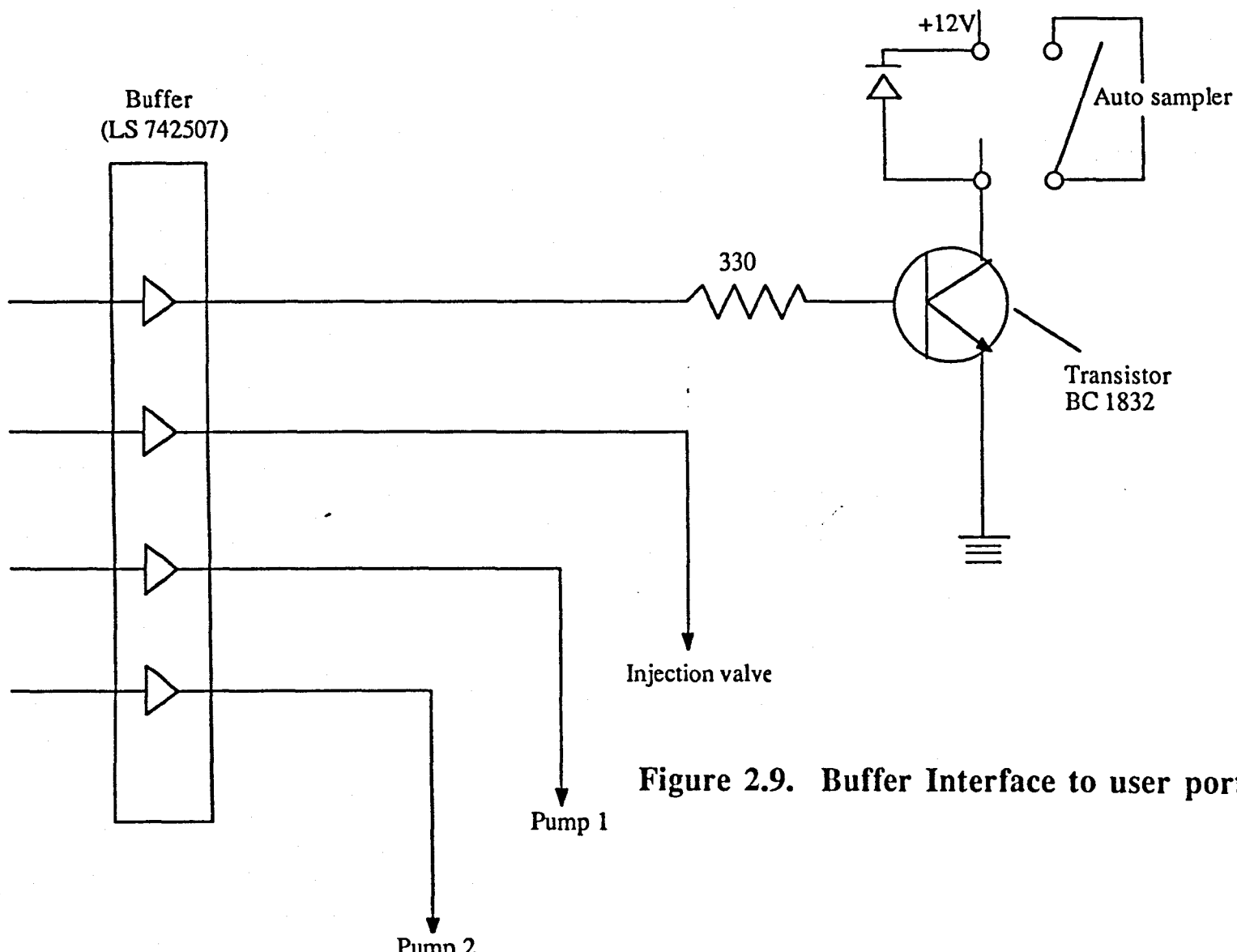


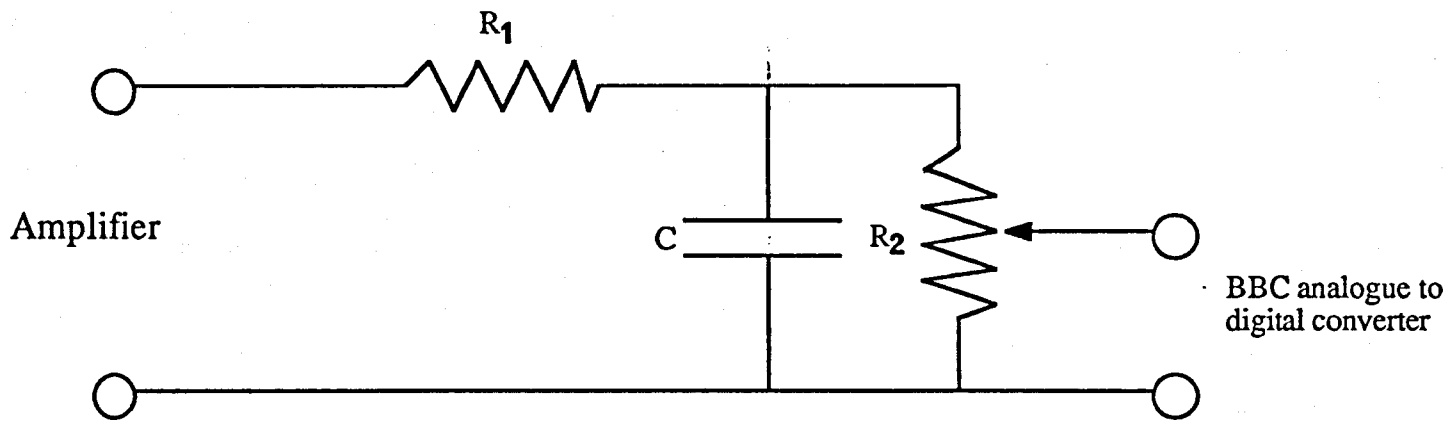
Figure 2.9. Buffer Interface to user port.

from a 12 V transformer, through the coil which energizes the switch of the autosampler causing it to close. This moves the autosampler into the sampling position. When the user port is low (0 V) the transistor is switched off, de-energizing the switch and causing the autosampler to move into the wash position. The turn-table then moves into the next position. A diode (4001) was used to protect the circuit from excessive voltages caused by induction.

The valve switching device (Anachem), also interfaced to the user port (PB0) via the buffer, was switched by setting the appropriate channel to high, followed by a 10 ms delay, then setting it to low. This voltage pulse caused the device to move into the next position, thus moving the position of the injection valve.

Pump 1 (Gilson Minipuls) and pump 2 (Ismatec S840) for propelling the streams through the flow injection manifold and filling the sample loop respectively, were interfaced via relay switches to channel PB 1 and PB 2 of the user port, also via the buffer. The pumps were then switched on by setting the appropriate channel to low, and off by setting to high.

The output from the PMT was amplified by an inverting amplifier within the PMT housing (BBH Power Products). The signal from the amplifier was processed by the analogue to digital converter (ADC) of



R_1 = 100 Ω resistor

R_2 = 1kΩ 20 turn variable resistor

C = 0.1μF capacitor

Figure 2.10 Amplifier interface to the analogue to digital converter.

the computer, via the circuit shown in Fig. 2.10. R1 is a 100Ω resistor, present in case of short circuit across the capacitor (C1), and to prevent overload of the amplifier at high frequency. Capacitor C1 (0.1 μF) smoothed the output from the amplifier prior to data collection, and R2, a 1kΩ variable resistor (20 turn) which acted as a potential divider to fine tune the output to gain maximum amplification, at the same time as preventing more than 1.8 V from reaching the ADC.

2.5.3. Software

The ADC is sampled, and the resulting peaks are displayed on the screen. User-defined values fix the maximum time in which the system will wait for a peak. A flow chart showing the operation of the software is shown in Fig 2.11.

A modular system comprising three programs was employed. The first program defined the sample order in the autosampler, the second program carried out the experiment, and was responsible for running the hardware and data capture. The third program was responsible for computing the results.

The common data file was in standard MICA format, enabling the data to be manipulated externally, using a program library module. The first program in the modular system defines the order in which the standards and samples are to be presented by the autosampler. The standards (and blanks) are user defined by a "*", hence

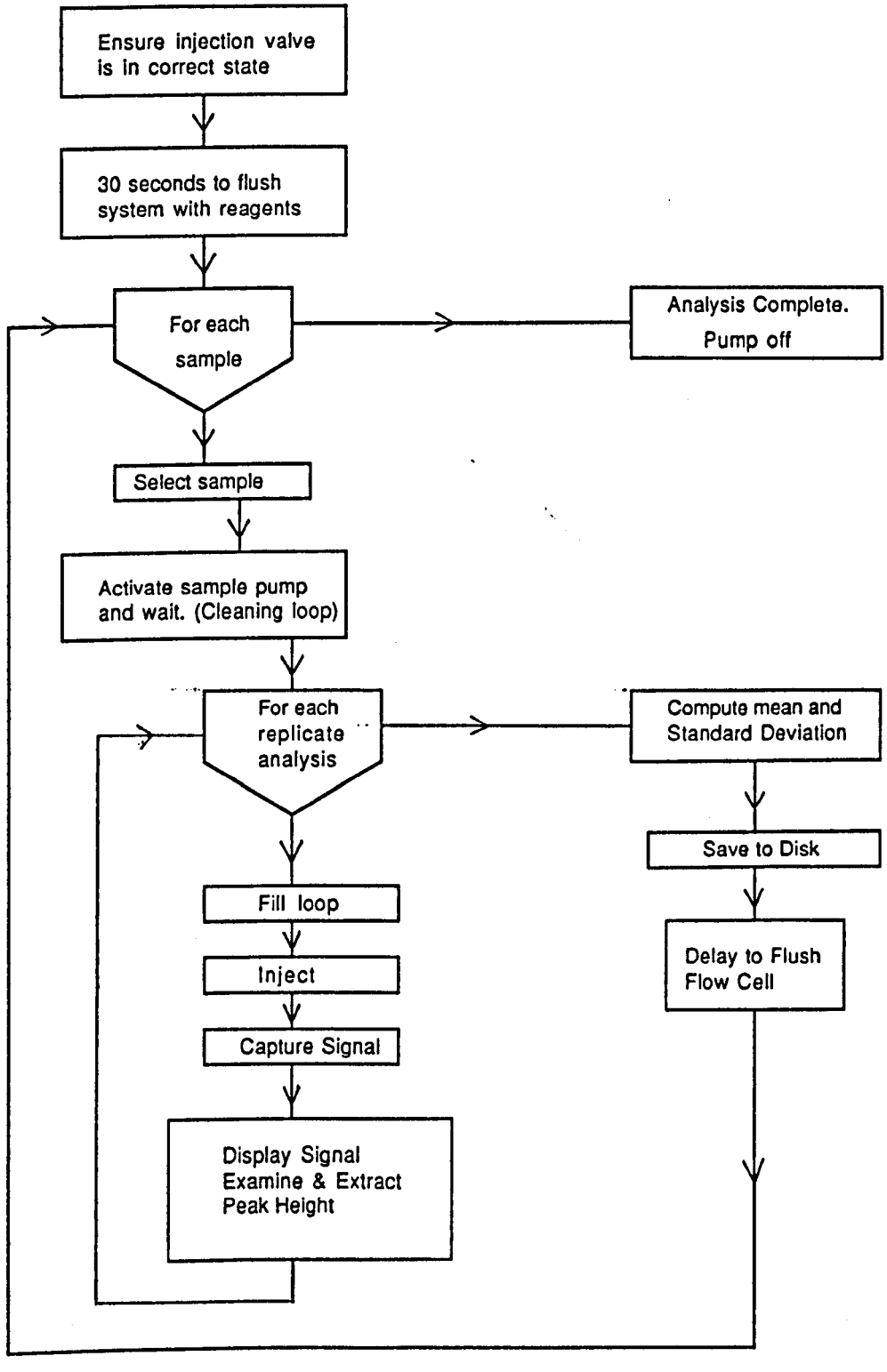


Figure 2.11. Software Flow Diagram.

if the first two characters in the definition are "*0", the solution will be taken to be a blank and any signal measured is regarded as a background signal and subtracted from the subsequent samples and standards. The absence of a "*" at the beginning of the name implies that the solution is an unknown for analysis. Each solution is therefore defined prior to analysis as:

1) Its position in the autosampler (1 to 22).
2) An unknown or a standard.
3) In the case of standard solutions, the concentrations are user defined.

The analysis program module calculates the mean values and sample standard deviation values for the peaks from each sample and standard. The concentrations of the standards (defined in module 1) are used, together with the values of the peak heights (in volts) to construct the calibration graph in module 3. Module 3 then uses the calibration graph to compute the concentrations of the unknowns, together with the 95% confidence intervals. The results are given in section 5.3.2.

Chapter Three

Determination Of Tertiary Amines In Water And Sea Water By FIA With CL Detection

3.1. Introduction

Tertiary amines have many industrial applications, e.g., as surfactants, corrosion inhibitors, organic reaction intermediates and in pharmaceutical preparations. A variety of reagents for the derivatization of primary and secondary amines have been reported. Among these are ninhydrin [158], for determination by absorption spectrophotometry, o-phthalaldehyde (OPA) [159], fluorescamine [160] and 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-CL) [161], which form fluorescent derivatives. Primary amines have been determined by the chemi-excitation of their fluorescent derivatives by the peroxyoxalate reaction, including the derivatives of 5-dimethylaminonaphthalene-1-sulphonylchloride (Dns-Cl), NBD-CL and OPA [162]. The peroxyoxalate reaction has been used for the detection of protonated amines following ion-pair extraction of the amine into an organic phase with a fluorescent counter-ion [163]. Very few derivatization reactions are available for tertiary amines, however. A non-aqueous flow-injection procedure with fluorescence detection selective for tertiary amines has been reported. The procedure involves reacting the tertiary amine with malonic acid and acetic anhydride to yield a fluorescent adduct [164]. A non-aqueous CL procedure for the determination of triethylamine has been reported, using benzoyl peroxide in acetone as the oxidant with a limit of detection of $1X10^{-4}$ M [165], though the CL emission

from this reaction could not be reproduced. An electrogenerated CL procedure for the detection of some nitrogen containing compounds, including primary, secondary and tertiary amines has been reported. This procedure involves the generation of tris(2,2'-bipyridine)ruthenium(III) by oxidizing tris(2,2'bipyridine)ruthenium(II) at the anode of a three electrode voltametric analyzer [166]. The ruthenium(III) complex oxidizes the nitrogen containing compound to yield a radical cation and an excited state ruthenium(II) complex cation which is thought to be the emitting species. This procedure has been used as a detector for the determination of tertiary amines by HPLC, and a theoretical limit of detection for triethylamine of $2X10^{-6}$ M has been reported [167]. The procedure is not selective for tertiary amines and is susceptible to interferences from other reducing species [166].

For the detection of tertiary amines in the aquatic environment, e.g., residual fatty amine ethoxylate surfactants in water and sea water, there remains a need for a sensitive and selective aqueous based analytical procedure.

This chapter presents an aqueous CL procedure for the determination of triethylamine and some other water soluble tertiary amines, using sodium hypochlorite as the oxidizing reagent, and rhodamine B as a sensitizer. A flow-injection procedure is described for the selective

determination of the tertiary amines in water and sea water.

3.2. Experimental

3.2.1. Reagents

All solutions were prepared with distilled-deionised water, and all tertiary amine solutions except trimethylamine were redistilled prior to use. Stock trimethylamine solution (1.716 M; standardized against 0.1 M hydrochloric acid with phenolphthalein indicator) was prepared by diluting 250 ml of trimethylamine (GPR grade, BDH) to 1 l with water. Stock triethylamine (0.01 M), tripropylamine (0.01 M), ethylamine (0.01 M) and diethylamine (0.01 M) solutions were prepared by dissolving each reagent (GPR grade, Aldrich) in water. Tripropylamine solutions were standardized by an acid-base titration with 0.1 M hydrochloric acid using phenolphthalein as an indicator. All standards were prepared daily by serial dilution of the stock solutions. Stock formaldehyde solution (1000 mg l^{-1}) was prepared by diluting formaldehyde (37% m/v; Aldrich) in water. Stock sodium hypochlorite solution (GPR grade; BDH) was standardized by an iodimetric titration [168] as 1.74 M (12.18% available chlorine). Borate buffer (0.1 M) was prepared from boric acid (AnalaR; BDH) and the pH adjusted with 10 M sodium hydroxide. Rhodamine B (BDH) and fluorescein solutions (Sigma) were prepared daily in borate buffer at the required pH. Artificial sea water

was prepared by dissolving 40 g sea water corrosion test mixture (BDH) in water and making up to 1 l.

3.2.2. Instrumentation And Procedures

3.2.3. Batch Experiments

Preliminary experiments and kinetic studies were done on a batch luminometer (Berthold Biolumat LB9500T; Fig. 2.2) In all experiments an aliquot of oxidant (100 µl) was injected into an aqueous solution of triethylamine ($1X10^{-3}$ M; 0.5 ml). For the preliminary experiments the output signal was integrated for 10 s following injection of the oxidant, and for the kinetic experiments a continuous analogue signal was recorded on a strip chart recorder (Chessell BD4004).

3.2.4. Flow-Injection Experiments

The flow-injection manifold used for all experiments is shown in Fig. 3.1. The sensitivity of the manifold was optimized using a modified simplex optimization program with the following variables (and ranges); pH (9-12), total flow rate (2.0-3.0 ml min^{-1}), sodium hypochlorite concentration ($1X10^{-4}$ M-$1X10^{-2}$ M) and rhodamine B concentration ($1X10^{-5}$ M-$1X10^{-2}$ M).

For the optimization experiments aqueous standards (50 µl) were automatically injected into a stream of borate buffer (0.1 M) via a solenoid activated rotary

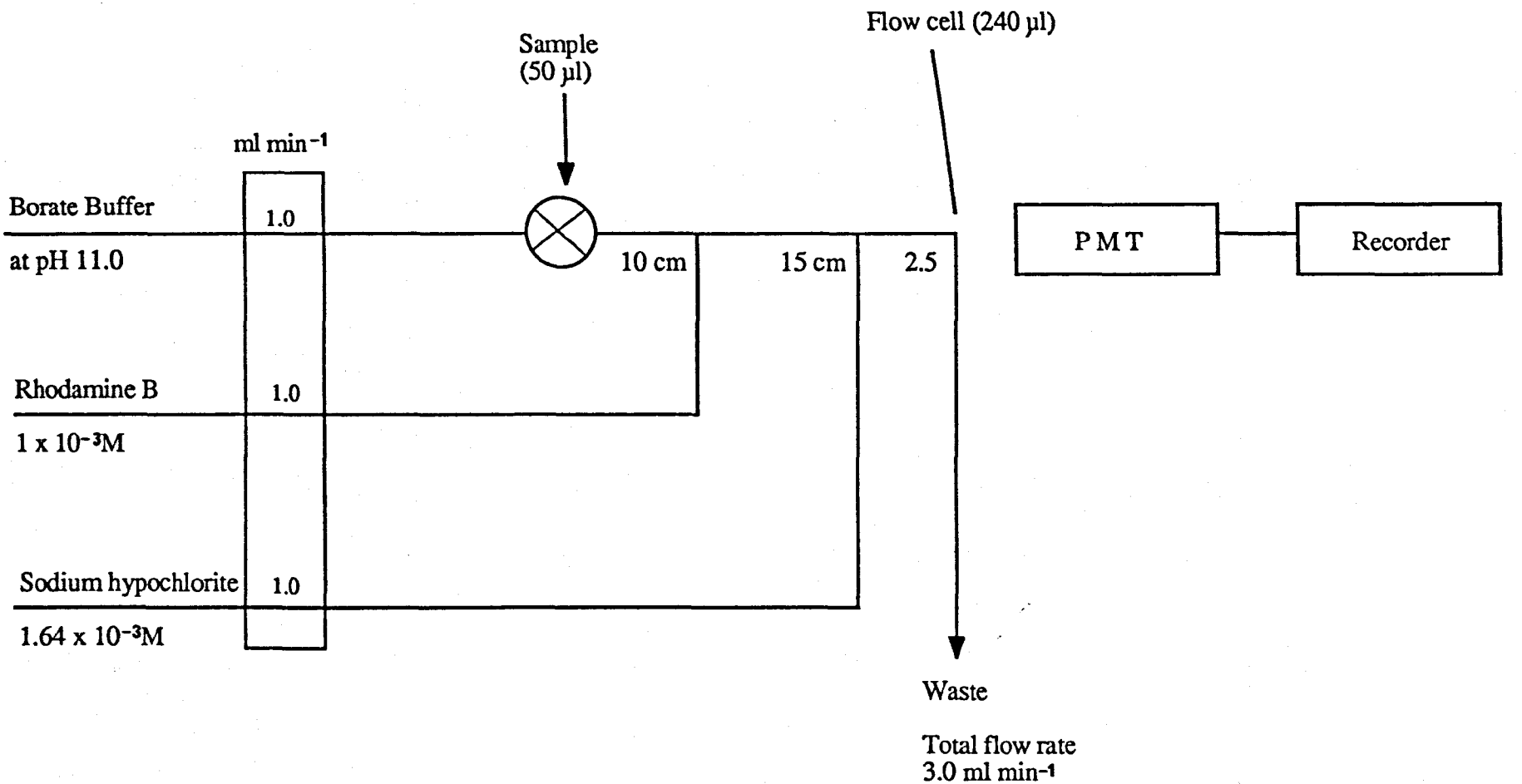


Figure 3.1. Manifold for tertiary amine determination.

valve (Rheodyne 5020). This stream was merged 10 cm downstream with a stream of rhodamine B at a PTFE T-piece and merged again 15 cm downstream with a sodium hypochlorite stream, at a second PTFE T-piece. All three streams were pumped at equal flow rates by a peristaltic pump (Gilson Minipuls 2) and PTFE tubing (0.5 mm i.d.) was used throughout the remainder of the manifold. The merged streams then travelled through a short length of PTFE tubing (2.5 cm) before passing into a glass coil flow cell (1.5 mm i.d.; 240 µl volume). The detector was an end window PMT (Thorn EMI 9789QB) operated at 1.1 KV and located in a light-tight housing. A red LED was used to calibrate the PMT response. The output from the PMT was recorded on a strip chart recorder (Chessell BD4004). All the results are the mean of five replicate injections.

Two samples of an aqueous scrubbing medium used to remove gaseous trimethylamine from an industrial discharge were analysed for trimethylamine content. The calibration procedure was based on the method of standard addition to minimise any matrix interferences. A 100 µg ml^{-1} standard was prepared from a sample of scrubbing medium known to be free from trimethylamine and this was analysed in the same way as the unknowns to asses the accuracy of the procedure.

The calibration standards for each of the samples and the 100 µg ml^{-1} standard were prepared by transferring 1 ml aliquots to 25 ml volumetric flasks.

Each was then spiked with 0, 0.6, 1, 2, 4, 8 and 20 ml respectively of a $1X10^{-3}$ M trimethylamine standard prepared in pH 11.0 buffer solution and made up to the mark with buffer.

3.3. Results And Discussion

3.3.1. Preliminary Work

The batch luminometer was used to screen a range of oxidants for the possible generation of chemiluminescence with triethylamine. No detectable emission was observed with the following oxidizing reagents in either 0.1 M ammonium chloride (pH 0.1) or 0.1 M borate buffer (pH 10); sodium iodate, potassium dichromate, iron (III) chloride, potassium persulphate, potassium periodate, potassium chlorate, sodium nitrate, hydrogen peroxide and potassium permanganate. Only sodium hypochlorite gave an appreciable response. A $1X10^{-3}$ M solution in borate buffer at pH 10.0 gave a reading of 3120 counts over the 10 s integration period (rsd=27%, n=5) when injected into a $1X10^{-3}$ M aqueous triethylamine solution, relative to a blank reading of 889 (rsd=83%, n=5). No detectable signal was observed when sodium hypochlorite solution was added to ethylamine or diethylamine. The CL intensity of triethylamine was increased by adding rhodamine B as a sensitizer. The response increased to 19129 counts over the 10 s period of integration (rsd=8%, n=5).

3.3.2. Optimization Of Flow-Injection Conditions

The results obtained using the batch luminometer showed that a weak CL emission is produced during the oxidation of triethylamine with alkaline sodium hypochlorite, and that the emission intensity is increased by the addition of rhodamine B which acts as a sensitizer. The major problem of using a batch luminometer, however, particularly when the CL emission is weak and the lifetime short is poor repeatability, due to irreproducible mixing of the oxidant and the sample. This is illustrated by the high rsd values, particularly for the blank measurements. The batch procedure was therefore adapted to a flow-injection procedure using the manifold shown in Fig. 3.1. In addition to rhodamine B, fluorescein was also investigated as a sensitizer. Both were investigated over the range $1X10^{-3}$ M to $1X10^{-6}$ M, and rhodamine B gave twice the intensity obtained with fluorescein at their respective optimum concentrations of $8X10^{-4}$ M and $8X10^{-5}$ M. Rhodamine B was used in subsequent experiments.

The optimum conditions of pH, total flow rate, rhodamine B concentration and sodium hypochlorite concentration were determined by a multivariate procedure, using a modified simplex optimisation program. The parameters were optimized to give a maximum CL emission of a $1X10^{-3}$ M triethylamine standard.

3.3.3. Multivariate Procedure

The major disadvantage of the univariate or "one at a time" approach to optimization is that it relies on the assumption that all the experimental variables are independent. In many cases this assumption is invalid, and may be overcome by adopting a multivariate or simplex optimization procedure in which all the variables are altered simultaneously to find the optimum response [169]. For experimental simplicity the flow rates of the three steams were kept equal. The starting conditions were pH 10.0, total flow rate 2.6 ml min^{-1}, $1.0X10^{-3}$ M rhodamine B and $1.0X10^{-3}$ M sodium hypochlorite. The optimum conditions were found to be pH 11.0, total flow rate 3.0 ml min^{-1}, rhodamine B concentration $1.0X10^{-3}$ M and sodium hypochlorite concentration, $1.64X10^{-3}$ M.

The effect of a range of metal ions (Al^{3+}, Cr^{3+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+} and Pb^{2+}) on the CL emission was also investigated by incorporating the chloride or nitrate salt in the sample solution. No significant changes in response were observed.

A simplified two channel manifold was also investigated with the rhodamine B stream incorporated into the buffer stream. This was found to result in periodic blockage of the injection valve due to precipitation of the rhodamine B.

3.3.4. Calibration Data

All data were obtained using the simplex optimized flow-injection manifold described above. Due to the variable response of the PMT, the mean dark current, the mean peak-to-peak noise and the reference signal of the red LED were determined before each experiment. The dark current and peak-to-peak noise increase with ambient temperature and PMT age and, because of the absence of a blank emission, this directly affects the limit of detection. All results are given relative to the dark current signal. A resolution of 0.05 mV was obtained with the chart recorder. The signal from the LED gives an indication of the day to day sensitivity of the PMT and is related to ambient temperature and operating voltage.

Tables 3.1 and 3.2 show the CL emission (mV) and rsd (n=5) for triethylamine standards in water and artificial sea water respectively, over the range 0-$1X10^{-2}$ M. There is no detectable blank emission in either case. The theoretical limit of detection (3 σ) is $1X10^{-6}$ M and $8X10^{-7}$ M in water and sea water respectively. Though in each case the practical limit of determination is $1X10^{-5}$ M as below this concentration the rsd is unacceptably high. The linear range is 0-$2X10^{-4}$ M ($r=0.9927$) in water and 0-$1X10^{-4}$ M ($r=0.9974$) in sea water, though in both cases analytically useful data can be obtained up to $1X10^{-2}$ M. The procedure is also suitable for the determination of trimethylamine and

tripropylamine in water. Calibration data for trimethylamine and tripropylamine over the range 0-1X10^{-2} M is shown in tables 3.3 and 3.4. The theoretical limits of detection (as defined above) are 6X10^{-6} M and 2X10^{-6} M for trimethylamine and tripropylamine respectively. The calibration data for trimethylamine is linear over the range 0-5X10^{-4} M, and for tripropylamine, 0-1X10^{-3} M, though, as in the case of triethylamine, analytically useful data can be obtained up to 1X10^{-2} M.

3.3.5. Analysis Of An Industrial Scrubbing Medium

In all cases the standard addition calibration data was linear up to the addition of 4 ml of spike solution and the final two points were omitted from the calculations. The results for the 100 µg ml^{-1} standard and the two unknown samples are shown in tables 3.5 to 3.7. In the case of the 100 µg ml^{-1} standard the x-axis intercept was extrapolated back to 4.5 µg ml^{-1} trimethylamine in the unspiked sample. The original standard was diluted by a factor of 25, hence the trimethylamine concentration of the original standard was found to be 113 ± 3.5 µg ml^{-1} at the 95% confidence level. Similarly the x-axis intercept was extrapolated back to 6.9 µg ml^{-1} for sample 1 and 1.3 µg ml^{-1} for sample 2 indicating a trimethylamine concentration in each original sample of 172 ± 2 µg ml^{-1} and 32 ± 1 µ ml^{-1} respectively. The presence of small quantities of other tertiary amines in the sample matrix may be responsible for the slightly high result of the 100 µg ml^{-1} standard,

as the standard addition calibration procedure is insufficient to remove the effect of other chemiluminescent species present in the sample matrix.

Table 3.1. Calibration Data For Triethylamine In Water

Concentration (mol l^{-1})	Signal (mV)	rsd (n=5) (%)
0	0.0	0.0
$1X10^{-5}$	0.8	20.3
$5X10^{-5}$	10.8	1.4
$8X10^{-5}$	17.6	0.6
$1x10^{-4}$	22.2	2.3
$2x10^{-4}$	37.4	1.4
$5x10^{-4}$	63.8	0.4
$1x10^{-3}$	84.8	0.3
$5x10^{-3}$	153.6	1.2
$1x10^{-2}$	193.0	0.7

Dark current = 1.0 mV; peak-to-peak noise = 0.2 mV; reference signal = 130 mV

$y=1.92X10^{5}$ mV/M(x)+0.7 mV

r=0.9927

Table 3.2. Calibration Data For Triethylamine In Sea Water

Concentration (mol l^{-1})	Signal (mV)	rsd (n=5) (%)
0	0.0	0.0
$1X10^{-5}$	1.6	17.7
$5X10^{-5}$	14.2	1.7
$8X10^{-5}$	22.6	1.2
$1X10^{-4}$	26.3	0.6
$2X10^{-4}$	41.4	1.1
$5X10^{-4}$	61.2	0.5
$1X10^{-3}$	80.8	0.6
$5X10^{-3}$	161.7	1.0
$1X10^{-2}$	225.8	1.7

Dark current = 1.7 mV; peak-to-peak noise = 0.2 mV; reference signal = 125 mV

$y=2.75X10^{5}$ mV/M(x)-0.3 mV

r=0.9974

Table 3.3. Calibration Data For Trimethylamine In Water

Concentration (mol l^{-1})	Signal (mV)	rsd (n=5) (%)
0	0.0	0.0
$5X10^{-5}$	1.0	4.0
$8X10^{-5}$	2.0	2.1
$1X10^{-4}$	2.7	3.1
$2X10^{-4}$	6.7	2.1
$5X10^{-4}$	16.0	0.8
$1X10^{-3}$	25.7	1.1
$5X10^{-3}$	43.6	0.5
$1X10^{-2}$	54.3	0.8

Dark current = 1.7 mV; peak-to-peak noise = 0.2 mV;
reference signal = 125 mV

$y=3.29X10^{4}$ mV/M(x)-0.4 mV

r=0.9984

Table 3.4. Calibration Data For Tripropylamine In Water

Concentration (mol l^{-1})	Signal (mV)	rsd (%)
0	0.0	0.0
$5X10^{-5}$	0.4	22.9
$8X10^{-5}$	4.2	4.2
$1X10^{-4}$	5.9	1.5
$2X10^{-4}$	14.0	1.3
$5X10^{-4}$	36.8	2.3
$1X10^{-3}$	76.4	1.2
$5X10^{-3}$	140.6	1.7
$1X10^{-2}$	210.6	2.2

Dark current = 1.7 mV; peak-to-peak noise = 0.2 mV;
reference signal = 125 mV

$y=7.80X10^{4}$ mV/M(x)-1.8 mV

r=0.9993

Table 3.5. Standard Addition Calibration Data For A 100 µg ml^{-1} Trimethylamine Matrix Matched Standard

Added spike (µg ml^{-1})	Signal (mV)	rsd (n=6) (%)
0.0	43.7	1.4
1.4	47.3	2.6
2.4	74.5	5.1
4.7	98.0	5.7
9.4	132.1	5.0
18.9	145.4	14.9
47.2	189.2	1.6

$y=9.76(x)+44.1$ mV

$r=0.9771$

Table 3.6. Standard Addition Calibration Data For Sample 1.

Added spike ($\mu g\ ml^{-1}$)	Signal (mV)	rsd (n=6) (%)
0.0	111.2	2.8
1.4	146.2	0.9
2.4	163.8	2.8
4.7	211.2	3.0
9.4	278.3	1.7
18.9	341.2	1.7
47.2	424.6	0.6

$y=17.4(x)+119.7$ mV

$r=0.9939$

Table 3.7. Standard Addition Calibration Data For Sample 2

Added spike ($\mu g\ ml^{-1}$)	Signal (mV)	rsd (n=6) (%)
0.0	21.9	3.0
1.4	41.7	5.0
2.4	78.7	5.0
4.7	120.7	4.3
9.4	197.1	4.4
18.9	271.7	4.0
47.2	374.2	3.4

$y=18.76(x)+24.7$ mV

$r=0.9930$

3.3.6. Interference Studies

Ethylamine was found to suppress the CL emission of a $1X10^{-3}$ M triethylamine solution by 80% when present at a tenfold excess, by 25% when present at the same concentration and not at all when present at lower concentrations (Table 3.8). Diethylamine suppressed the CL emission by 80% when present at a tenfold excess. Lower concentrations did not significantly reduce the emission (Table 3.9). Diethylamine and ethylamine gave no detectable CL emission when $1X10^{-3}$ M aqueous solutions were injected. The procedure was used to measure the

concentration of trimethylamine in a gaseous industrial effluent, as described above. The gaseous emissions were scrubbed with water to remove any trimethylamine present. The trimethylamine content in the scrubbing medium was determined. Gas chromatographic investigation has indicated that trimethylamine is the only amine present. The scrubbing medium was also found to contain formaldehyde at the mg l^{-1} level [170]. The effect of formaldehyde was therefore investigated and quenched the emission of a 5 mg l^{-1} trimethylamine solution by 20% when present in a one hundred fold excess and by 5% when present in a tenfold excess. Lower concentrations did not reduce the CL intensity (Table 3.10). Formaldehyde itself gave no emission.

Table 3 8. Effect Of Ethylamine On The Emission Intensity Of A $1X10^{-3}M$ Aqueous Triethylamine Standard

Concentration (mol l^{-1})	Signal (mV)	rsd (n=5) (%)
0	55.7	1.2
$1X10^{-6}$	54.2	0.8
$1X10^{-5}$	50.7	1.5
$1X10^{-4}$	50.4	0.7
$1X10^{-3}$	36.8	0.7
$1X10^{-2}$	10.8	2.6

Table 3.9. Effect Of Diethylamine On The Emission Intensity Of A $1X10^{-3}$ M Aqueous Triethylamine Standard

Concentration (mol l^{-1})	Signal (mV)	rsd (n=5) (%)
0	55.7	1.2
$1X10^{-6}$	47.9	0.5
$1X10^{-5}$	50.0	0.7
$1X10^{-4}$	49.2	1.2
$1X10^{-3}$	51.1	0.7
$1X10^{-2}$	9.4	3.2

Table 3.10. Effect Of Formaldehyde On The Emission Intensity Of A 5 mg l^{-1} Aqueous Trimethylamine Standard

Concentration (mg l^{-1})	Signal (mV)	rsd (n=5) (%)
0	37.0	2.3
1	37.1	4.1
5	37.6	1.7
50	35.1	1.6
500	29.6	1.5

3.3.7. Reaction Kinetics

The CL emission intensity with respect to time was investigated using the batch luminometer; 100 µl of sodium hypochlorite ($1.64X10^{-3}$ M) was injected into 0.5 ml of triethylamine ($1X10^{-3}$ M) and 0.5 ml rhodamine B ($1X10^{-3}$ M) in water at pH 11.0. The analogue output was fed to a chart recorder. The emission - time profile is shown in Fig. 3.2. Maximum emission was reached 1.1 s after injection and this decayed by 90% after 8.3 s. These results are compatible with the optimized flow-injection conditions of 3.0 ml min^{-1} (50 µl s^{-1}) total flow rate and a flow cell volume of 240 µl.

The kinetics of the reaction between trimethylamine and hypochlorous acid have been investigated by Ellis and Soper [171], and it was found that simple tertiary amines react with hypochlorous acid and the hypochlorite anion to yield the tertiary chlorammonium ion which decomposes to form the dialkyl chloramine and an aldehyde. (Formaldehyde in the case of the oxidation of trimethylamine). The overall reaction is shown below:

$$(CH_3)_3N + 2HOCl \longrightarrow (CH_3)_2NCl + HCHO + H_2O + HCl \quad (1)$$

The presence of formaldehyde and the secondary N - chloramine has been verified experimentally [172]. The initial step in the reaction is the formation of the trimethylchlorammonium cation by electrophilic attack on

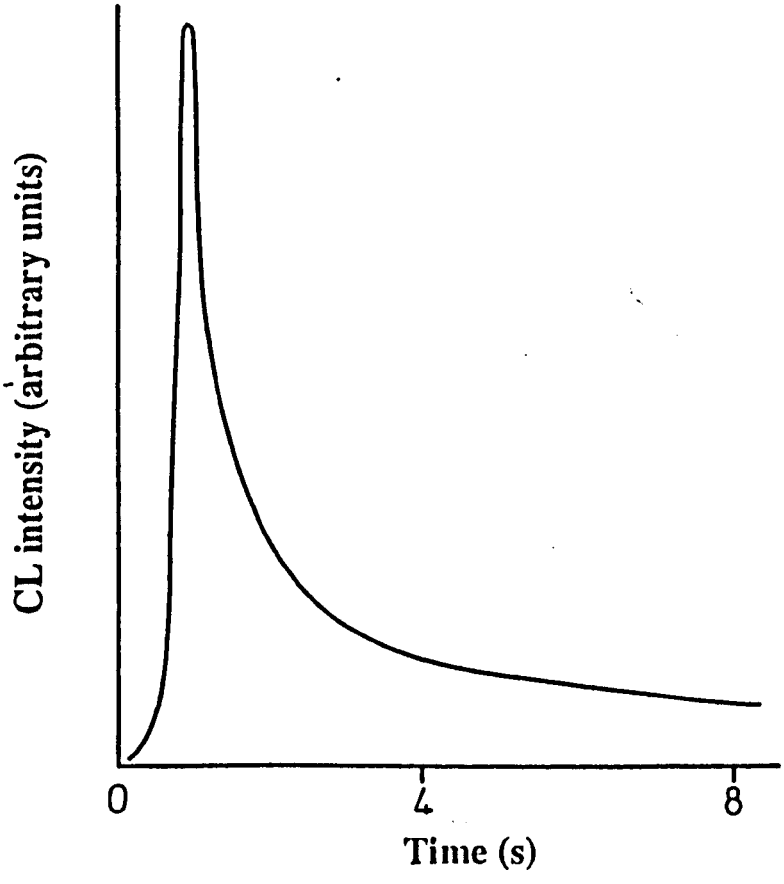


Figure 3.2. Chemiluminescence emission versus time profile for 1×10^{-3}M triethylamine in water.

the tertiary amine nitrogen atom by hypochlorous acid, shown below:

$$(CH_3)_3N + HOCl \longrightarrow (CH_3)_3N^+\text{-}Cl + OH^- \quad (2)$$

The rate of this reaction is directly related to the basicity of the nitrogen atom [172], and kinetic studies have shown that tertiary amines react much faster than primary or secondary amines [173]. This explains the better sensitivity observed for triethylamine relative to trimethylamine, and the lack of response for ethylamine and diethylamine. The lower response for tripropylamine may be due to the steric effect of the larger propyl groups.

The rate determining step in the overall reaction scheme is the conversion of the trimethylchlorammonium cation to dimethylamine and formaldehyde. The secondary amine is then rapidly converted to dimethylchloramine by the hypochlorous acid, but further conversion to methylamine is relatively slow.

The thermal decomposition of 1,2-dioxetanes is known to generate excited state carbonyl compounds (Section 1.5.5) which may give a chemiluminescence emission on relaxation to the ground state. The gas phase chemiluminescence emission of alkenes on oxidation with ozone is due to the formation of excited state formaldehyde [137]. Since the reaction with hypochlorite produces aldehydes it is possible that the CL emission is

due to the formation of the aldehyde in an electronically excited state.

3.4. Conclusions

The oxidation of trimethylamine, triethylamine and tri-n-propylamine with alkaline sodium hypochlorite at pH 11.0 generates a CL emission. The emission intensity can be increased by the addition of rhodamine B which acts as a sensitizer. Triethylamine can be determined in water and sea water over the range $0-1X10^{-2}$ M. The calibration data is linear over the range $0-2X10^{-4}$ M in water and $0-1X10^{-4}$ M in sea water. The theoretical detection limits (3 σ) for triethylamine in water and sea water were $1X10^{-6}$ M and $8X10^{-7}$ M respectively. The CL reaction is also suitable for the determination of trimethylamine and tripropylamine and the limits of detection (3 σ) in water were $6X10^{-6}$ M and $2X10^{-6}$ M respectively. In addition, the technique was used for the determination of trimethylamine in an aqueous industrial scrubbing medium, though the result for a 100 μg ml^{-1} control standard was 13% high. This may be due to the presence of other tertiary amines in the sample matrix, as the standard addition procedure is not sufficient to eliminate the effect of other chemiluminescent species present. Further work may improve this procedure by using HPLC with CL detection, to remove interfering chemiluminescent species such as different tertiary amines prior to detection.

Ethylamine, diethylamine and formaldehyde did not give any CL emission, though ethylamine and diethylamine quenched the emission of a $1X10^{-3}$ M triethylamine standard by 80% when present in a tenfold excess. Formaldehyde quenched the emission of a $1X10^{-4}$ M trimethylamine standard by 20% when present in a 100 fold excess and by 5% when in a tenfold excess.

Chapter Four

Determination Of A Non-Ionic Surfactant (Nonidet AT 85) In Aqueous Environmental Samples By FIA With CL Detection

4.1. Introduction

Non-ionic surfactants are the second most widely used class of surfactants after anionic surfactants [174]. Industrial applications of non-ionic surfactants include textile and fibre manufacture, cosmetics, pharmaceuticals, mining, paper manufacture, oil drilling and food and animal feed production. Non-ionic surfactants used in the food industry are based primarily on fatty acid esters and glycerides. For most other industrial applications they are based on ethylene oxide polymers, including alkyl phenol ethoxylates, fatty acid ethoxylates, fatty alcohol ethoxylates and fatty amine ethoxylates. The general structures of these compounds are shown in Fig. 4.1 [175].

Nonidet AT 85 is a non-ionic surfactant of the fatty amine ethoxylate type (Fig. 4.1), in which each ethoxy chain contains an average of five units. It is used in oil drilling operations as a mud surfactant [176]. In carrying out the drilling operation the drilling mud is pumped down the hollow drill string to pass out through the ports in the drill bit. This then returns to the surface in the annulus formed between the borehole wall and the drill string, bringing the debris to the surface and lubricating the drill string and bit [177]. Large volumes of mud are required during drilling operations, 50-60,000 gallons being common. Nonidet AT 85, and related fatty amine ethoxylates are used in drilling muds to control the viscosity, emulsion

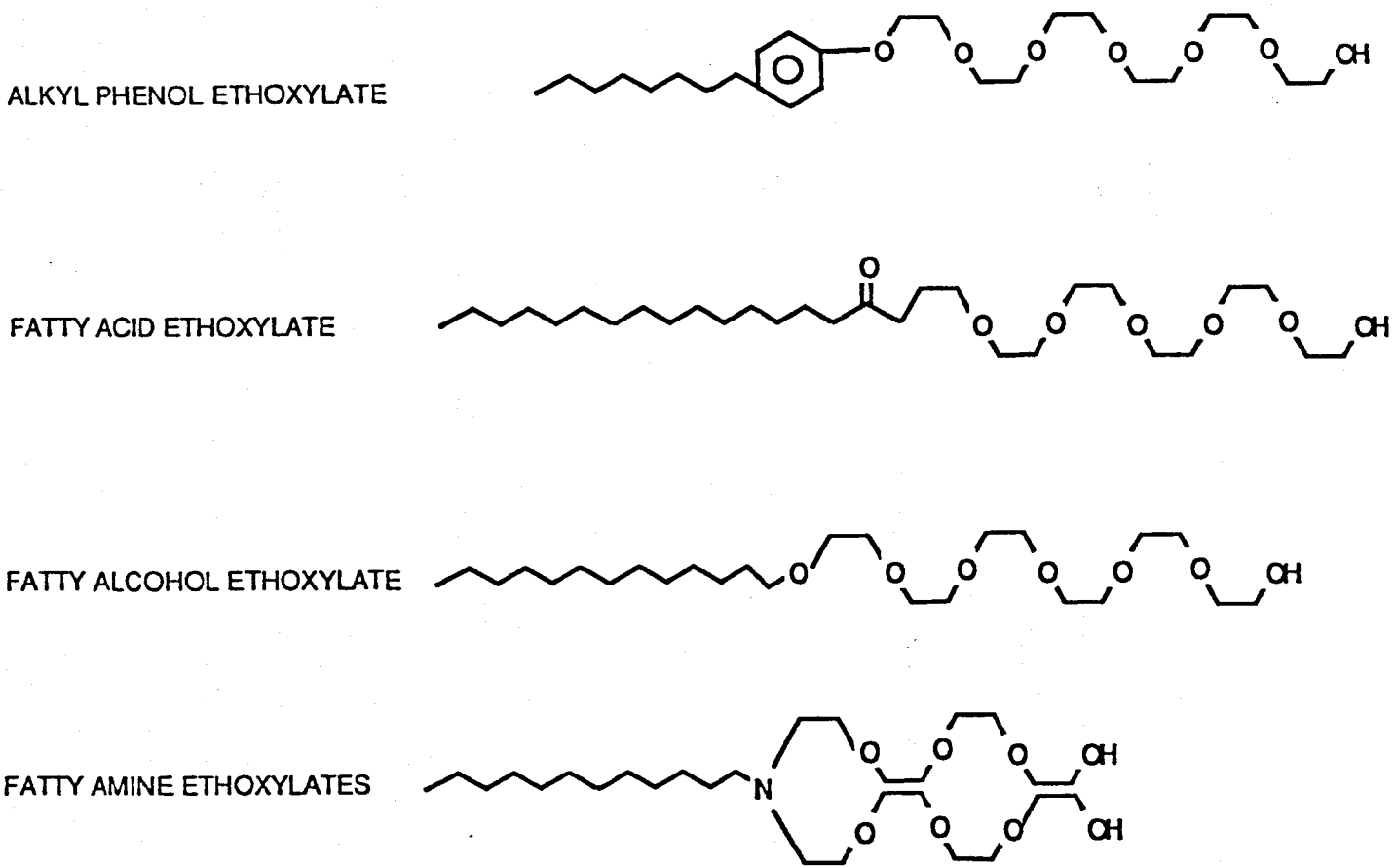


Figure 4.1. **General structures of Polyethoxylate based non-ionic surfactants.**

stability and wettability and as a lubricant for the drill string and bit.

There is a need to monitor ethoxylate based non-ionic surfactants in the aquatic environment due to their toxicity and poor biodegradability, particularly in cold conditions [178]. Their selective determination at low mg l^{-1} levels is problematic, particularly in the presence of other non-ionic surfactants, biodegradation products and endogenous materials [179]. Methods for their determination often depend on the formation of complexes of the polyethoxylate chain with cobalt, tungsten, molybdenum or bismuth compounds [179]. Current EC legislation on the biodegradability of non-ionic surfactants (EC directive 82/242) recommends the Wickbold method (bismuth active substances) [180] for analysis. It is however susceptible to interference from other organic compounds and the sensitivity is poor for low chain length ethoxymers. Several gas chromatographic methods have been reported but they are not suitable for compounds containing more than 6 ethylene oxide units due to their involatile nature [181]. Such compounds require derivatization [182,183]. High performance liquid chromatography can also be used although it is often limited in sensitivity because ethoxylates do not posses a suitable chromophore or fluorophore (with the exception of alkyl phenol ethoxylates [184-186]), and refractive index [187] flame ionization [188] and mass spectrometric detectors [189] have been used. The applicability of

HPLC is therefore rather limited with respect to the analysis of environmental samples, which often require preconcentration techniques such as steam distillation - solvent extraction [190], or gaseous stripping into ethyl acetate [191].

In response to concerns regarding the possible environmental impact of Nonidet AT 85 (e.g., it has a reported LD_{50} value (oral) of 500 - 2000 mg l^{-1} in rats [192]) there is a requirement for its detection in sea water at the low mg l^{-1} level.

This chapter describes the adaptation of the procedure for the determination of tertiary amines (Chapter 3) to the determination of this surfactant in sea water.

4.2. Experimental

4.2.1. Reagents

Distilled, deionised water was used throughout. Synthetic sea water was prepared by dissolving 40 g of sea-water corrosion test mixture (BDH) in 1 l of water. Nonidet AT 85 (Akzo Chemie) and Nonidet P 40 (BDH) stock solutions (1000 mg l^{-1}) were prepared in water and standard solutions were prepared by dilution with synthetic sea water. Borate buffer (0.1 M) was prepared from boric acid (AnalaR; BDH), and the pH adjusted with 10 M sodium hydroxide. Oxidant solutions were prepared by dissolving sodium hypochlorite (GPR grade; BDH)

(standardized by an iodimetric titration as 1.74 M) in buffer. Sensitizer solutions were prepared by dissolving rhodamine B (BDH) in buffer. Stock trimethylamine solution (1.576 M; standardized against 0.1 M hydrochloric acid) was prepared by diluting 250 ml trimethylamine (GPR grade, BDH) to 1 l with water. Stock triethylamine (0.01 M) and tripropylamine (0.01 M) solutions were prepared by dissolving each reagent (GPR grade; Aldrich) in water. The tripropylamine and trimethylamine stock solutions were standardised against 0.1 M HCl with phenolphthalein as the indicator.

4.2.2. Instrumentation And Procedures

4.2.3. Kinetic Studies.

Kinetic studies on the surfactant were carried out using a batch luminometer (Berthold Biolumat LB 9500T). In these experiments hypochlorite (100 µl, 0.46 M) in pH 10.5 buffer was injected into a cell containing surfactant (0.5 ml, 1318 mg l^{-1}) and rhodamine B (0.5 ml, 4.45×10^{-3} M in pH 10.5 buffer). Kinetic studies were also carried out on trimethylamine, triethylamine and tripropylamine. For these studies hypochlorite (100 µl, 1.64×10^{-3} M) in pH 11.0 buffer was injected into the appropriate amine solution (0.5 ml, 1×10^{-3} M) in water, in the presence of rhodamine B (0.5 ml, 1×10^{-3} M) in pH 11.0 buffer. The analogue output was recorded on a strip chart recorder (Chessell BD 4004).

4.2.4. Surfactant Determination.

The optimum flow-injection manifold used, based on a glass coil flow cell is shown in Fig. 4.2. The sensitivity was simplex optimized using a 20 mg l^{-1} surfactant standard. The following variables (and ranges) were used; total flow rate (1.0-4.0 ml min^{-1}), pH (9.0-12.0), rhodamine B concentration ($5x10^{-3}$-$1x10^{-5}$ M) and sodium hypochlorite concentration (0.5-$1x10^{-3}$ M). For experimental simplicity the flow rates of the three streams were kept equal.

Standards (100 µl) were automatically injected into the buffer carrier stream via a solenoid activated rotary valve (Rheodyne 5020). This stream was merged 10 cm downstream with the rhodamine B stream at a PTFE T-piece and again a further 15 cm downstream with a stream of sodium hypochlorite. All three carrier streams were pumped at equal rates via a peristaltic pump (Gilson Minipuls 2) and PTFE tubing (0.5 mm i.d.) was used throughout the remainder of the manifold. The three merged streams travelled 2.5 cm before passing into the glass flow cell (1.5 mm i.d.; 240 µl volume). The detector was an end-window photomultiplier tube (Thorn EMI 9789QB) operated at 1.05 KV and located in a light-tight housing.

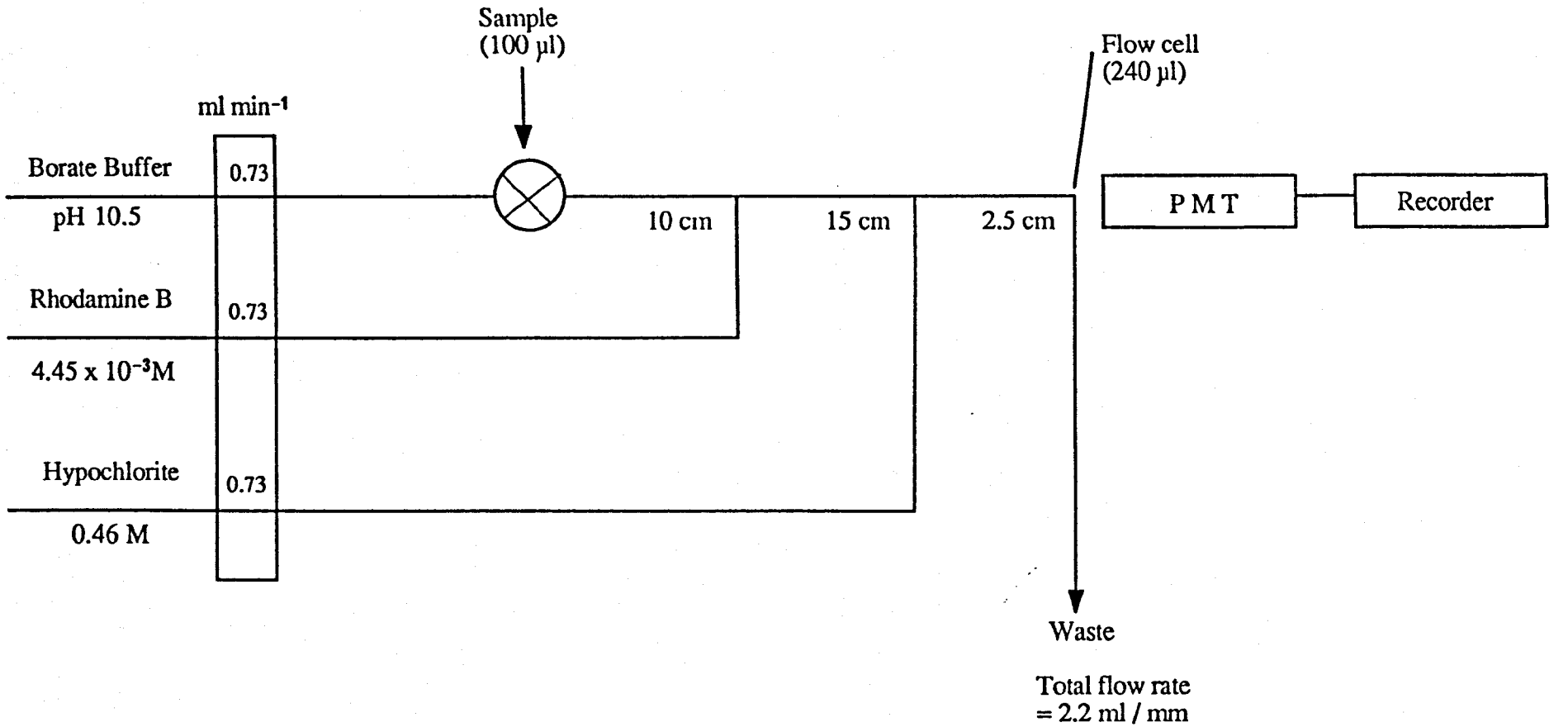


Figure 4.2 Manifold for Nonidet AT 85 determination.

4.2.5. Comparison Of Lamina And Glass Coil Flow Cells

Because of the disadvantages associated with the use of glass coil flow cells in an industrial environment (Section 2.3), the glass coil flow cell was compared with the lamina flow cell (Fig. 2.4.) using the emission generated by the oxidation of triethylamine with sodium hypochlorite in aqueous solution [107]. The Nonidet AT 85 CL reaction was also investigated with both flow cell designs. The glass coil was made from 1.5 mm i.d. borosilicate glass tubing and was 240 µl volume. The flow-injection manifold for the triethylamine CL reaction is shown in Fig. 3.1. All three carrier streams were pumped at equal flow rates. The experimental conditions were pH 11.0, total flow rate 3.0 ml $min.^{-1}$, $1X10^{-3}$ M rhodamine B and $1.64X10^{-3}$ M sodium hypochlorite. Peak heights and widths of the CL emission profiles for the two flow cell designs were compared using a $1X10^{-3}$ M triethylamine standard. The CL emission profiles were obtained using the glass coil flow cell (volume 240 µl) and various lamina flow cells (volumes 126 µl, 234 µl and 440 µl).

4.3. Results And Discussion

4.3.2. Kinetic Studies

The CL emission with respect to time was investigated using the batch luminometer. The emission - time profiles for a 1318 mg l^{-1} surfactant standard in

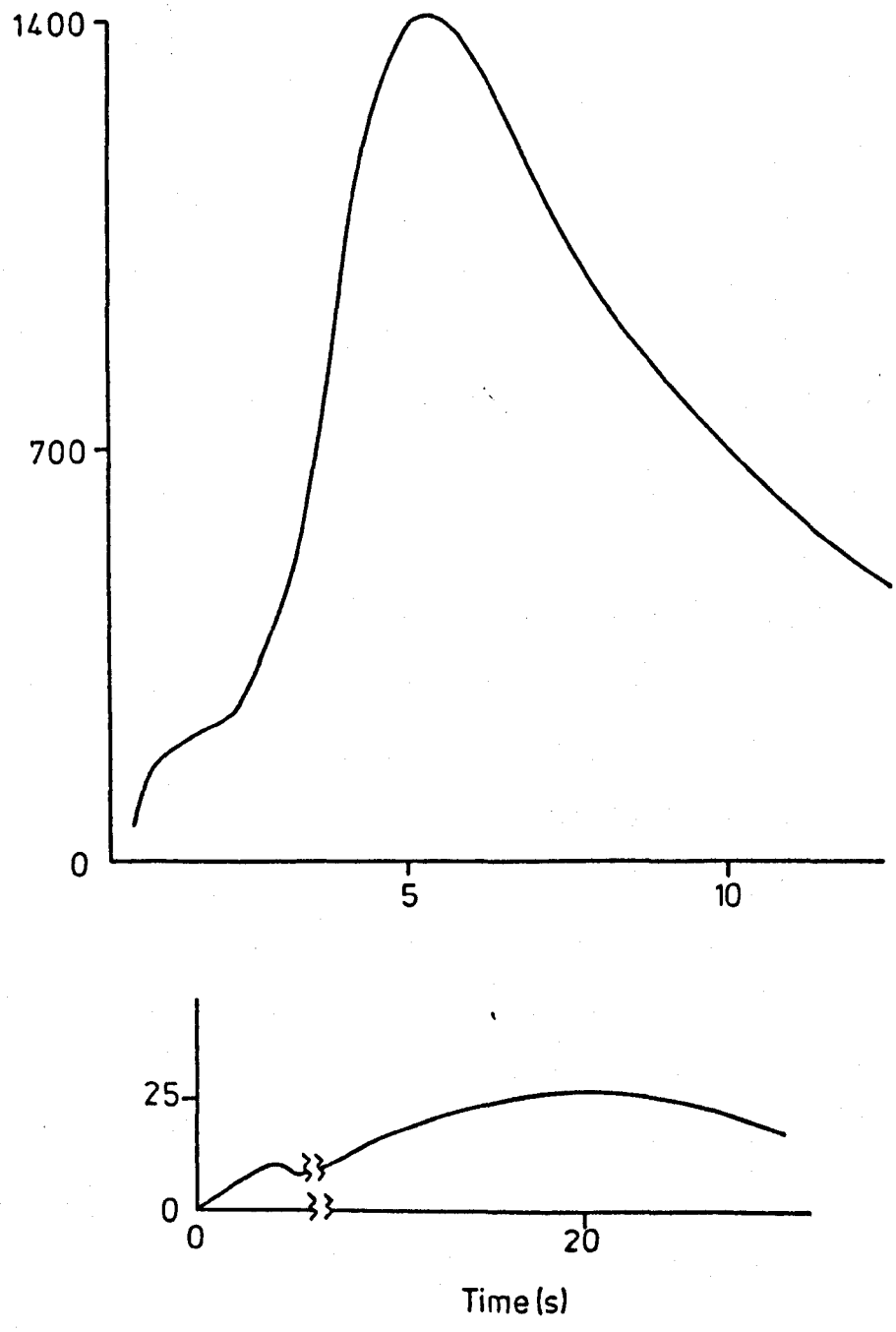


Figure 4.3. **Chemiluminescence emission versus time profiles for 1318 mg l^{-1} surfactant solution in sea water and a sea water blank.**

sea water and a sea water blank are shown in Fig. 4.3. For the standard the maximum CL intensity was reached 4.9 s after the oxidant was injected, and decayed to 10% of its maximum after a further 20.6 s. A shoulder on the rising curve of the emission profile occurred 1 s after injection of the oxidant. CL emission from the sea water blank gave a similar shoulder after 1 s and a maximum intensity after 17.8 s. The origin of the blank emission is uncertain but decomposition of hypochlorite by metal ions in the sea water matrix may be responsible [193].

The rate of hypochlorite oxidation of tertiary amines is related to the basicity of the amine group [172]. As discussed in chapter three, this explains the lack of emission when primary and secondary amines are oxidised [107]. Table 4.1 shows kinetic data for the hypochlorite oxidation of trimethylamine, triethylamine, tri-n-propylamine, surfactant and a sea water blank. The slower reactions with tripropylamine and surfactant as compared with triethylamine are probably due to steric effects resulting from the larger alkyl groups present. In the case of the present surfactant electrostatic repulsion of the hypochlorite anion by the ethoxy groups may also be a factor.

Table 4.1. Kinetic Data For The Hypochlorite Oxidation Of Tertiary Amines

Amine	T_{max}[a] (s)	$T_{10\%max}$[b] (s)
Trimethylamine	1.0	6.6
Triethylamine	1.1	8.3
Tripropylamine	2.2	12.2
Nonidet AT 85	4.9	25.5
Sea water blank	17.8	61.8[c]

[a] time to achieve maximum emission.

[b] time to decay to 10% of maximum emission.

[c] time to decay to 50% of maximum emission.

4.3.3. Comparison Of The Lamina And Glass Flow Cells

The maximum CL emission intensity and the peak width at half height for the standard triethylamine solution were 48 mV and 8 s for the glass flow cell and 22 mV and 16 s for the equivalent (234 µl) lamina flow cell, indicating the superior flow characteristics of the former. When different lamina flow cell volumes were investigated it was found that the peak width at half height increased with increasing cell volume due to increased dispersion of the sample slug. The flow cell volume that gave maximum CL emission was 234 µl (peak height and width at half height were 22 mV and 16 s respectively). This is due to the combined effects of dispersion and the lifetime of the CL emission. For all

flow cell volumes investigated, there was little change in repeatability, and the rsd values varied between 0.9% and 1.5% (n=10). When the volume was increased to 440 µl, the peak height decreased to 20 mV and the peak width at half height increased to 23 s. The CL emission - time profile for the triethylamine reaction is shown in Fig. 3.2, and shows that the CL reaction is fast, reaching a maximum intensity after 1.1 s, and decaying to 10% of its maximum after 8.3 s. The larger volume flow cells give rise to increased dispersion of the sample within the flow cell and result in a more dilute portion of the sample being presented to the detector at its maximum emission intensity, hence, in the case of short lived CL emissions, increasing the flow cell volume from 234 µl to 440 µl resulted in a decrease in CL intensity.

An interesting observation with the lamina flow cell was that the maximum CL intensity for Nonidet AT 85 (1000 mg l^{-1}) increased by 80%, from 19 mV to 34 mV on increasing the cell volume from 225 µl to 372 µl. This can be explained by the fact that the surfactant has a longer lived CL emission than triethylamine and the larger volume flow cell therefore enables a greater portion of the sample slug to be presented to the detector, which, in the case of the slower CL emission will remain near to its maximum for a longer time period. The larger volume cell will therefore integrate a larger portion of the emission-time profile. When the lamina flow cell was used for the determination of the non-ionic

surfactant using the optimized conditions for this reaction described below, the precision was poor (10-20% r.s.d., n=10) for all concentrations and cell volumes used. This is in contrast to the results for triethylamine and is directly attributable to the increased viscosity of the reagents and reflects the inferior mixing within the lamina flow cell relative to the glass coil flow cell. The r.s.d. was much improved (<10%; n=10) by incorporating a knitted coil (optimum length 35 cm) in front of the flow cell [194]. The optimum flow rate was found not to change from 2.2 ml $min.^{-1}$, although the sensitivity was slightly reduced due to increased dispersion.

4.3.4. Quantification

The signal to blank ratio for a 20 mg l^{-1} surfactant standard in sea water was maximized using a simplex program (Section 3.3.3). The optimum conditions were: total flow rate 2.2 ml min^{-1}., sodium hypochlorite concentration 0.46 M, rhodamine B concentration $4.45x10^{-3}$ M and pH 10.5. Calibration data (Table 4.2) for sea water solutions using the glass coil flow cell and the optimum conditions stated above were linear over the range 0-50 mg l^{-1}, and the theoretical limit of detection (3σ) was 4 mg l^{-1}, though poor repeatability below 10 mg l^{-1} dictates a practical limit of detection of 5 mg l^{-1}. The detection limit (3σ) obtained with the lamina flow cell was slightly higher due to increased dispersion and was 12 mg l^{-1}. The CL emission from the

surfactant is more intense in sea water than in distilled water, (the latter giving a limit of detection > 50 mg l^{-1}). The effect of the following metal ions on the CL emission of the surfactant was investigated ($1x10^{-3}$ M as the chloride or nitrate); Cr^{3+}, Co^{2+}, Al^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+}, but individually none gave any significant increase relative to deionized water. The enhancement by the sea water matrix is probably due to the combined catalytic effect of certain cations and anions present.

The non-ionic surfactant Nonidet P 40 was investigated for CL emission. Nonidet P 40 is an octyl phenol ethoxylate containing an average of 9 units of ethylene oxide per molecule and no amine group. This gave no CL emission under the optimized flow conditions, indicating that the presence of the tertiary amine group is necessary for CL emission, but quenched the emission of a 50 mg l^{-1} fatty amine ethoxylate surfactant solution by 22% when present at the same concentration. There was no attenuation of the CL emission when 5 mg l^{-1} Nonidet P 40 was present.

Two sediment samples were analyzed for the possible presence of the fatty amine ethoxylate surfactant. Aqueous extracts of the sediments were injected into the optimised flow-injection manifold and the surfactant concentration was determined by standard addition. The calibration data is shown in table 4.3. Preliminary investigation showed that one extract (sample 1) was

below the detection limit and the other (sample 2) gave a signal of 1.4 mV.

The standards were prepared by spiking 0.9 ml aliquots of sample 2 with 0.1 ml of Nonidet standard in distilled - deionised water, containing 3000, 2000, 1000, 500, 200 and 0 mg l^{-1} Nonidet respectively, giving final spike concentrations of 0, 20, 50, 100, 200 and 300 mg l^{-1}. These were injected into the optimized flow-injection manifold and the concentration of the spike was plotted against the emission intensity. The concentration of Nonidet AT 85 in the unspiked sample was found by extrapolation. The data was linear up to a final spike concentration of 200 mg l^{-1}. The extrapolated intercept was -271.4, indicating a concentration of 271 mg l^{-1}. The 95% confidence limits were 271 mg l^{-1} ± 52 mg l^{-1}. The slope for this data is less than that for Nonidet AT 85 in sea water. This illustrates the need for good matrix matching of samples and standards, or the use of a standard addition procedure.

Table 4.2. Calibration Data For Nonidet AT 85

Surfactant concentration (mg l^{-1})	Peak height (mV)	rsd(n=6) (%)
0	0.6	12.2
5	1.3	26.8
10	1.5	9.7
20	2.0	12.7
30	2.1	5.4
40	2.9	4.3
50	3.6	2.9

$y=5.3X10^{-2}(x)+0.8$ mV

r=0.9796

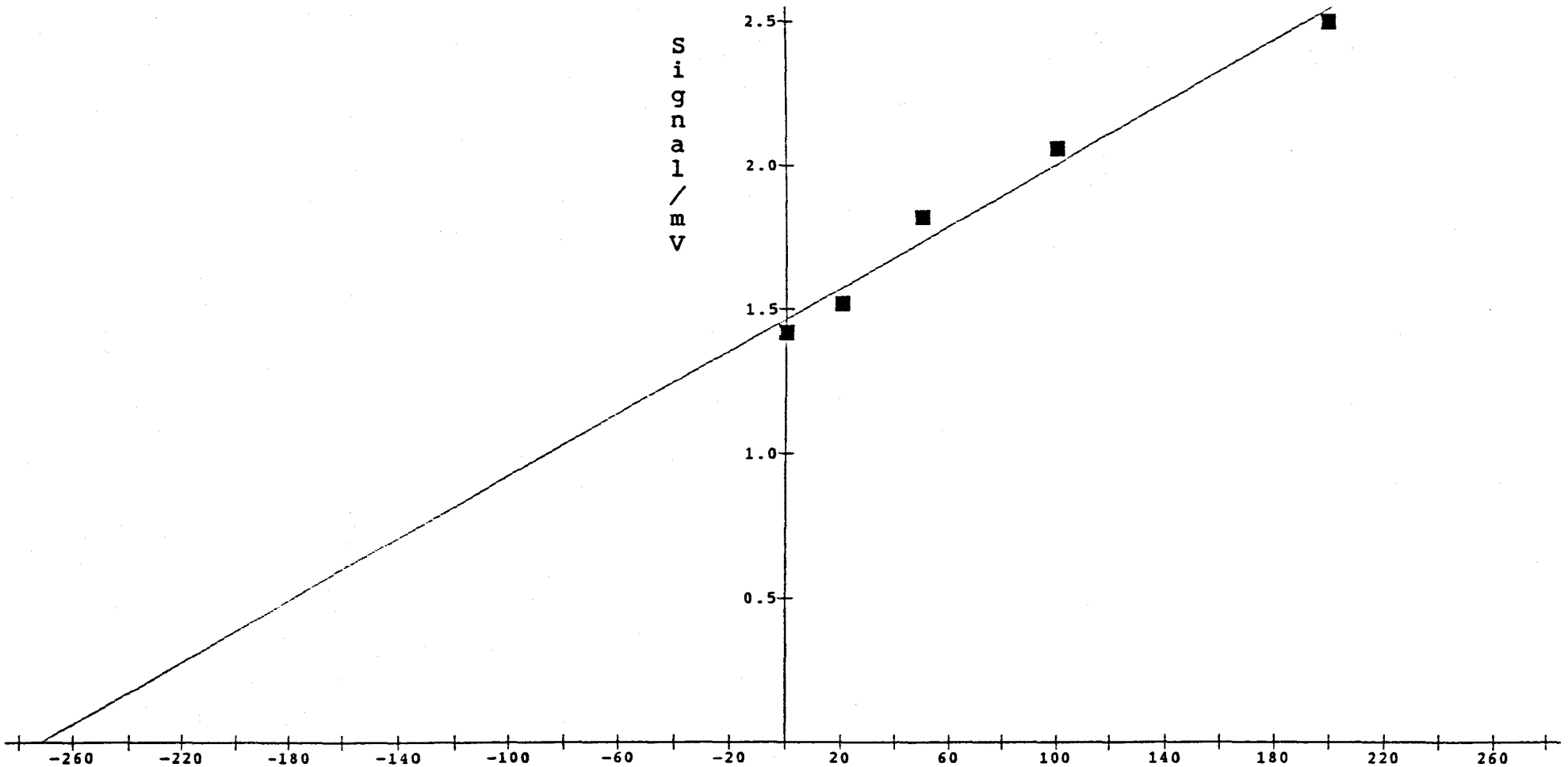
Table 4.3. Standard Addition Calibration Data For The Aqueous Nonidet AT 85 Extract.

Final spike concentration (mg l^{-1})	Signal (mV)	rsd (n=5) (%)
0	1.42	10.4
20	1.52	11.8
50	1.82	8.1
100	2.06	6.5
200	2.50	6.3
300	2.62	6.8

$y=5.4X10^{-3}(x)+1.46$ mV

r=0.9887

Figure 4.3.A. Standard addition calibration graph
for the aqueous Nonidet AT 85 extract.



Although it was not possible to corroborate these results with another technique, the proposed method is nonetheless rapid and simple and therefore appropriate for semi-quantitative on-site analysis.

4.4. Conclusions

The CL procedure for the determination of water-soluble tertiary amines has been adapted for the determination of the fatty amine ethoxylate surfactant, Nonidet AT 85. The oxidation of this surfactant with sodium hypochlorite at pH 10.5 in the presence of rhodamine B, generates a CL emission. This enables the surfactant to be determined in sea water with a theoretical limit of detection (3σ) of 4 mg l^{-1}. Nonidet P 40 (an octyl phenol ethoxylate) did not give a detectable CL emission, indicating that the tertiary amine group is essential for chemiluminescence.

This work also shows that the lamina flow cell results in reduced CL emission intensities as compared with the glass coil flow cell and a limit of detection ($3\ \sigma$) of 12 mg l^{-1} was obtained. The robust nature of the lamina flow cell, however, and the ease with which the flow cell can be dismantled for cleaning, and the cell volume changed makes the use of the lamina flow cell advantageous in an industrial laboratory, or in the field where maximum CL intensity may not be as important as the requirement for a robust cell.

Chapter Five

Peroxyoxalate Chemiluminescence

5.1 Introduction.

The detection of fluorophores by peroxyoxalate CL using separation by thin layer chromatography (TLC) was first demonstrated by Curtis and Seitz [195]. Dansyl derivatives of amino acids were separated by TLC and the components were detected by spraying on bis(2,4,6-trichlorophenyl)oxalate (TCPO) and hydrogen peroxide solutions. Since then, many analytical applications of the peroxyoxalate CL reaction have been reported [196]. For example, bis(2,4-dinitrophenyl)oxalate (DNPO) has been used for the flow injection determination of dansylalanine with a detection limit of 5 fmol [197]. Peroxyoxalate CL has also been used to detect fluorescent compounds after separation by HPLC; detection limits can be one or two orders of magnitude lower than those obtained with conventional fluorescence detection and matrix interferences can be reduced. One example is the determination of amino polycyclic aromatic hydrocarbons in oils. The detection limits for 1-aminoanthracene, 6-aminochrysene and 3-aminofluoranthene were 16 pg, 1.2 pg and 18 pg respectively with fluorescence detection and 0.15 pg, 0.3 pg and 0.09 pg respectively with CL detection [43]. A range of polycyclic aromatic hydrocarbons have been determined using TCPO in aqueous micellar media by FIA [123] and after separation by reversed phase HPLC [96], with detection limits ranging from 0.77 pg for perylene to 25 ng for the less efficient chemiluminophore, triphenylene. The TCPO reaction has

been used to detect dansylated amino acids after separation by reversed phase HPLC, with detection limits of 2-5 fmol [198]. Glucose has been determined using the TCPO reaction to measure the hydrogen peroxide generated by immobilized glucose oxidase [199].

In the initial studies described in this chapter, the peroxyoxalate reaction was investigated using perylene as the fluorophore and the automated flow injection manifold, described in section 2.5, was compared with the equivalent manual system. Peroxyoxalate CL was compared with a fluorimetric procedure for the determination of primary amines in non-aqueous media [200].

5.2. Experimental

5.2.1. Reagents

All solvents used were either HPLC grade or AnalaR grade unless otherwise stated. Bis(2,4,6-trichlorophenyl) oxalate (TCPO) and bis(2,4-dinitrophenyl) oxalate (DNPO) were obtained from Fluka chemicals, and working solutions ($1X10^{-3}$ M) were prepared by dissolving 0.0211 g and 0.0224 g of DNPO and TCPO respectively in acetonitrile (50 ml). Stock perylene solution ($1X10^{-4}$ M) was prepared by dissolving 0.0252 g perylene (Aldrich) in 1 l of acetone. 0.1 M hydrogen peroxide (AnalaR; BDH) was prepared by dissolving 100 volume hydrogen peroxide (1.12 ml) in 100 ml of acetonitrile. 2-mercaptoethanol (BDH), o-phthaladehyde

(Aldrich) and n-hexylamine (Aldrich) stock solutions (0.1 M) were prepared in acetonitrile, working solutions were prepared by dilution of the stock solutions.

5.2.2. Preliminary Experiments

Preliminary experiments were carried out using TCPO or DNPO (1 mM) and hydrogen peroxide (0.1 M) in acetonitrile; the analyte was perylene. The flow injection manifold was as shown in Fig. 2.8. In order to keep the reaction background to a minimum the manifold was flushed with dilute hydrochloric acid, followed by distilled - deionised water to remove any water soluble material, followed by acetone to remove any remaining material and the water.

5.2.3. Comparison Of Automated And Manual Flow Injection Systems

Standards and samples (100 µl) were injected into the acetonitrile carrier stream. This stream was merged with the hydrogen peroxide stream and then the peroxyoxalate stream. The manifold is as shown in Fig. 2.8. The three streams were pumped at equal flow rates via a peristaltic pump (Gilson Minipuls 2; P1), the total flow rate was 2.0 ml min^{-1}. Silicone pump tubes (Altec Ltd) were employed for their resistance to corrosion by many organic solvents, and PTFE tubing (0.5 mm i.d.) was used throughout the remainder of the manifold. The merged streams then travelled a further 5 cm before

passing into a glass coil flow cell (240 µl). The detector was an end window PMT (Thorn EMI 9789QA) operated at 1180 V. In the case of the manual system, the standards and samples were injected using a rotary valve (Rheodyne 5020) switched by an electrically activated switching device and the signals were recorded on a strip chart recorder (Chessell BD 4004). In the case of the automated system (Section 2.5) the valve switching device was interfaced to a microcomputer. Standards and samples were drawn into the sample loop by means of a second peristaltic pump (Ismatec Mini S 840; P2) which was operated by the computer. Sample introduction was carried out by the autosampler. The output from the PMT was amplified by an inverting amplifier (BBH Power Products) and was interfaced to the internal analogue to digital converter of the computer. An overload protection device was employed to protect the computer from transient voltage spikes. The signals were smoothed prior to their capture by the computer.

5.2.4. Determination Of Primary Amines

A non-aqueous procedure for the determination of primary amines has been reported [200]. The procedure is based on the derivatization of the amine with o-phthalaldehyde (OPA) and 2-mercaptoethanol (2-ME) [159] to form a highly fluorescent isoindole. The manifold used for the determination of primary amines using the

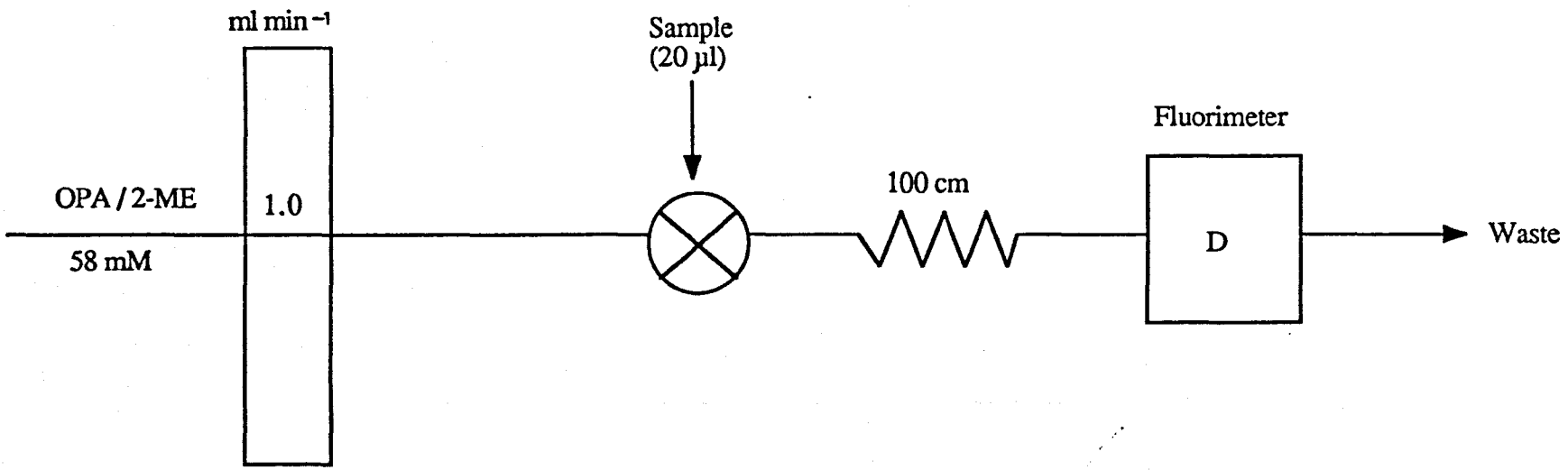


Figure 5.1. Manifold for primary amine determination with fluorescence detection.

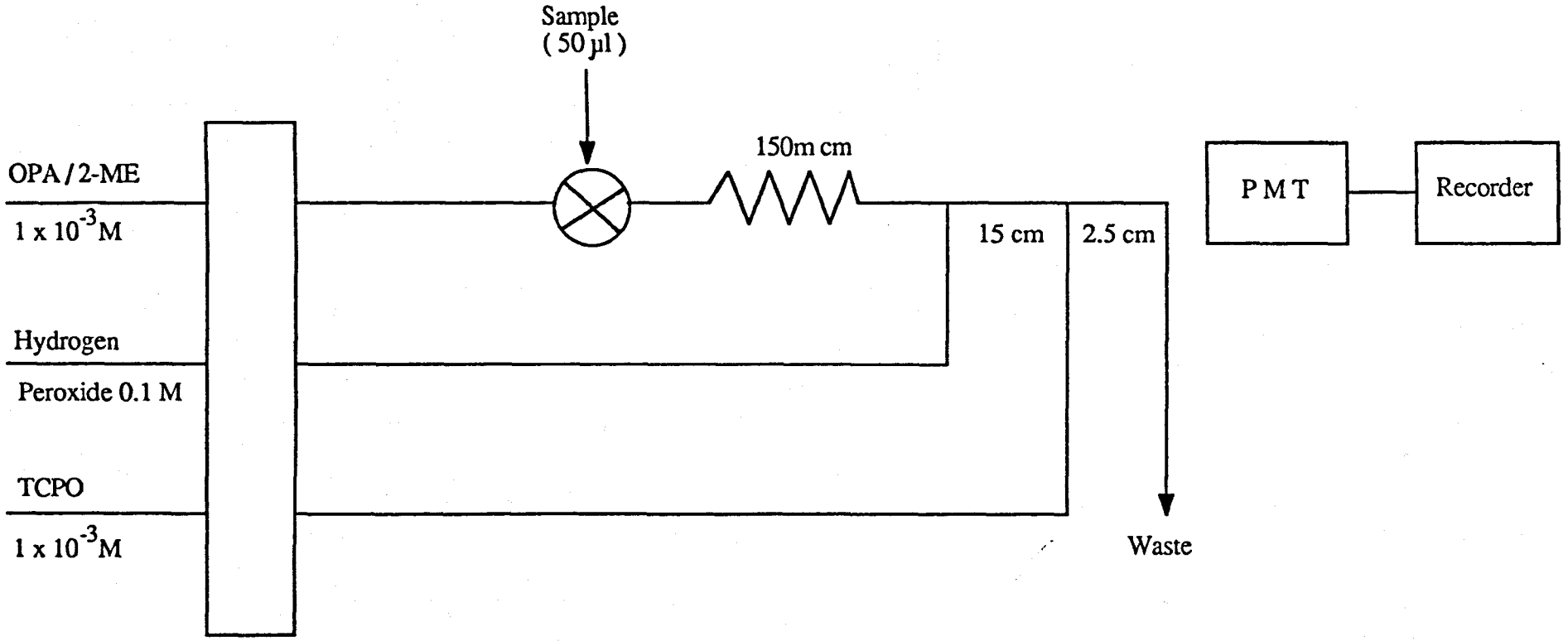


Figure 5.2 Manifold for primary amine determination with chemiluminescence detection.

OPA procedure with fluorescence detection is shown in Fig. 5.1 [201]. This manifold was modified to facilitate the use of CL detection, and this is shown in Fig. 5.2.

With fluorescence detection the best solvent was found to be methanol [202]. This quenched the CL reaction, however, and so acetonitrile was chosen as a solvent for the CL reagents. The OPA and 2-ME concentrations were also reduced from 58 mM to 1 mM for the CL determination.

5.3. Results And Discussion

5.3.1. Preliminary Experiments

Long range calibration data for perylene were obtained using the flow injection manifold shown in Fig. 2.8. Calibration data are shown in Tables 5.1 and 5.2 for TCPO and DNPO respectively, over the range 0 to $1X10^{-5}$ M. The limits of detection (3 σ) were $2X10^{-9}$ M and $7X10^{-10}$ M for TCPO and DNPO respectively. TCPO and DNPO are the most widely used aryl oxalates, though recently other aryl oxalates have been evaluated for use as CL reagents [202,203]. TCPO proved to be more stable than DNPO, and solutions were stable for several hours when prepared in acetonitrile in the presence of 1 mM hydrogen peroxide [202]. In contrast, DNPO decomposed rapidly in the presence of hydrogen peroxide [202]. Of the aryl oxalates investigated the DNPO reaction was found to generate the most intense CL emission, and is

potentially two orders of magnitude more sensitive than TCPO [202]. The solubility of DNPO in acetonitrile was also found to be greater than TCPO [202]. Other aryl oxalates have been synthesized and exhibit a range of solubilities, stabilities and CL intensities [202,203].

Table 5.1. Calibration Data For Perylene Using TCPO

Concentration (mol l^{-1})	Signal (mV)	rsd (n=6) (%)
0.0	0.0	0.0
$1X10^{-9}$	0.0	0.0
$1X10^{-8}$	2.7	15.3
$1X10^{-7}$	23.1	5.4
$1X10^{-6}$	289.6	2.7
$1X10^{-5}$	3283.3	3.2

$y=3.3X10^{8}(x)-16.1$ mV

$r = 0.9999$

Table 5.2. Calibration Data For Perylene Using DNPO

Concentration (mol l^{-1})	Signal (mV)	rsd (n=6) (%)
0.0	0.0	0.0
$1X10^{-9}$	19.6	11.2
$1X10^{-8}$	34.4	3.7
$1X10^{-7}$	575.8	4.9
$1X10^{-6}$	3887.5	0.4

$y=3.84X10^{9}(x)+64.0$ mV
r = 0.9910

5.3.2. Comparison Of Automated And Manual Flow-Injection Systems

Calibration data were obtained for perylene using the TCPO reaction. The signals for the standards and the unknowns were corrected by subtracting the blank signal. Tables 5.3 and 5.4 show the calibration data obtained using the automated and manual systems respectively. In the case of the automatic system, the inverting amplifier was set on the maximum gain. A comparison of the two sets of results shows that the repeatability obtained with the automatic system is superior to that of the manual system. Since the samples were injected in each case using the same valve switching device (though not interfaced to the computer in the case of the manual system), the superior precision of the automated system

is due to the data collection hardware. The analogue to digital converter is able to respond faster to the transient CL signal than the chart recorder, and the relatively slow response of the chart recorder results in a degradation of the precision of the data [204].

The limits of detection for perylene obtained using TCPO as the CL reagent on the two systems were compared, and the concentrations of perylene giving a signal three standard deviations of the blank (obtained by taking 7 blank readings) were $7X10^{-9}$ M and $3X10^{-9}$ M for the manual and the automated systems respectively. The lower detection limit obtained with the automated system is due to the higher precision of the data.

Table 5.3. Calibration Data For Perylene Obtained With The Automated System.

Concentration (nM)	Signal (V)	Corrected Signal (V)	rsd (n=7) (%)
0	0.395	0.000	3.7
10	0.600	0.205	2.3
20	0.823	0.428	1.7
0	0.403	0.000	4.5
40	1.255	0.851	2.0
60	1.762	1.358	1.5

$y=0.023(x)-0.017$ V

$r=0.9991$

Table 5.4. Calibration Data For Perylene Obtained With The Manual System.

Concentration (nM)	Signal (V)	Corrected Signal (V)	rsd (n=7) (%)
0	0.176	0.000	9.2
10	0.437	0.261	13.7
20	0.669	0.493	6.9
40	0.971	0.795	8.5
60	1.389	1.213	5.5

y=0.019(x)+0.045 V

r=0.9962

5.3.3. Determination Of Primary Amines

The derivatization reaction scheme for primary amines using OPA and 2-ME is shown in Fig. 5.3. Initial studies on the CL detection of n-hexylamine using the manifold shown in Fig. 5.2. proved disappointing as no detectable CL was obtained using a carrier stream of 58 mM OPA/2-ME in methanol. It has been observed previously that methanol reduces the CL intensity of the peroxyoxalate reaction [205]. The methanol was therefore replaced with acetonitrile, resulting in greatly enhanced emission. Calibration data is shown in Table 5.5.

Table 5.5. Initial Calibration Data for n-Hexylamine

Concentration (mol l^{-1})	Signal (mV)	rsd (n=5) (%)
0	0.0	0.0
$1X10^{-5}$	0.3	44.3
$5X10^{-5}$	8.6	24.1
$1X10^{-4}$	54.0	27.3
$5X10^{-4}$	1365.0	4.0
$1X10^{-3}$	3370.0	1.5

The calibration data was non-linear, curving away from the x-axis. The absence of any blank emission means that the practical limit of detection is around $5X10^{-5}$ M, and is determined by the repeatability of the procedure. Background emission was not detectable.

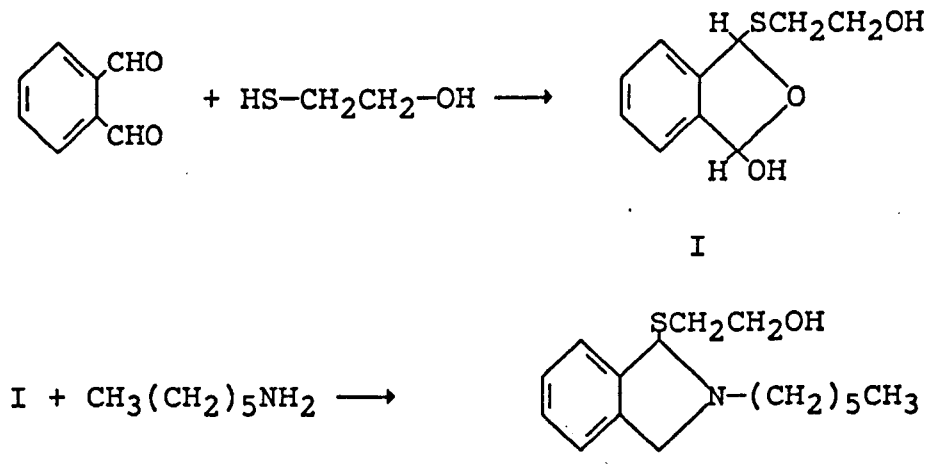
The absence of a detectable background emission led to an investigation of the use of an OPA/2-ME carrier stream for the detection of perylene using the TCPO reaction, and a 58 mM OPA/2-ME stream in acetonitrile was found to totally suppress the emission. To investigate this the OPA and 2-ME concentrations were varied independently. A $1X10^{-2}$ M OPA stream did not quench the emission on its own, $1X10^{-2}$ M 2-ME quenched the emission of a $1X10^{-5}$ M perylene solution by a factor of 10, and a $1X10^{-2}$ M OPA/2-ME carrier stream, which had been standing

for 24 hours quenched the emission by a factor of 100. A freshly prepared solution of $1X10^{-2}$ M OPA/2-ME quenched the emission by a factor of 20.

The background emission, (a) in the absence of OPA and 2-ME, (b) with $1X10^{-2}$ M OPA/2-ME (24 hours old), (c) with $1X10^{-2}$ M 2-ME alone and (d) with $1X10^{-2}$ M OPA alone was 12.3 mV, 0.5 mV, 5.6 mV and 14.8 mV respectively.

From these results, it was found that 2-ME quenches both the analyte CL and the background emission, OPA alone has essentially no effect, and OPA/2-ME has a larger quenching effect than 2-ME alone, possibly due to the formation of an intermediate sulphide which is known to form when OPA and 2-ME are mixed and allowed to stand [159] (Fig. 5.3).

Fig. 5.3. Formation of the Fluorescent Product



I

The quenching effect of 2-ME, and the intermediate sulphide may be due to the formation of a charge transfer complex with the sulphide (I), rather than the fluorescer. The observation that easily oxidizable compounds quench peroxyoxalate CL has been reported previously [206].

In an attempt to lower the limit of detection for n-hexylamine using CL detection, the effect of lowering the OPA/2-ME concentration was investigated. The concentration of both OPA and 2-ME were kept equal and varied simultaneously. The results are shown in Table 5.6. An optimum concentration of $1X10^{-3}$ M was found. The n-hexylamine standard used was $5X10^{-2}$ M.

Table 5.6. Investigation Of The Effect Of OPA/2-ME Concentration

Concentration of OPA/2-ME (mol l^{-1})	Background emission (mV)	Signal (mV)
$1X10^{-2}$	10.2	381.0
$4X10^{-3}$	12.2	958.0
$1X10^{-3}$	20.3	2022.5
$5X10^{-4}$	29.5	1865.0
$1X10^{-4}$	23.8	343.5

Due to the optimum concentration of the reagent being reduced to $1X10^{-3}$ M for CL detection, the 100 cm knitted mixing coil was replaced with a 150 cm coil to increase the amount of fluorescent product formed, and the flow rate was optimised. The optimum flow rate was 2.3 ml min^{-1} and calibration data for n-hexylamine obtained under these conditions is shown in Table 5.7.

Table 5.7. Calibration Data For n-Hexylamine Using The Optimized Derivatization Conditions.

Concentration (mol l^{-1})	Signal (mV)	rsd (n=6) (%)
0	10.4	10.3
$5X10^{-7}$	29.3	7.7
$1X10^{-6}$	91.3	7.3
$5X10^{-6}$	259.2	2.8
$1X10^{-5}$	533.3	6.7
$5X10^{-5}$	2035.4	2.4
$1X10^{-4}$	3872.9	0.3

The calibration data was linear over the range investigated.

$$y=3.85X10^{7}(X)+61.2 \text{ mV}$$

$$r=0.9993$$

The limit of detection was calculated using the standard deviation of the fitted line [207] as twice the standard deviation of the blank, and was $3X10^{-6}$ M. This method was used to directly compare the detection limits of the CL procedure with the fluorimetric procedure which was calculated in this way due to the absence of a blank emission [201]. The LOD was $8X10^{-5}$ M [201]. The limit of detection for the CL method is therefore 26 times lower than that obtained with the fluorimetric method.

5.4. Conclusions

Preliminary experiments on the peroxyoxalate chemi-excitation reaction compared the use of TCPO and DNPO for the determination of perylene. In each case the calibration data was linear over the range investigated, and detection limits (3σ) using TCPO and DNPO were $2X10^{-9}$ M and $7X10^{-10}$ M respectively.

In addition, the TCPO reaction was used for the determination of the fluorescent o-phthaladehyde derivative of n-hexylamine in non-aqueous media. This gave a limit of detection of $3X10^{-6}$ M, as compared with $8X10^{-5}$ M with conventional fluorescence detection. The use of DNPO as CL reagent would give a detection limit lower than that obtained with TCPO.

Direct comparison of the sensitivities of the automated and the manual systems showed that the limits of detection (3σ) for perylene, using the TCPO reaction were $3X10^{-9}$ M and $7X10^{-9}$ M respectively. The lower detection limit obtained with the automated flow injection manifold is due to the rapid response of the data collection hardware in comparison to the chart recorder used with the manual system. This results in improved precision of the data.

Chapter Six

Determination Of Carboxylic Acids In Non-Aqueous Media By HPLC With CL Detection

6.1. Introduction

One of the ageing processes in lubricating oils resulting in a degradation in the performance of the oil is that of oxidation. Oxygen is sufficiently soluble in oil to provide favourable conditions for oxidation, particularly at the elevated temperatures occurring in internal combustion engines.

The oxidation of petroleum based hydrocarbons proceeds by a radical chain mechanism via alkyl and peroxy radicals [208]. The predominant oxidation reaction is that of hydrogen abstraction at tertiary carbon atoms and carbon atoms α to aromatic rings. These carbon-centred radicals then react with oxygen to give peroxy radicals.

$$RH + O_2 \longrightarrow R^{\cdot} + H\text{–}O_2^{\cdot}$$

$$R^{\cdot} + O \longrightarrow R\text{–}OO^{\cdot}$$

The radical chain reaction is then propagated by reaction of the peroxy radicals with hydrocarbon molecules [208].

$$R\text{–}O\text{–}O^{\cdot} + RH \longrightarrow RO_2H + R^{\cdot}$$

This leads to the formation of alkyl and aryl carboxylic acids including benzoic acid. The oxidation products then form sludges which can cause blockages in

oil filters and oil lines. The deposits can precipitate on engine walls, reducing heat transfer and the efficiency of the lubrication process. The acids also promote chemical corrosion of the engine leading to excessive wear.

Certain compounds which occur naturally in petroleum based oils, particularly sulphur and nitrogen containing compounds can interrupt the propagation of such radical reactions by removing the radicals or combining with the peroxides. Phenols and amines can function as radical acceptors by transferring a hydrogen atom from the oxygen or nitrogen atom to the radical. The resulting inhibitor radicals then either combine or undergo electron transfer to give ionic species [209]. Lubricating oils therefore possess a natural stability to oxidation. Synthetic additives are also used to enhance the inherent stability of oils, and to modify their properties [210]. There is therefore a need to monitor the susceptibility of the oil to oxidation.

6.1.1. Oxidation Testing

The oxidation stability of oils can be determined by several standard methods. These tests involve the oxidation of the oil by air or oxygen at different temperatures and pressures over a specific period of time. A metal catalyst may be present [208]. Oxidation of the oil is slow until the anti-oxidant compounds present have been exhausted. This is known as the

induction period, after which the oil oxidizes rapidly. Methods of monitoring the oxidation of the oil include monitoring the neutralisation number and viscosity or carbon forming tendency [208]. The neutralisation number of an oil is determined by dissolving the oil in a mixture of toluene and iso-propyl alcohol containing a small amount of water. The resulting single phase solution is titrated with an alcoholic base standard and the end point is monitored by the colour change of the added indicator (p-naphtholbenzein) [211]. In the case of highly coloured oils the colour change may be obscured, and a potentiometric titration [211], or the measurement of oxygen uptake [212] can be used. The autoxidation of oils has been reported to give a chemiluminescent emission and this has been evaluated as a method for monitoring the oxidative stability of lubricants [213]. This procedure, however, was found to give anomalous results, and was subject to many interferences. The methodologies currently in use do not differentiate between the carboxylic acids formed and other acids such as the sulphonic acids and phenols which are also present. There is a need, therefore for a procedure which can monitor the formation of individual carboxylic acids selectively in the presence of these compounds in oils.

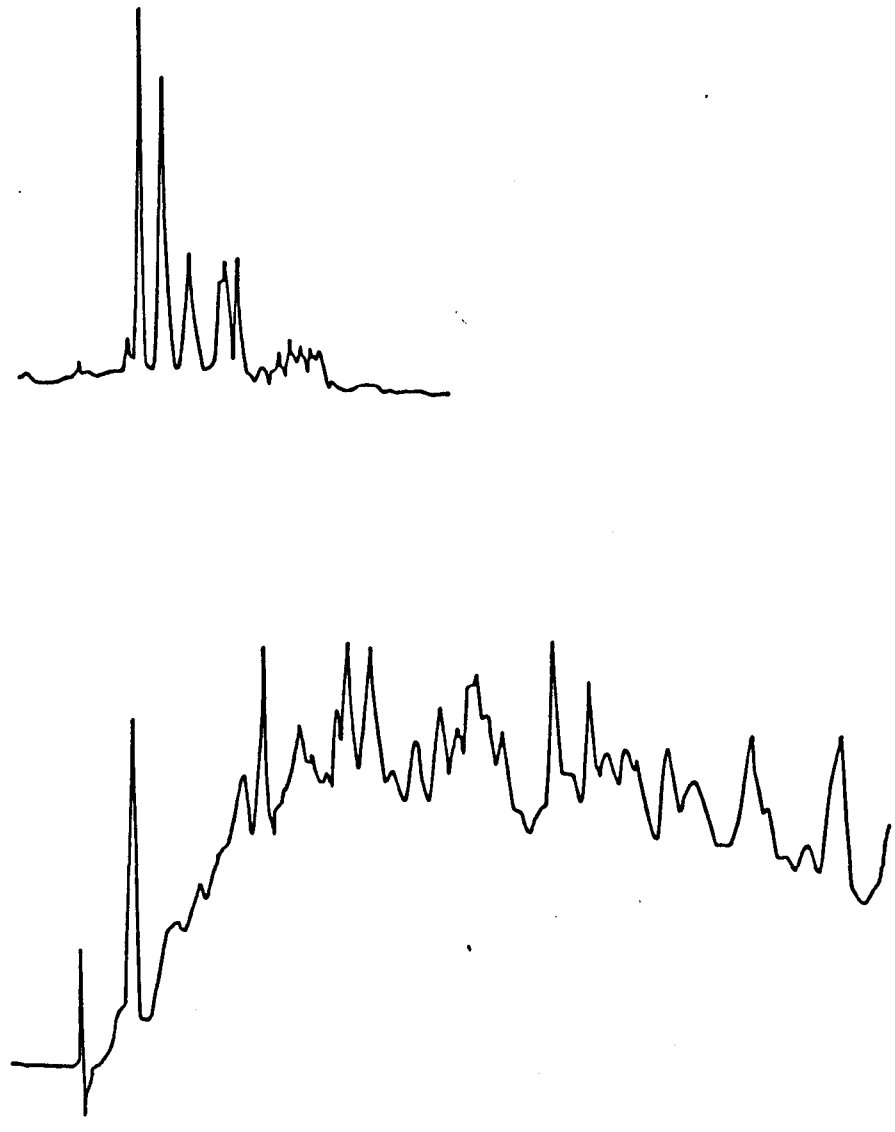


Figure 6.1. **HPLC determination of a range of amino polyaromatic hydrocarbons with CL and fluorescence detection [43].**

Peroxyoxalate CL has been used to detect fluorescent compounds after separation by HPLC. The detection limits can often be lower than those obtained with conventional fluorescence detection, and matrix interferences can be reduced. Fig. 6.1. compares chromatograms for the HPLC determination of a range of amino polycyclic aromatic hydrocarbons in oil using chemiluminescence detection and fluorescence detection [43].

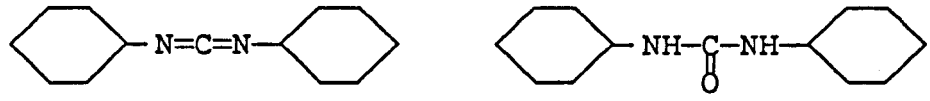
A number of fluorimetric procedures for the detection of carboxylic acids following separation by HPLC have been reported, including derivatization with coumarin derivatives [214,215], fluorescent halogen compounds [216-218], fluorescent diazo compounds [219,220] and fluorescent hydrazides [223]. Chemiluminescence procedures have been reported for the determination of carboxylic acids in biological fluids [221-222,224-225].

The measurement of the degradation of lubricating oils requires a procedure which can selectively monitor the formation of oxidation products in the oil matrix. This chapter reports the development of a pre-column non-aqueous fluorimetric derivatization procedure for the detection of straight chain carboxylic acids over the range C_2 to C_{20} and benzoic acid using the peroxyoxalate reaction as a HPLC detection technique.

The derivatization of carboxylic acids in non-aqueous media was carried out using

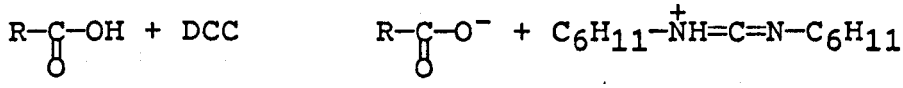
dicyclohexylcarbodiimide (DCC) as a coupling reagent for the formation of the fluorescent esters or amides by reaction of the acids with the fluorescent alcohol or amine. The DCC reaction has been widely used for the large scale synthesis of peptides [226,227]. Diimides have also been used for the fluorimetric derivatization of carboxylic acids [228]. In DCC mediated esterification reactions the acid is converted into a compound with a better leaving group. The overall reaction consists of four steps (Fig. 6.2.) [229]. The first step involves the protonation of a nitrogen atom of DCC. In the second step the acyl oxygen of the conjugate base of the acid attacks the DCC to form the acid-DCC derivative. The second nitrogen is protonated in the third step. Finally the carbonyl carbon of the acid-DCC derivative is attacked by the fluorescent alcohol and the substituted urea derivative is eliminated to yield the ester and dicyclohexylurea (DHU).

Fig. 6.2. DCC Mediated Esterification Of Carboxylic Acids

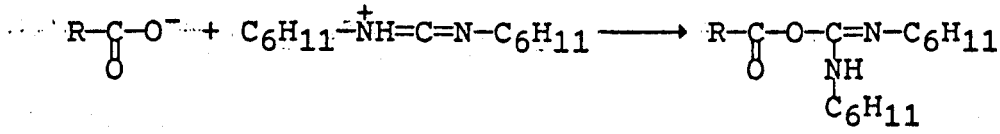


DCC DHU

Step 1

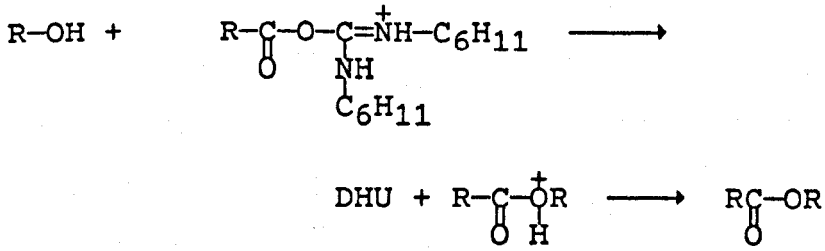


Step 2



Step 3

Step 4



6.2. Experimental

6.2.1. Reagents

All the solvents used were AnalaR or HPLC grade unless otherwise stated. DNPO was obtained from Fluka chemicals, and working solutions were prepared by dissolving the appropriate amount in acetonitrile (May & Baker). Hydrogen peroxide solutions were prepared in acetonitrile from 100 volume stock (AnalaR; BDH). Perylene stock solution ($1X10^{-4}$ M) was prepared by dissolving perylene (Aldrich) in acetonitrile. The other fluorescent molecules investigated: 2-aminoanthraquinone, 1-aminoanthraquinone, 2-aminochrysene, tryptophol, 7-hydroxycoumarin, 4-hydroxycoumarin and 9-hydroxymethyl anthracene (9-anthracenemethanol) were obtained from Lancaster Synthesis. All working solutions were $1X10^{-4}$ M in acetonitrile with the exception of perylene and 2-aminochrysene which were $1X10^{-6}$ M and $1X10^{-5}$ M respectively. Acid solutions were prepared in n-heptane except for phthalic acid which was dissolved in acetone. The acids used were: acetic (Koch-Light); benzoic, hexanoic, octanoic, decanoic, stearic, (BDH); lauric, eicosanoic,(Aldrich); salicylic, phthalic, butyric, myristic and palmitic, (Hopkin & Williams). DCC (Aldrich) was used as the solid, and 4-pyrrolidinopyridine solutions (Aldrich) were prepared in dichloromethane. 2-bromopyridine and triethyl oxonium tetrafluoroborate were obtained from Lancaster Synthesis.

6.2.2. Preliminary Investigations

Candidate fluorescent labels were investigated for their CL efficiency with the peroxyoxalate reaction. Fluorescent molecules containing either hydroxy or primary amine groups were investigated with the intention of converting the carboxylic acids to fluorescent esters or amides. The fluorescent alcohols and amines (above) were screened using the 3-line flow-injection manifold shown in Fig. 6.3, and the CL intensities were recorded.

6.2.3. Derivatization Experiments

Initial experiments were carried out using 2-bromo-1-ethylpyridinium tetrafluoroborate as the coupling reagent. This reaction has been reported to proceed rapidly and give good yields under mild conditions [228,230]. Initial experiments indicated that the reaction gave many products, in addition to the desired product. The resulting chromatograms were thus complicated by many interfering peaks. Further experiments were carried out using DCC as coupling reagent.

The carboxylic acids were derivatized with a molar excess of label and DCC in the presence of the base catalyst, 4-pyrrolidinopyridine. The acid solutions (5 ml in n-heptane) were added to round bottomed flasks, together with the catalyst (0.4 ml, 0.1 M) in dichloromethane, DCC (0.2 g, $9.7X10^{-4}$ Mol) and the label (2 ml, $1X10^{-2}$ M) in dichloromethane. 10 ml aliquots of

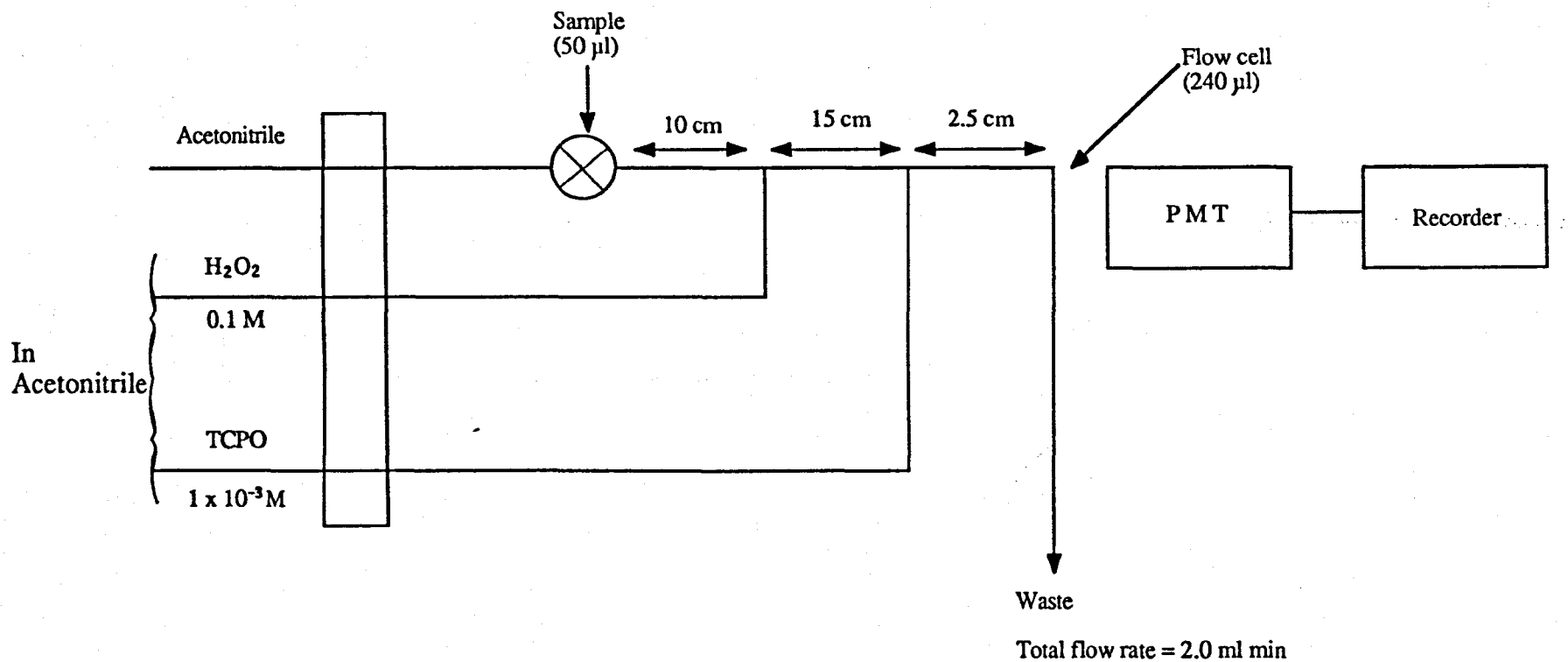


Figure 6.3. Manifold for screening fluorescent labels.

n-heptane were added and the solutions refluxed on a water bath at 97° C for 30 minutes and made up to 25 ml with dichloromethane.

6.2.4. Isolation and Identification

To investigate the DCC mediated derivatization reaction of butyric acid with 9-anthracene methanol, a large scale preparation was carried out. To a round bottomed flask 4-pyrrolidinopyridine (10 ml, 0.1 M) in dichloromethane was added together with butyric acid (25 ml, 0.5%, 0.057 M) in heptane, DCC (7.5 g, 0.039 Mol) and 9-anthracene methanol (25 ml $1X10^{-2}$ M) in dichloromethane. This was then refluxed for 30 minutes. The reaction mixture was analysed by TLC, using a mobile phase of 4:1 dichloromethane : 40° - 60° petroleum spirit. The reaction mixture was compared with an unreacted solution of the label. The above procedure was repeated with palmitic acid, though the volume of the 0.5% solution was increased from 25 ml to 72 ml to maintain the number of moles at the same level as that of the butyric acid. Similarly the volume of a 0.5% benzoic acid solution was increased to 34 ml. The TLC plates were visualised using UV light at 254 nm.

The above reaction mixtures were purified by column chromatography to remove any non-fluorescing by-products and excess starting materials. A silica gel stationary phase was used together with a mobile phase of 4:1 dichloromethane : 40° - 60° petroleum spirit. The

elution of the product band was followed by UV light. The product was collected and the solvent removed by rotary vacuum distillation. Samples of the isolated derivatives were analysed by mass spectrometry to confirm their identity. Solutions of the derivatives ($3.12X10^{-6}$ M in tetrahydrofuran) were prepared and the fluorescence spectra of each was recorded and compared with the fluorescence spectrum of 9-anthracene methanol under the same conditions. The CL spectrum of 9-anthracene methanol was obtained using the monochromator-detector assembly (shown in Fig. 2.5). The flow-injection manifold used to obtain the CL spectrum was as shown in Fig. 6.3.

6.2.5. HPLC Procedures

The derivatized solutions were initially made up to 25 ml with dichloromethane and injected (20 µl) via a high pressure rotary injection valve (Rheodyne 7010). The column used was a Spherisorb ODS 2 (25 cm X 0.46 cm i.d.). The particle size of the solid support was 5 µm. Initial experiments involving the chromatography of the derivatives of the short chain length acids (below C_{10}) used a mobile phase of 90:10 acetonitrile : water. A mobile phase of 60:30:10 acetonitrile :THF : water was used to elute derivatives above C_{10}. For the initial experiments involving the elution and detection of individual acids, the mobile phase was pumped by a single piston pump (HPLC Technology RR/035). For the separation

of mixtures (from C_2 to C_{20}) it was necessary to employ gradient elution, and a ternary gradient of acetonitrile, THF and water was delivered by a Spectraphysics model SP8200 HPLC pump.

The CL detector was as described in chapter 2, and the lamina flow cell, illustrated in Fig. 2.4. was used. The DNPO chemi-excitation reaction was employed to detect the fluorescent derivatives as they eluted. The DNPO stream and the hydrogen peroxide streams were pumped via a second single piston HPLC pump (Magnus Scientific). The two streams merged at a Y-piece prior to the pump, this stream merged again with the column eluent at a low dead volume stainless steel T-piece. The merged streams travelled 5 cm through 0.5 mm i.d. PTFE tubing into the lamina flow cell, where the CL emission was detected by the PMT (Thorn EMI 9789QA, operated at 1180 V). The column eluent and the reagent stream were both pumped at 1.0 ml min^{-1}. The HPLC system with CL detection is shown in Fig. 6.4.

The sensitivity of the CL detector was optimized using a modified simplex optimization program with the following variables (and ranges); reagent flow rate (0.8 ml min^{-1}-2.0 ml min^{-1}), hydrogen peroxide concentration ($1X10^{-2}$ M-1.00 M) and DNPO concentration ($1X10^{-4}$ M-$5X10^{-3}$ M). The eluent flow rate was kept at 1.0 ml min^{-1}. throughout. The optimum parameters were found after 9 experiments and were: reagent flow rate, 1.5 ml min^{-1}.; hydrogen peroxide concentration, 0.23 M; and DNPO

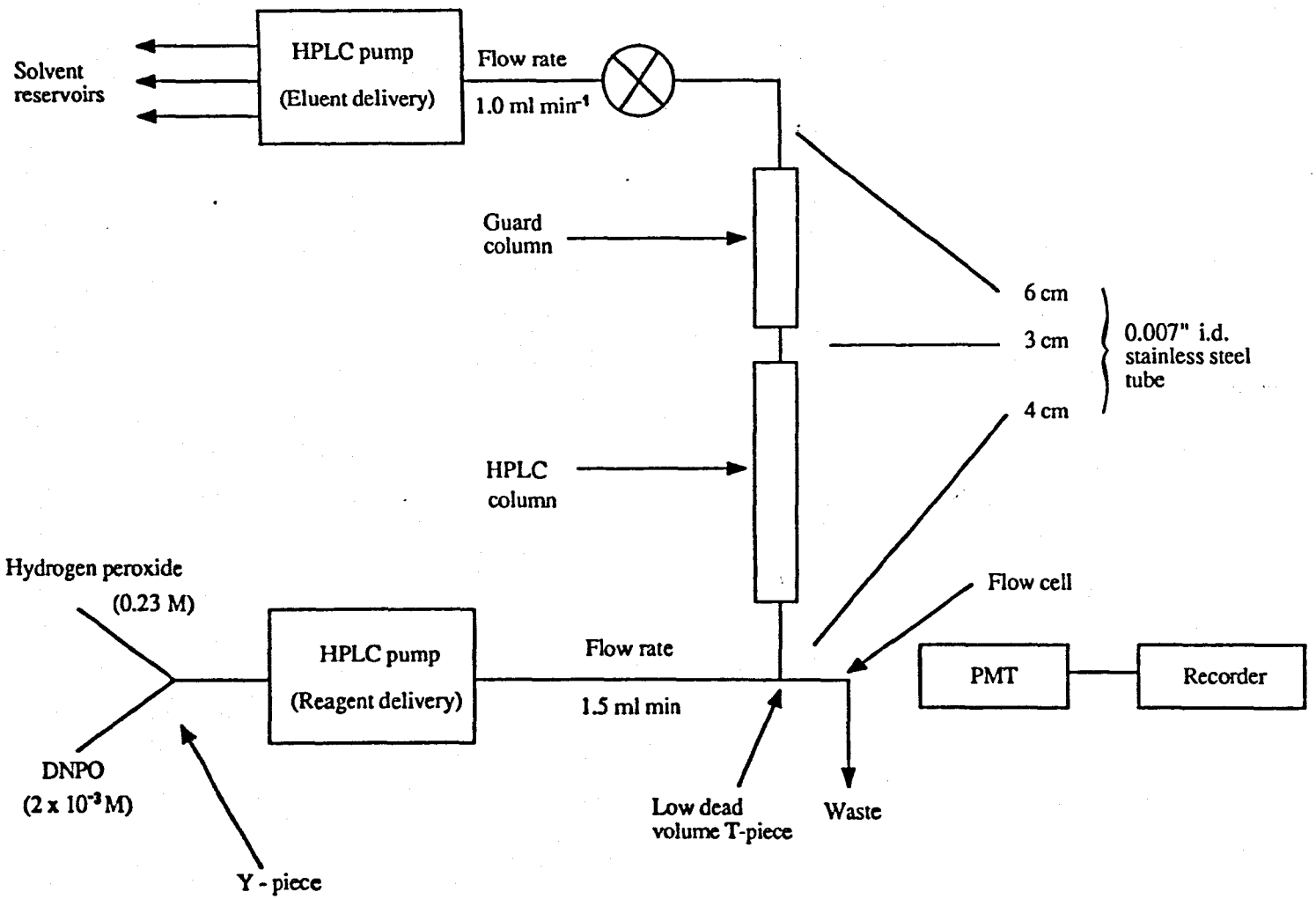


Figure 6.4. HPLC - CL System.

concentration, $2X10^{-3}$ M. These conditions were used in all subsequent experiments.

To optimise the chromatographic separation of straight chain carboxylic acids, from C_2 to C_{20} and benzoic acid, a mixture containing 25 mg l^{-1} of each acid was prepared in n-heptane. 5 ml of the mixture was derivatized as described above, and made up to 25 ml with dichloromethane. A 2.5 ml portion of this was diluted a further ten times with dichloromethane.

6.2.6. Calibration Experiments

Initial calibration experiments were carried out using n-heptane as a model sample matrix. Calibration data for benzoic, butyric and palmitic acids were obtained using both CL and fluorescence detection. For the fluorescence calibration experiments a Perkin-Elmer LS-2B filter fluorimeter was used. Each standard (5 ml) was derivatized as previously described. After refluxing, each of the reaction mixtures was made up to 25 ml with dichloromethane, and then diluted a further ten times with dichloromethane.

To investigate the effect of a lubricating oil matrix on the derivatization reaction, 5 ml aliquots of an unoxidized mineral oil sample were spiked with 5 ml, 2 ml, 1 ml, 0.1 ml and 0 ml of 0.05% butyric acid standard. The samples were then derivatized as above.

The accuracy of the procedure was assessed by spiking an oxidized oil sample (2 ml) (which did not contain detectable amounts of any carboxylic acid below C_{20}) with a 0.1% benzoic acid standard in heptane (2 ml). This was diluted to 50 ml with heptane. 5 ml aliquots of the spiked oil sample were transferred to four round bottomed flasks together with 5 ml of an internal standard (0.1% hexanoic acid in heptane) and 0 ml, 1 ml, 2 ml and 4 ml of the 0.1% benzoic acid standard. The standards were derivatized as described above and the amount of benzoic acid was determined by standard addition calibration.

6.2.7. Interference Studies

The effect of varying concentrations of a sulphonic acid on the derivatization of a 0.05% ($5.7X10^{-3}$ M) butyric acid standard was investigated. A solution of naphthalene sulphonic acid ($5.7X10^{-2}$ M) was prepared in THF. 5 ml, 2.5 ml, 0.5 ml and 0.1 ml were added to subsequent flasks, together with 5 ml of the butyric acid standard and derivatized as previously described.

The effect of phenol was also investigated. A solution of phenol ($5.7X10^{-2}$ M) was prepared in dichloromethane. 5 ml of the butyric acid standard, together with 5 ml, 2.5 ml, 0.5 ml and 0.1 ml of the phenol solution were each added to round bottomed flasks. The solutions were derivatized as above.

6.3. Results And Discussion

6.3.1. Preliminary Investigations

Preliminary investigations were carried out on several fluorescent molecules containing hydroxy or amino groups, with the intention of forming fluorescent esters or amides. The CL intensities of these molecules are shown in Table 6.2.

Table 6.2. CL Intensities of Some Hydroxy and Amino Compounds

Compound	Mean Signal (mV)
2-aminochrysene	4191.7[a]
4-hydroxycoumarin	1595.8[b]
perylene	1166.7[c]
9-anthracene methanol	810.0[b]
1-aminoanthraquinone	180.4[b]
7-hydroxycoumarin	112.1[b]
2-aminoanthraquinone	107.9[b]

a $1X10^{-5}$ M in acetone

b $1X10^{-4}$ M in acetone

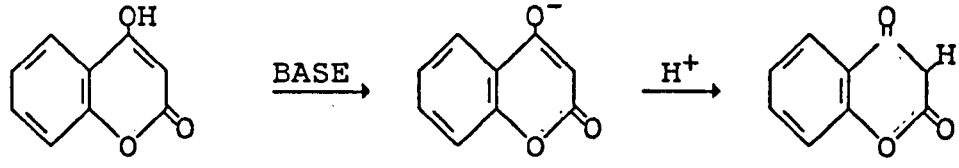
c $1X10^{-6}$ M in acetone

Perylene is known to be one of the most efficient chemiluminophores [36]. A $1X10^{-4}$ M solution gave a

signal greater than 5 V which could not be accommodated on the recorder and a $1X10^{-6}$ M solution was screened for comparison with the other compounds.

Preliminary derivatization experiments were carried out using benzoic acid with 2-aminochrysene. The coupling reagent was DCC. After several experiments using a range of reagent concentrations at room temperature and elevated temperatures the derivatization reaction proved unsuccessful. The DCC mediated reaction of carboxylic acid groups and amine groups has been used extensively in peptide synthesis [226,227], and the reason for the failure of the DCC reaction with 2-aminochrysene may be the much lower basicity of the nitrogen lone pair, as compared with the amine groups in amino acids and other non-aromatic amines. For example, the basicity of aniline (pK_a=4.62) is much lower than that of the aliphatic cyclohexylamine (pK_a=10.68) [231].

The next most efficient chemiluminophore was 4-hydroxycoumarin, and the derivatization procedure was attempted using the DCC reaction at both ambient and elevated temperatures, though the reaction again proved unsuccessful. The reason for this is not clear but may be due to the base catalysed tautomerisation of the 4-hydroxycoumarin to give the keto form [232].



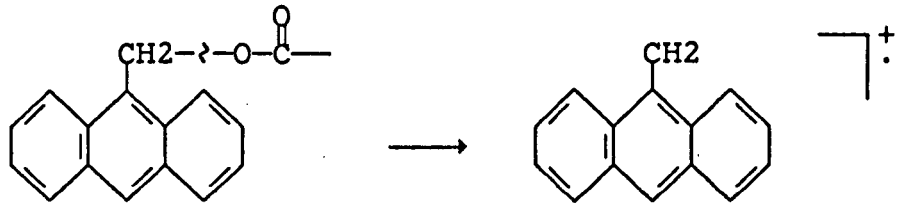
The third most efficient chemiluminophore was 9-anthracene methanol, and the derivatization of benzoic acid using DCC was found to proceed rapidly and cleanly. The reaction was carried out as previously described, the optimum reflux time was found to be 30 minutes.

6.3.2. Isolation And Identification Of The Derivative

Analysis of the products of the large scale derivatization reactions of benzoic, butyric and palmitic acids by TLC indicated that after refluxing for 30 minutes the reaction had proceeded to completion. The unreacted label had an R_f value of 0.14. The chromatographic conditions are described in Section 6.2.4. Analysis of the reaction mixtures of butyric, benzoic and palmitic acids showed the formation of the derivative, with R_f values of 0.79, 0.74 and 0.77 respectively. In each case a spot at R_f 0.14, corresponding to the unreacted 9-anthracene methanol was absent.

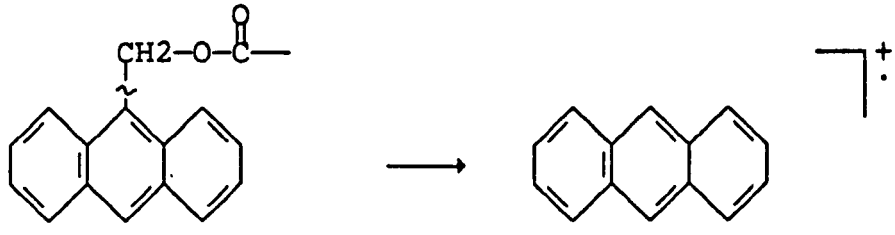
The esters of the above acids were then purified by column chromatography. Samples of the isolated esters were analysed by mass spectrometry to confirm their structures. In all cases the molecular ion for each

ester, though small, was clearly visible. A feature present in each spectrum was a peak at m/z 191, due to cleavage at the acyl oxygen bond.



m/z=191

A feature of all the spectra except that of the palmitic acid derivative was a peak at m/z 177 due to the radical cation of the naphthalene molecule.



m/z=177

Fluorescence studies indicated that the formation of the ester had little effect on the excitation and emission spectra of 9-anthracene methanol. Figures 6.5 and 6.6 show the fluorescence spectra of 9-anthracene methanol and the butyric acid derivative (excitation was at 260 nm). Each spectrum exhibits three major bands

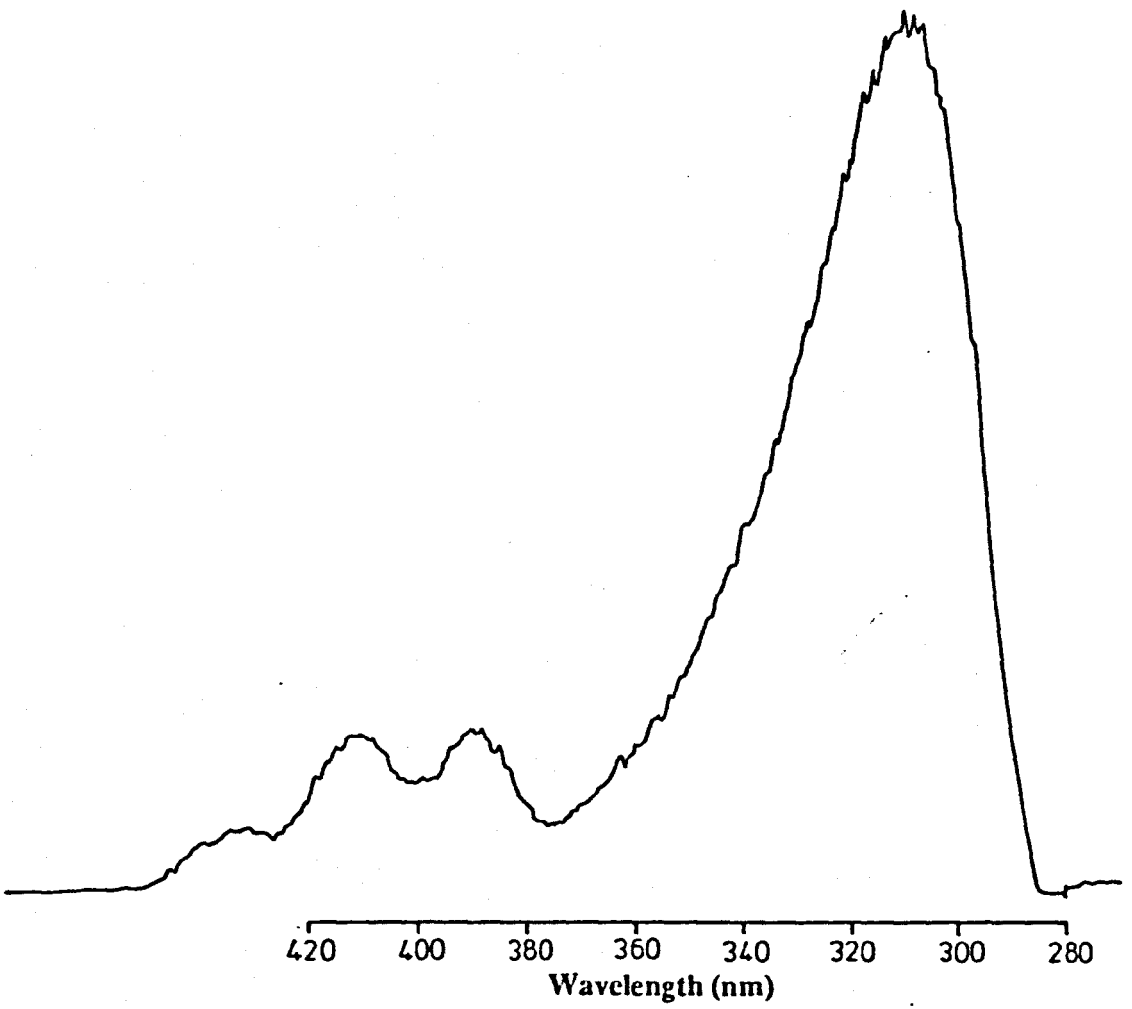


Figure 6.5. **Fluorescence spectrum of 9-anthracene methanol excitation wavelength 260 nm.**

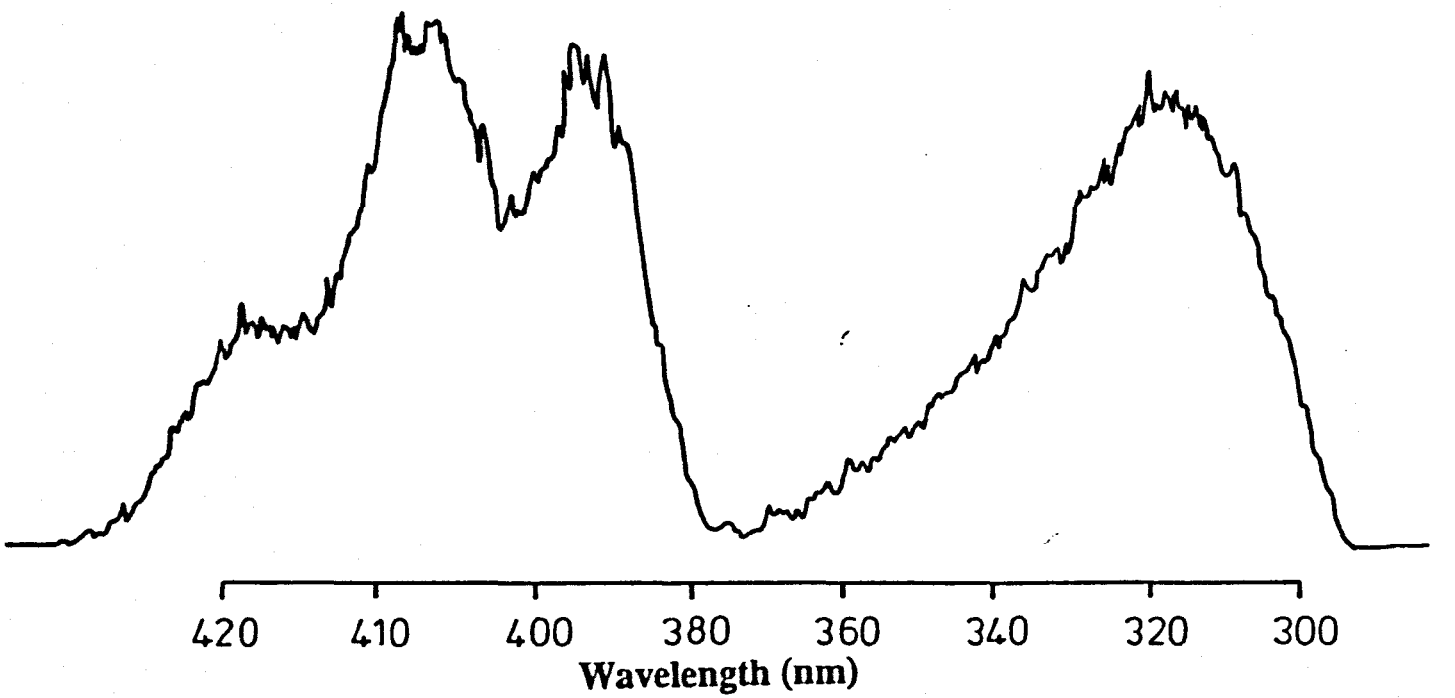


Figure 6.6. **Fluorescence spectrum of the butyric acid derivative of 9-anthracene methanol excitation wavelength 260nm.**

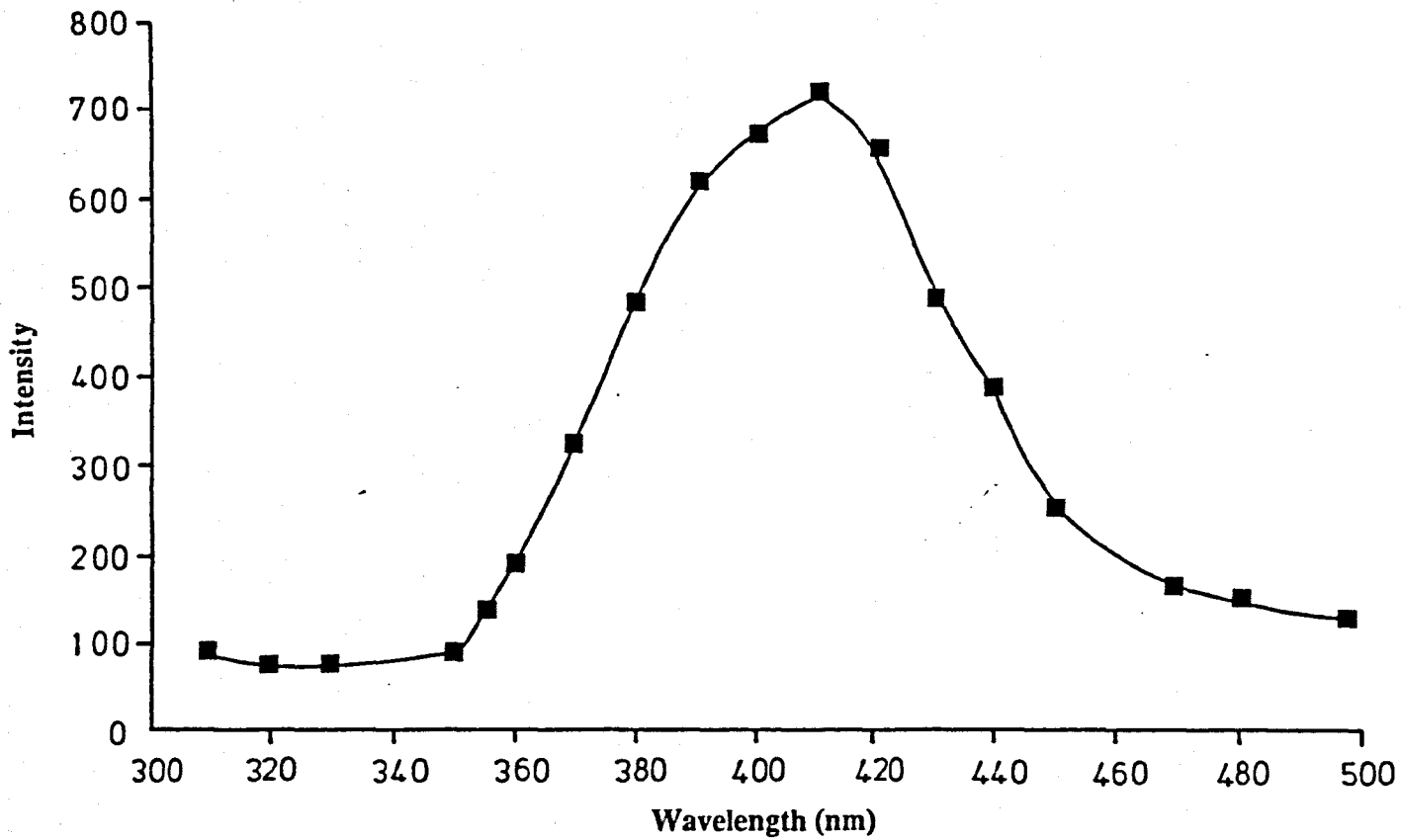


Figure 6.7. **CL spectrum of 9-anthracene methanol.**

with maxima at 310 nm, 390 nm and 412 nm, though in the case of the C_4 derivative the bands at 390 nm and 412 nm are small relative to the band at 310 nm. The corrected CL spectrum of 9-anthracene methanol was recorded (Fig. 6.7). The maximum CL intensity occurs at 420 nm. The maximum at 310 nm is not seen in the CL spectrum, possibly due to inefficient excitation at the lower wavelengths (Section 1.5.3.) The fine structure seen in the fluorescence spectrum is not observed in the CL spectrum, and this is due to the inferior resolution obtained when the slits are removed from the CL monochromator.

6.3.3. HPLC Procedures

Initial experiments were carried out using a mobile phase of 90:10 acetonitrile : water. The derivatized acids were injected individually to asses the retention times. The acetic acid derivative had a retention time of 3 min, and the lauric acid derivative (C_{12}) was eluted after 32 min. The higher acid derivatives (C_{14} to C_{20}) required a stronger mobile phase, and with 60:30:10 acetonitrile : THF : water, the retention times were over the range 8 min to 29 min.

Gradient experiments were carried out on a mixture of straight chain acids, from C_2 to C_{20} (increasing in chain length by two carbon intervals) and benzoic acid in n-heptane. Each was present at a concentration of 25 mg l^{-1}. The mixture was derivatized as previously

described, and the solvent gradient used is shown in Table 6.1.

Table 6.1. Solvent Gradient

	% Water	% Acetonitrile	% THF	Time (min)
1	30	70	-	0
2	20	80	-	10
3	20	20	60	25
4	20	20	60	40
5	30	70	-	42

The resulting chromatogram is shown in Fig. 6.8. The first peak is the unreacted label. The benzoic acid derivative was eluted at t_R 16 minutes, and was not completely resolved from the hexanoic acid derivative. Extensive peak tailing and peak splitting was also a problem, and this was due to the presence of heptane and (to a lesser extent) dichloromethane in the injected sample solution. These are strong solvents in reversed phase HPLC, though usually with injection volumes less than 25 µl the solvent does not adversely affect the peak shape [233]. In this case, however, the heptane present in the sample slug was not miscible with the initial mobile phase composition, and was solvating the sample components, thus preventing them from equilibrating between the stationary and mobile phases. The result of this was the peak broadening and splitting seen in the chromatogram. To overcome this problem the solvent was

removed by heating under a stream of nitrogen after completion of the derivatization reaction. The residue was then dissolved in a minimum volume of dichloromethane and made up in acetonitrile. The same mixture was derivatized following this modified procedure, and the resulting chromatogram is shown in Fig. 6.9. The peak shape was greatly improved, showing no tailing or splitting. The benzoic acid was also completely resolved from the hexanoic acid. Table 6.2 Shows the retention times of the acid derivatives using the above gradient program.

Table 6.2. Retention Times Of The Carboxylic Acid Derivatives

Carboxylic Acid	Retention Time (Min)
Acetic	9
Butyric	15
Benzoic	18.5
Hexanoic	22
Octanoic	27
Decanoic	30
Lauric	32.5
Myristic	34.5
Palmitic	37
Stearic	40
Eicosanoic	43

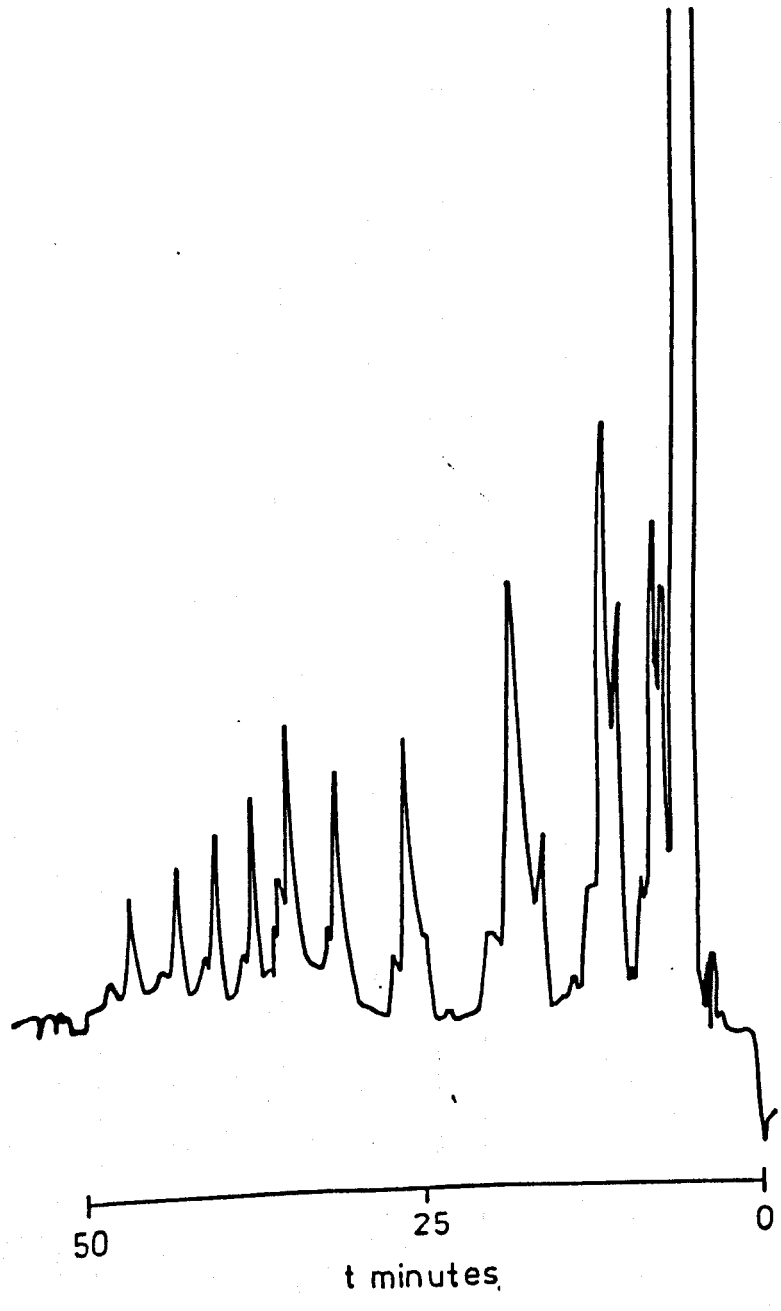


Figure 6.8. **Initial chromatogram of a derivatized mixture (C_2-C_{20}) of carboxylic acids.**
See text for gradient conditions.

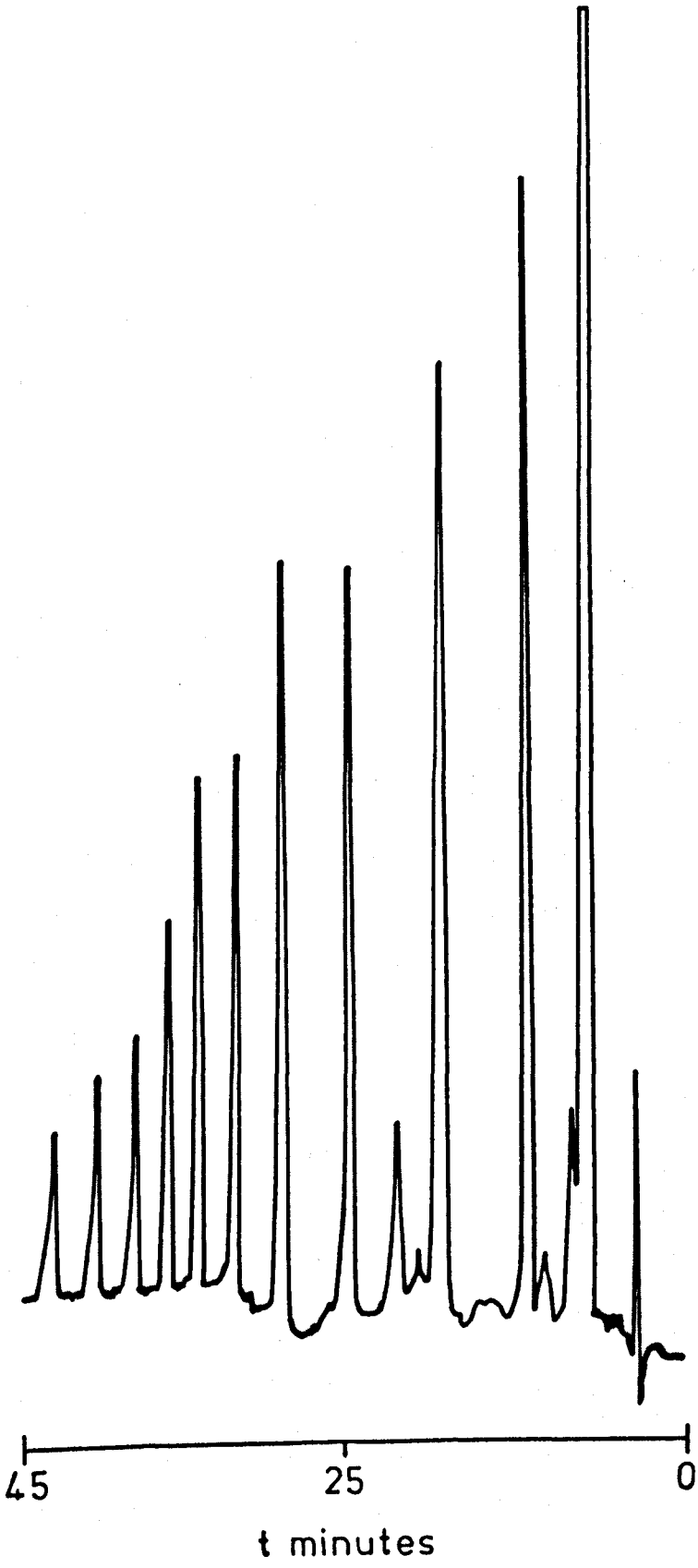


Figure 6.9. Chromatogram of a derivatized mixture (C_2-C_{20}) of carboxylic acid after stripping off the heptane. *See text for gradient conditions.*

6.3.4. Interference Studies

The effect of a sulphonic acid and phenol were investigated as both species are present in oils and may interfere with the derivatization reaction. The sulphonic acid reduced the CL intensity of a 0.05% m/v butyric acid standard by 7% when present at equimolar concentrations, and by 90% when present in a 5-fold molar excess. Phenol reduced the emission by 27% when present at one fifth of the butyric acid molar concentration, by 68% when present at equimolar concentrations and by 84% when present in a 5-fold molar excess. The reason for the reduction in signal in the case of phenol is the esterification reaction of phenol with the carboxylic acids. In the case of the sulphonic acids, the reduction is probably due to the formation of the sulphonate esters with the label. This illustrates the need for calibration procedures such as standard addition or matrix matching to allow for the reduction of the formation of the desired fluorescent derivative by other species present in the matrix.

6.3.5. Calibration Data

Calibration data was obtained using the optimised CL detection system. Initial studies were carried out using benzoic, butyric and palmitic acids in n-heptane. The results are shown in Tables 6.3, 6.4 and 6.5.

Table 6.3. Calibration Data For Benzoic Acid In n-Heptane

Concentration (%)	Signal (mV)	%rsd (n=3)
0.05	2875.0	3.8
0.02	816.7	1.8
0.01	203.3	2.8
0.008	146.7	3.9
0.005	38.3	7.5

$y=6.4X10^4(x)-382.7$ mV

r=0.9980

Table 6.4. Calibration Data For Butyric Acid In n-Heptane

Concentration (%)	Signal (mV)	%rsd (n=3)
0.05	4166.7	2.1
0.02	1566.7	1.8
0.01	545.8	1.3
0.008	320.0	3.1
0.005	130.0	0

$y=8.74X10^4(x)-233.6$ mV

r=0.9963

Table 6.5. Calibration Data For Palmitic Acid In n-Heptane

Concentration (%)	Signal (mV)	%rsd (n=3)
0.1	762.5	2.3
0.08	675.0	5.2
0.06	412.5	12.9
0.04	237.5	7.4
0.02	102.5	3.4

$y=8.15X10^3(x)-42.5$ mV

r=0.9896

In all cases the calibration data was linear over the range investigated. On column detection limits (3σ) were 200 pmol, 140 pmol and 95 pmol for benzoic, butyric and palmitic acids respectively.

Calibration data for the above carboxylic acids were also obtained using fluorescence detection. The excitation and fluorescence wavelengths were 260 nm and 392 nm respectively. Calibration data for benzoic, butyric and palmitic acids are given in tables 6.6, 6.7 and 6.8.

Table 6.6. Calibration Data For Benzoic Acid With Fluorescence Detection.

Concentration (%)	Signal (mV)	%rsd (n=3)
0.05	379.2	2.7
0.02	109.2	1.3
0.01	23.0	0.0
0.008	15.7	1.8
0.005	5.4	2.7

$y=8.5X10^{3}(x)-52.5$ mV

r=0.9978

Table 6.7. Calibration Data For Butyric Acid With Fluorescence Detection

Concentration (%)	Signal (mV)	%rsd (n=3)
0.05	811.7	3.6
0.02	213.3	1.4
0.01	67.5	0.0
0.008	38.2	2.0
0.005	16.0	0.0

$y=1.8X10^{4}(x)-107$ mV

r=0.9966

Table 6.8. Calibration Data For Palmitic Acid With Fluorescence Detection.

Concentration (%)	Signal (mV)	%rsd (n=3)
0.1	157.5	2.3
0.08	143.8	1.2
0.06	86.2	2.0
0.04	43.8	4.0
0.02	17.0	0.0

$y=1.9X10^3(x)-24.6$ mV

$r=0.9861$

As with CL detection, the calibration data were linear over the range investigated. The on column detection limits (3σ) were 200 pmol, 270 pmol and 225 pmol for benzoic, butyric and palmitic acids respectively.

To investigate the effect of lubricating oil on the CL procedure for the determination of carboxylic acids, a base oil sample known to be free of carboxylic acids was spiked with varying amounts of a 0.05% butyric acid standard. The derivatization procedure was carried out, and the results are given in Table 6.9. The emission intensity for the 5 ml spike was 62% of that obtained with the same amount of butyric acid in n-heptane, and

this reflects the presence of endogenous compounds such as sulphonic acids which compete with the carboxylic acids for the fluorescent label.

Table 6.9 Data Showing The Effect Of Oil On The CL Intensity Of Added Butyric Acid Standards

Added spike (ml)	Signal (mV)	%rsd (n=3)
0	0.0	0.0
0.1	0.0	0.0
1	133.3	2.2
2	366.7	7.9
5	1016.7	3.8

6.3.6. Assessment Of The Accuracy of The Procedure

The accuracy of the procedure was assessed by spiking an oxidised oil sample which did not contain a detectable concentration of benzoic acid with a known amount of benzoic acid, to give a concentration of 0.1%. Because of the suppression effect of the oil matrix, a standard addition calibration procedure was employed, together with the use of an internal standard of hexanoic acid, used to minimise the effects of any differences in the derivatization reactions of the standards. The ratio of analyte to internal standard was plotted against the amount of added spike. The calibration graph is shown in

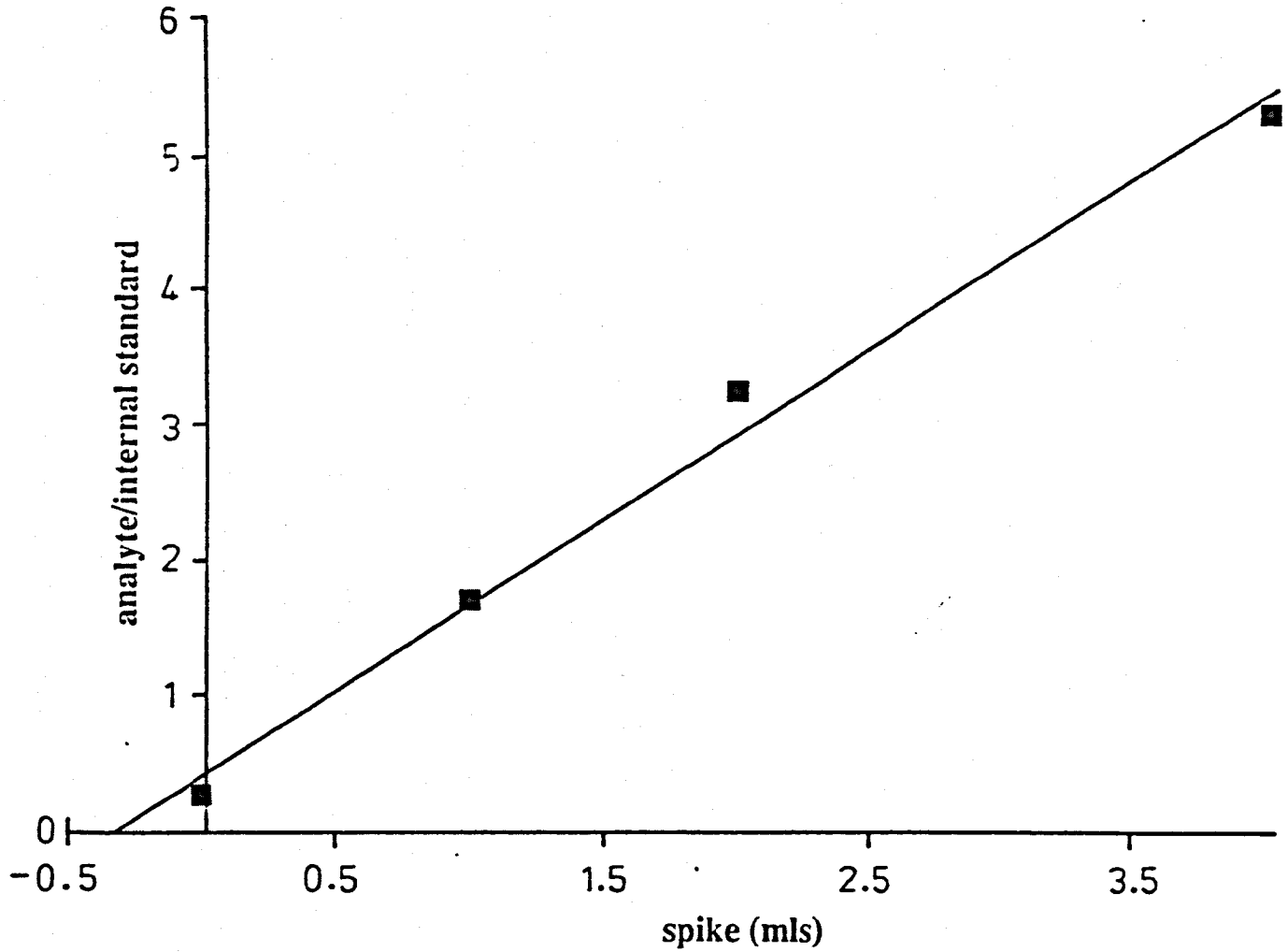


Figure 6.10. Standard addition calibration graph for benzoic acid.

Fig. 6.10. The concentration of benzoic acid found was 0.0825%±0.03% at the 95% confidence level. The acid recovery was therefore 82%.

6.4. Conclusions

This procedure provides a selective means of determining carboxylic acids in non-aqueous matrices. The pre-column derivatization reaction proceeds rapidly and selectively to completion to yield fluorescent esters of the carboxylic acids over the range C_2 to C_{20}, and benzoic acid. The acid derivatives were separated by reversed phase HPLC, and were eluted within 43 minutes using a ternary gradient.

Sulphonic acid and phenol were found to quench the emission intensity of the fluorescent esters due to competing reactions during the derivatization reaction.

Calibration data with both CL and fluorescence detection were linear over the range investigated (0 to 0.05% for benzoic and butyric acids and 0 to 0.1% for palmitic acid. On column detection limits (3σ) of 200 pmol, 140 pmol and 95 pmol were achieved for benzoic, butyric and palmitic acids respectively with CL detection. Detection limits with CL detection were a factor of two lower than with fluorescence detection for butyric and palmitic acids. The detection limit for benzoic acid was similar with both CL and fluorescence detection. The recovery of benzoic acid in an oxidized oil sample was 82%.

The procedure can be used to monitor the oxidation stability of lubricating oils by the selective measurement of the resulting carboxylic acids.

Chapter Seven

General Conclusions

This work has shown that the oxidation of some water soluble tertiary alkyl amines with alkaline sodium hypochlorite solution generates a CL emission. The emission intensity was enhanced by the use of a fluorescent sensitizer (rhodamine B). The CL emission was used to determine tertiary amines in water and sea water by FIA. The detection limits (3σ) for triethylamine in water and sea water were $1X10^{-6}$ M and $8X10^{-7}$ M respectively. Trimethylamine and tripropylamine were also determined in water with detection limits (3σ) of $6X10^{-6}$ M and $2X10^{-6}$ M respectively.

This reaction was used to determine Nonidet AT 85, a commercially available non-ionic surfactant containing a tertiary amine group. The surfactant was determined by FIA in sea water with a limit of detection (3σ) of 4 mg l^{-1}, and in aqueous extracts of a marine sediment by a standard addition calibration procedure.

A lamina flow cell was compared with a glass coil flow cell. This proved to be robust and the volume was easily variable. The lamina flow cell was therefore chosen for use in an industrial laboratory to monitor the environmental impact of off shore operations which discharge Nonidet AT 85 into the aquatic environment. The disadvantage of the lamina flow cell as compared with the conventional glass coil flow cell was the inferior flow characteristics, resulting in increased dispersion of the sample slug. Hence, the detection limits (3σ) for

the surfactant with the glass coil and with the lamina flow cell were 4 mg l^{-1} and 12 mg l^{-1} respectively.

The primary amine n-hexylamine was determined in non-aqueous media by FIA with CL detection. On line fluorimetric derivatization was carried out using the o-phthaladehyde, 2-mercaptoethanol derivatization reaction. The fluorescent derivative was detected using the peroxyoxalate reaction and a detection limit (2σ) of $3X10^{-6}$ M was achieved compared with $8X10^{-5}$ M with conventional fluorescence detection.

Carboxylic acids (straight chain C_2 to C_{20} and benzoic acid) were determined in non-aqueous media. A pre-column derivatization procedure was employed and the resulting fluorescent esters were separated by reversed phase HPLC. The fluorescent derivatives were detected by the peroxyoxalate chemi-excitation reaction. Gradient elution was employed to separate the acid derivatives within 43 minutes. On column detection limits (3σ) for butyric and palmitic acids were 140 pmol and 95 pmol, a factor of two lower than with fluorescence detection. The on column detection limit for benzoic acid was 200 pmol, and was similar to that obtained with fluorescence detection. The procedure was selective for carboxylic acids in lubricating oils and interferences from other acidic species present were eliminated by the use of a standard addition calibration procedure.

The flow-injection manifold was automated. The injection valve, peristaltic pumps and the autosampler were interfaced to a microcomputer. The data capture and manipulation was also carried out by the computer. Comparison of the manual and automated systems for the determination of perylene using the TCPO reaction showed that the detection limits (3σ) were $7X10^{-9}$ M and $3X10^{-9}$ M respectively. The lower detection limit obtained with the automated system is due to the higher precision of the data.

Chapter Eight

Suggestions For Future Work

The major limitation of the FIA procedure for the determination of tertiary amines with CL detection is that other tertiary amines present in the sample matrix will interfere and give results which are high. Because of this, the FIA procedure is limited to samples containing only one tertiary amine. In the case of samples containing more than one tertiary amine or other chemiluminescent species, a separation step would be needed prior to detection. This may be achieved by using the CL reaction as a HPLC detection procedure. The individual tertiary amines would then be determined by mixing the column eluent with the CL reagent stream and monitoring the emission produced for each chemiluminescent component.

The oxidation of lubricating oils to yield carboxylic acids proceeds via a number of intermediate compounds including aldehydes and ketones [234]. It is therefore desirable to measure these intermediate compounds during stability tests to establish the point at which the oil looses its resistance to oxidation. A number of fluorimetric derivatization procedures have been reported for carbonyl compounds [235-237]. Future work may therefore involve the investigation of the fluorimetric derivatization of these compounds in an oil based matrix. Sensitive and selective detection of these compounds would then be possible using the peroxyoxalate reaction with separation by HPLC analysis.

Organosulphur compounds, including diethyl sulphide, polysulphides, diaryl sulphides, disulphides, thiols, mercaptobenzimidazoles, thiophenes and thioaldehydes are employed as oxidation inhibitors in lubricating oils [210]. To monitor these compounds in oils a sensitive and selective procedure is desirable. A number of gas phase CL detectors are available which are selective for sulphur containing compounds. Of these the flame photometric detector is widely used as a detector for gas chromatography. This is based on the formation of sulphur atoms in a cool hydrogen/oxygen flame. The sulphur atoms recombine to form S_2 in an electronically excited state [238]. Ozone also reacts with certain sulphur compounds to yield electronically excited sulphur dioxide. Detection limits in the parts per billion range have been reported [239,240]. The reaction of molecular fluorine with organosulphur compounds also gives a CL emission [241]. This reaction is highly selective and 7 orders of magnitude of selectivity over many other compounds has been achieved [241,242]. Solution phase CL of sulphite [243-246], sulphur dioxide [243] and sulphide [247] has been reported. Preliminary work has indicated that certain organosulphur compounds undergo solution phase chemiluminescence when reacted with aqueous sodium hypochlorite [157]. These compounds include 2-(ethylthio)phenol, 2-ethyl thiophene, p-(ethylthio)benzoic acid and some disulphides. Iodosobenzene was also found to generate a CL emission when used to oxidize 2-(ethylthio)phenol in non-aqueous

media [248]. Further work may enable this solution phase chemiluminescence to be used for the determination of these compounds in oil based matrices.

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