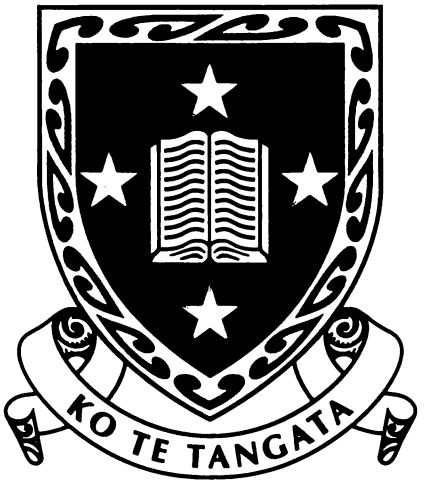
Porphyrins and Phthalocyanines as Chemical Visualisation Reagents for Latent Fingerprint Development



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Te Whare Wānanga o Waikato

A thesis submitted in partial fulfilment of the requirements of the Degree of Doctor of Philosophy in Chemistry

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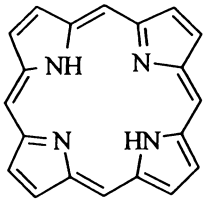
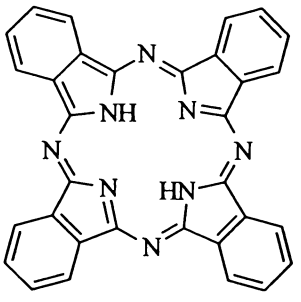
ABSTRACT

The focus of the research presented in this thesis was the investigation of porphyrins and phthalocyanines as potential reagents for the development of latent fingerprints. Porphyrins and phthalocyanines are structurally related compounds which have many features in common, including the ability to substitute a wide variety of metal ions into the core of the macrocyclic ring, along with the ability to attach a vast range of functional groups to the periphery of the macrocyclic ring and to the metal centre. These compounds also exhibit diverse absorption and emission spectra, which are sensitive to the metal ions and functional groups incorporated. Porphyrins are the most abundant colouring matter found in nature, and phthalocyanines are used extensively as colouring agents. These qualities indicate that such compounds are good candidates for fingerprint reagents.



porphin



phthalocyanine

A porphyrin and several phthalocyanines were synthesised, characterised, and appraised as potential fingerprint reagents. All compounds examined showed reactivity towards one or more fingerprint components. In particular it was found that dihydroxytetraphenylporphyrinatotin(IV) was capable of developing prints via reaction with water and non-water soluble fingerprint components. This compound may have application for the development of fingerprints on substrates such as thermal paper. It may also be a useful prior treatment to Physical Developer, a currently used technique for the development of fingerprints that have been exposed to water. Another compound, copper(II) tetrachlorosulfonylphthalocyanine was found to develop prints by reaction with water soluble fingerprint components. This compound may be of use on substrates where currently available methods give low contrasting fingerprints. Overall it was determined that porphyrins and phthalocyanines show genuine promise as fingerprint reagents and that further research in this area is clearly warranted.

ACKNOWLEDGEMENTS

I'm finally going to finish University - YIPPEE!! Which isn't to say that I haven't enjoyed my time here, for I have. However it is the end of a large chapter of my life, full of those infamous learning experiences, that have made me a wiser, or a least a less foolish person! Anyway before I move on to new and interesting challenges I'd like to acknowledge the following people for their contribution to this thesis and to my life in Hamilton.

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Touch passion when it comes your way
It is rare enough as it is
Don't walk away when it calls you by name

'Marcus', "Babylon 5"

I dedicate this thesis with much love and affection to my
Mum & Dad, and to my late Aunty Thelma
For encouraging me to aspire

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LIST OF SCHEMES

LIST OF ABBREVIATIONS

analytical reagent	AR
Angstrom	Å
Celsius	°C
centimetre	cm
1,8-diazofluorenone	DFO
electrospray mass spectra	ESMS
emission	em
ethanol	EtOH
excitation	ex
extinction coefficient	ε
general purpose reagent	GPR
gram	g
Kelvin	K
kilobar	kB
kilovolt	kV
laboratory reagent	LR
litre	m
mass/charge	m/z
methanol	MeOH
microgram	µg
microsecond	µs
milliamperes	mA
millilitre	mL
minute	min
molarity	M
mole	mol
nanometres	nm
physical developer	PD
phthalocyanine	Pc
second	s
tetrahydrofuran	THF
tetraphenylporphyrin	TPP
ultraviolet	UV
visible	Vis
volt	V
volume	v
watt	W
wavelength	λ
weight	w

CHAPTER ONE

INTRODUCTION

1.1 Forensic Science

The term forensic comes from the Latin word *forum* which means "the market-place" and this is because justice in Roman society was administered in the market-place [1]. In New Zealand and in other countries the essence of this is visible today, with an adversarial system of justice which allows a jury to examine opposing opinions and make a decision based on the evidence presented by the prosecution and the defence [2].

However, what is forensic science? Edmond Locard (1877-1966) advanced the theory that 'every contact leaves a trace'. By this he proposed that criminals will carry with them some trace from the scene of the crime, and will leave some trace of their presence behind them [3]. This is the principle at the foundation of forensic science, which in simple terms is the application of science to the solving of crime.

Historically forensic science had only a few techniques at its disposal. Last century visual recognition was still the major method in use for the identification of habitual criminals. A common method of establishing identity was for detectives to regularly visit prisons and watch prisoners to imprint their faces on their memories [4]. Bertillonage, otherwise known as anthropometry, a criminal classification system based on body measurements, enjoyed some international success. However it was rapidly superseded by the introduction of what at the time was the single greatest advance in scientific criminal investigation - the classification of fingerprints [3].

Today the number of forensic science techniques available is vast and ever-growing, unfortunately largely due to continually increasing crime rates. By 1995 the offence rate

in New Zealand stood at 141 per 1,000 people, more than seven times the rate in 1950 [5]. Similar trends are seen around the world. In Australia over the 13 year period 1973/74 to 1986/87 a 117% increase in crime rate per 100,000 people was seen [6]; and in Europe by 1986 the registered crime rate per 100,000 was approximately 7500, more than five times that seen in 1950 [7].

Clearly there is a demand for forensic science and for new and improved techniques, to help combat growing crime rates. Fingerprints are still the world's most commonly used means of identifying felons [3], enjoying unparalleled success in the scope of physical evidence [8]; and with the advent of computerised latent fingerprint systems the value of a single latent fingerprint has significantly increased [8]. In New Zealand, fingerprint evidence is said to account for clearance of up to 85% of all violent crime [9]. Therefore progress in this area is of fundamental interest.

1.2 History of Fingerprints

Fingerprints are the impressions made by the ridges on the fingers and thumbs. Fingerprint patterns are established on an unborn child's hands in the mother's womb and remain constant from the sixth month of inter-uterine life until death; apart from when gross destruction of the tissue occurs. They are unique to each individual, including identical twins, and therefore they offer an infallible means of personal identification. [1, 3, 4, 10, 11]

The earliest use of fingerprints can be dated back to circa 3,000 B.C. where it is suggested that imprints were purposely impressed in clay bricks used for construction of a king's storehouse in Mesopotamia [10]. However the Chinese can probably be credited with first recognising the individuality of fingerprints, as for centuries they used thumb impressions on document clay seals [1, 10, 11].

The significance of fingerprints to forensic matters though, was not realised until much later. In 1880, Dr Henry Faulds' letter to *Nature* on October 28 entitled 'On the Skin

Furrows of the Hand' stated "When bloody finger marks or impressions on clay, glass, etc., exist, they may lead to the scientific identification of criminals". This letter generated much interest and led to further research. Some of this culminated in the Henry system, a method of classifying fingerprints based upon ridge patterns, developed by Sir Edward Henry. The Henry system was introduced at Scotland Yard in 1901, and thus dactyloscopy or fingerprinting as it is more commonly known officially became a forensic technique that was subsequently adopted around the world. [3, 10, 11]

It should be noted that Dr Juan Vucetich also developed a fingerprint classification system, now called the Vucetich system, at a similar time in Argentina. However this system is not in use outside of South America. Vucetich is however credited with securing the first ever fingerprint conviction for murder. [3, 10, 11]

1.3 The Nature of Fingerprints

1.3.1 Types of Fingerprints

Fingerprints encountered in a criminal investigation fall into three categories [3, 11, 12]:

(i) impressions left in soft materials such as wax, wet paint, soap, putty, or chocolate,
(ii) visible prints made by some contamination on the finger such as blood, paint, oil, grease, or dirt and,
(iii) latent or 'hidden' prints which are those deposited by skin secretions.

Fingerprints of type (i) and (ii) are usually obvious and do not require any treatment; though weak type (ii) fingerprints may be enhanced. Type (iii) fingerprints however range from barely visible to completely invisible and do require development. Consequently there are a variety of methods for visualising contaminated and latent fingerprints drawing on a wide area of science, from surface physics to chemistry. [12]

In choosing a method for developing a latent fingerprint one must consider the composition of the fingerprint, the surface upon which it is deposited, and the processes occurring after its deposition [13, 14].

1.3.2 Composition of Latent Fingerprints

Human skin contains three types of secreting glands, these being eccrine, apocrine and sebaceous glands. Both eccrine and apocrine glands are sudoriferous glands, which are also known as sweat glands. The major features of each type of gland are as follows [12, 15-17].

Eccrine Glands Widely distributed throughout the body and particularly numerous on the palms of the hands and the soles of the feet, where their surface density reaches approximately 400-465 glands per cm^2.

Apocrine Glands Concentrated in the axillary regions of the body but are also found in the genital regions and mammary areolea. These glands begin functioning at puberty.

Sebaceous Glands Associated with hair follicles and mainly located on the face and scalp, where their surface density reaches approximately 600 glands per cm^2, being 100 glands per cm^2 elsewhere. Absent on the palms of hands, soles of feet, and lower lip.

The main constituents of the secretions of the different glands are listed in Table 1.1. The volume of a fingerprint is of the order of 10^{-5} mL [18] and contains no more than approximately 10 μg of material [19], which consists of a mixture of eccrine sweat and sebaceous material, with the latter being transferred to the fingers by the touching of the face or the head. Apocrine sweat does not usually contribute to latent fingerprints.

Table 1.1 Constituents of human sudoriferous and sebaceous glands [20]

Gland	Inorganic Constituents	Organic Constituents
Eccrine[†]	chlorides metal ions e.g. Na^+, K^+, Ca^{2+} ammonia sulfate phosphate	amino acids urea lactic acid sugars creatinine choline uric acid
Apocrine	iron	proteins carbohydrates cholesterol
Sebaceous		glycerides (mono-, di-, and tri-) free fatty acids wax esters squalene sterol esters sterols hydrocarbons

[†]Note: eccrine constituents contribute approximately 1% of eccrine sweat, the other 99% is water

Fingerprints therefore contain many components and vary widely in composition both from one individual to another and with one individual from minute to minute [13]. This results from the varying amount of material discharged from the glands, which is influenced by mental, sensory, thermal, emotional, and psychic stimuli [12, 21].

1.3.3 Surface and Post-Deposition Considerations

The surface upon which a latent fingerprint has been deposited must be considered when choosing a fingerprint development technique, as no one technique develops all prints on all surfaces. The surfaces likely to be encountered in the investigation of a crime can be divided into the following categories [13].

- smooth, non-porous
- rough, non-porous
- paper and cardboard
- plastic packaging material
- soft vinyl (PVC), rubber and leather
- adhesive coated surfaces
- metal (untreated)
- raw wood (untreated)
- wax and waxed surfaces
- fabric

What has happened to a latent fingerprint since deposition must also be considered, as factors such as temperature, exposure to light and water, and relative humidity can alter

the chemical and physical nature of a fingerprint. Components may decompose, evaporate, or diffuse [13]. For example the chloride component of a latent fingerprint will migrate if exposed to high humidity; it has been shown that significant deterioration occurs after 15 days at 60% relative humidity [12, 22]. Another example is a loss of water soluble components. Clearly a surface that is rained upon or submerged in water will be unlikely to have latent fingerprints that contain many of the constituents of eccrine sweat.

Hence the selection of an appropriate technique is to a large extent governed by the surface encountered and knowledge of any contributing environmental factors. There are more than one hundred techniques for the development and visualisation of latent fingerprints reported in the literature [22], with different methods having specific advantages and/or disadvantages depending upon the circumstances. Therefore the choice of development technique is vitally important. The application of more than one technique can often increase the total number of fingerprints detected or improve the quality of those partially developed. This requires a range of methods that work sequentially. It should be noted that sequential processing of exhibits is the general procedure adopted by most forensic laboratories. A flowchart for a very general approach to the detection of latent fingerprints is shown in Figure 1.1, and in Figure 1.2 a specific example of a processing chart is shown. The following section describes some of the more common techniques encountered in the development of latent fingerprints.

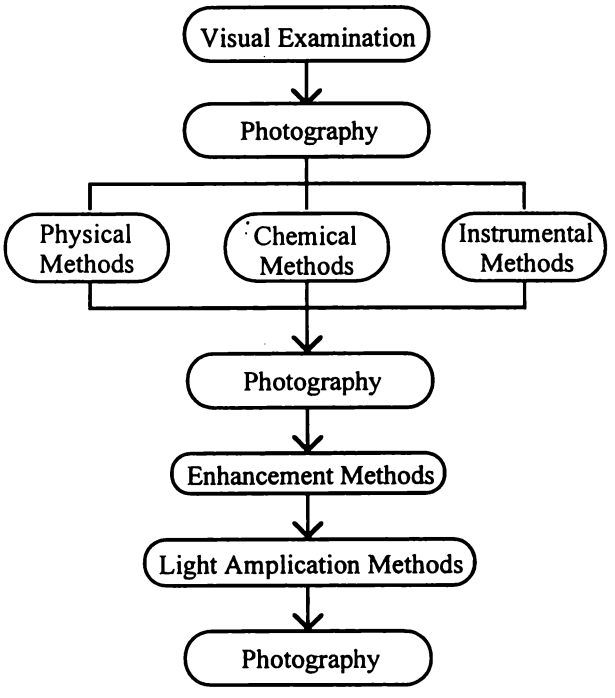


Figure 1.1 Flowchart showing a general approach for the detection of latent fingerprints [22]

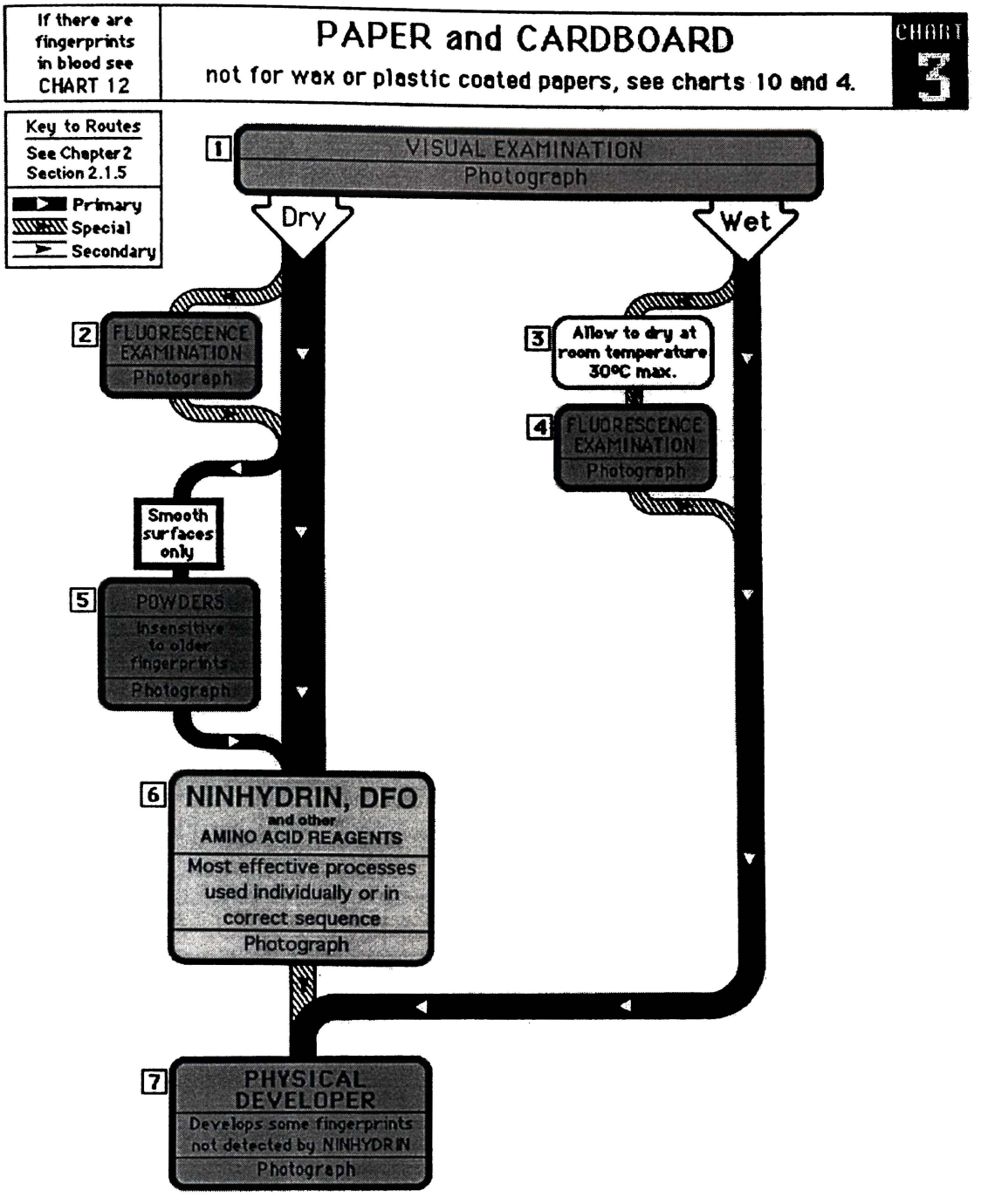


Figure 1.2 A processing chart showing a sequence of fingerprint development techniques for the detection of latent fingerprints on paper [13]

1.4 Methods of Fingerprint Development

1.4.1 Powders

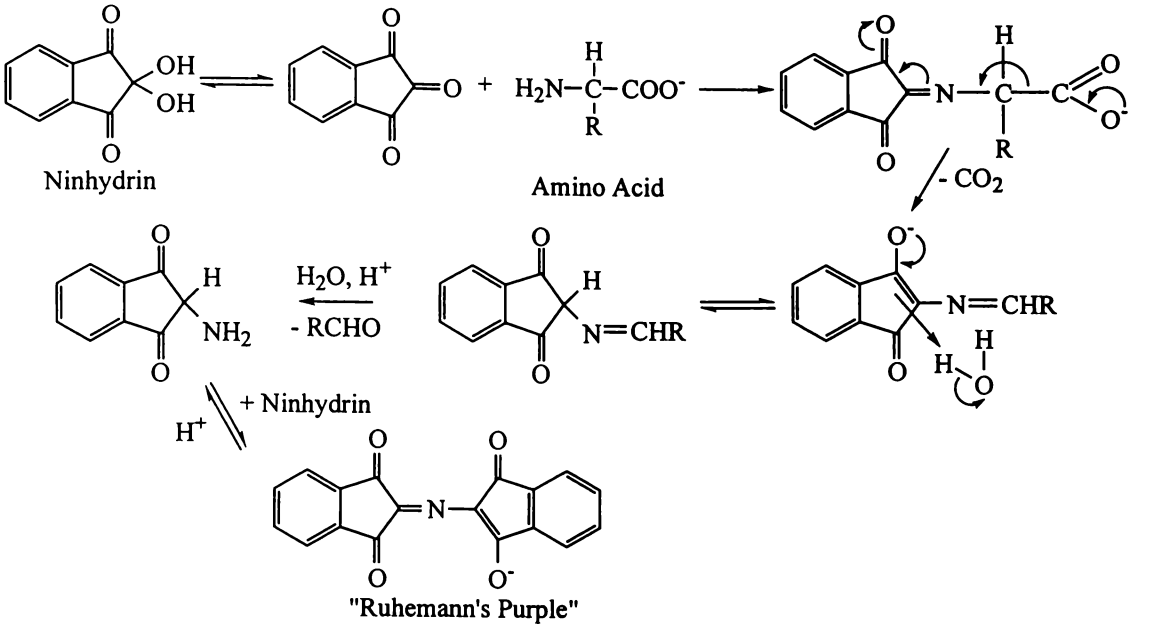
The use of dusting powders is one of the simplest and most widely used techniques for developing latent fingerprints on non-porous surfaces. This procedure relies on the mechanical adhesion of the powder to the fingerprint deposit. Many substances and

formulations have been investigated as to their suitability as fingerprint powders [12, 22]. One of the most sensitive powders for general use is considered to be aluminium flake. This powder is manufactured by grinding a paste of aluminium, stearic/palmitic acids, and mineral oil in a ball mill. During the process the particles acquire a flat plate-like structure and a surface layer of fatty acids which aids adhesion to the fingerprint [12, 23].

Other powders that are of general use are magnetic powders and luminescent powders. Magnetic powders are particularly useful for surfaces where the adhesive nature of brushing may damage the fingerprint [12, 23]. Luminescent powders are useful for situations such as multi-coloured surfaces where contrast of the fingerprint with the background would be a problem [22].

1.4.2 Ninhydrin

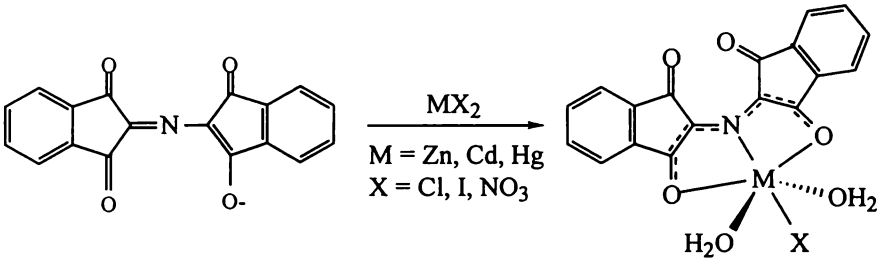
Ninhydrin is probably the most routinely used chemical technique for the development of latent fingerprints on porous surfaces such as paper. It was first prepared by Ruhemann in 1910, who documented its reaction with α-amino acids; an α-amino acid having the amine group located on the carbon atom adjacent to the carboxyl group [24]. In the following years it became, and still is, a colourimetric reagent for the detection of amino acids in chromatography. However the use of ninhydrin as a fingerprint reagent was not suggested until 1954 when Oden and von Hofsten noted that purple coloured fingerprints were revealed on paper chromatograms that had been handled. The mechanism for its reaction with the amino acid component of a fingerprint is outlined in Scheme 1.1. [12, 22, 25]



Scheme 1.1 Mechanism of the reaction between ninhydrin and an amino acid to form Ruhemann's Purple [12, 25]

Fingerprints developed by this technique are usually purple, though some variation in colour is seen [13]. However this robust technique does suffer from some limitations, including poorly contrasting fingerprints on dark surfaces and low sensitivity to weak fingerprints. This can be overcome by further treating the item with zinc chloride to yield fingerprints that are luminescent when illuminated by an appropriate light source. [12, 22, 25]

Luminescent fingerprints result from metal complexation with Ruhemann's Purple, this being an active chelating agent that forms coloured coordination complexes with a variety of metals (Scheme 1.2) [26]. Operationally, zinc is the preferred metal for complexation, due largely to toxicity and cost considerations. The zinc complex has a broad excitation maximum between 480 and 490 nm, which coincides nicely with the 488 nm line of an argon ion laser; and emission maxima at approximately 545, 565, and 595 nm [13, 25].



Scheme 1.2 Formation of Ruhemann's Purple-metal complexes (group IIb metals) [26]

1.4.3 1,8-Diazofluorenone

Another compound that has wide use for developing latent fingerprints on porous surfaces such as paper is 1,8-diazofluorenone (DFO), (Figure 1.3). DFO reacts with α-amino acids and their esters via imine formation to give decarboxylated azomethine ylides and ester substituted azomethine ylides respectively. In the absence of dipolarophiles, α-amino acids and DFO react to give a red fluorescent dye product whose structure (Figure 1.3) is closely related to that of protonated Ruhemann's Purple. [27]

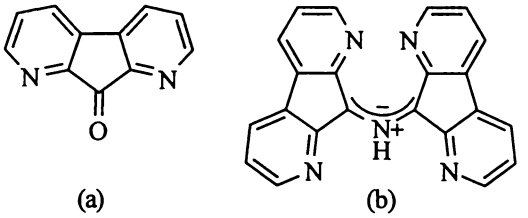


Figure 1.3 (a) Structure of DFO, (b) structure of reaction product between an α-amino acid and DFO [27]

Fingerprints developed by this method are sometimes visible in ambient light. When this is the case they have a magenta colouration [13]. Generally however they are detected by illumination with an appropriate light source. The excitation maximum occurs at 568 nm with less intense excitation maxima occurring at 470 and 525 nm; emission occurs over a wide wavelength range with a maximum at approximately 578 nm [13]. This reagent is considered to be far more sensitive than ninhydrin and further only requires one step to obtain a luminescent fingerprint. However as stated in Section 1.3.3 the sequential processing of items can increase the total number of fingerprints detected. Operationally DFO and ninhydrin can be used, in that order, to detect a greater number of fingerprints than if they were employed on their own.

1.4.4 Physical Developer

Physical Developer (PD) is a reagent that is primarily used to develop latent fingerprints on porous surfaces such as paper, that have been exposed to damp or wet conditions. It is a photographic process which works by depositing nascent silver,

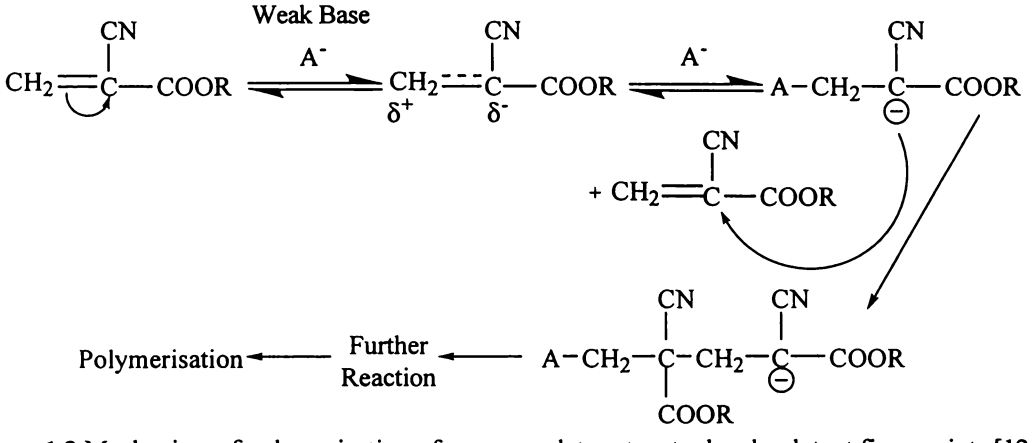
formed in solution by a ferrous/ferric redox couple, on to the latent fingerprint to form a grey silver deposit. The generalised reaction taking place can be expressed as follows:

$$Fe^{2+}_{(aq)} + Ag^{+}_{(aq)} \rightarrow Ag_{(s)} + Fe^{3+}_{(aq)}$$

PD reacts with sebaceous material present in the fingerprint, although the mechanism of the reaction is not fully understood. However as it does react with non-water soluble sebaceous material, the ability to develop fingerprints on exhibits that have been in contact with moisture in some way, is self evident. A further advantage is that PD can be used subsequently to ninhydrin and DFO to yield enhanced or further fingerprints. [12, 13, 22]

1.4.5 Cyanoacrylate Fuming

Cyanoacrylate ester fuming is a robust technique that is used to develop fingerprints on a wide range of non-porous surfaces. The use of cyanoacrylate esters as adhesives (Super Glue™ etc.) is well established, surfaces being bonded together upon polymerisation of the cyanoacrylate ester when atmospheric water vapour acts on the acidic stabiliser present in the glue. The mechanism of cyanoacrylate ester vapours developing fingerprints is given in Scheme 1.3, the weak base being supplied by the latent fingerprint itself. [12, 22]



Scheme 1.3 Mechanism of polymerisation of cyanoacrylate esters to develop latent fingerprints [12, 22]

Fingerprints developed by this technique are white. Consequently on some surfaces the contrast between background and fingerprint is poor. Enhancement in such situations is generally achieved by staining the fingerprints with dyes, although the more traditional technique of fingerprint powders can also be used. A wide variety of dyes, which are most often luminescent, are utilised for enhancing cyanoacrylate developed fingerprints. Some routinely used dyes include Basic Yellow 40, Coumarin 540, Gentian Violet, Rhodamine 6G, Thenoyl Europium Chelate (TEC), and more recently Europium ThenoylTrifluoroAcetone ortho-Phenanthroline (EuTTAPhen). [12, 22, 28-30]

1.4.6 Small Particle Reagent

Small particle reagent (SPR) is a technique that can be used to develop fingerprints on a wide range of surfaces from paper to metal to rock to plastic. It also has the advantage of being able to develop fingerprints on surfaces that are wet or covered in dust. SPR itself is a suspension of finely divided molybdenum disulfide particles in a surfactant solution. While the exact mechanism is unknown, the reagent appears to associate with water insoluble unsaturated lipid material, possibly by physical adsorption. [12, 13, 22, 31]

Fingerprints developed by this technique are usually dark to light grey in colour. Alternative SPR formulations are available to overcome instances when contrast of a grey print with the background is poor. There is a white SPR formula based on zinc carbonate particles that is appropriate for dark surfaces. For multi-coloured surfaces where neither grey or white fingerprints are particularly useful a luminescent SPR can be employed. As with cyanoacrylate fuming, dyes are incorporated. However in this case the dyes are added to the standard molybdenum disulfide SPR solution, enabling a luminescent fingerprint to be obtained in one step. [13, 22, 31]

1.4.7 Vacuum Metal Deposition

Vacuum metal deposition (VMD) is a technique that is particularly successful on polythene, is effective on other smooth surfaces, and may be capable of detecting

fingerprints on synthetic fabric or thin cotton. VMD utilises vacuum-coating technology for the evaporation of metals and the deposition of thin metal films. [1, 12, 13, 22]

To develop fingerprints by VMD, an exhibit is suspended in a vacuum chamber above a pair of molybdenum containers, one containing gold and the other containing zinc. The exhibit is then treated successively by vacuum evaporation of gold followed by zinc. The gold penetrates or diffuses into the fingerprint, most likely the sebaceous material, rather than into the background. Nucleation by the zinc onto the gold surface then occurs to reveal the fingerprint. Originally cadmium was used, however as it is highly toxic it has been largely replaced by zinc. [12, 13, 22]

1.4.8 Iodine

Iodine vapour is one of the oldest techniques known to reveal latent fingerprints, with its use being described as early as 1891. Two mechanisms are proposed, one physical and one chemical, for the development of fingerprints by iodine. The suggested physical mechanism is the absorption of iodine into the sebaceous material of a fingerprint, while the alternative chemical mechanism is the iodination of unsaturated lipids such as oleic acid, with research tending to support the physical process of absorption. [12, 13, 22]

Fingerprints developed by this technique are yellow/brown in colour with development being rapid, however their lifetime is short and the prints also fade rapidly. This fading is attributed both to resublimation of the iodine, and to chemical reaction of the iodine with unsaturated lipids to give colourless compounds. Fingerprints can however be 'fixed' so they do not fade. Suggested fixing agents include p,p'-tetramethyldiaminodiphenylmethane which gives green/blue prints and 7,8-benzoflavone which gives dark purple prints. [12, 13, 22]

Iodine can be used on a variety of surfaces both porous and non-porous, including skin, which is an exceptionally difficult substrate. However this technique suffers from several disadvantages, these being that the vapours are highly toxic and corrosive,

secondary treatment is required to prevent prints fading, and old prints are difficult to develop. Consequently on most surfaces this technique has largely been superseded by other methods. [12, 13, 22]

1.4.9 Sulfur Dioxide

The use of sulfur dioxide, SO_2, is a radioactive method for the development of fingerprints. Exhibits are exposed to SO_2 gas, of which a small proportion is radioactive $^{35}SO_2$. The SO_2 dissolves in the water present in fingerprints and is also thought to react with some of the unsaturated compounds found in fingerprints by addition across double bonds to form cyclic sulphones. This converts the fingerprint into a radioactive form which is visualised by autoradiography. This technique involves the treated exhibit being 'sandwiched' between two sheets of film for a period of time (up to several days). The radioactive areas emit radiation which develops the film and hence the fingerprint. [1, 12, 13, 22]

This technique is viable on several surfaces, however it requires specific facilities that have a high capital and running cost. Consequently this technique is not widely used, though it is valuable, as it is currently the only known method for the development of fingerprints on fine fabric. Other radioactive gases have also been examined, however only SO_2 has had operational success. [1, 12, 13, 22]

1.5 Current Fingerprint Research

Conventional fingerprint development techniques are effective in the recovery of latent fingerprints under ordinary circumstances. However latent fingerprints are often deposited on surfaces with unique characteristics that can and do pose problems. When this occurs traditional methods of latent fingerprint detection are often ineffective. Consequently research efforts focus on the development of techniques that may be successfully applied to these unique and difficult surfaces. Research also focuses on techniques that offer increased sensitivity over the current methods that are available. An overview of the recent research in latent fingerprint visualisation follows. [22]

1.5.1 Ninhydrin Analogues

Until the early 1980s the majority of improvements made to the ninhydrin method of fingerprint development involved modification of the reagent solution formulation and process conditions. No chemical modifications had been considered. This latter approach was initially undertaken by Almog and coworkers. They synthesised three analogous compounds containing the same functional group, the vicinal triketone, with the expectation that these compounds would also develop latent fingerprints, and further the hope that they might perform better than ninhydrin. Their expectations were met; all three analogues developed latent fingerprints on paper with a sensitivity similar to that of ninhydrin, and one, benzo[f]ninhydrin (Figure 1.4) developed dark green fingerprints, an outcome which was regarded as promising as this could provide increased contrast on coloured surfaces. [12, 22, 25]

Investigation into the luminescent properties of the zinc chloride complexes of the analogue developed fingerprints was also carried out. It was found that only the benzo[f]ninhydrin complex fluoresced as intensely as the ninhydrin complex. The excitation maximum occurs at 530 nm, which conveniently coincides with the NdYAG (Neodymium Yttrium Aluminium Garnet) laser emission maximum at 532 nm. A small portable version of the frequency-doubled NdYAG laser has been specifically designed for crime scene work. [12, 22, 25]

These results led to further and currently ongoing research in the area of ninhydrin analogues. There is a general desire to synthesise analogues which may lead to [12]:

(i) an increased rate of reaction with amino acids
(ii) the ability to select an analogue which produces maximum contrast with a given surface and
(iii) the potential of improved detection sensitivity by enhancing the luminescence of the metal complexes.

Figure 1.4 shows the ninhydrin analogues that to date have shown the most promise as possible alternatives or complements to ninhydrin.

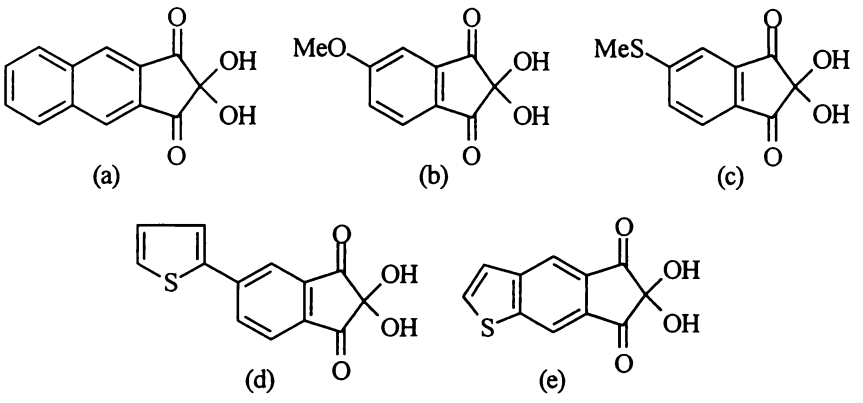


Figure 1.4 Analogues of ninhydrin (a) benzo[f]ninhydrin, (b) 5-methoxyninhydrin, (c) 5-methylthioninhydrin, (d) 5-thienylninhydrin, (e) thieno[f]ninhydrin [12, 22, 25, 32-35]

The luminescence of Ruhemann's Purple complexes has also been investigated. It has been shown that cooling the developed fingerprints with liquid nitrogen (-196°C) will increase the fluorescence [12, 13, 25, 36]. A range of metals have also been complexed with Ruhemann's Purple to see what luminescent properties the different complexes might have. Other group IIb metals have shown some promise [12, 25], with cadmium and mercury complexes both having different emission maxima to the zinc complex. This may be useful if the emission obtained with the zinc complex is not resolvable from the background luminescence. However the high toxicity of cadmium and mercury limits practical use to a large extent.

Lanthanide complexes, in particular those of europium and terbium, have also been investigated [37-39]. The idea in this case is to make use of lanthanide luminescence, which can be enhanced via the intramolecular energy transfer that occurs when a lanthanide ion is complexed with a suitable organic ligand, such as Ruhemann's Purple (see Section 1.5.3 for further explanation). This alternative has different excitation and emission wavelengths to the standard zinc complex, which again might be useful if background luminescence at the excitation wavelength of the zinc complex is causing problems. This type of complex also lends itself to time-resolved imaging as the lifetimes of the lanthanide complexes are of the millisecond order. Though this would require specialised equipment, which is relatively expensive. To date however, the zinc

complex is still that used operationally as it appears to be the most safe, inexpensive, and widely applicable option.

1.5.2 Luminescent Reagents

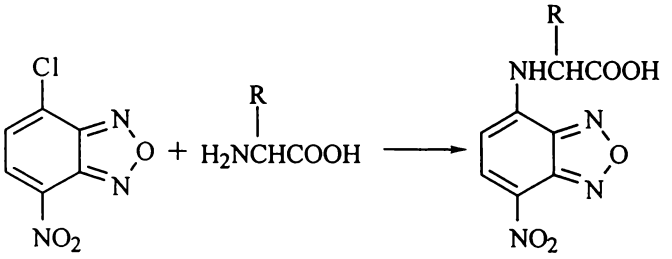
Luminescent fingerprints, such as those developed with ninhydrin/$ZnCl_2$ or DFO, are highly desirable as luminescent visualisation is potentially more sensitive than standard coloured visualisation. Ideally, a successful reagent for developing luminescent fingerprints should meet three criteria [12]:

(i) the reagent itself should not be luminescent,
(ii) the luminescence of the developed fingerprint should not coincide with any background luminescence, and
(iii) the reagent should react specifically with a particular fingerprint component.

An overview of the luminescent reagents that have been investigated follows. Note that none of the reagents fulfil all three of the ideal criteria. Also note that lanthanide reagents fall into the category of luminescent reagents but are covered separately in the following section, 1.5.3.

1.5.2.1 NBD-Cl

NBD-Cl (7-chloro-4-nitrobenzo-2-oxa-1,3-diazole) was first reported as a luminescent reagent for amino acids and other amines (Scheme 1.4). Its applicability for luminescent fingerprint development was reported later. A detailed study comparing this reagent to ninhydrin was undertaken [40]. The study found that for fresh (up to one month) and old (17 months) fingerprints there was little difference in performance of the two techniques. For fingerprints of intermediate age (3 to 9 months) NBD-Cl was the superior reagent to use. However a major disadvantage of NBD-Cl is that there can be non-specific reaction with some constituents of paper. This causes emission similar to that of the developed fingerprint, i.e. poor resolution between fingerprint and background. A further problem is that NBD-Cl is now a suspected carcinogen. [12]

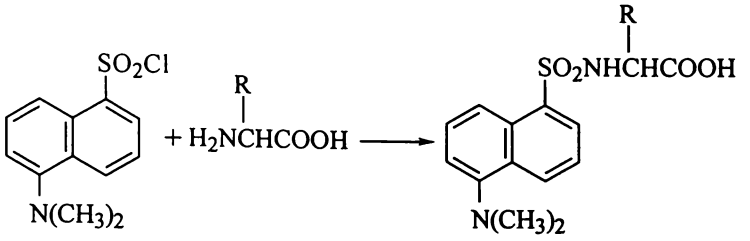


Scheme 1.4 Reaction of NBD-Cl with amino acids [12]

Other nitrobenzofurazanyl ethers have also been prepared and examined as potential fingerprint reagents, these being 4-methoxy-7-nitrobenzofurazan (NBD-OCH_3), 4-ethoxy-7-nitrobenzofurazan (NBD-OCH_2CH_3), 4-(methoxy-ethoxy)-7-nitrobenzofurazan (NBD-$OCH_2CH_2OCH_3$), and 4-phenoxy-7-nitrobenzofurazan (NBD-OC_6H_5). All show similar sensitivity and unfortunately, similar background emission problems to NBD-Cl. However these reagents may be suitable for vapour phase development techniques (Section 1.5.5). [25]

1.5.2.2 Dansyl Chloride

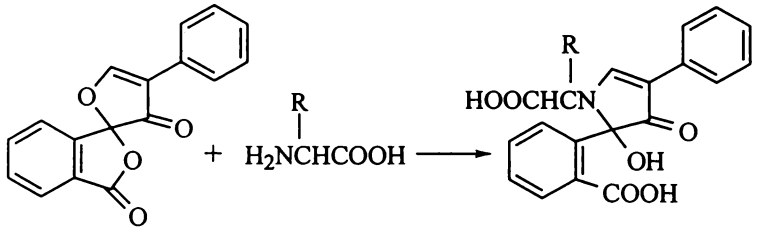
Dansyl chloride was originally used to prepare luminescent conjugates of albumin. Its reaction with amino acids to produce luminescent derivatives has been established (Scheme 1.5). The extension of this compound to develop fingerprints came later. [12] Treatment with dansyl chloride yields fingerprints that are intensely luminescent under UV light. Having excitation at UV wavelengths makes this reagent suited to surfaces which suffer from high background luminescence under the more commonly used blue-green wavelengths, surfaces such as cardboard and darkly coloured papers. Indeed, this reagent was compared with ninhydrin/$ZnCl_2$ on cardboard and brown papers and was found to be the far superior reagent to use, regardless of the age of the fingerprint. It should be noted that some care is needed with this technique. Background luminescence, which will reduce sensitivity, will occur if the article is sprayed too heavily or if it is heated. Also the developing fingerprint should be closely monitored to ensure overdevelopment does not occur, as this results in photodecomposition. This procedure can be considered to be complementary to existing techniques rather than a replacement, as it succeeds in an area in which current techniques fail to perform satisfactorily. [41]



Scheme 1.5 Reaction of dansyl chloride with amino acids [12]

1.5.2.3 Fluorescamine

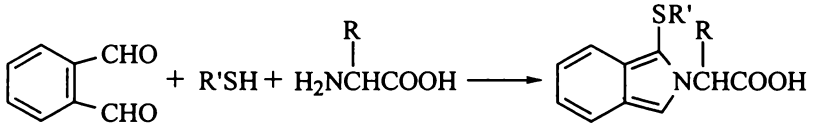
Fluorescamine (4-phenylspiro(furan-2-(3H),1'-phthalan)-3,3'dione) is another compound that reacts with amino acids to give luminescent products (Scheme 1.6). This reagent develops fingerprints that are luminescent under UV illumination, although triethylamine is needed to stabilise the luminescent fingerprint. This reagent however does not appear to be used operationally. [12]



Scheme 1.6 Reaction of fluorescamine with amino acids [12]

1.5.2.4 o-Phthalaldehyde

o-Phthalaldehyde reacts with amino acids, in the presence of a thiol reducing agent, to give luminescent products (Scheme 1.7). The sensitivity of this reagent in derivatising amino acids was studied and found to be five times greater than fluorescamine. This reagent develops fingerprints that are luminescent under UV illumination. However emission from optical brightners, present in paper, is quite similar and causes interference and thus reduced sensitivity. This might be overcome by *o*-phthalaldehyde derivatives which have different excitation and emission maxima. [12]



Scheme 1.7 Reaction of *o*-phthalaldehyde with amino acids [12]

1.5.3 Lanthanide Reagents

Lanthanide luminescence has been of interest in other scientific disciplines, and has shown utility in biochemical probes, fibre optic communication, and laser construction [42, 43]. Lanthanides already have some application in fingerprint development techniques; they are used in stains for further developing cyanoacrylate treated fingerprints (Section 1.4.5); they have also been suggested as alternative complexing agents for use with ninhydrin (Section 1.5.1). However recent research [29, 42-44] indicates that lanthanide reagents may have potential as stand-alone fingerprint development techniques.

Europium is the more fully investigated lanthanide in terms of fingerprint development and has luminescence properties which make it favourable for such use. The two major features are, a large Stokes shift and a long luminescence lifetime. Stokes shift is the difference between the excitation wavelength and the emission wavelength, with a large shift making optical filtering both easy and variable. Europium excitation occurs in the near-UV and emission occurs in the red. Long luminescence lifetime, of the order of milliseconds, makes time-resolved imaging more accessible. Current luminescent techniques available have in comparison microsecond lifetimes which make time-resolved imaging not only difficult but highly costly due to the sophisticated optics and controllers required. It should be noted that lanthanide luminescence intensities are low due to poor inherent absorption by the metal. However this is overcome by chelation with organic ligands that absorb readily and intramolecularly transfer excitation energy to the complexed lanthanide ion. [29, 30, 42-44]

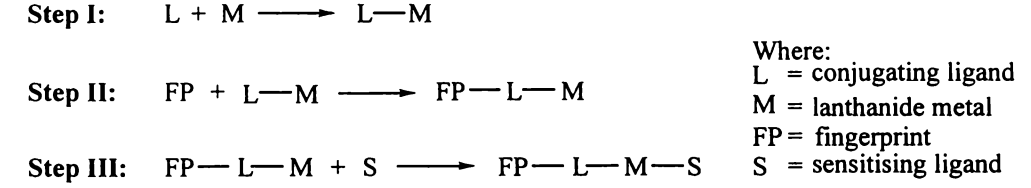


Figure 1.5 General scheme for developing fingerprints with lanthanide based reagents (adapted from [42-44])

Outlined in Figure 1.5 is the general scheme put forward by Allred and coworkers for the development of fingerprints with lanthanide based reagents. Step I involves reacting

europium with a conjugating ligand, ethylenediaminetetraacetic acid (EDTA, Figure 1.6), to form a non-luminescent complex. In step II the complex reacts with the lipid component of the fingerprint, with the speculated mechanism being either trans-esterification or acid anhydride formation. At this stage the bonds between the europium and EDTA are partially disrupted. This allows sensitising ligands, such as 1,10-phenanthroline and thenoyltrifluoroacetone (Figure 1.6), to be bound to the europium to form a luminescent complex, this being step III. [42-44]

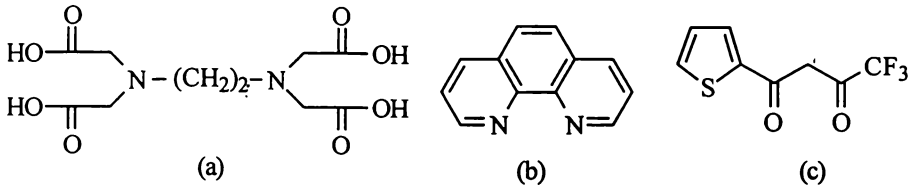


Figure 1.6 Ligands currently suggested for use in conjugation with europium; (a) EDTA, (b) 1,10-phenanthroline, (c) thenoyltrifluoroacetone (adapted from [43])

This technique differs from other lipid sensitive methods, such as physical developer (Section 1.4.4), in that it involves a chemical reaction rather than a physical interaction (e.g. particle adherence). On many surfaces substantial background luminescence can develop. This is caused by sensitising ligands reacting with Eu-EDTA complexes that are not bound to any lipid material. However this can be reduced by a vigorous washing step. It should also be noted that fingerprints developed by this method can fade over a time span of a day or two. Further optimisation, coupled with the fact that this technique is suitable for both porous and smooth surfaces indicates however that lanthanide based reagents may have an operational future. [42-44]

The fingerprint development scheme and mechanism of reaction proposed by the authors has not been thoroughly established. Further work in this area may be required to determine the exact nature of the chemistry occurring.

Research in the area of lanthanide reagents has also been carried out at the University of Waikato [45], with the investigation of coordinatively unsaturated (six coordinate) europium and terbium complexes. The most promising complex was tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato) europium(III), $Eu(fod)_3$, with the

reactivity of the reagent attributed to 'unsaturation'; europium prefers 8-coordination and as the compound is only 6-coordinate further ligands can be bound. $Eu(fod)_3$ was found to be less effective than DFO on paper, however it showed definite promise on two substrates, galvanised iron and aluminium drink cans.

1.5.4 Biological Detection Methods

In the early 1940s it was demonstrated that bacteria could be grown upon fingerprints deposited on nutrient agar, though at the time it was not thought to have a practical application. However this idea was explored when a non-damaging technique was required to develop fingerprints on a valuable oil painting. Organisms were isolated and screened to see if any were capable of growing on compounds present in the sebaceous material of fingerprints; one was found, *Acinetobacter calcoaceticus*. To develop fingerprints by this method an agar gel incorporating the bacterium is spread over the site of possible fingerprints and then allowed to incubate. The gel is then removed and subsequently stained to reveal any fingerprints. Clearly in the case of an oil painting this technique is favourable as the gel can be removed without any damage being caused to the exhibit. [1, 12, 22, 46]

Another biological detection method is the use of antisera and lectins, which can be used to detect the presence of ABH blood group substances in fingerprint deposits. The original work focused on determining blood group types but demonstrated that the technique was also capable of revealing fingerprints. This work was followed up with a specific evaluation of anti-A, anti-B, various monoclonal antisera, and a wide range of lectins as fingerprint reagents. Monoclonal anti-H and several lectins were found to be successful at developing fingerprints on non-porous surfaces. [12, 22]

Biochemical techniques involving antibodies, enzymes, and micro-organisms are renowned for great sensitivity. Very little research has been done in to the application of these techniques for developing fingerprints. It may well be that this field could offer development techniques of far greater sensitivity than those which are currently available. [12]

1.5.5 Vapour Phase Reagents

1.5.5.1 Dimethylaminocinnamaldehyde

Dimethylaminocinnamaldehyde (DMAC, Figure 1.7) is already used in various forensic procedures including the detection of drugs such as benzodiazepines, and for the identification of urine stains; DMAC reacts with urea, a urine component, to give a red coloured product. As urea is also a component of eccrine sweat (Table 1.1) it should also develop fingerprints. Indeed, DMAC has already been considered as a fingerprint reagent. Studies determining the scope and limitations, using solutions of DMAC, were carried out. It was concluded that its only real advantage was the rapid development of relatively fresh fingerprints (up to 72 hours) where the application of heat for fingerprint development was either undesirable or not possible. [12, 47]

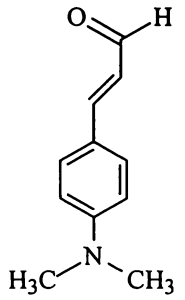


Figure 1.7 Structure of DMAC [47]

More recent work has been carried out using this reagent in the vapour phase [47]. It was found that immediately after fuming, faintly luminescent fingerprints with good ridge detail could be observed under illumination at 530 nm. Leaving the fumed samples overnight at ambient conditions substantially increased the luminescence without any loss of ridge detail or interference from the background. It was also found that DMAC did not interfere with some routine methods; DMAC can be used prior to the common sequence DFO, ninhydrin, physical developer, without any loss of detail; it can also be used prior to metal deposition but not after; it however can not be used in conjunction with cyanoacrylate fuming. [47]

There is also a particular application for the use of DMAC. Thermal papers, such as facsimiles, EFT-POS receipts, and bus tickets, cannot be treated by conventional

methods. Solvents, heat, and acids cause the colour formers in the paper to mix, resulting in the blackening of the paper. This problem was partially overcome by the synthesis of alkyl analogues of ninhydrin that are soluble in non-polar solvents. Fuming with DMAC gives luminescent fingerprints with no background interference and therefore shows particular promise as a reagent for such paper. [47]

Similar compounds that have been tested as potential vapour phase reagents include 4-nitrocinnamaldehyde, *trans*-cinnamaldehyde, 4-dimethylaminobenzaldehyde, 4-diethylaminobenzaldehyde, *o*-phthalaldehyde (Section 1.5.2.4, as solution based reagent), and 4-diethylaminocinnamaldehyde. The first two failed to develop any luminescent marks, the next three developed only faintly luminescent marks, but the last gave comparable results to DMAC. As 4-diethylaminocinnamaldehyde has a lower melting point and may be more volatile than DMAC it may offer some advantages. Further work in this area is likely. [47]

1.5.5.2 Ruthenium Tetroxide

Ruthenium tetroxide (RuO_4) has been proposed as a method for developing fingerprints previously. RuO_4 fumes react readily with various organic compounds, including those found in the sebaceous material of fingerprints, to produce brownish-black ruthenium dioxide (RuO_2). However practical use was limited due to both an inability to consistently produce fumes when required, and an inability to produce a sufficient quantity of fumes for use. However this problem has been overcome by a new formulation. A saturated RuO_4 solution is prepared in a halogenated solvent such as tetradecafluorohexane (C_6F_{14}). This solution can then be used to develop fingerprints by fuming, or even as a 'soaking' solution if this is preferred. [48]

This method shows utility on a wide variety of surfaces including bond paper, thermal sensitive paper, cloth, leather, vinyl, wood, glass, stainless steel, and human skin. Development of fingerprints is both easy and sensitive and does not preclude the use of other techniques, though fingerprints on porous surfaces which absorb and diffuse fats

and oils can become unclear with time. [48] It should be noted however that RuO_4 is rather toxic, a factor which could preclude operational use of this reagent [49, 50].

By analogy, osmium tetroxide (OsO_4) should give similar results to RuO_4, by reacting to form dark coloured osmium dioxide (OsO_2) [51]. However OsO_4 is extremely toxic, so it is unlikely to be of practical use.

1.5.5.3 Anthraquinone Dyes

1-Amino-2-phenoxy-4-hydroxy-anthraquinone and 1,4-bis(ethylamino)anthraquinone (Figure 1.8) have recently been investigated as latent fingerprint reagents and show dual application. Upon sublimation, the dyes, within 5 to 10 minutes of treatment, will develop prints of up to 10 to 15 days age on non-porous surfaces such as plastics, metals, and glass. It is thought that the dyes adhere to the fatty acid components of the fingerprints, with 1-amino-2-phenoxy-4-hydroxy-anthraquinone giving red/purple prints and 1,4-bis(ethylamino)anthraquinone giving blue prints. These dyes can also be used to enhance cyanoacrylate treated prints (Section 1.4.5). Again upon sublimation, the dyes, within 15 minutes of treatment, enhance the already cyanoacrylate developed prints. In this case it is thought that the dyes are adhering to the cyanoacrylate polymers. [52]

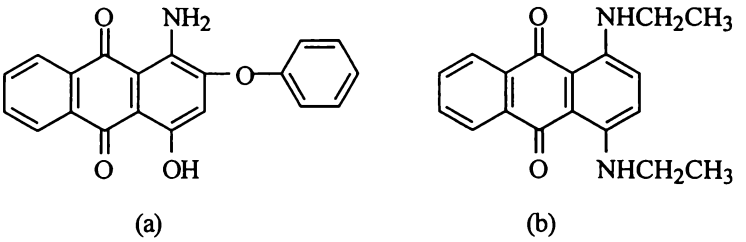


Figure 1.8 Structure of (a) 1-amino-2-phenoxy-4 hydroxy-anthraquinone and (b) 1,4-bis(ethylamino)anthraquinone (adapted from [52])

1.5.6 Other Reagents

1.5.6.1 Colliodal Gold

The use of colloidal gold for the development of latent fingerprints is yet another example of the adaptation of a previously established biochemical method for the

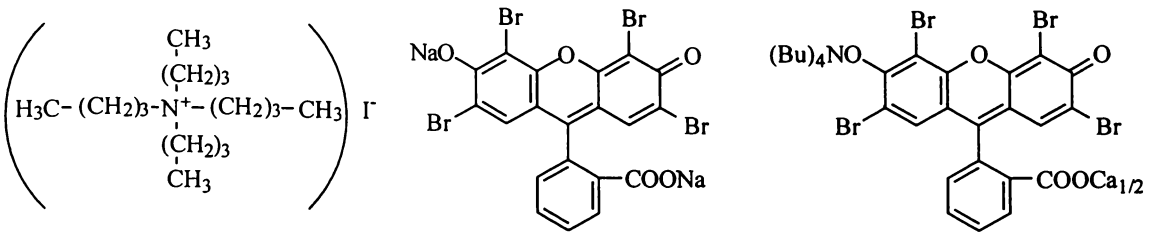
detection of proteinaceous material. Gold sols are used for the detection of proteins on nitrocellulose blots as the suspended gold particles are highly sensitive to proteins. The detection can be further enhanced by subsequent treatment with silver, consequently this technique is sometimes referred to as multi-metal deposition rather than just colloidal gold. [1, 53, 54]

To develop latent fingerprints, items are treated with a colloidal gold solution followed by a modified physical developer solution (silver treatment) and in preliminary investigations this treatment was found to be most effective on non-porous surfaces. In comparison with cyanoacrylate fuming (Section 1.4.5) and vacuum metal deposition (Section 1.4.7) it was found to be at least equal and often superior to both these methods on several common plastics and polythenes. Furthermore it showed promise on difficult surfaces including cling film, masking tape, and beer bottle labels. It should be noted that prints developed with this technique are of a fragile nature and do require careful handling. However prints can be lifted in a manner analogous to powdered prints, this preserves them more securely and also conveniently eliminates any background problems. This method has already been used with success operationally, and with further study may become a more routine method. [1, 53, 54]

1.5.6.2 Phase Transfer Catalysis Based Reagent

Calcium ions, deposited in fingerprints, are converted into largely insoluble oxide or carbonate species. Subsequently any reaction with these salts is likely to be too slow to have any practical use. Employing an acidic media would not be appropriate, as while it would redissolve the calcium it would also be likely to cause migration of the calcium ions and therefore ruin the fingerprint. To overcome this a new approach was devised using a phase transfer catalyst, which is an agent that is primarily used to bring together species present in separate phases. Success with this approach has been shown using t-tetrabutylammonium iodide[†] and sodium eosin (2',4',5',7'-tetrabromo-3',6',-dihdroxyspiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one disodium salt), (Figure 1.9). [16]

[†]Note the article states t-tetrabutylammonium iodide, however it is probable that tetra-n-butylammonium iodide was actually meant



(a) (b) (c)

Figure 1.9 Structure of (a) tetrabutylammonium iodide, (b) sodium eosin, (c) calcium complex after fingerprint development (adapted from [16])

The t-tetrabutylammonium iodide†, also acting as a precipitating reagent, was the phase transfer catalyst used to accelerate the reaction between the insoluble calcium and an aqueous solution of a complexing reagent, sodium eosin. Pink-red fingerprints were developed by a 2-3 minute immersion in a solution containing both the phase transfer catalyst and the complexing reagent. Good quality fingerprints were obtained on a variety of surfaces, though this technique was unsuccessful in developing fingerprints older than one week on paper. [16]

The mechanism proposed by the authors has not been established definitively and their interpretation of the likely chemistry involved poses some questions. For example it is unclear why a calcium-based reagent should give good resolution between prints and paper when paper itself contains significant amounts of calcium carbonate, added during manufacture. Further work may be required to determine the actual chemistry occurring.

1.5.6.3 Corona Discharge Technique

This technique is based on developing fingerprints inside a corona discharge induced plasma. A corona discharge is a partial electrical discharge which is characterised by a high voltage (several kV) and a low current (a few mA). The corona discharge can be generated using the arrangement shown in Figure 1.10. Fingerprints are developed within a few seconds to a few minutes of voltage application, depending on the discharge parameters; the resulting images are 'negative' with respect to an inked fingerprint. [55]

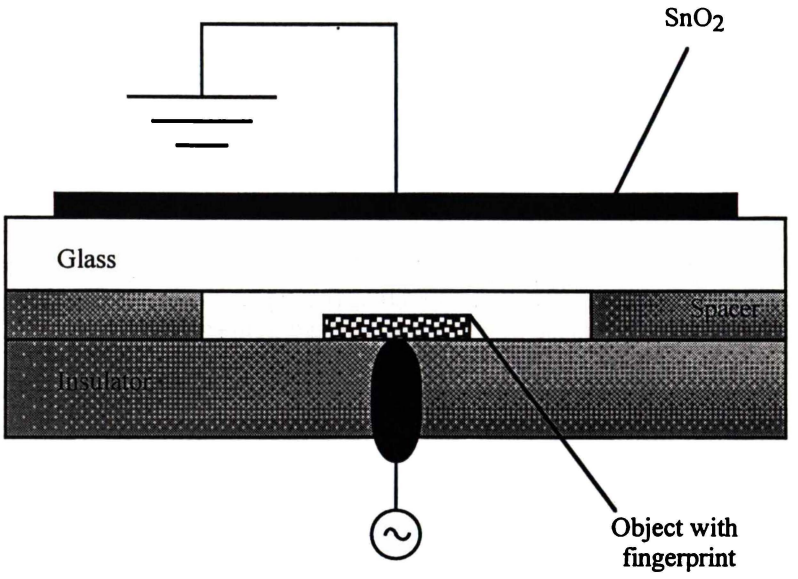


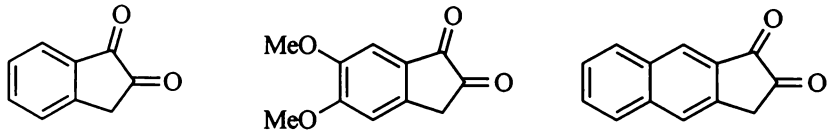
Figure 1.10 Experimental arrangement for the development of fingerprints using a corona discharge (adapted from [55])

X-ray photoelectron spectroscopy (XPS) was used to investigate the physical and chemical processes causing fingerprint development. The results obtained were consistent with the following mechanism. The corona discharge process, in air, generates ozone and other oxidising species. These species are far more chemically reactive than O_2 molecules and easily oxidise the substrate upon which the fingerprint is deposited. Sebaceous components of the fingerprint, in particular the saturated fatty acids, protect the substrate from oxidation. When the substrate changes colour upon oxidation, the change in colour between the oxidised areas (fingerprint valleys) and the protected areas (fingerprint ridges) results in a developed fingerprint that is visible to the naked eye. It is important to note that this work was undertaken on metallic substrates, however the same mechanism should apply for any substrate that undergoes colour change upon oxidation. [55]

As this process relies on oxidation the need to use an electrical discharge, instead of an alternative ozone source (or another oxidising species) has been questioned. Currently however the only commercial method for ozone generation is an electrical discharge. Ozone is highly explosive and decomposes rapidly upon generation, so can not be easily stored. This also holds for other oxidising species generated by electrical discharge. [55]

1.5.6.4 1,2-Indanediones

1,2-Indanediones, which could be loosely considered as pseudo-ninhydrin analogues, have recently been investigated as latent fingerprint reagents. While undertaking research to devise new synthetic strategies for ninhydrin analogues, utilising a 1,2-indanedione precursor rather than the routinely used 1,3-indanedione, it was observed that a substituted 1,2-indanedione had developed a luminescent fingerprint. This discovery resulted in a more thorough investigation of the parent 1,2-indanedione (Figure 1.11) along with a variety of substituted 1,2-indanediones as possible fingerprint reagents. [56]



(a) (b) (c)

Figure 1.11 Structure of (a) 1,2-indanedione (b) 5,6-dimethoxy-1,2-indanedione (c) benzo[f]indane-1,2-dione [56, 57]

The 1,2-indanediones that have been examined have given the following results. None develop amino acid spots or fingerprints with the same intensity of colour as ninhydrin, the colour obtained is usually a pale pink or purple, similar to that obtained with DFO (Section 1.4.3). Further treatment with zinc salts generally changes the colour to a dark pink/pale purple and also enhances the luminescence. The best results were obtained with 5,6-dimethoxy-1,2-indanedione (Figure 1.11). Prior to zinc treatment the intensity of luminescence was marginally better than that of DFO, after zinc treatment the intensity of luminescence clearly surpassed that of DFO. From preliminary investigation this compound also appears to have a slightly lower detection limit than DFO, suggesting that this reagent may reveal weak fingerprints that were previously undetectable with DFO and ninhydrin. Prints without zinc treatment have a relatively short lifetime, losing both colour and luminescence rapidly over a few days. After zinc treatment the lifetime of the prints is increased, with decomposition now taking a number of weeks or months. The mode of action and the exact nature of the fluorescent products with amino acids is currently unknown. Determination of the mechanism, along with further studies of 1,2-indanediones is currently underway. [56]

More recent investigations of 1,2-indanediones by other researchers [57] show some discrepancies with the results previously obtained. They are however currently attributed to the different testing methods employed until the reaction mechanism is established. Of more interest are the other results that were obtained. It was found that unlike the sequential use of DFO and ninhydrin, where ninhydrin can develop additional prints, this does not occur with the sequential use of 1,2-indanedione and ninhydrin. It was suggested that this may indicate a more complete reaction of the 1,2-indanedione with amino acids than that which occurs with DFO. It was also found that the fusion of a second benzene ring, to give benzo[f]indane-1,2-dione (Figure 1.11), did not give the anticipated deepening of colour that is exhibited by the same modification with ninhydrin. Overall however, both research groups give the impression that with optimisation, 1,2-indanediones are likely candidates as a routine technique for the development of fingerprints.

1.6 Porphyrins

1.6.1 Structural Features of Porphyrins

The porphin nucleus (Figure 1.12) is a macrocycle that consists of four 'pyrrole-type' rings joined by four methine bridges. Porphyrins are formally derived by substitution at some or all of the outer positions, 1-8 being peripheral positions and α–δ being 'interpyrrolic' methine positions usually termed 'meso'. [59, 60]

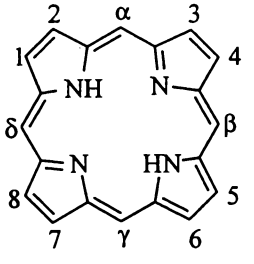


Figure 1.12 Structure of porphin [59, 60]

Porphyrins readily form complexes with a wide variety of metals, generally by displacing the two NH protons. The complexed metal ion is situated in an almost symmetrical electrostatic field consisting of four nitrogen atoms, with which it may form

four almost identical coordinate donor-acceptor bonds. If the interaction between the porphyrin and the metal is primarily electrostatic, labile complexes are formed, such as those with Group 1 and 2 metals. If the electrostatic interaction involves filling of the vacant orbitals of the metal by the electrons of the donor nitrogen atoms, stable, predominantly covalent complexes are formed, such as those with transition metals. [59, 60]

The porphyrin macrocycle is aromatic in nature, being largely planar and highly conjugated. There are nominally 22 π electrons in the macrocycle, but only 18 are included in any one delocalisation pathway, conforming with Hückel's 4n+2 rule for aromaticity. A number of electrophilic substitution reactions occur on the unsubstituted positions, which is consistent with an aromatic heterocycle. The conjugation is such that any metal that is coordinated is highly involved in the macrocyclic ring, that is it may stabilise or destabilise it. This direct contact with the conjugated system influences all, even the most distant parts of the molecule, and alters the oxidation-reduction, acid-base, electro-optical, and all other properties of a porphyrin. [58-60]

The structural diversity of porphyrins is directly attributable to the fact that substitution by almost any functional group can occur at any position on the periphery of the macrocyclic ring. This diversity, along with the specific features of porphyrins, is the reason for their importance and wide distribution in nature. Two fundamental examples are chlorophyll and heme, which respectively make leaves green and blood red and bring life to plants and animals [61].

Chlorophylls (Figure 1.13) are part of the protein-lipid system which initiate photosynthesis in green plants. In photosynthetic bacteria the function of chlorophyll is performed by its structural analogue, bacteriochlorophyll. Chlorophylls are highly involved in all aspects of the conversion of sunlight into chemical energy, being the primary photon-acceptors, the principal energy-transfer agents, and the primary electron donors in the process of photosynthesis. [59, 60, 62, 63]

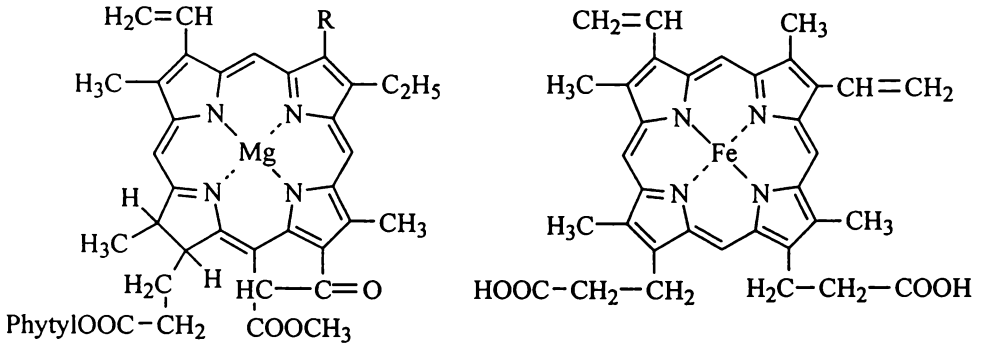


Figure 1.13 (a) Chlorophyll *a*, R = CH_3, chlorophyll *b*, R = CHO, Phytyl = $CH_2CHC\text{-}CH_3[(CH_2)_3CH\text{-}CH_3]_2(CH_2)_3CH(CH_3)_2$;(b) heme [59, 60, 62, 63]

Heme (Figure 1.13), also known as iron protoporphyrin, is the prosthetic group (non-peptide portion of a protein molecule) found in both haemoglobin and myoglobin; a peripherally modified form is also contained in the cytochromes and the enzymes peroxidase and catalase. In haemoglobin, heme groups bind oxygen so that it can be transported through the bloodstream from the lungs to the tissues. Heme groups in myoglobin store cellular oxygen until it is required for metabolic action. [24, 59, 60, 61]

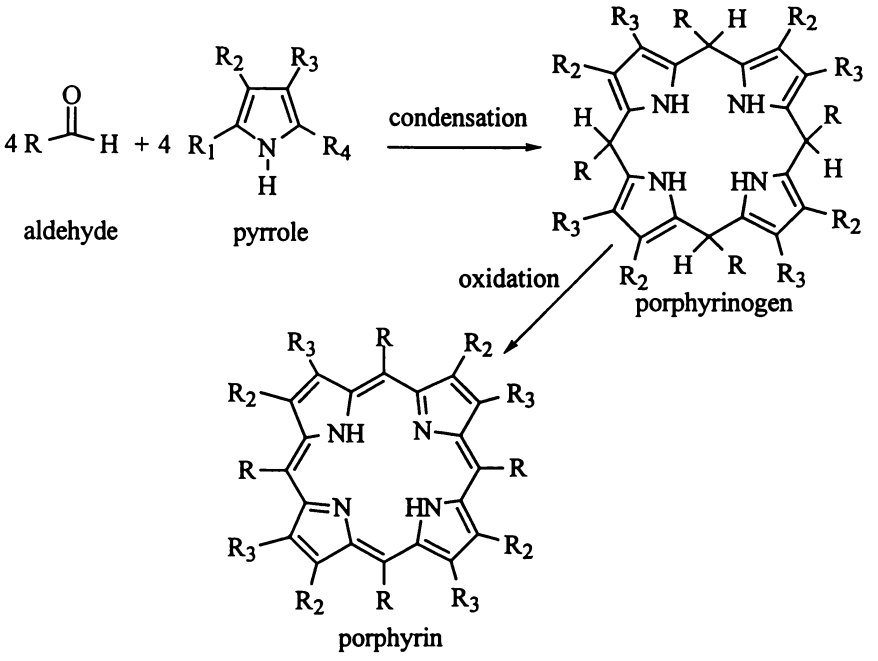
1.6.2 Synthesis of Porphyrins

As previously stated, porphyrin compounds are widely distributed in nature. Consequently they can be isolated from green leaves, blood and other natural sources. Porphyrins can also be synthesised and indeed synthesis is essential to obtain porphyrins that do not occur naturally. [59, 64]

The most traditional method for synthesising porphyrins is by monopyrrole tetramerisation. The original procedure is that of Rothemund [65, 66], which was modified by Adler and Longo [67], and more recently improved by Lindsey [68]. With this technique isomerisation occurs, so it is primarily for the synthesis of porphyrins which bear both identical pyrrolic and *meso* substituents, the two most well known being *meso*-tetraphenylporphyrin (TPP) and β-octaethyl-porphyrin (OEP).

The synthetic procedure involves the condensation of an aldehyde with a pyrrole to give an intermediate porphyrinogen which is then oxidised to the porphyrin (Scheme 1.8).

Rothemund's original conditions were quite severe, 220°C for 48 hours in a sealed bomb, resulting in both low yields and limited applicability as few substituted benzaldehydes could be converted to the corresponding porphyrin.



Scheme 1.8 Synthesis of symmetrical porphyrins by monopyrrole tetramerisation; R = Ph, $R_1 = R_2 = R_3 = R_4 = H$ gives TPP; $R_1 = H$, $R_2 = R_3 = Et$, $R_4 = CH_2NMe_2$ gives OEP (no aldehyde used)

Under Adler and Longo's modifications, porphyrins are synthesised by refluxing aldehyde and pyrrole in propionic acid (141°C) for 30 minutes, open to the air. These comparatively milder reaction conditions allow a greater selection of substituted benzaldehydes to be converted to the subsequent porphyrins, in yields of up to 20%. The reaction conditions are also amenable to preparing porphyrins on a large scale. However the reaction conditions are still too harsh for benzaldehydes with sensitive functional groups and isolation of the desired product is not always straightforward.

The synthetic strategy of Lindsey has aimed to overcome these problems. With this technique, pyrrole and the desired benzaldehyde react reversibly at room temperature with trace acid catalysis to form the porphyrinogen at thermodynamic equilibrium. An oxidant is then added to irreversibly convert the porphyrinogen to the porphyrin. Boron trifluoride etherate (BF_3) and trifluoroacetic acid (TFA) are both suitable as the acid catalyst, while the oxidants favoured are either *p*-chloranil or 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ). *p*-Chloranil affords higher yields but does require an

exposure time of one hour whereas DDQ gives an almost instantaneous conversion of porphyrinogen to porphyrin. Under these mild conditions a wide range of porphyrins may be routinely prepared from the corresponding substituted benzaldehydes in yields of 30-40%. Suggestions for improvements to the current synthetic strategies for preparing porphyrins occur regularly in the literature, including methods for obtaining porphyrins that do not have symmetrical substitution [69-77].

As mentioned previously a wide variety of metals have been complexed with porphyrins. Metalloporphyrins are formally obtained by 'inserting' a metal into a porphyrin, and indeed almost every metal has been 'inserted'. No general method exists for the insertion of all metal ions as metal ions behave differently. Outlined in Table 1.2 are eight general methods for the preparation of mono-metalloporphyrins, though these are not the only methods available. It should also be noted that the conditions of metallation may affect any peripheral substitution. [78]

Table 1.2 General procedures for the insertion of metals into porphyrins (adapted from [78])

Metalating System	Temperature (°C)	Metals Inserted
MX_mL_n/HOAc	100	Ag, Co, Cu, Fe, Hg, In, Ir, Mn, Ni, Pt, Rh, Sn, Tl, V, Zn
MX_m/Py	115-185	Ag, As, Au, Ba, Bi, Ca, Cd, Ge, Hg, Mg, Pb, Sb, Sc, Si, Sn, Sr, Tl, Zn
$M(acac)_n$/solvent	180-240	Al, Co, Cr, Cu, Eu, Fe, Ga, Hf, In, Mn, Mo, Ni, Pr, Sc, Th, Ti, V, Y, Yb, Zn, Zr
MX_m/PhOH	180-240	Mo, Os, Re, Ta, W (X = O, OPh, acac, Cl)
MCl_m/PhCN	191	Cr, In, Mo, Nb, Pd, Pt, W, Zr
MCl_m/DMF	153	Ag, As, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Hg, In, Mg, Mn, Ni, Pb, Pd, Rh, Sb, Sn, Tl, V, Zn
MR_m/solvent	25	Al, Mg, Ti
$MX_m(CO)_n$/solvent	80-200	Co, Cr, Fe, Ir, Mn, Mo, Ni, Os, Re, Rh, Ru, Tc

1.6.3 Absorption Spectra of Porphyrins

As mentioned previously porphyrins are responsible for making leaves green and blood red. Indeed porphyrins are the most abundant colouring matter found in nature. This unique feature is due to the diversity of electronic absorption and emission spectra that porphyrins exhibit.

In 1883, Soret discovered an intense absorption band in haemoglobin at approximately 400 nm. This was later observed by Gamgee in porphyrins. This 'Soret' band, which is also referred to as the Q band, is the most intense band in porphyrins, with molar extinction coefficients (ε) around 400, 000 L mol^{-1} cm^{-1} often being observed. It is found in all tetrapyrroles in which the nucleus is fully conjugated and therefore can be regarded as characteristic of this type of macrocyclic conjugation. [59, 60]

Typical absorption spectra observed in neutral solvents are illustrated in Figure 1.14. Apart from the Soret band there are four satellite bands, often referred to as B bands, numbered I to IV as shown. In some porphyrins a further small band, Ia, located between bands I and II, can be discerned. A further band, termed the γ band, is also present in porphyrins and is found at approximately 280 nm. Symmetrically substituted porphyrins generally have the etio-type spectrum where the intensity of IV > III > II > I. Other spectroscopic patterns exist and include rhodo-type, oxorhodo-type and phyllo-type. Characteristically the position and intensity of the bands varies from one porphyrin to another. [59, 60]

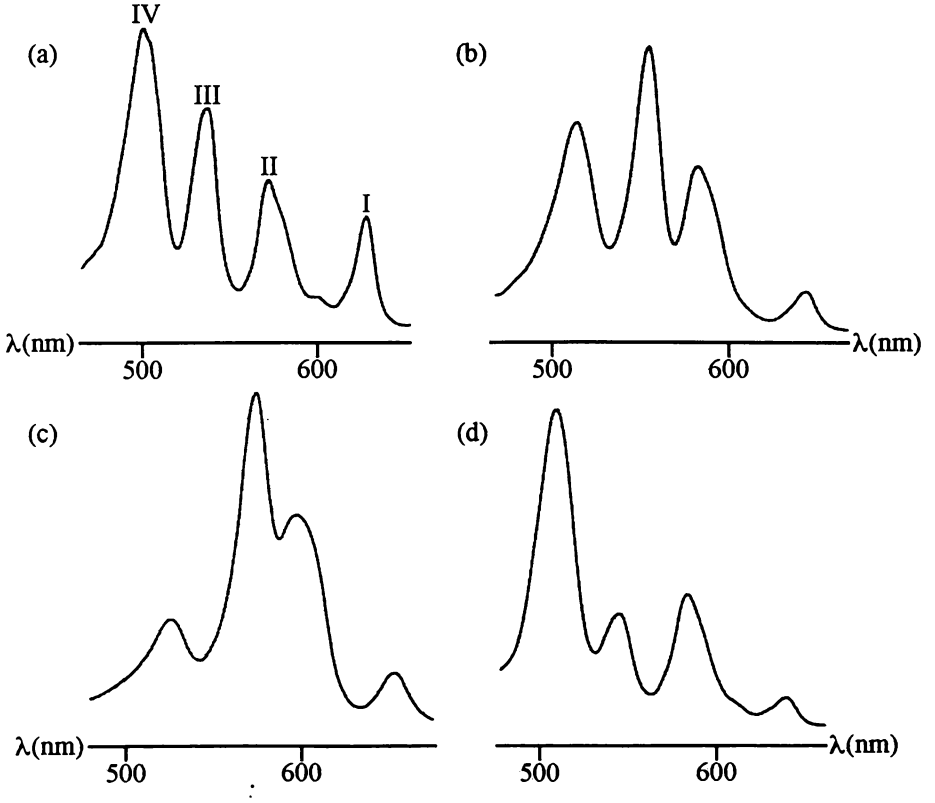


Figure 1.14 Typical UV-Vis absorption spectra of porphyrins in chloroform, note Soret band omitted; (a) etio-type, (b) rhodo-type, (c) oxorhodo-type, (d) phyllo-type [59, 60]

Metalloporphyrin complexes, with divalent metal ions, have a Soret and two visible bands, usually termed the α and β bands (Figure 1.15). The wavelengths of the α and β bands appear to be related to the porphyrin bands I and III, and II and IV, respectively. It should also be noted that metals exert considerable effects upon absorption wavelength. [60]

It is generally accepted that the prominent absorption bands of porphyrins and metalloporphyrins are $\pi \rightarrow \pi^*$ transitions, associated with the porphyrin ring. The Soret band is an allowed transition to the highest energy vacant π^* orbital ($^1A_{1g} \rightarrow {}^1E'_u$) which is why it exhibits such high intensity. [59, 79]

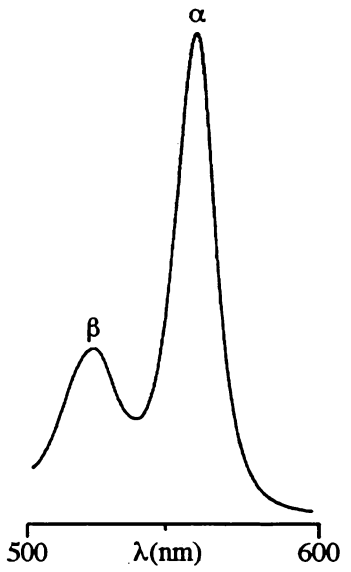


Figure 1.15 UV-Vis spectrum of a typical square planar metalloporphyrin in chloroform; note Soret band omitted (adapted from [60])

1.6.4 Emission Spectra of Porphyrins

Electronic excitation occurs when an electron, usually from an electron pair bond or a lone pair, is excited to a higher state. If the spin of the excited electron is antiparallel to its partner the excited state is a singlet, if however the spin of the excited electron is parallel to its partner then the excited state is a triplet. Generally the absorption of a quantum of light by a molecule leads to a transition from a singlet ground state to a singlet excited state, even though there will usually be a triplet state that is of lower energy. [80, 81]

The photochemical processes that can occur subsequent to an absorption of a quantum of light are represented in Figure 1.16. Initially there is internal conversion, whatever upper singlet state is reached there is often rapid radiationless energy transfer to the lowest excited singlet state. Also shown is intersystem crossing, where some of the energy is transferred to the triplet state also by a radiationless process, which results in competition between the lowest excited singlet and triplet states. Luminescent emission, from either state, can then occur. [80, 81]

This emission can either be fluorescence or phosphorescence, which are two distinct forms of luminescence. Fluorescence is defined as emission between two states of the same multiplicity, generally singlet, whereas phosphorescence is emission between two states of different multiplicity, generally triplet to singlet. These different transitions have practical implications. Fluorescent emission ceases when the light source is removed whereas phosphorescent emission will continue after the light source has been removed. This practical difference can be attributed to the lower energy triplet state being metastable. [51, 80, 81]

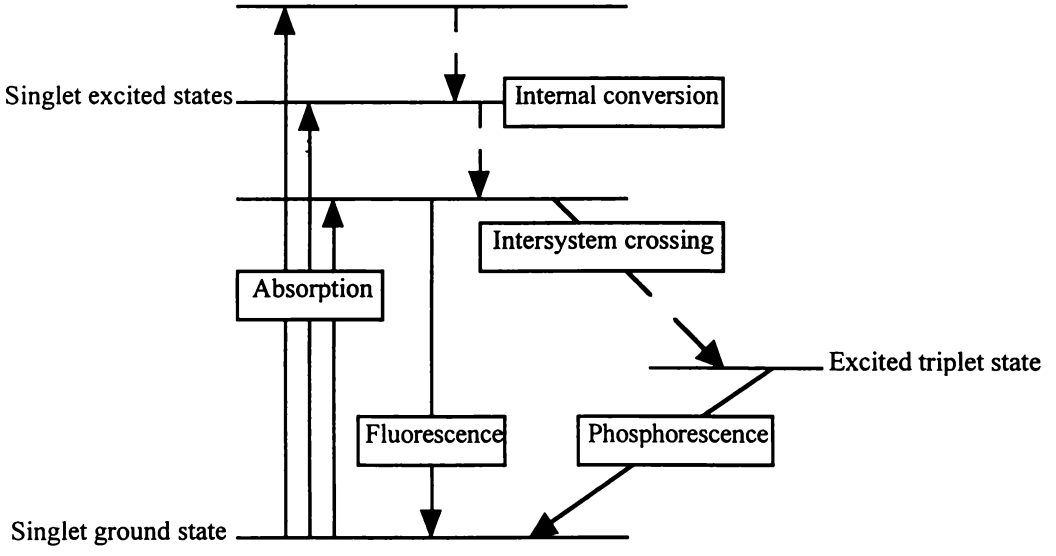


Figure 1.16 A generalised Jablonski diagram representing the molecular energy levels in photochemical processes (adapted from [80])

For both fluorescence and phosphorescence there is a difference between the absorbed and emitted light. The emitted light is almost always of lower energy, i.e. longer wavelength, than that which was absorbed, with the difference known as the Stokes

shift [Stokes shift (cm^{-1}) = $10^7\{(\lambda_{ex})^{-1} - (\lambda_{em})^{-1}\}$, λ in nm]. Luminescent compounds have two characteristic spectra, the excitation spectrum and the emission spectrum. The excitation spectrum is the relative efficiency of different wavelengths to cause luminescence, while the emission spectrum is the relative intensity of luminescence at differing excitation wavelengths. The shape of the excitation spectrum should match that of the absorption spectrum and be independent of the emission wavelength measured, though this is seldom the case due to instrumental artifacts. The quantum efficiency, also referred to as the quantum yield, Φ, is the ratio of the total energy emitted per quantum absorbed, with the higher the value of Φ_F the greater the fluorescence of the compound. It should be noted that a variety of factors affect Φ, including intensity of incident light source, concentration of the compound, molar absorptivity of the compound, and the temperature. [81]

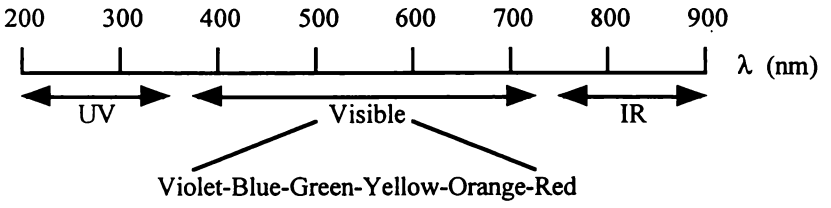


Figure 1.17 UV-Vis-IR region of the electromagnetic spectrum

Like the absorption bands of porphyrins and metalloporphyrins, the luminescence of these compounds is associated with the porphyrin π and π^* states, though the lifetime and reactivity of these states can vary greatly depending on the metal ion incorporated. In general, porphyrins show strong fluorescence at room temperature, while metalloporphyrin luminescence falls into several categories largely dependent on the electronic structure of the metal. Generally porphyrins and metalloporphyrins are excited in the visible and emit in the red and infrared (see Figure 1.17). Summarised in Table 1.3 are the luminescence properties of a variety of metalloporphyrins.

Table 1.3 Luminescent properties of porphyrins in solution at room temperature (adapted from [79])

Non-luminescent	Fluorescent	Phosphorescent	Fluorescent and Phosphorescent
Ag, Co(II), Cu(II), Ni(II), Ru(II)L_2, Ru(III), Sn(II), VO	Al, Ba, Be, Cd, Ge(IV), H_2, Hf(IV), Mg(II), Nb(V), Pb(II), Sc(II), Sr, Si(IV), Sn(IV), Ta(V), Ti(IV), Zn(II), Zr(IV)	Co(III), Rh(III), Ir	Pd(II), Pt(II), Ru(II)CO

1.7 Phthalocyanines

1.7.1 Structural Features of Phthalocyanines

Phthalocyanine (Figure 1.18) is a structural analogue of porphyrin, where the four methine bridges (—CH=) have been replaced by four aza bridges (—N=). It also differs from porphyrin in that it has benzene-type groups attached to the pyrrole-type groups. Therefore it can also be considered as a macrocycle consisting of four isoindole units linked by four aza bridges. [59, 82, 83]

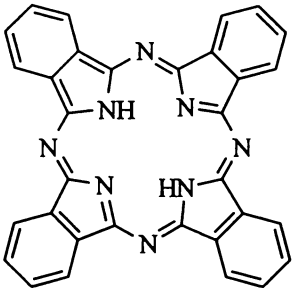


Figure 1.18 Structure of phthalocyanine [59, 82, 83]

Phthalocyanines, unlike porphyrins, are not naturally occurring compounds. Phthalocyanine was first obtained in 1907 by Braun and Tcherniac at the South Metropolian Gas Company, London, as a by-product in the synthesis of *o*-cyanobenzamide. The next recorded preparation of a phthalocyanine was in 1927 by de Diesbach and von der Weid, where a Cu phthalocyanine was obtained. The third observation of a phthalocyanine was in 1928 during the industrial production of phthalimide, at the works of Messrs. Scottish Dyes Ltd., Grangemouth, Scotland, who were in 1929, granted the first patent with respect to phthalocyanines. The dark blue insoluble complex that was noticed in the iron vessels used for phthalimide production

was subsequently found to be ferrous phthalocyanine. As it appeared that such substances might prove of academic interest, study was taken up and research into this area commenced. [82, 84, 85]

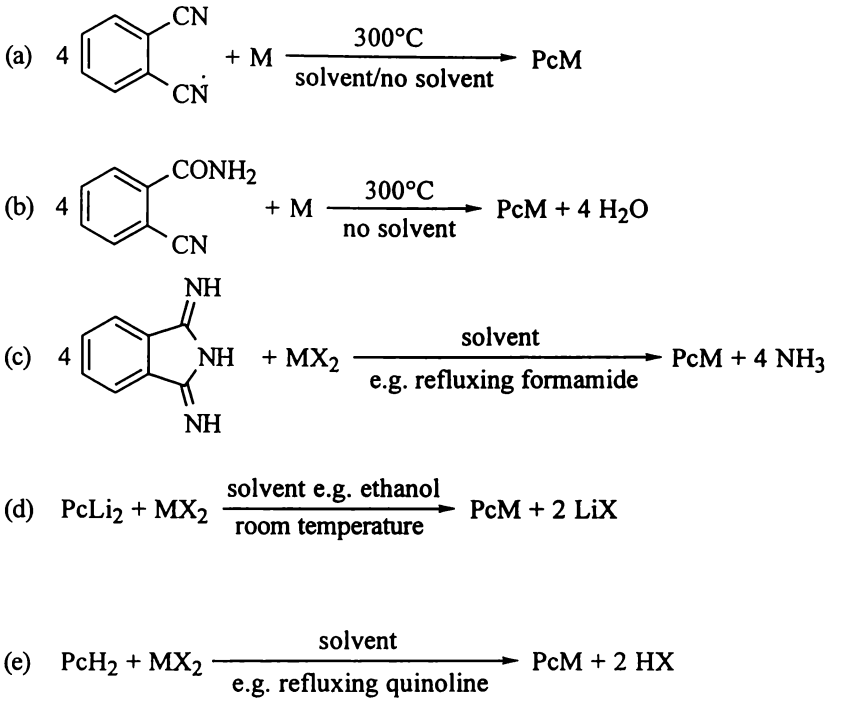
Phthalocyanines are planar molecules. The π electrons of the benzene rings participate in a weak interaction with the π electrons of the macrocyclic ring, which itself has 18 π electrons, fulfilling Hückel's 4n + 2 rule for aromaticity. Phthalocyanines often exist in polymorphic modifications with metal-free phthalocyanine existing in three polymorphs, α, β, and γ, while most metallophthalocyanines exist in two polymorphs, α and β. These forms may be distinguished by infrared spectroscopy and their X-ray diffraction patterns, with the β modification generally being the more stable form. [59, 82]

Like porphyrins, metal complexes are formed by the removal of the two central hydrogens, resulting in the formation of four equivalent N→M bonds. The difference in kinetic stability of stable phthalocyanines compared to their respective porphyrins is evidence of the higher strength of these N→M bonds. Metal complexes are known in all oxidation states from 0 to VI, though complexes with a metal ion of oxidation state II are the most common. To date about 70 different elements have been used as the central atom in phthalocyanines, with the stability of the complex depending upon the central metal atom. For example Cu, Ni, and Zn phthalocyanines are highly stable whereas Fe phthalocyanine is not stable in the presence of oxygen. [59, 82, 83]

1.7.2 Synthesis of Phthalocyanines

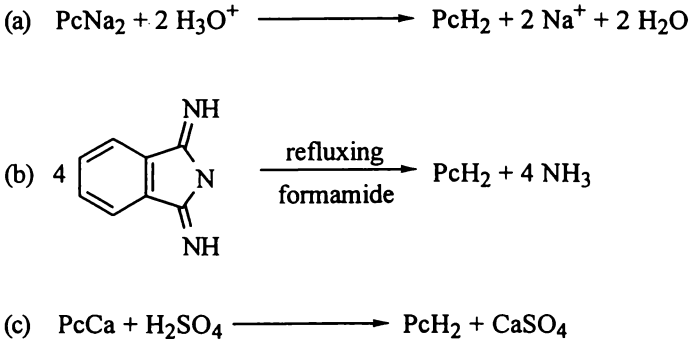
Phthalocyanines and metallophthalocyanines may be prepared by a variety of methods, with their synthesis differing from that of porphyrins and metalloporphyrins. Metals are normally 'inserted' into a preformed porphyrin to yield a metalloporphyrin, whereas metallophthalocyanines are generally obtained by 'wrapping' the reagents around a metal ion, which is acting as a template for the condensation. It should be noted however that metals can also be subsequently inserted into phthalocyanine. Phthalocyanine is generally obtained by the demetallation of different

metallophthalocyanines, though it may also be prepared directly. Outlined in Scheme 1.9 are the main methods for synthesising metallophthalocyanines and outlined in Scheme 1.10 are the main methods for obtaining phthalocyanine. [78, 82, 83]



Scheme 1.9 Synthetic strategies for the synthesis of metallophthalocyanines; note for MX_2, X generally Cl; also note that for (a) and (b) M can also be MO_x or MCl_x [82, 83]

Reaction (a) in Scheme 1.9, between phthalonitrile and a finely divided metal, metal hydride, oxide, or chloride is probably the most commonly used. The reaction is vigorous at 250° - 300°C with sufficient heat being generated to maintain the reaction temperature. High boiling solvents such as 1-chloronaphthalene or quinoline can be employed. Milder conditions can also be used by heating in an alcohol (e.g. 1-pentanol) in the presence of 1,8-diazabicyclo[5.4.0]undec-1-ene. [82, 83]



Scheme 1.10 Synthetic strategies for the preparation of phthalocyanine [82, 83]

Purification of metallophthalocyanines can be obtained by sublimation at 400°C *in vacuo*, though not all metallophthalocyanines will sublime. For those, recrystallisation from chlorobenzene, quinoline, or chloronaphthalene may be used. With some more soluble phthalocyanines, Soxhlet-extraction with lower boiling solvents such as acetone can be carried out. [82, 83]

The preparation of peripherally substituted metallophthalocyanines can be achieved by two distinct synthetic routes. Substituents can be attached either prior, or after the formation of the phthalocyanine macrocyclic ring. Prior attachment generally leads to derivatives with substitution in all four rings, whereas for subsequent attachment, the degree of substitution can be variable and is determined by the reaction conditions. [86]

The exact placement of the substituted groups on the benzene rings can also vary, giving rise to positional isomerism. A substituent in one ring may occupy position 3 (= 6, *ortho* to the imino bridge) or position 4 (= 5, *meta* to the bridge), (Figure 1.19). For a tetra-substituted compound with a substituent in all four rings, differences between positions 3 and 4, or 5 and 6, are recognised. However differences between 3 and 6, and 4 and 5, are often ignored. Hence a mixed tetra-substituted phthalocyanine in which the substituents randomly occupy positions 4 and 5, or 3 and 6, can be considered as a symmetrically substituted derivative; though there will obviously be chemical and physical difference between such positional isomers. It should also be pointed out that a more systematic numbering of substituted phthalocyanines does occur, and this is shown in Figure 1.19. From this we can see that 3,3',3",3'" is equivalent to 1,8,15,22. It is important to be aware of the two styles of numbering as they both occur synonymously in the literature. [86]

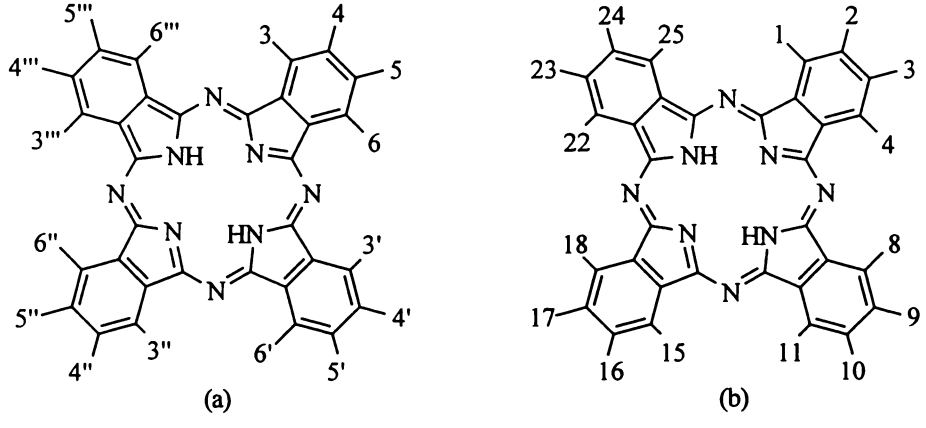


Figure 1.19 Diagram showing the two styles of numbering substituted phthalocyanines

Tetra- and octa-substituted phthalocyanines can be synthesised from mono- and di-substituted phthalonitriles respectively, as previously mentioned. Mono-substituted phthalonitriles always produce a mixture of four positional isomers of tetrasubstituted phthalocyanines. Shown in Figure 1.20 are the positional isomers obtained for 1,4-substituted phthalocyanines prepared from 3-substituted phthalonitriles. Similar isomers occur for 2,3-substituted phthalocyanines obtained from 4-substituted phthalonitriles. [83]

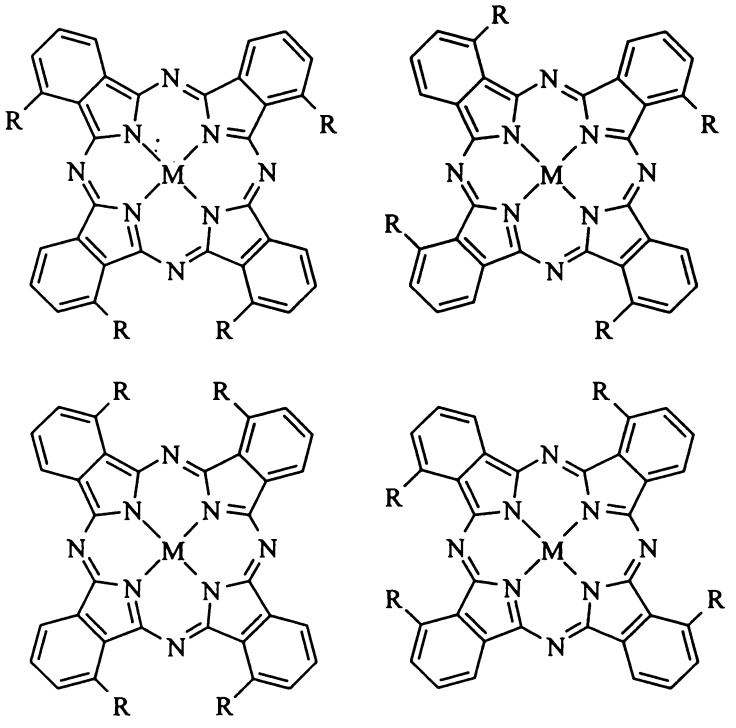


Figure 1.20 Isomers of 1,4 substituted phthalocyanines (adapted from [83])

Some suggestions for improving the synthesis of phthalocyanines and metallophthalocyanines have appeared in the literature. One is the use of high pressure conditions, approximately 10 kbar [87] and another more novel approach is the use of microwave irradiation [88]. The latter technique involves the simple mixing and grinding of reactants and irradiating the reaction mixture in a domestic microwave oven for 4 to 7 minutes in the absence of any solvent. To date Cu, Co, Ni, and Fe phthalocyanines have been prepared by this method in very high yield [88].

1.7.3 Absorption Spectra of Phthalocyanines

Like porphyrins, phthalocyanines have characteristic absorption spectra. Typical metallophthalocyanine spectra (Figure 1.21) have two major bands, an intense Q-band in the 600-750 nm region and a B-band in the 300-350 nm region. The B-band is equivalent to the Soret band in porphyrins, though it is less intense and blue shifted with respect to the Soret band. In addition weaker satellite bands are also exhibited. These generally occur in the 450-600 nm region, though they can be found over the 200-1000 nm range, which does result in some overlap occuring. Metal-free phthalocyanines have very similar absorption spectra, the difference being two Q-bands in the red region as opposed to one (Figure 1.21). The Q-band absorption results in solutions being generally deep blue or green and the intensity of absorption is so great that films as thin as 30Å have been reported to be visible to the naked eye. This intensity of absorption coupled with their deep colour has ensured both a great reputation and a wide application in painting, printing, textile, and paper industries, as well as in chemical fibre and plastic dyeing processes, as superior quality blue, blue-green, and green pigments. [82, 89, 90]

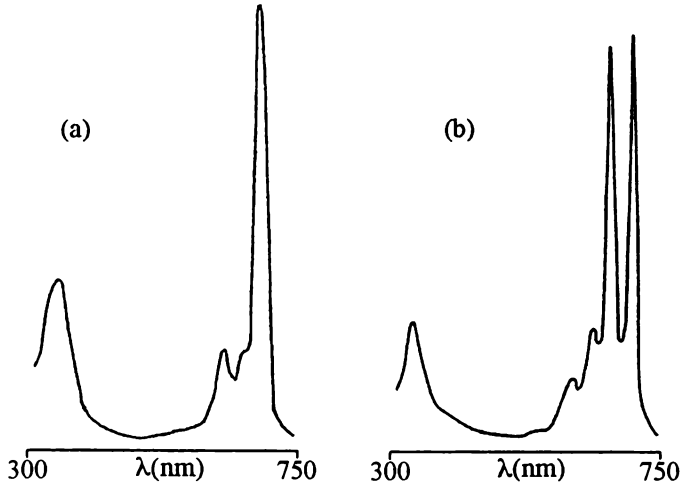


Figure 1.21 Typical UV-Vis absorption spectra of (a) metallophthalocyanines and (b) metal-free phthalocyanines

Phthalocyanines and metallophthalocyanines are quite susceptible to dimerization, polymerization, and aggregation in solution, with peripherally substituted phthalocyanines often associating even at low concentrations. This is easily monitored

by the position of the Q-band. There is a shift to shorter wavelength, a decrease in molar extinction coefficient, and a broadening of the band as aggregation occurs. [89, 91, 92]

Variation in the Q-band absorption wavelengths of metallophthalocyanines with different metal ions is less pronounced than the variation in the Soret band absorption wavelengths seen with metalloporphyrins. The other bands are however more sensitive to changes in the complexed metal ion, axial ligands, and peripheral substitution. [82, 89]

Like porphyrins, the absorption bands in phthalocyanines and metallophthalocyanines are $\pi \rightarrow \pi^*$ transitions. The Q-band is attributed to the transition from the highest occupied molecular orbital to the lowest unoccupied molecular orbital of the phthalocyanine ring. The satellite bands are usually assigned as metal to ligand, or ligand to metal charge transfer. [59, 82, 89, 91]

1.7.4 Emission Spectra of Phthalocyanines

Like porphyrins, the luminescence of phthalocyanines is associated with the π and π^* states. However $n\pi^*$ states, arising from filled non-bonding orbitals from the aza bridging groups of the phthalocyanine macrocycle, are also thought to be involved. The nature of the central metal ion also has a significant effect on the luminescence properties. [93, 94]

Phthalocyanines can exhibit very high quantum yields; for instance phthalocyanine itself has $\Phi_F = 0.7$ and magnesium(II) phthalocyanine has $\Phi_F = 0.6$, both at 77 K, while chloroaluminium(III) phthalocyanine has $\Phi_F = 0.58$, at room temperature. Comprehensive data on the room temperature luminescence properties of metallophthalocyanines was not readily available. However the data found indicated that it is not completely unreasonable to be of the opinion that Table 1.3, on room temperature luminescence properties of porphyrins, would provide a rough guide,

though it is noted that some differences are likely. Analogy is made as certain trends are exhibited by complexed metal ions with regard to luminescence. For instance the complexes of closed-shell metal ions, such as Mg^{2+}, Zn^{2+}, and Cd^{2+}, often exhibit fluorescence. Another trend is that the complexes of paramagnetic ions can show phosphorescence, as such ions often aid intersystem crossing, that is transition from the singlet to the triplet state. [81, 85, 93-95]

1.8 Aims of this Research Project

As stated earlier, research in the area of fingerprint development techniques is of fundamental interest. Any method that offers increased sensitivity over, or is complementary to current techniques is worth further investigation. At the recent International Symposium on Fingerprint Detection and Identification (Ne'urim, Israel, 1995) several specific suggestions were put forward for future research and included the following [96]:

- Synthesis of a fluorescent amino acid reagent that is as sensitive as DFO, but which does not require heating
- Determination of a specific, sensitive, non-toxic, easy to use, method for the detection of fingerprints in blood, both in the laboratory and at scenes of crime
- Synthesis of reagents which exhibit phosphorescence, rather than fluorescence to overcome the problems of background fluorescence
- Determination of a suitable solvent to replace Freon
- Determination of a method which will allow the detection of fingerprints on fired cartridge cases
- Determination of a suitable method for the detection of fingerprints on skin
- Synthesis or detection of sensitive reagents which will react with fingerprint constituents other than amino acids or lipids, for example chlorides
- Determination of a reagent which will react with lipids to give a fluorescent product that can be used on papers.

Porphyrins and phthalocyanines, by virtue of their features, are both used and investigated for a truly vast range of applications including catalysis, dyes and pigments, light harvesting systems, non-linear optics, and photodynamic therapy [86, 97-106]. In New Zealand they are even going to be used to 'capture' the first rays of sunlight of the new millennium [107]. Considering some of the features of porphyrins and phthalocyanines, such as the ability to complex with a wide variety of metals, the ability to substitute almost any functional group on the periphery of the macrocyclic ring, the ability to attach functional groups to substituted metal centres, their intense colours, and their luminescence properties, there is a strong indication that these compounds may have an application as reagents for the development of fingerprints. They may even meet some of the desired research aims listed previously. Hence the general aim of this research was to investigate the potential of porphyrins and phthalocyanines to be used as fingerprint reagents, by synthesising and examining a number of representative compounds.

The following chapters document this investigation with the structure of the thesis as follows. In each of Chapters Two, Three, and Four a different type of porphyrin or phthalocyanine is examined. Specifically, axially substituted metalloporphyrins are treated in Chapter Two, axially substituted metallophthalocyanines in Chapter Three, and peripherally substituted metallophthalocyanines in Chapter Four. Each chapter covers the synthesis, relevant characterisation, reactivity towards fingerprint components and fingerprints, optimisation of development conditions, any subsequent comparison to relevant established fingerprint detection methods, and conclusions and recommendations. A summary of the conclusions and recommendations reached, along with suggestions for further research are presented in Chapter Five.

CHAPTER TWO

AXIALLY SUBSTITUTED METALLOPORPHYRINS

2.1 Introduction

In devising reagents of this class, the aim is to have a functional group attached in the axial position associated with the metal centre of a metalloporphyrin that is either itself reactive, or alternatively, sufficiently labile to allow the metal to be reactive, towards one or more fingerprint components. The targeted metalloporphyrin was dihydroxytetraphenylporphyrinatotin(IV), $TPPSn(OH)_2$, (Figure 2.1). This particular compound was selected as, according to the literature [108], a range of bis(acido) derivatives may be prepared by treatment of $TPPSn(OH)_2$ with excess acid as follows:

$$TPPSn(OH)_2 + 2HX \rightarrow TPPSnX_2 + 2H_2O$$

where HX = ROH, ArCOOH, RCOOH, etc. This indicates that $TPPSn(OH)_2$ should react with the carboxyl groups of both amino acids and fatty acids, suggesting that it could react with both water and non-water soluble fingerprint components.

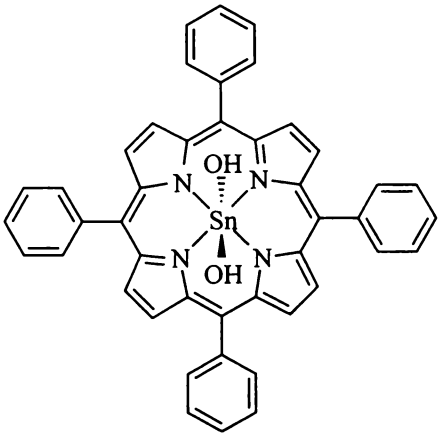


Figure 2.1 Structure of $TPPSn(OH)_2$

Please note that an article on the work presented in this chapter entitled "Appraisal of the Porphyrin Compound, $(TPP)Sn(OH)_2$, as a Latent Fingerprint Reagent" was published in the Journal of Forensic Identification, Volume 49, Issue 3, May/June 1999. A copy is included (pocket, inside back cover).

2.2 Synthesis

2.2.1 Tetraphenylporphyrin

Tetraphenylporphyrin (TPP), the precursor of $TPPSn(OH)_2$, was synthesised according to the literature method [109] as follows. Benzaldehyde (4.7 mL, BDH Chemicals Ltd, GPR grade) and pyrrole (6.7 mL, BDH Chemicals Ltd, LR grade) were added simultaneously to refluxing propionic acid (250 mL, Merck-Schuchardt, 99%) and the mixture refluxed for 30 minutes before being allowed to cool and stand at room temperature overnight. The mixture was then filtered and the crystalline product washed with distilled water and methanol to give sparkling purple crystals, approximate yield 16.9%. Please note that unless otherwise stated, all solvents used in the work-up of the compounds in this chapter, and in subsequent chapters, were of AR grade.

The TPP synthesised in this manner generally contains a small impurity of *meso*-tetraphenylchlorin (Figure 2.2), which is easily detected by the UV-Vis spectrum [109]. This impurity can be readily removed according to the following literature method [109]. TPP in refluxing chloroform is treated with a solution of 2,3-dichloro-5,6-dicyanobenzoquinone in benzene and the mixture refluxed for 3 hours. The hot solution is vacuum filtered through a sintered glass funnel containing alumina. The alumina is washed with dichloromethane and the combined filtrates rotary evaporated to reduce the volume. Methanol is then added and filtration affords pure TPP in high yield. The subsequent synthesis and clean up of $TPPSn(OH)_2$ however rendered this step largely unnecessary.

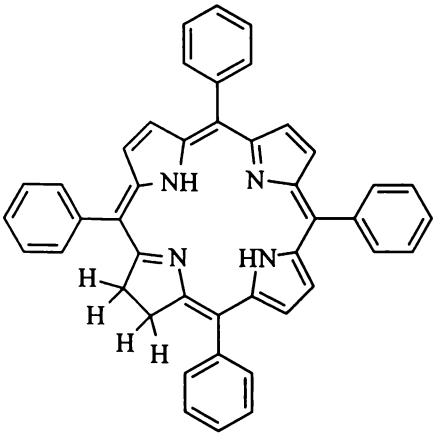


Figure 2.2 Structure of *meso*-tetraphenychlorin

2.2.2 TPPSn(OH)$_2$

$TPPSn(OH)_2$ was synthesised according to the literature method [108] as follows. TPP (1 g) and powdered $SnCl_2.2H_2O$ (0.8 g, BDH Chemicals Ltd, GPR grade) were stirred and refluxed in pyridine (100 mL, BDH Chemicals Ltd, AR grade) for 1 hour. The solution was then cooled to 50°C, concentrated ammonia (50 mL, Ajax Chemicals, AR grade) was added, and heating and stirring was continued for a further hour. Distilled water (600 mL) was added, the solid collected by vacuum filtration, washed with distilled water, and dried by suction. The solid was digested *in situ* with chloroform which dissolved the purple product leaving behind a brown residue of tin salts. The filtrate was dried with anhydrous sodium sulphate (BDH Laboratory Supplies, GPR grade) and then concentrated on a rotary evaporator. A column of alumina (BDH Laboratory Supplies, Brockmann Grade II) was prepared in chloroform and the concentrate was applied to the column and eluted with chloroform. The purple band collected was then rotary evaporated to dryness and the shiny purple crystals collected, approximate yield 78.0%.

2.3 Characterisation of TPPSn(OH)$_2$

2.3.1 Absorption Spectra of TPPSn(OH)$_2$

All absorption spectra were collected on a Varian Cary 1 UV-Vis Spectrophotometer in quartz cells and unless otherwise stated all solvents used for the collection of UV-Vis

spectra in this chapter, and in subsequent chapters, were of AR grade. UV-Vis spectra of $TPPSn(OH)_2$ were collected from chloroform solutions and the results are shown in Table 2.1, with the wavelengths of the α and β bands being in excellent agreement with those given in the literature [108]. Note that the molar extinction coefficient, $\varepsilon = A/(c.\ell)$, where A is absorbance, c is concentration in mol L^{-1}, and ℓ is path length in cm.

Table 2.1 UV-Vis data for $TPPSn(OH)_2$

Band	λ (nm)	ε (L mol^{-1} cm^{-1})
α	599.4	12700 ± 700
β	560.2	21000 ± 1000
Soret	426.2	590000 ± 40000

2.3.2 Excitation and Emission Spectra of $TPPSn(OH)_2$

All excitation and emission spectra were collected on a Perkin Elmer LS50B Luminescence Spectrometer in quartz cells using 10 nm excitation and emission slit widths unless otherwise stated. Also all solvents used for the collection of luminescence spectra in this chapter, and in subsequent chapters, were of AR grade unless otherwise stated. Spectra of $TPPSn(OH)_2$ were collected from chloroform solutions.

$TPPSn(OH)_2$ exhibits six excitation maxima of varying intensities at approximately 330, 405, 435, 518, 555, and 600 nm (Figures 2.3 and 2.4). Excitation at all these wavelengths gave very similar emission spectra with maxima at approximately 606 and 652 nm (Figure 2.5), with excitation at the different wavelengths causing changes only in the relative intensities of the two emission maxima. Excitation at 405 nm gave the most intense emission spectrum, and all excitation wavelengths, except 518 nm, gave a more intense 606 nm emission.

The most significant result is that $TPPSn(OH)_2$ has a variety of excitation wavelengths, which is an advantangeous property for a fingerprint reagent. The practical application is that if excitation at a particular wavelength causes background luminescence which masks any developed prints, it may be possible to avoid that by simply shifting to an alternative excitation wavelength that does not cause background luminescence. It

should be noted however that not all six excitation wavelengths of $TPPSn(OH)_2$ may be of practical use. The emission spectra indicate that the fluorescence of this compound should be red in colour; no phosphorescence was detected.

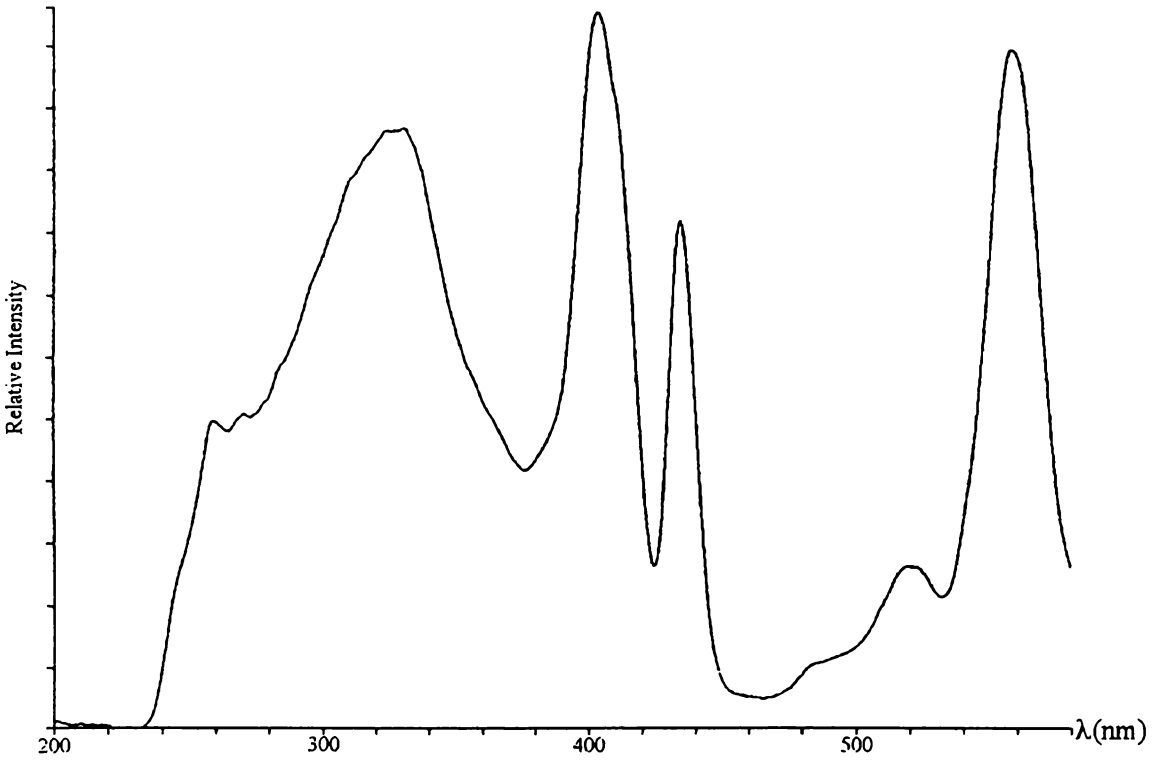


Figure 2.3 Excitation spectrum for the 606 nm emission of $TPPSn(OH)_2$ in chloroform

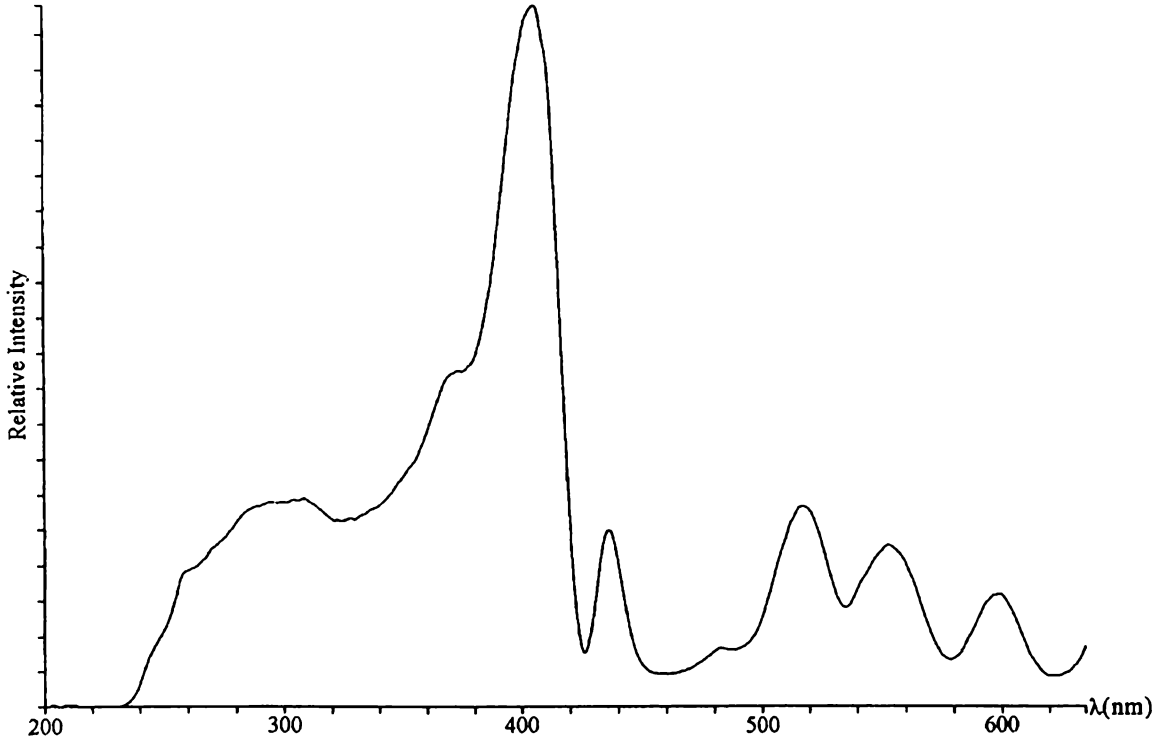


Figure 2.4 Excitation spectrum for the 652 nm emission of $TPPSn(OH)_2$ in chloroform

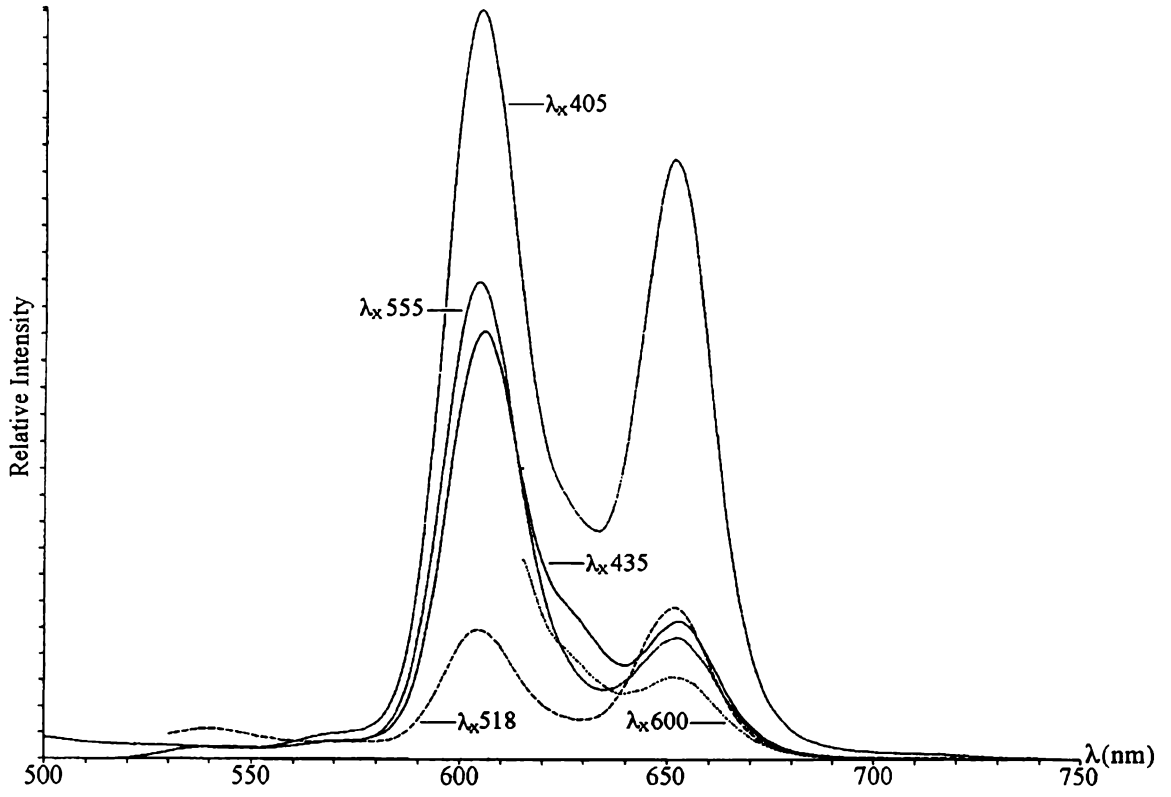


Figure 2.5 Emission spectra for excitation of $TPPSn(OH)_2$ in chloroform at 405, 435, 518, 555, and 600 nm

2.3.3 Electrospray Mass Spectra of $TPPSn(OH)_2$

All electrospray mass spectra (ESMS) were collected on a VG Platform II ES Mass Spectrometer. ESMS of $TPPSn(OH)_2$ were collected in acetonitrile/water at a cone voltage of 80 V. The spectrum in Figure 2.6 shows only one ion, $TPPSn(OH)^+$. In Figure 2.7 a high resolution spectrum is shown, along with the calculated isotope pattern [110]. There is excellent agreement between the experimental and calculated data and hence confirmation of $TPPSn(OH)^+$. Spectra were also collected in methanol. These were very similar to those obtained in acetonitrile/water except that $TPPSn(OCH_3)^+$ was seen rather than $TPPSn(OH)^+$. However this is not unexpected considering the lability of the hydroxy groups.

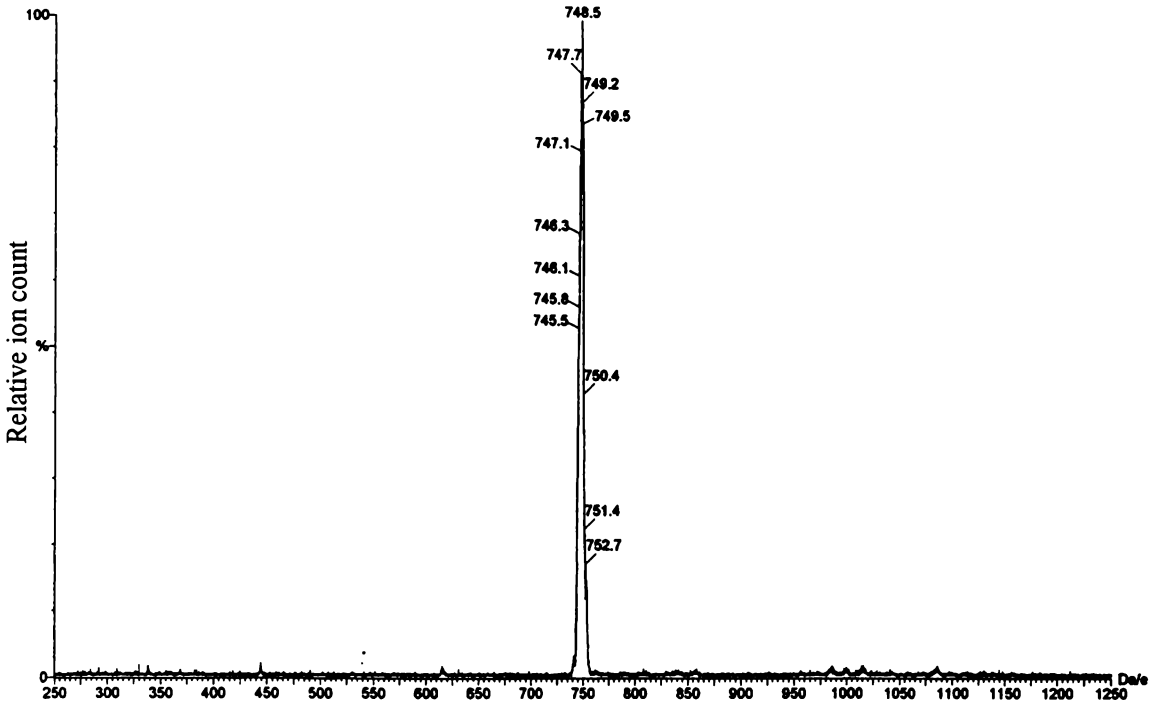


Figure 2.6 ESMS spectrum of $TPPSn(OH)_2$ in acetonitrile/water at a cone voltage of 80V

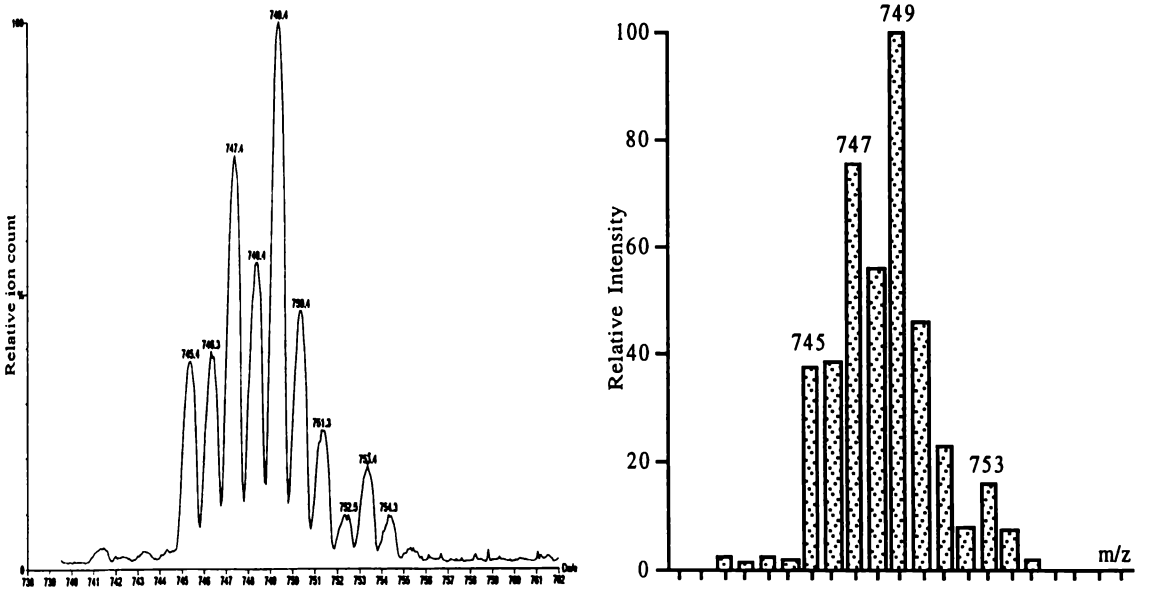


Figure 2.7 Comparison of high resolution ESMS spectrum of $TPPSn(OH)^+$ with calculated isotope pattern of $TPPSn(OH)^+$

2.4 Preliminary Reactivity Study of $TPPSn(OH)_2$

According to the literature [108] a change in the axial ligands of $TPPSn(OH)_2$ affects the visible absorption bands. The β band shifts from 560 nm to 557 nm and the α band shifts from 600 nm to 596 nm when then the hydroxy groups are replaced by carboxylato groups. A study was undertaken to see if a similar effect could be obtained by attempting to replace the hydroxy groups with amino acid groups. A positive result would give a preliminary confirmation that this compound was reactive towards amino acids and therefore perhaps fingerprints. A similar kind of approach has been utilised by others [33].

$TPPSn(OH)_2$ was reacted with three different amino acids: glycine, L-β-phenylalanine and 4-amino-n-butyric acid. In small conical flasks was placed 5 mL of a 5 x 10^{-5} M $TPPSn(OH)_2$ solution in chloroform (the concentration being appropriate for collecting UV-Vis spectra). To each was added a small spatula full of the respective amino acid. The solutions were magnetically stirred for 30 minutes then filtered through a pasteur pipette containing glass wool to remove excess amino acid. The UV-Vis spectra were then collected. In all cases a shift in the α and β bands occurred, though not as great as the shifts seen for the carboxylato complex. The shifts in the bands however indicated that some reaction had taken place and so a fingerprint screen was undertaken.

2.5 Fingerprint Screen of $TPPSn(OH)_2$

The following method was devised, in a manner similar to methods employed by other workers [34], to enable possible fingerprint reagents to be screened simply and quickly. Using an autopipette, 10 μL spots of a 0.25 % (w/v) glycine solution were placed on No. 2 Whatman filter paper. Eight spots were placed on 11 cm diameter filter paper for reagent tests (Figure 2.8) and four spots were placed on 9 cm diameter filter paper for reagent solvent blanks. Once glycine had been applied the filter papers were hung up

and left to air-dry for a minimum of 15 minutes. The filter papers were then treated with the reagent solution to be screened for three different times:

(i) a drag,

(ii) a 2 minute soak, or

(iii) a 10 minute soak.

The filter papers were then cut in half. One half was hung up and left to air-dry and the other half was oven-dried at 70°C for 10 minutes. The filter papers were then examined in ambient light and under a light source if appropriate.

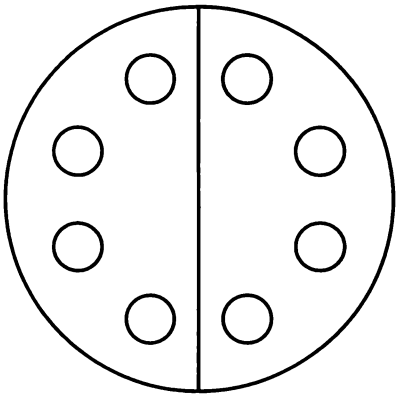


Figure 2.8 Representation of 11 cm diameter filter paper with glycine spots; note spots not visible in ambient light as shown

The initial screen was carried out with a 5 x 10^{-5} M solution of $TPPSn(OH)_2$ in chloroform. Note that all solvents used for fingerprint work in this chapter, and in subsequent chapters, were of AR grade unless otherwise stated. The following results were obtained. In ambient light the filter papers were a pale green colour and no developed spots were apparent. Filter papers were then examined under laser illumination at 488 nm. All laser examinations were carried out with a Spectra Physics 164 Argon Ion Laser fitted with an Ultrafine Technology Liquid Light Guide, and with the use of appropriate goggles. There appeared to be a faint yellow ring around all spots whether they were treated or untreated (reagent solvent blank). This is likely to be due to migration of the glycine. More importantly however, was the fact that red spots were visible on all the filter papers, especially for the sample that had been soaked for 10 minutes and oven dried. It was also noted that all of the filter paper fluoresced red, but that the developed spots were darker in colour, indicating that some kind of

background wash might be required. Overall though this was seen as an early positive result and also as confirmation that $TPPSn(OH)_2$ reacts with glycine.

A further fingerprint screen was then carried out. This time a more concentrated $TPPSn(OH)_2$ solution was used, 1.322×10^{-3} M, in chloroform. In ambient light the filter paper was quite lime green in colour and darker than that obtained with the weaker solution, though again spots were not visible in ambient light. Under laser illumination at 488 nm spots were visible, with those soaked for 10 minutes being the most obvious, with little difference between the oven and air-dried samples. Background fluorescence was also high.

A comparison of both fingerprint screens under laser illumination showed that the more concentrated solution had given a more intense red colour and had better visual contrast between the spots and the background. These results showed that the reagent had some promise but the high background colour under laser illumination was not favourable. It was decided to see if this problem could be minimised by a washing step.

2.6 Background Wash Screen for $TPPSn(OH)_2$

2.6.1 Solubility of $TPPSn(OH)_2$

In order to determine the most appropriate solvents for the background wash studies, it was necessary to determine what solvents $TPPSn(OH)_2$ were soluble in. Test-tubes containing a small amount of $TPPSn(OH)_2$ were prepared. To these was added approximately 1-2 mL of solvent. The results obtained are shown in Table 2.2.

Table 2.2 Solubility of $TPPSn(OH)_2$

Solvent	Solubility	Colour of Resulting Solution
Distilled Water	X	-
Methanol	√	purple
Ethanol	√	purple
Acetonitrile	√	pale green
Acetone†	√	pale green
Tetrahydrofuran	√	pale green going purple quite rapidly
Chloroform	√	purple
Toluene	√	pale green going pinky/purple slowly
n-Hexane	X	-
Dichloromethane	√	purple

†drum grade

2.6.2 Background Wash Experiment

From the solubility results six solvents were selected for the background wash experiment:

(i) chloroform,
(ii) dichloromethane,
(iii) ethanol,
(iv) methanol,
(v) tetrahydrofuran, and
(vi) toluene.

All six solvents were used as both the reageant solvent and the rinsing solvent. Filter paper was treated with glycine as for a fingerprint screen (Section 2.5), then soaked in reagent solution for 10 minutes. In all instances the concentration of the $TPPSn(OH)_2$ solutions was 5.2×10^{-4} M, with this concentration primarily selected for convenience, (0.01 g in 25 mL). Washing was then undertaken either immediately after treatment with the reagent solution or 10 minutes after treatment with the reagent solution, i.e. after 10 minutes air-drying of the reagent treated filter paper. Three different rinsing times were also used, which gave the following combinations:

(i) immediate drag rinse,
(ii) immediate 2 minute rinse,
(iii) immediate 10 minute rinse,
(iv) 10 minute dry then drag rinse,
(v) 10 minute dry then 2 minute rinse, and
(vi) 10 minute dry then 10 minute rinse.

After rinsing, filter papers were then left to air dry. It should be noted that one 11 cm diameter filter paper was divided into four quarters (Figure 2.9) with three of the quarters being used for the three rinsing times and the fourth quarter being a non-rinsed reagent blank.

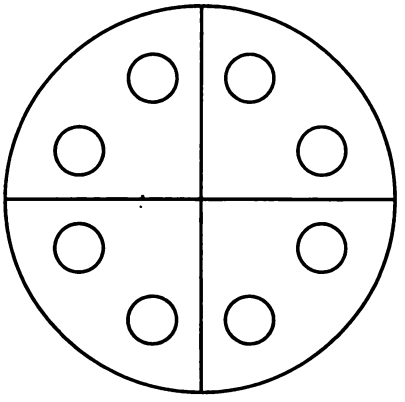


Figure 2.9 Representation of 11 cm filter paper divided into quarters

2.6.3 Background Wash Results

All filter papers were examined in ambient light, and under laser illumination at both 488 nm (0.3 W) and 514.5 nm (0.2 W), with examination at 514.5 nm being carried out approximately 3 months after the background wash experiment was conducted. The results obtained are shown in Table 2.3.

Table 2.3 Background wash results for $TPPSn(OH)_2$ with glycine on filter paper, key below

Reagent	Wash Solvent		$CHCl_3$			MeOH			EtOH			THF			Toluene			CH_2Cl_2		
	Solvent		AL	488	514	AL	488	514	AL	488	514	AL	488	514	AL	488	514	AL	488	514
$CHCl_3$	Imm	Drag	X	P	A	√	A	A	√	A	A	S	P	A	√	P	P	S	P	A
		2 min	X	P	P	S	P	P	S	A	A	√	A	A	√	A	P	S	P	A
		10 min	X	P	P	X	P	P	X	P	P	S	P	P	√	P	P	X	P	A
	Dry	Drag	X	P	A	√	A	G	S	A	A	√	A	A	S	P	A	√	P	A
		2 min	X	P	A	S	P	P	X	P	P	√	A	A	√	P	P	√	P	A
		10 min	X	P	P	X	P	P	X	P	P	√	A	G	√	P	P	S	P	A
MeOH	Imm	Drag	√	P	P	√	P	A	√	A	A	√	A	A	√	P	P	√	A	A
		2 min	X	P	A	S	P	P	√	P	P	X	G	G	√	P	A	S	P	A
		10 min	X	P	P	X	P	P	S	P	P	X	A	A	√	P	A	S	P	A
	Dry	Drag	√	A	A	√	P	P	√	A	P	√	G	G	√	P	P	√	P	P
		2 min	√	G	G	√	P	P	√	A	A	√	G	G	√	P	P	√	A	A
		10 min	√	G	G	X	P	P	√	A	A	√	G	G	√	P	P	√	G	G
EtOH	Imm	Drag	√	G	G	√	G	G	√	G	G	√	G	V	√	G	V	√	A	P
		2 min	S	P	A	S	P	A	S	A	G	S	A	G	√	G	G	√	G	V
		10 min	X	P	A	X	P	P	X	P	A	X	A	G	S	A	G	√	G	V
	Dry	Drag	√	G	G	√	A	A	√	A	G	√	V	G	√	A	A	√	A	A
		2 min	√	V	V	S	P	P	√	G	G	√	V	V	√	A	G	√	G	A
		10 min	√	V	V	X	P	P	√	G	G	√	V	V	√	G	G	√	G	G
THF	Imm	Drag	√	G	G	√	P	P	√	G	G	√	G	G	√	V	V	√	V	V
		2 min	√	V	V	√	P	A	√	A	G	√	V	V	√	V	V	√	V	V
		10 min	√	G	G	S	P	P	√	G	G	√	V	V	√	V	V	√	V	V
	Dry	Drag	√	G	G	√	A	A	√	P	A	√	G	G	√	V	V	√	V	V
		2 min	√	V	V	√	P	A	√	G	G	√	V	V	√	V	V	√	V	V
		10 min	√	V	V	S	P	P	√	G	V	√	V	V	√	G	V	√	V	V
Toluene	Imm	Drag	√	P	P	S	P	A	√	A	A	S	P	P	√	P	P	S	P	P
		2 min	X	P	P	√	A	P	√	G	A	S	A	P	√	A	P	S	A	P
		10 min	X	P	P	X	P	P	X	A	P	√	A	P	√	A	P	S	A	A
	Dry	Drag	√	A	P	√	A	A	√	A	A	√	A	A	√	A	P	√	A	P
		2 min	S	A	P	S	P	P	√	G	G	√	A	A	√	P	P	√	A	A
		10 min	X	P	P	X	P	P	S	P	A	√	A	A	√	A	A	S	A	A
CH_2Cl_2	Imm	Drag	√	A	P	√	P	A	√	G	G	S	P	A	√	P	P	√	A	P
		2 min	√	A	P	√	A	A	√	G	G	√	G	P	√	G	A	√	G	A
		10 min	√	A	P	X	P	P	S	A	A	√	P	P	√	G	A	√	G	G
	Dry	Drag	√	A	P	√	A	P	√	A	A	√	A	A	√	P	P	√	A	P
		2 min	√	A	P	S	P	P	S	A	A	√	A	A	√	A	P	√	A	A
		10 min	√	P	P	X	P	P	X	P	P	√	G	G	√	A	P	√	A	A

Key: AL = ambient light
Imm = immediate
X = no visible spot
S = slightly visible spot
√ = clearly visible spot
P = poor
A = average
G = good
V = very good
X, S, √ referring to visibility of spot in ambient light
P, A, G, V referring to quality of resolution between spot and background under laser illumination

The best individual result obtained under laser illumination was for treatment with a tetrahydrofuran reagent solution that was air-dried and then given a 10 minute

chloroform wash (Figure 2.10). The best overall results under laser illumination however were obtained when tetrahydrofuran was used as the reagent solvent and dichloromethane or toluene were used as the washing solvent. From these results it was decided to attempt to develop fingerprints using the tetrahydrofuran reagent solution/dichloromethane wash solvent combination.

Figure 2.10 Glycine spots developed by a 10 minute soak in a 5 x 10^{-3} M $TPPSn(OH)_2$ tetrahydrofuran reagent solution, followed by 10 minute air-drying and then a 10 minute chloroform wash

2.7 Fingerprint Development with $TPPSn(OH)_2$

2.7.1 Initial Experiments

Fingerprints were collected on CopyRight 80 gsm white paper. The samples were to be soaked in a 5 x 10^{-3} M $TPPSn(OH)_2$ tetrahydrofuran solution for one of three different times:

(i) 10 minutes,

(ii) 20 minutes, or

(iii) 30 minutes.

The samples were then to be rinsed with dichloromethane in the same manner as for the background wash screen:

(i) immediate drag rinse,

(ii) immediate 2 minute rinse,

(iii) immediate 10 minute rinse,

(iv) 10 minute dry then drag rinse,

(v) 10 minute dry then 2 minute rinse, or

(vi) 10 minute dry then 10 minute rinse.

If this was unsuccessful it was to be repeated by increasing the reagent concentration in 10-fold increments. While glycine spots on filter paper had been developed at lower concentrations, the quantity of amino acid present had been greater than the actual concentration found in fingerprints (Section 1.3.2). However a problem was immediately encountered. At the selected concentration the required mass of $TPPSn(OH)_2$ was not completely soluble in tetrahydrofuran. The next best set of results obtained in the background wash screen were when ethanol was used as the reagent solvent and tetrahydrofuran was used as the washing solvent (Table 2.3). Also under these conditions, better results had been obtained when there had been 10 minutes drying time prior to rinsing, rather than immediate rinsing. Hence attempts were made to develop fingerprints using a 5×10^{-3} M $TPPSn(OH)_2$ ethanol solution and a tetrahydrofuran wash, with soaking times and rinsing times as previously stated, but with the omission of the immediate rinses.

The following results were obtained. No fingerprints were detected in ambient light. Under laser illumination at 514.5 nm (0.3 W) some ridges of a fingerprint were visible on one sample. The interesting point was that the ridges were detected where the wash procedure had not been completely successful, that is the ridges were seen where there was a higher concentration of $TPPSn(OH)_2$. It was possible that the change in substrate, filter paper to white paper, had altered the reactivity of $TPPSn(OH)_2$, that is, it had become, in some way, more selective towards the fingerprint than the background. It was therefore decided to try and develop fingerprints with just a soaking step.

Three solvents were selected for this experiment: ethanol, tetrahydrofuran, and methanol. These solvents were chosen as they had exhibited the best non-rinsed blank results in the background wash screen, i.e. the best resolved spots without rinsing. Concentrations used were 5×10^{-3} M for the ethanol solution and for one methanol

solution, 5 x 10^{-4} M for the tetrahydrofuran solution and for the other methanol solution. Five soaking times were also selected:

(i) 2 minutes,
(ii) 5 minutes,
(iii) 10 minutes,
(iv) 20 minutes, and
(v) 30 minutes.

The treated papers were examined in ambient light and under the laser at 514.5 nm (0.3 W). The results obtained are shown in Table 2.4.

Table 2.4 Results from initial soaking of fingerprints experiment, key below

Solvent	EtOH		THF		MeOH ($5x10^{-4}$)		MeOH ($5x10^{-3}$)	
Time	AL	514.5	AL	514.5	AL	514.5	AL	514.5
2 minutes	√	√	?	?	?	X	X	X
5 minutes	√	√	?	?	X	X	?	?
10 minutes	√	√	X	?	X	X	X	X
20 minutes	?	√	?	?	X	?	X	X
30 minutes	?	√	X	X	X	X	X	X

Key: AL = ambient light
√ = fingerprint ridges definitely visible
? = fingerprint ridges possibly visible
X = no fingerprint ridges visible

Positive results were obtained, fingerprints had been successfully developed with the use of the ethanol solution. Optimisation of the development conditions was now required.

2.7.2 Solvent Optimisation

While methanol had not successfully developed any fingerprints in the initial experiment some further work was carried out with this solvent, because it had previously shown the best non-rinsed blank results on filter paper. It was decided to see if a mixed solvent system of ethanol and methanol would give better results than ethanol alone. Three mixed solvent solutions of $TPPSn(OH)_2$ were prepared, with solvent ratios by volume, and all at a concentration of 5 x 10^{-3} M:

(i) 90:10 EtOH/MeOH,
(ii) 80:20 EtOH/MeOH,
(iii) 70:30 EtOH/MeOH.

Fingerprints were collected on white paper, cut in half, treated, and then 'rejoined' (Figure 2.11), as the best way to detect any difference in development is by direct comparison.

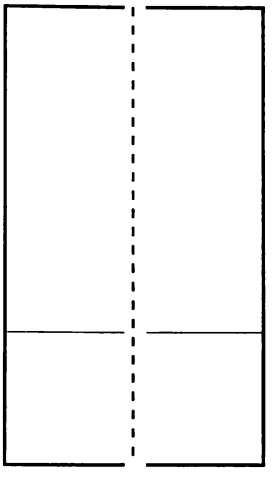


Figure 2.11 Representation of ruled paper for comparison work, fingerprint is collected on this and then cut in half using pre-ruled dividing line, lower box is for pegging and noting any details

Fingerprints were treated with the reagent solution for one of six different times:

(i) a drag,
(ii) 2 minute soak,
(iii) 5 minute soak,
(iv) 10 minute soak,
(v) 20 minute soak, or
(vi) 30 minute soak.

Samples were left to air dry and then examined in ambient light and under the laser at 514.5 nm (0.3 W). Results obtained are shown in Table 2.5.

Table 2.5 Results of ethanol/methanol solvent comparison for $TPPSn(OH)_2$, key below

Solvent	EtOH vs 90:10				EtOH vs 80:20				EtOH vs 70:30			
Time	AL		514.5		AL		514.5		AL		514.5	
Drag	√B	√	√B	√	X	X	√B	√	X	X	√	√B
2 min	√=	√=	√=	√=	√B	√	√B	√	√	X	√B	√
5 min	√=	√=	√=	√=	√	√B	√=	√=	√	√B	√B	√
10 min	√	√B	√	√B	√	√B	√=	√=	√B	√	√B	√
20 min	X	X	√	√B	X	X	√=	√=	X	X	X	X
30 min	X	X	√	√B	X	X	√	√B	X	X	√=	√=

Key: AL = ambient light
X = no fingerprint developed
√ = full or partial fingerprint developed
B = better contrast between developed print and background
'=' = no difference between developed prints

While the sample size was small, clear trends could be seen. Ethanol was a better solvent than 70:30 ethanol/methanol, there was little difference between ethanol and 80:20 ethanol/methanol, and 90:10 ethanol/methanol appeared to perform better than ethanol alone. To confirm these results an examination of these prints was carried out under the Polilight®. While the laser is an adequate light source, the Polilight® enables excitation at the actual excitation maxima of $TPPSn(OH)_2$. This gives more ideal conditions under which to view the developed fingerprints and thus more accurate appraisals can be made.

All prints were viewed under the Polilight® at the excitation wavelengths obtained spectroscopically (Section 2.3.2), these being 330, 405, 435, 518, and 550 nm (noting that 555 nm was actually determined, but that under the Polilight 550 nm gave better results). Examination at 600 nm was not possible as this excitation wavelength was not available with the Polilight®. The appearance of the developed fingerprints differs with excitation wavelength. At 330 nm the background was very pale and the fingerprints were not very clear, at 405 nm the fingerprint ridges were dark red in colour compared to a light red background, 435 nm was similar to 405 nm but the background was more of a dull red, 518 nm was similar again but the background was starting to glow more, 550 nm was similar to 518 nm. Overall, all the wavelengths were useable though 330 nm was of the least practical use. Clearly though, several excitation wavelengths were useable which as stated previously is a potential asset considering background interference can occur at different excitation wavelengths. It was also noted that at longer excitation wavelengths the developed prints were darker in colour than those at shorter excitation wavelengths, and the same trend was seen for the background colour. In Table 2.6 are the results obtained for the comparisons made under the Polilight® at all wavelengths.

Table 2.6 Results of the ethanol/methanol comparisons under Polilight examination, key below

λ (nm)	Solvent	Drag	2 min	5 min	10 min	20 min	30 min
330	E:10	E	=	=	=	=	=
	E:20	=†	E	=	M	=	M
	E:30	=†	E	M	E*	=	=
405	E:10	E	=	=	=	M	M
	E:20	=†	E	=	=	E	M
	E:30	=†	=	M	=	=	=
435	E:10	=	=	M	=	M	M
	E:20	E	E	E*	=	=‡	M
	E:30	E*	=‡	=‡	E	=	M
518	E:10	=	=	M	=	=	=
	E:20	E	E	=	=	=	M
	E:30	=	E	=	E	=	=
550	E:10	=	=	=	=	=	=
	E:20	E	E	=	=	E	M
	E:30	=	E	=	E	=	=

Key:
- E:10 = ethanol versus 90:10 ethanol/methanol
- E:20 = ethanol versus 80:20 ethanol/methanol
- E:30 = ethanol versus 70:30 ethanol/methanol
- E = best contrast between fingerprint and background developed by ethanol solution
- M = best contrast between fingerprint and background developed by methanol containing solution
- '=' = equivalent performance by both solutions
- † = only a very partial print
- ‡ = equal contrast but different appearance (colour)
- * = only marginally better

From these results it was concluded that the addition of methanol to the reagent solution did not sufficiently improve the quality of the developed fingerprints to warrant its addition. For 60% of the prints there is no difference in resolution between those developed with a 100% ethanol reagent solution and those developed with a mixed solvent reagent solution. For 23% of the prints those developed with a 100% ethanol solution are actually of better quality, particularly for shorter soaking times, e.g. 2 minutes. However for 17% of prints the mixed solvent reagent solutions do perform better, especially for longer soaking times, e.g 30 minutes.

From an operational viewpoint a single solvent solution is easier to prepare. These results indicate that little advantage is gained from a mixed solvent reagent solution. Also from an operational viewpoint a short soaking time is preferred. These results indicate that ethanol is most suited in meeting these preferences. Therefore it is concluded that ethanol is the solvent of choice for fingerprint development with

$TPPSn(OH)_2$. It is noted that ethanol is not necessarily an ideal solvent. The main disadvantage is that it can cause ink to run on documents. Another disadvantage is that is a highly flammable solvent.

While viewing these prints under the Polilight® it was noted that at this stage a few of the prints, without complete optimisation of the development conditions were almost of a quality required for evidential matching purposes [111].

2.7.3 Concentration and Soaking Time Optimisation

A survey of different $TPPSn(OH)_2$ concentrations and soaking times was then undertaken to determine their optimum conditions for the development of fingerprints on paper.

Initial comparisons were made using a 5×10^{-3} M solution against both a 2.5×10^{-3} M solution and a 1.25×10^{-3} M solution, at the same six soaking times used for the solvent comparison (drag, 2, 5, 10, 20, and 30 minutes). Fingerprints were collected on white paper, cut in half, treated and then 'rejoined' (Figure 2.11) for examination in ambient light and under laser illumination at 514.5 nm (0.3 W). The results obtained under laser illumination are shown in Table 2.7.

Table 2.7 Results from initial concentration comparisons under laser examination, key below

Time	5×10^{-3} vs 2.5×10^{-3} M		5×10^{-3} vs 2.5×10^{-3} M	
Drag	√B	√	√B	X
2 min	√	√B	√B	√
5 min	√=	√=	√=	√=
10 min	√	√B	√	√B
20 min	√†	√†	√=	√=
30 min	√†	√†	√	√B

Key: X = no developed print
√ = full or partial developed print
B = better quality print, i.e. better contrast between print and background
'=' = equivalent prints
† = very partial prints, difficult to distinguish any difference

While the sample size was small some general trends were observed. At weaker concentrations, longer soaking times produced better results and at stronger concentrations, shorter soaking times produced better results. From an operational

viewpoint a short soaking time is favourable. Therefore further experiments were carried out using the shorter soaking times of a drag and a 2 minute soak.

The first experiment compared a drag versus a 2 minute soak for both the 5 x 10^{-3} M and the 2.5 x 10^{-3} M concentrations. The second experiment compared a drag and a 2 minute soak at a 5 x 10^{-3} M concentration versus a 2 minute soak at a 2.5 x 10^{-3} M concentration. The third experiment compared a 20 second soak with a 5 x 10^{-3} M concentration versus a 2 minute soak with both the 5 x 10^{-3} M and 2.5 x 10^{-3} M concentrations. The last experiment compared a drag with a 1 x 10^{-2} M concentration versus a 2 minute soak with both the 5 x 10^{-3} M and 2.5 x 10^{-3} M concentrations. The results from all these experiments are shown in Table 2.8, noting that samples were examined in ambient light, under laser illumination at 514.5 nm (0.3 W), and under the Polilight®.

Table 2.8 Results obtained for time and concentration comparisons for $TPPSn(OH)_2$, key below

Concentration	5 x 10^{-3} M, Drag vs 2 min						2.5 x 10^{-3} M, Drag vs 2 min					
Sample	AL		514.5		Polilight		AL		514.5		Polilight	
1	√	√	√	√B^j	√=	√=	?	?	√	√B	-	-
2	√	√	√	√B^j	√	√B^j	X	X	?	?	-	-
3	?	?	√=	√=	√=	√=	?	X	√	√B	-	-
Concentration	5 x 10^{-3} M Drag vs 2.5 x 10^{-3} M 2 min						5 x 10^{-3} M 2 min vs 2.5 x 10^{-3} M 2 min					
Sample	AL		514.5		Polilight		AL		514.5		Polilight	
1	?	?	√	√B	√	√B^j	?	?	√=	√=	√	√B^j
2	√	?	√	√B	√	√B	X	X	√=	√=	√	√B^j
3	?	X	√B	√	√=	√=	?	X	√B	√	√B	√
Concentration	5 x 10^{-3} M 20 s vs 2 min						5 x 10^{-3} M 20 s vs 2.5 x 10^{-3} M 2 min					
Sample	AL		514.5		Polilight		AL		514.5		Polilight	
1	X	X	√=	√=	√=	√=	?	?	√=	√=	√=	√=
2	√	√	√=	√=	√=	√=	?	?	√=	√=	√=	√=
3	?	?	√	√B	√=	√=	X	X	√=	√=	√=	√=
Concentration	1 x 10^{-2} Drag vs 5 x 10^{-3} 2 min						1 x 10^{-2} Drag vs 2.5 x 10^{-3} 2 min					
Sample	AL		514.5		Polilight		AL		514.5		Polilight	
1	?	?	√†	√B	√†	√B	?	?	√†	√B^j	√†	√B^j
2	X	X	√=†	√=	√=†	√=	?	?	√†	√B	√†	√B
3	X	X	√=†	√=	√†	√B^j	√	√	√†	√B	√†	√B

Key: AL = ambient light
X = no developed print
? = possible ridge detail
√ = full or partial developed print
'=' = equivalent prints
B = better quality, i.e. better contrast between print and background
j = just/marginally (qualification of quality)
- = not examined
† = prints slightly blurry

The interpretation of these results is as follows. It was clearly evident that at a concentration of 2.5 x 10^{-3} M a 2 minute soak was necessary to develop good quality fingerprints. However at a concentration of 5 x 10^{-3} M a 20 second soak was sufficient to develop good quality fingerprints. A drag with a concentration of 1 x 10^{-2} M did develop fingerprints, however they had a tendency towards bluriness (possibly due to oversaturation and too rapid a reaction) and were clearly not as good as those developed with either the 5 x 10^{-3} M or 2.5 x 10^{-3} M solutions. It was also noted that prints were sometimes partially visible to the naked eye under ambient light conditions (i.e. it could be seen where prints were but there was not clear contrast between ridge detail and background) for the 5 x 10^{-3} M solution. Hence the best conditions for developing fingerprints with $TPPSn(OH)_2$ can be considered to be:

(i) a 2 minute soak in a 2.5 x 10^{-3} M solution or
(ii) a 20 second soak in a 5 x 10^{-3} M solution.

Neither regime requires heating and fingerprint development is immediate, although viewing of the fingerprints does require a light source, e.g. a Polilight®, with excitation wavelengths of 330, 405, 435, 518, or 550 nm, with the optimum wavelength being background dependent. Both regimes produce good quality fingerprints and examples are given in Figure 2.12. Overall, regime (i) is the optimum and produces the best contrasting and quality fingerprints. However regime (ii) on some occasions produces fingerprints that are partially visible to the naked eye under ambient light conditions, which could be an advantage operationally if an exhibit to be treated is large.

Having determined that optimum conditions for developing fingerprints with $TPPSn(OH)_2$ it was now possible to investigate how this reagent compared with currently used reagents.

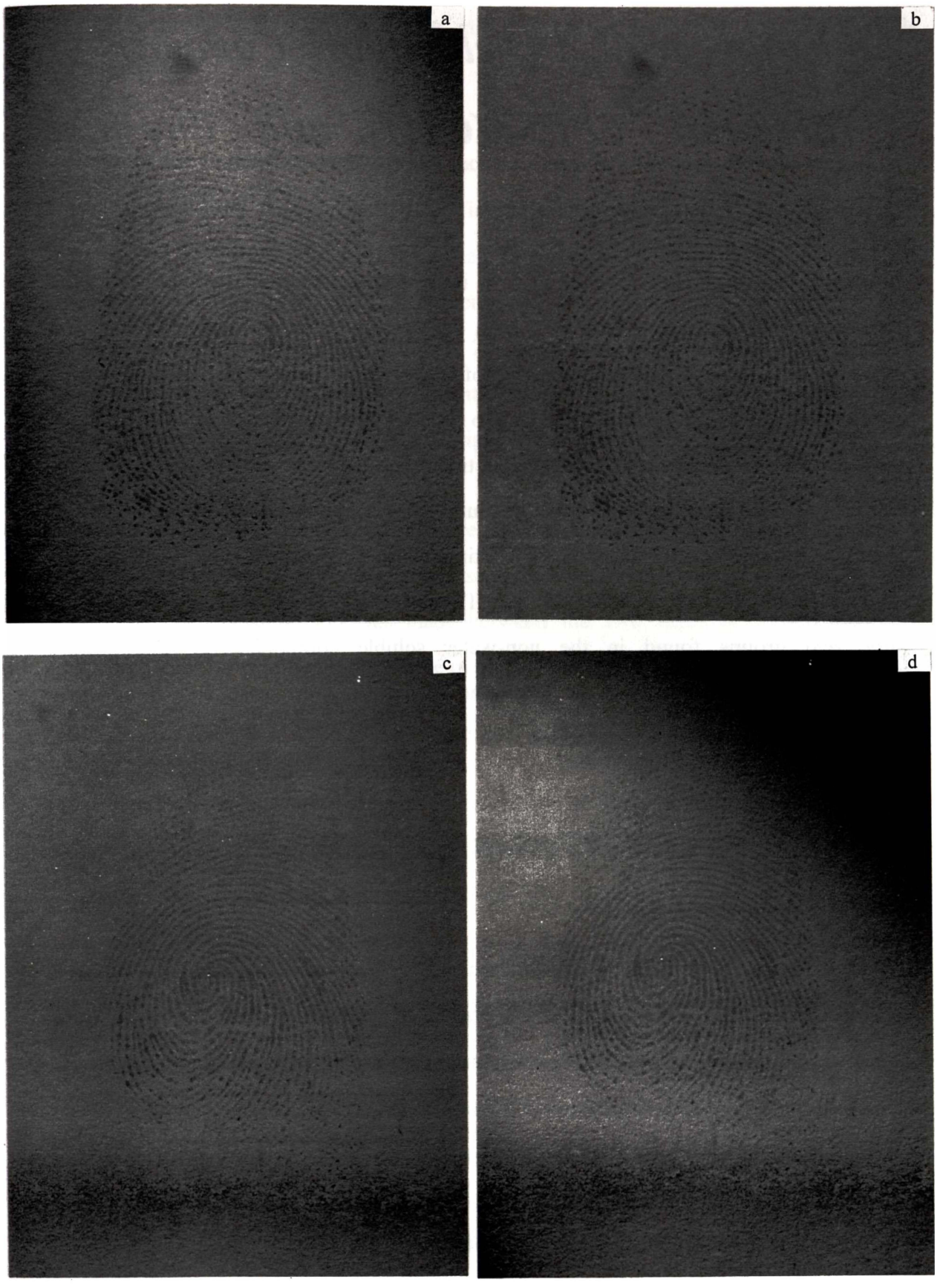


Figure 2.12 Fingerprint developed with a 20 second soak in a 5 x 10^{-3} M $TPPSn(OH)_2$ solution with (a) excitation at 550 nm and (b) excitation at 518 nm, fingerprint developed with a 2 minute soak in a 2.5 x 10^{-3} M $TPPSn(OH)_2$ solution with (c) excitation at 550 nm and (d) excitation at 518 nm; all photos taken under Polilight® excitation using a 600 nm barrier filter

2.8 Fingerprint Development Comparison between $TPPSn(OH)_2$ and Physical Developer

Physical Developer (PD, Section 1.4.4) is a technique primarily used for the development of fingerprints that have been exposed to water, for example items that may have been left in the bush for a period of time. It is generally only used in serious cases as it is an involved procedure [13]. To process just one item requires 55 minutes and this does not take into account the time required for solution and glassware preparation. Further, the main reagent solution has a limited lifetime once prepared. Therefore it would be advantageous if a less time-consuming method could be developed which was both easy to prepare and use. $TPPSn(OH)_2$ should, in theory, react with fatty acid groups found in the non-water soluble component of fingerprints. Experiments were carried out to determine if this was the case and if so how $TPPSn(OH)_2$ performed in comparison with PD.

2.8.1 Reaction of $TPPSn(OH)_2$ with Water-Exposed Fingerprints

To determine whether $TPPSn(OH)_2$ would develop fingerprints that had been exposed to water the following experiment was undertaken. Fingerprints were collected on white paper, soaked in distilled water, with some agitation, for one of three different times:

(i) 2 minutes,

(ii) 10 minutes, or

(iii) 30 minutes.

The samples were air-dried for 2 hours, treated with a 5×10^{-3} M 20 second soak and then examined in ambient light and under the laser at 514.5 nm (0.3 W).

No fingerprint ridges were detected in ambient light and only two partial prints were detected under laser illumination. It was thought that this low success rate may have

been due to the fingerprints containing only a very small fraction of non-water soluble components.

To see if the fingerprint development success rate could be improved the experiment was repeated with two changes. The first alteration was to have donors rub their thumbs on their faces prior to placing their fingerprints on paper. This was done to ensure that the fingerprints were 'sebum-enriched' [18, 19]. The other alteration was to treat samples with either the 5×10^{-3} M 20 second soak or the 2.5×10^{-3} M 2 minute soak. As there is likely to be a low quantity of material for $TPPSn(OH)_2$ to react with it was reasoned that a longer soaking time might be more favourable.

Again in ambient light no fingerprints were detected. However under laser illumination a high success rate was achieved, only two samples did not contain any ridge detail. Also while no direct comparison was made between the two different treatments there appeared to be no significant difference in performance between the two.

This experiment showed that $TPPSn(OH)_2$ could successfully develop fingerprints that had been exposed to water for up to 30 minutes and therefore that $TPPSn(OH)_2$ was likely to be reacting with the fatty acid components of fingerprints. With these results it was decided to undertake a comparison with PD.

2.8.2 Initial Comparison with Physical Developer

Fingerprints were collected on white paper from donors who had rubbed their thumbs on their faces. Samples were then soaked and occassionally gently agitated in distilled water for 30 minutes, air-dried for 2 hours, cut in half, treated with $TPPSn(OH)_2$ and PD, 'rejoined' for comparison (Figure 2.11), and examined as appropriate. For $TPPSn(OH)_2$ both the 20 second 5×10^{-3} M and the 2.5×10^{-3} M 2 minute soak were used. PD was used according to established methods [13], (Appendix I). The 30 minute water-soaking time was selected in an attempt to represent a worst case scenario, as PD has a solid reputation in such circumstances [112].

The results obtained were as follows. No prints were developed with PD, apart from two control prints to confirm that PD was working; it was noted that the control prints were weak. Three prints were detected under laser examination at 514.5 nm (0.3 W) for those treated with $TPPSn(OH)_2$. While there was a low development rate these results suggested that $TPPSn(OH)_2$ might out-perform PD, an outcome which was unexpected and seemed a little too good to be true. Hence the experiment was repeated with new PD solutions.

The results obtained from the repeated experiment were almost the complete opposite of those obtained in the initial experiment. More prints were developed with PD than with $TPPSn(OH)_2$, and further those developed with PD were generally of a better quality than those developed with $TPPSn(OH)_2$. The only difference in these two experiments was the age of the PD working solution. In the first experiment the PD working solution was 6 days old whereas for the second experiment it was fresh (i.e. prepared the same day it was used). While it is known that the PD working solution does deteriorate with age it was not expected to deteriorate so rapidly. From these results it was decided to undertake a more thorough comparison of $TPPSn(OH)_2$ with PD to look at the difference in performance of these two techniques as the age of the PD working solution increases.

2.8.3 Comparison with Physical Developer Over Time

In order to compare the relative performance of $TPPSn(OH)_2$ with PD over time the following experiment was conducted. Fingerprints were collected on white paper and Croxley 100% recycled manilla envelope paper, from donors who had rubbed their thumbs on their faces. Samples were soaked and occasionally gently agitated in distilled water for 30 minutes, air-dried for 2 hours, cut in half, treated with $TPPSn(OH)_2$ and PD, and then 'rejoined' for comparison (Figure 2.11). For $TPPSn(OH)_2$ the 2.5×10^{-3} M 2 minute soak was used. For PD two identical batches of solutions were prepared and used (Appendix I). This experiment was carried out with fresh, 1 week old, 2 week old, and 3 week old PD solutions. It should be noted that samples were left in the PD working solution for 40 minutes rather than the standard 20 minutes for the 2 and the 3

week old solutions, as longer soaking times are recommended for aged working solutions [13]. A summary of the results obtained is shown in Table 2.9.

Table 2.9 Summary of results obtained for the comparison of $TPPSn(OH)_2$ with PD on water-exposed prints over time, key below

PD Solution Age	Physical Developer		$TPPSn(OH)_2$		Performance Ratio
	Total	Quality	Total	Quality	PD:$TPPSn(OH)_2$
Fresh	90%	83%G 17%P	50%	60%G 40%P	1.8
1 week	80%	100%G	60%	50%G 50%P	1.3
2 weeks	50%	60%G 40%P	70%	57%G 43%P	0.7
3 weeks	20%	100%P	70%	71%G 29%P	0.3

Key G = print or partial print with clear ridge detail
P = print or partial print with faint ridge detail
% refers to mean percentage of fingerprints developed (2 batches)

From these results it can be seen that PD is the superior reagent to use when the solutions have been freshly prepared. However at the one week stage the two reagents are starting to perform more equivalently, though PD develops better quality fingerprints. At the two week stage $TPPSn(OH)_2$ is starting to become the superior reagent to use as it now develops more fingerprints. At the three week stage $TPPSn(OH)_2$ has become the superior reagent to use developing substantially more and better quality fingerprints than PD. It is assumed that these changes are due to the progressive deactivation of the PD working solution, which will occur via slow reduction of Ag^+ to $Ag_{(s)}$. While surfactant is added to the PD working solution to aid stabilisation of the Ag^+, it does not halt reduction completely.

Given these results it could not be recommended that $TPPSn(OH)_2$ be a replacement for PD, however $TPPSn(OH)_2$ may be of use in combination with PD. Considering that developing fingerprints with $TPPSn(OH)_2$ is a far less involved technique than developing them with PD, $TPPSn(OH)_2$ treatment may be appropriate prior to PD treatment, though this would be dependant upon the compatibility of the two reagents. This issue was examined and is discussed in the following section.

2.9 Order of Treatment With Respect to Physical Developer

As written in Section 1.3.3 the application of more than one technique can often increase the total number of fingerprints detected or improve the quality of those partially developed. Further, considering the evidential value of fingerprints on exhibits it is unlikely that a new or novel technique is even going to be considered if there is the chance that it will preclude tried and trusted detection methods. Therefore it is useful to gauge how well a potential new reagent may fit in with current treatment schemes.

As the recommendation for $TPPSn(OH)_2$ with respect to water-exposed prints was as a prior treatment to PD (Section 2.8) an experiment were carried out to determine if these two reagents were compatible in this order

For the $TPPSn(OH)_2$ - PD order experiment fingerprints were collected on white paper, from donors who had rubbed their thumbs on their faces. Half of the fingerprints were reagent treated as is. The other half were soaked and occasionally gently agitated in distilled water for 30 minutes and then air-dried for 2 hours prior to reagent treatment. Samples were treated with either a 2.5×10^{-3} M 2 minute soak or a 5×10^{-3} M 20 second soak, examined in ambient light and under laser illumination at 514.5 nm (0.3 W). Samples were then treated with PD (Appendix I) and examined in ambient light.

It was found that $TPPSn(OH)_2$ and PD are completely compatible reagents. That is, PD develops prints without any compromise in performance after fingerprints have already been treated with $TPPSn(OH)_2$. For the fingerprints that had not been soaked in water, it was found that the developed prints had at times no clear ridge detail, that is they could appear as 'blobs'. However this was just due to an overloading of material from the rubbing of thumbs on faces.

2.10 Thermal Paper Fingerprint Development Comparison

The development of fingerprints on thermal paper, such as faxes, EFT-POS and credit card receipts, and bus and railway tickets, generally pose a problem, as the routinely used ninhydrin and DFO techniques tend to turn thermal paper a dark grey/black almost immediately. Consequently the likelihood of recovering fingerprints from such items is miminal which in turn lowers their evidential value. Hence a study was carried out comparing the performance of $TPPSn(OH)_2$ against ninhydrin and DFO on thermal paper.

Fingerprints were collected from a variety of donors on 'sent' Olympic thermal fax paper that had been ruled up as in Figure 2.11, with a proportion of the samples containing 'writing'. The fingerprints were cut in half, one group was treated with $TPPSn(OH)_2$, with both a 2.5×10^{-3} M 2 minute soak and a 5×10^{-3} M 20 second soak. The other group was divided into two separate groups with one group being treated with ninhydrin and the other group being treated with DFO, both according to established methods [13], (Appendix I). All samples were examined in ambient light, under laser illumination, and under the Polilight®. The results obtained are summarised in Table 2.10.

Table 2.10 Summary of results obtained from the comparison of $TPPSn(OH)_2$ with ninhydrin and DFO on thermal fax paper, key below

Prints Developed	$TPPSn(OH)_2$‡		Ninhydrin	DFO
	Total	Quality		
Winter	45%	79% G, 21% P	0%†	0%†
Summer	80%	83% G, 17% P	0%†	0%

Key G = print or partial print with clear ridge detail
P = print or partial print with faint ridge detail
% refers to percentage of fingerprints developed
† indicates a few fingerprints were detected, however the prints were of exceptionally poor quality, i.e. completely unsuitable for identification purposes
‡ results obtained from Polilight® examination

The first point to examine is the seasonal difference in the total quantity of fingerprints developed by $TPPSn(OH)_2$. While a development rate of 45% is a reasonable

performance it was thought that this might be low due to poor donor print quality, as the experiment was carried out during the winter months. Under these conditions reduced surface perspiration was evident from the donors. The experiment was repeated during the summer months and a substantial increase in the total number of fingerprints developed, to 80%, was obtained. This confirmed the seasonal variation in donor print quality and indicates that research of this nature should keep these kinds of issues in mind. The relevance in this instance being that good quality fingerprints are often left by criminals due to their increased rate of perspiration.

More important however was the superior performance of $TPPSn(OH)_2$ in developing fingerprints on thermal paper. The fingerprints developed were slightly visible in ambient light, and clearly visible under the Polilight® at excitation at 405 nm (Figure 2.13) and to a lesser extent at excitation at 435 nm. It should be noted that fingerprints were not visible at the longer Polilight® excitation wavelengths of 518 and 550 nm, nor under laser illumination at 514.5 nm. This is probably due to a component in the thermal fax paper giving a background emission at these excitation wavelengths that masks the emission of the developed fingerprint. In this instance the utility of having more than one excitation wavelength is clearly shown. While no direct comparison was undertaken between the 2.5×10^{-3} M 2 minute soak and the 5×10^{-3} M 20 second soak the latter did have a much higher failure rate, therefore a 2.5×10^{-3} M 2 minute soak is recommended for thermal paper. With these conditions the evidential value of the item is not compromised, that is the thermal paper does not turn black as it does when treated with ninhydrin or DFO. Ethanol also poses little problem with evidential integrity, with the 'writing' on thermal paper only partial fading and not running.

The longevity of the developed prints was also documented in this experiment. As can be seen in Figure 2.13, the prints are all clearly visible, though a substantial amount of time had elapsed between treatment and photographic recording. This result, while unintentionally gained, was an excellent one, meaning that there is no immediate pressure to record developed prints, which is an operational advantage.

Finally as the recommendation for $TPPSn(OH)_2$ on thermal papers is as a complete alternative to ninhydrin/$ZnCl_2$ and DFO no order of treatment experiment, as for PD (Section 2.9), was undertaken.

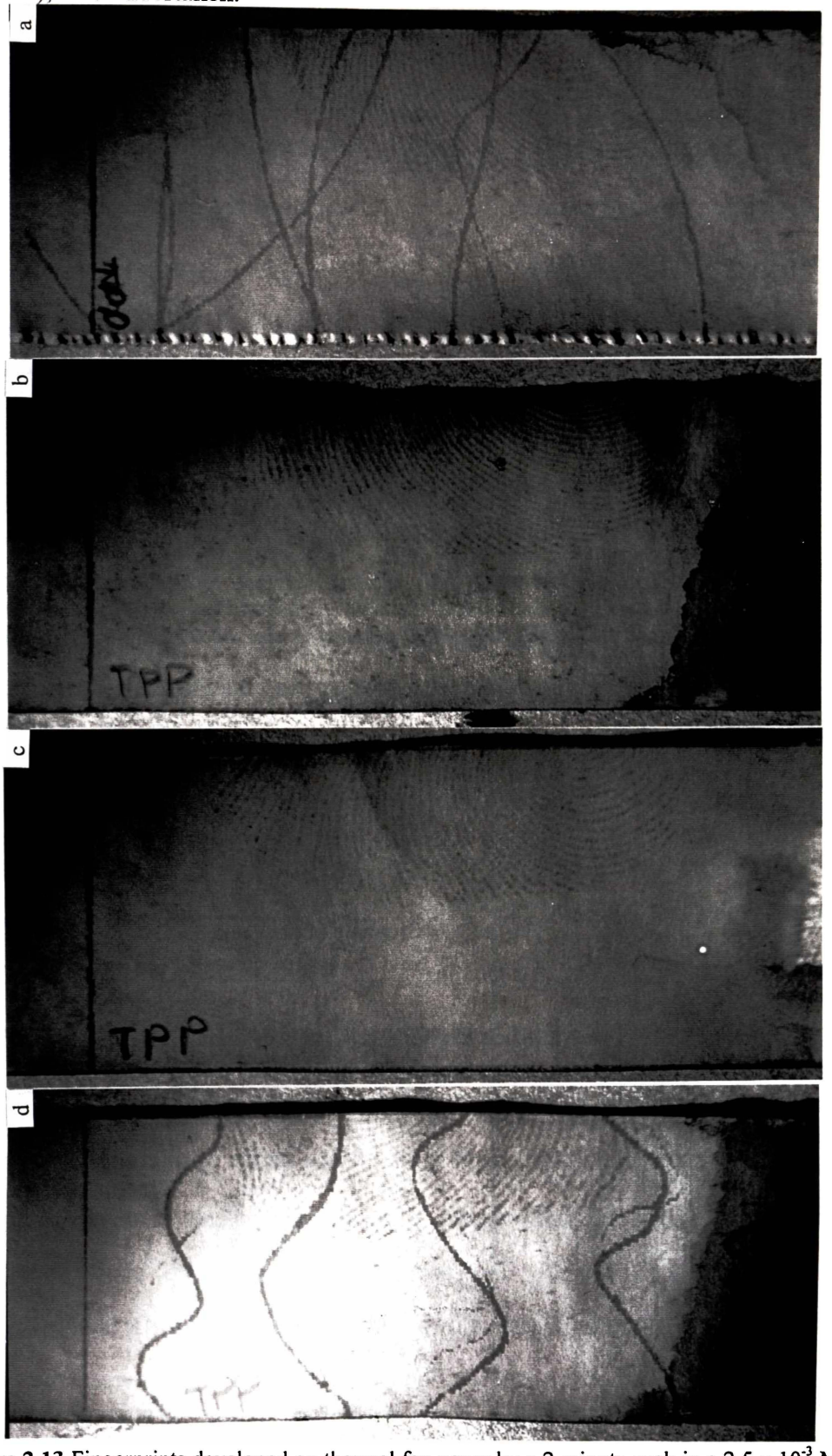


Figure 2.13 Fingerprints developed on thermal fax paper by a 2 minute soak in a 2.5 x 10^{-3} M $TPPSn(OH)_2$ solution, note that (a) was taken approximately 2 months after print development and that (b), (c), and (d) were taken approximately 9 months after print development; photos taken with Polilight® excitation at 405 nm using a Kodak CC20Y colour compensating gelatin filter

2.11 Mode of Action of $TPPSn(OH)_2$

It is likely that developed prints are a product of the reaction of $TPPSn(OH)_2$ with amino acids for the water soluble fingerprint component and with fatty acids for the non-water soluble fingerprint component according to:

$$TPPSn(OH)_2 + 2RCOOH \rightarrow TPPSn(OOCR)_2 + 2H_2O$$

with the amino and fatty acids likely to be bonded to the Sn metal centre through the oxygen of the carboxyl groups.

Support for these modes of action are provided in a number of ways. Reactivity with amino acids was shown with the glycine fingerprint screen results (Sections 2.5 and 2.6) and is illustrated in Figure 2.9. Reactivity with fatty acids was confirmed by the following experiment. $TPPSn(OH)_2$ was dissolved in dichloromethane to which oleic acid (May & Baker, 99%) was then added. Oleic acid, systematic name *cis*-9-octadecenoic acid (Figure 2.14), was selected as it is one of the fatty acids found in high proportion in secreted sebum [19]. The mixture was magnetically stirred for one hour and then examined by UV-Vis and ESMS.

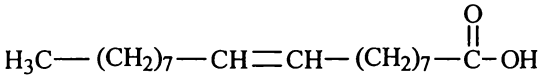


Figure 2.14 Structure of oleic acid

A significant shift was detected in the B bands, the α band shifted from 599.4 nm to 595.4 nm and the β band shifted from 560.2 nm to 556.4 nm. There was also a change in the intensity ratio β/α from 1.62 to 1.94. These shifts were consistent with a change in axial ligand [108]. ESMS results were clearly indicative of a reaction between $TPPSn(OH)_2$ and oleic acid. The spectrum in Figure 2.15 shows one major ion, $TPPSn(O_2C_{18}H_{33})^+$, and in Figure 2.16 a high resolution spectrum is shown, along with the calculated isotope pattern [110]. The excellent agreement between the experimental and calculated data confirmed $TPPSn(O_2C_{18}H_{33})^+$.

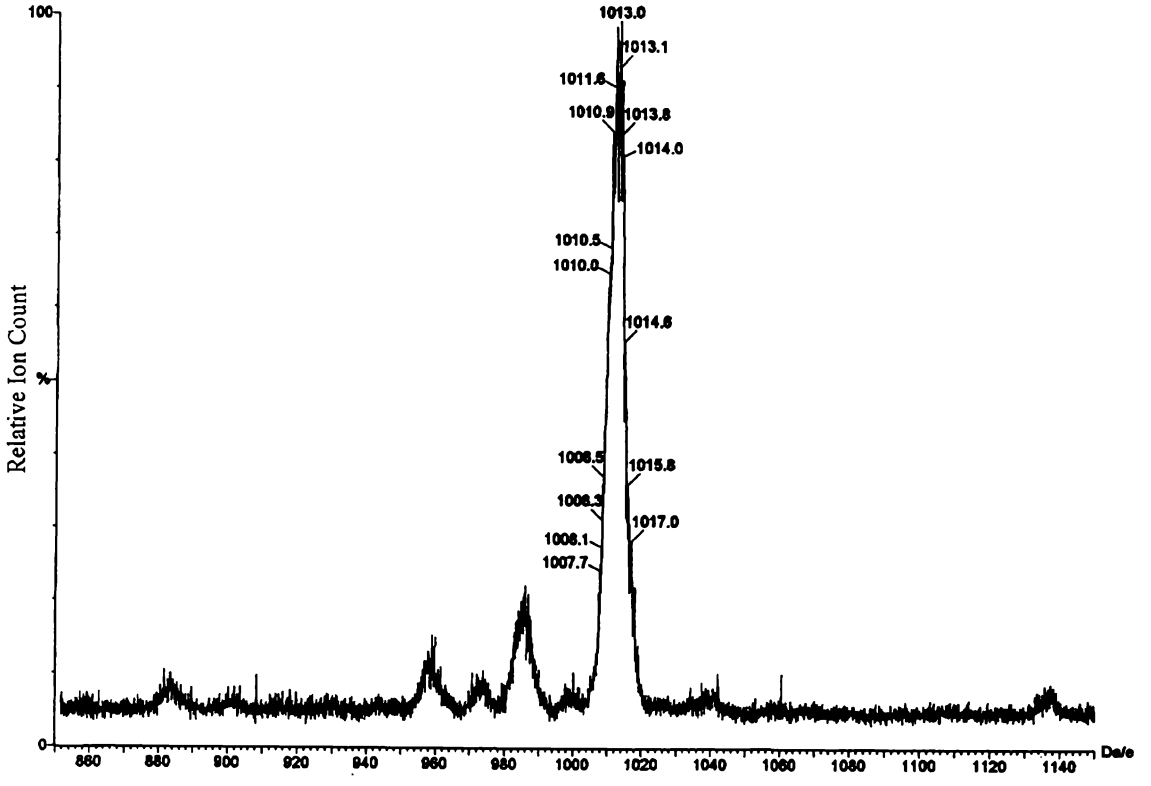


Figure 2.15 ESMS spectrum of $TPPSn(OH)_2$ - oleic acid derivative in methanol at a cone voltage of 75V

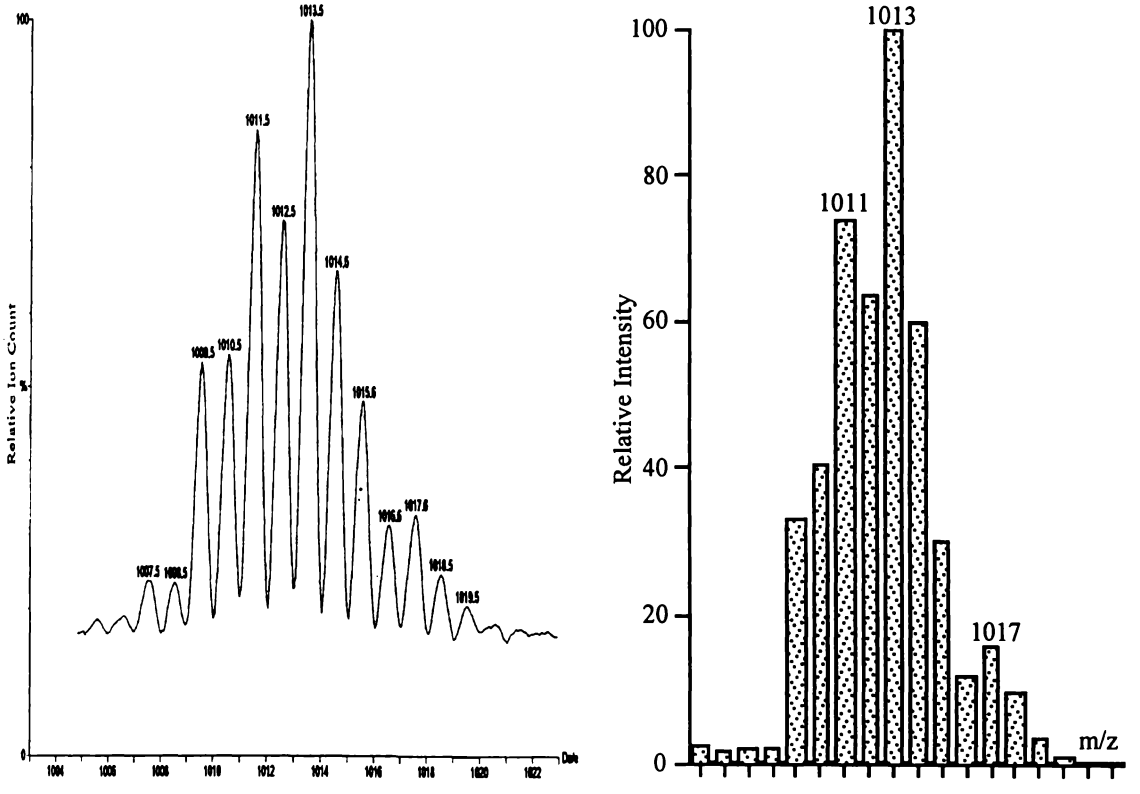


Figure 2.16 Comparison of high resolution ESMS spectrum of $TPPSn(O_2C_{18}H_{33})^+$ with calculated isotope pattern of $TPPSn(O_2C_{18}H_{33})^+$

Bonding attachment to the Sn metal centre via the oxygen of the carboxyl groups is suggested as a number of reported crystal structures, for a variety of $TPPSn(OH)_2$ derivatives (acetate, benzoate, salicylate, acetylsalicylate) all have this mode of attachment [113, 114].

It should be added that the dual reactivity of $TPPSn(OH)_2$, that is reaction towards water and non-water soluble fingerprint components, is fairly unique. Most reagents employed in the development of latent fingerprints target one type of component only. In this instance the double action is directly attributable to the mode of action of $TPPSn(OH)_2$.

Finally as an aside, the mode of action displayed by $TPPSn(OH)_2$ provokes some ideas regarding the development of new fingerprint reagents, regardless of whether they are porphyrin or phthalocyanine-based. Carboxyl groups are not a functional group frequently targeted in the development of fingerprints, however they may merit further attention for two reasons. Firstly, most of the methods for developing the sebaceous components of a fingerprint involve physical interaction of a reagent rather than chemical reaction of a reagent. Targeting carboxy groups, which occur in fatty acids, would provide a way of developing the sebaceous material by chemical reaction. Secondly, considering that carboxy groups occur in both water and non-water soluble fingerprint components, in theory, attaching a dye molecule to every carboxyl group present, may provide the opportunity to bind to a larger total quantity of the fingerprint, and therefore develop a highly coloured or highly luminescent print. Though consideration of how often carboxyl groups occur in commonly encountered substrates would have to be taken into account.

2.12 Conclusions and Recommendations

$TPPSn(OH)_2$ is capable of developing latent fingerprints. It reacts with both water soluble and non-water soluble latent fingerprint components to give a developed

fingerprint that is partially visible in ambient light and clearly visible under illumination with an appropriate light source.

The optimum conditions for developing good quality fingerprints with $TPPSn(OH)_2$ on paper were determined to be either:

(i) a 2 minute soak in a 2.5×10^{-3} M ethanol solution or

(ii) a 20 second soak in a 5×10^{-3} M ethanol solution.

Regime (i) gives the best contrasting and quality fingerprints overall, however regime (ii) can give fingerprints that are partially visible in ambient light, which may be an operational advantage if the item to be treated is large. Fingerprint development is rapid and requires no heating, although viewing of the fingerprints does require an appropriate light source, e.g. a Polilight®. There are six excitation wavelengths, these being 330, 405, 435, 518, 550, and 600 nm, with the optimum excitation wavelength for viewing fingerprints being background-dependent. Fingerprints, once developed, show excellent stability, with no noticeable deterioration seen over extended periods of time.

$TPPSn(OH)_2$ was found to be a useful alternative, though not complete replacement, for PD, in the treatment of fingerprints that have been exposed to water. In contrast to PD, $TPPSn(OH)_2$ is quick and easy both to prepare and use, and the reagent solution can be stored for long periods of time without deterioration. $TPPSn(OH)_2$ can also be used prior to PD without any effect on the subsequent performance of PD. Therefore suggest that $TPPSn(OH)_2$ might be an appropriate treatment step prior to PD that may result in more prints being recovered than would otherwise be the case.

For the development of fingerprints on thermal paper treatment with $TPPSn(OH)_2$ would be highly recommended and in particular would suggest use of regime (i) with illumination at 405 nm. These conditions gave a high development rate of good quality fingerprints on such paper, unlike ninhydrin and DFO, which are largely unuseable.

In terms of future work it might be useful if further comparisons were undertaken with $TPPSn(OH)_2$ against other reagents that have appeared in the literature that could be appropriate for thermal papers, such as DMAC. It would also be interesting to

investigate peripherally substituted derivatives of $TPPSn(OH)_2$. Such compounds might exhibit different solubilities, and different absorption and emission characteristics, that might make them more appropriate reagents than $TPPSn(OH)_2$ itself.

Finally while excellent results were obtained in a laboratory situation the real test is how a reagent is viewed by those people who are professionally involved. To try and gain some feedback, samples of the reagent, together with instructions of use and a survey form were prepared and sent out to interested parties (these are shown in Appendix II). Unfortunately only three replies (two survey forms and one email) were received (also shown in Appendix II), so it is currently difficult to determine conclusively whether $TPPSn(OH)_2$ has a genuine place in the reagents routinely used for the development of latent fingerprints. Further appraisal in casework situations is required.

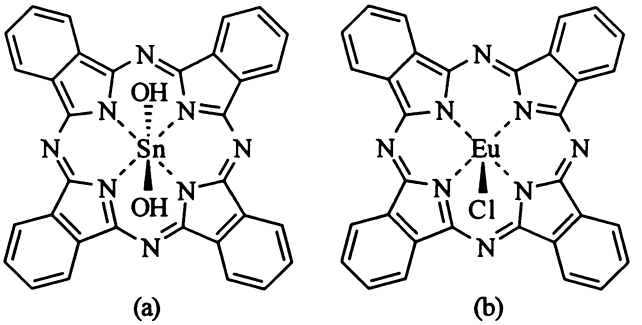
CHAPTER THREE

AXIALLY SUBSTITUTED METALLOPHTHALOCYANINES

3.1 Introduction

As with axially substituted metalloporphyrins the aim in devising reagents of this class is to have an axially substituted metallophthalocyanine whose functional group is either itself reactive, or alternatively sufficiently labile to allow the metal to be reactive, towards one or more fingerprint components. The targeted compound was dihydroxytin(IV)phthalocyanine, $PcSn(OH)_2$, (Figure 3.1), which was selected as it might show similar reactivity to $TPPSn(OH)_2$, (Chapter Two).

Another axially substituted metallophthalocyanine, chloroeuropium(III)phthalocyanine, PcEuCl, (Figure 3.1), underwent preliminary investigation. Interest in this particular compound was generated by the current research focus on lanthanide-based fingerprint reagents at this University [45] and elsewhere (Section 1.5.3). According to the literature [82] PcEuCl exhibits remarkable solubility in common solvents and strong luminescence, factors which indicate the potential promise of this compound.



(a) (b)

Figure 3.1 Structure of (a) $PcSn(OH)_2$ and (b) PcEuCl

3.2 Synthesis

3.2.1 $PcSnCl_2$

Dichlorotin(IV)phthalocyanine, ($PcSnCl_2$), the precursor of $PcSn(OH)_2$, was synthesised according to the literature method [115] as follows. 1,2-Dicyanobenzene (10.09 g, Aldrich Chemical Company Inc, 98%) and anhydrous stannous chloride (4.51 g, BDH Chemicals Ltd, GPR grade) were refluxed in 1-chloronaphthalene (125 mL, Aldrich Chemical Company Inc, ~90%) for 3 hours. The reaction mixture was left to cool and the solid subsequently collected by vacuum filtration. The solid was washed with benzene, 95% ethanol (drum grade), glacial acetic acid, and ethanol and then oven dried at 100°C, to give a sparkling purple/blue crystalline powder, approximate yield 84.6%.

3.2.2 $PcSn(OH)_2$

$PcSn(OH)_2$ was synthesised according to the literature method [115] with slight modification. $PcSnCl_2$ (1.50 g) was refluxed in ethanol (75 mL, BDH Laboratory Supplies, AR grade) and concentrated ammonia (75 mL, Ajax Chemicals, AR grade) for 5.75 hours. The reaction mixture was left to cool and the solid subsequently collected by vacuum filtration. The solid was washed with distilled water, ethanol, and benzene and then oven dried at 100°C, to give a blue/purple powder, approximate yield 96.2%.

3.2.3 PcEuCl

PcEuCl was synthesised according to reaction (a), Scheme 1.9 (Section 1.7.2). Europium chloride hexahydrate (0.82 g, Aldrich Chemical Company Inc, 99.99%) and 1,2-dicyanobenzene (1.62 g, Aldrich Chemical Company Inc, 98%) were heated gradually until refluxing in 1-chloronaphthalene (5 mL, Aldrich Chemical Company Inc, ~90%), refluxed for 4.5 hours and then left to cool overnight. The solid material was washed into a round bottom flask with ether, rotary evaporated to near dryness and subsequently oven dried at 100°C, to give a grey/green powder, approximate yield 45.2%.

3.3 Characterisation of $PcSn(OH)_2$

3.3.1 Absorption Spectra of $PcSn(OH)_2$

$PcSn(OH)_2$ exhibited a typical metallophthalocyanine absorption spectrum with an intense Q-band, a less intense Soret band, and another weak band. However $PcSn(OH)_2$ had very low solubility in a wide range of solvents, making molar extinction coefficient calculations unreliable. The results shown in Table 3.1 are the variation in absorption maxima wavelength with solvent.

Table 3.1 UV-Vis data for $PcSn(OH)_2$

Band	Q-band	Satellite band	Soret band
Solvent	λ (nm)	λ (nm)	λ (nm)
Chloroform	688.0	622.2	361.6
Dichloromethane	691.6	623.8	360.8
Dimethylsulfoxide†	698.8	628.4	357.2

†LR grade

3.3.2 Excitation and Emission Spectra of $PcSn(OH)_2$

Like the absorption spectra, collection of excitation and emission spectra were hampered by the very low solubility of $PcSn(OH)_2$. Spectra were collected in ethanol, noting that in this solvent there might have been some exchange between the hydroxy and ethoxy groups.

$PcSn(OH)_2$ exhibits three major excitation maxima at approximately 290, 418, and 444 nm (Figure 3.2), and a broad emission maximum at approximately 475 nm, along with a largely insignificant emission maximum at approximately 602 nm (Figure 3.3). Excitation at 418 and 444 nm gave the most intense emission spectra. However the 444 nm excitation band may be of limited practical use due to its small Stokes shift. Excitation at 290 nm gave a weak emission spectrum, which indicates that this excitation wavelength is probably of little or no practical use. The emission spectra indicate that the fluorescence of this compound should be blue in colour; no phosphorescence was detected.

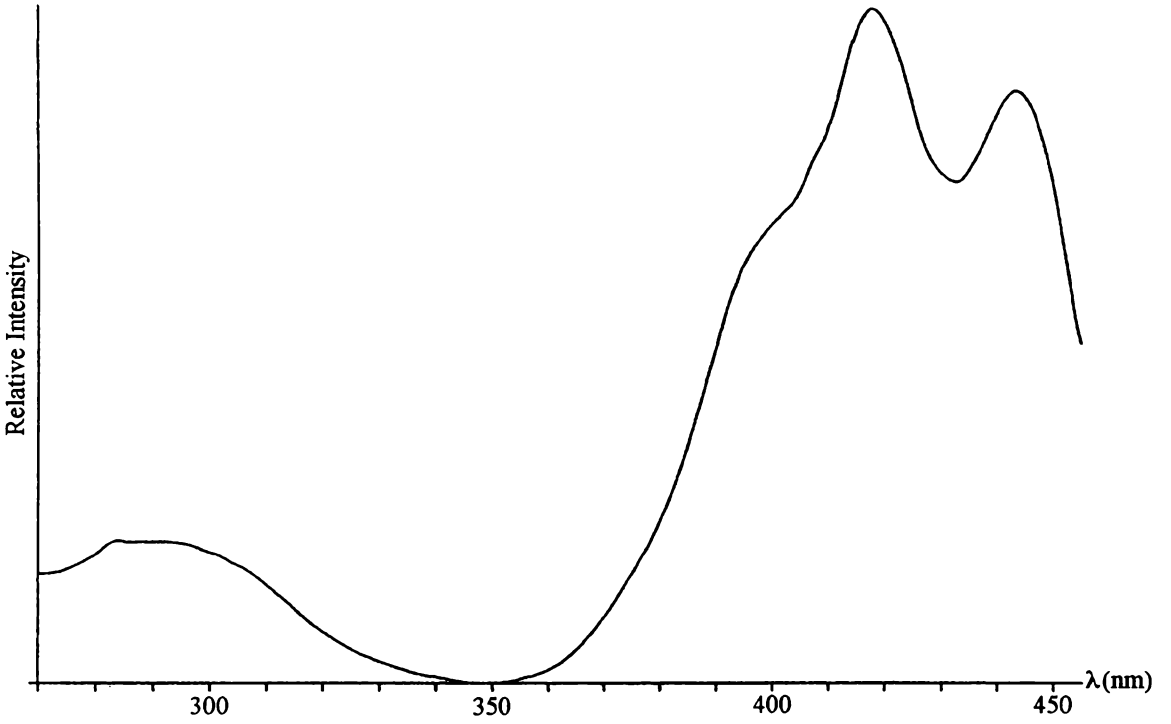


Figure 3.2 Excitation spectrum for the 475 nm emission of $PcSn(OH)_2$ in ethanol

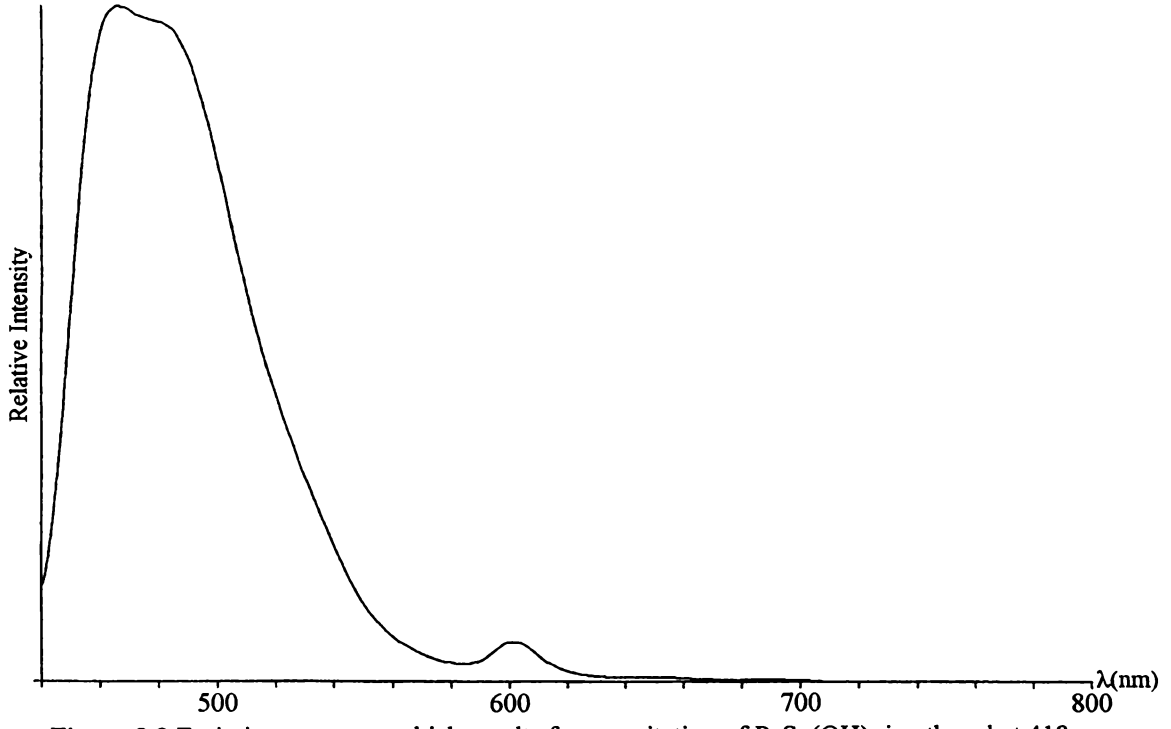


Figure 3.3 Emission spectrum which results from excitation of $PcSn(OH)_2$ in ethanol at 418 nm

3.3.3 ESMS of $PcSn(OH)_2$

ESMS of $PcSn(OH)_2$ were collected in methanol at cone voltages of 50 and 110 V. Unlike $TPPSn(OH)_2$, $PcSn(OH)_2$ did not exhibit sufficient solubility in acetonitrile/water for spectra to be collected. The spectrum in Figure 3.4 shows two major ions, $PcSn(OH)^+$ and $PcSn(OCH_3)^+$. In Figure 3.5 high resolution spectra of these two ions are shown, along with the calculated isotope patterns [110]. There is excellent agreement between the experimental and calculated data and hence confirmation of $PcSn(OH)^+$ and $PcSn(OCH_3)^+$.

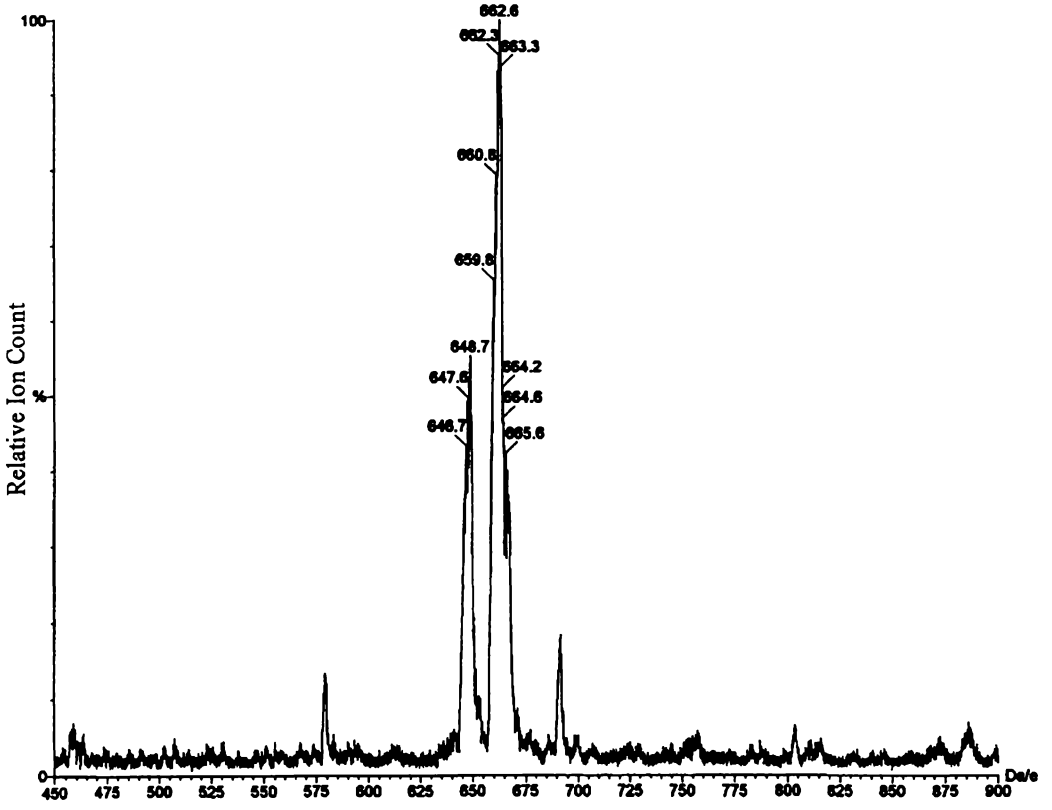


Figure 3.4 ESMS spectrum of $PcSn(OH)_2$ in methanol at a cone voltage of 50 V

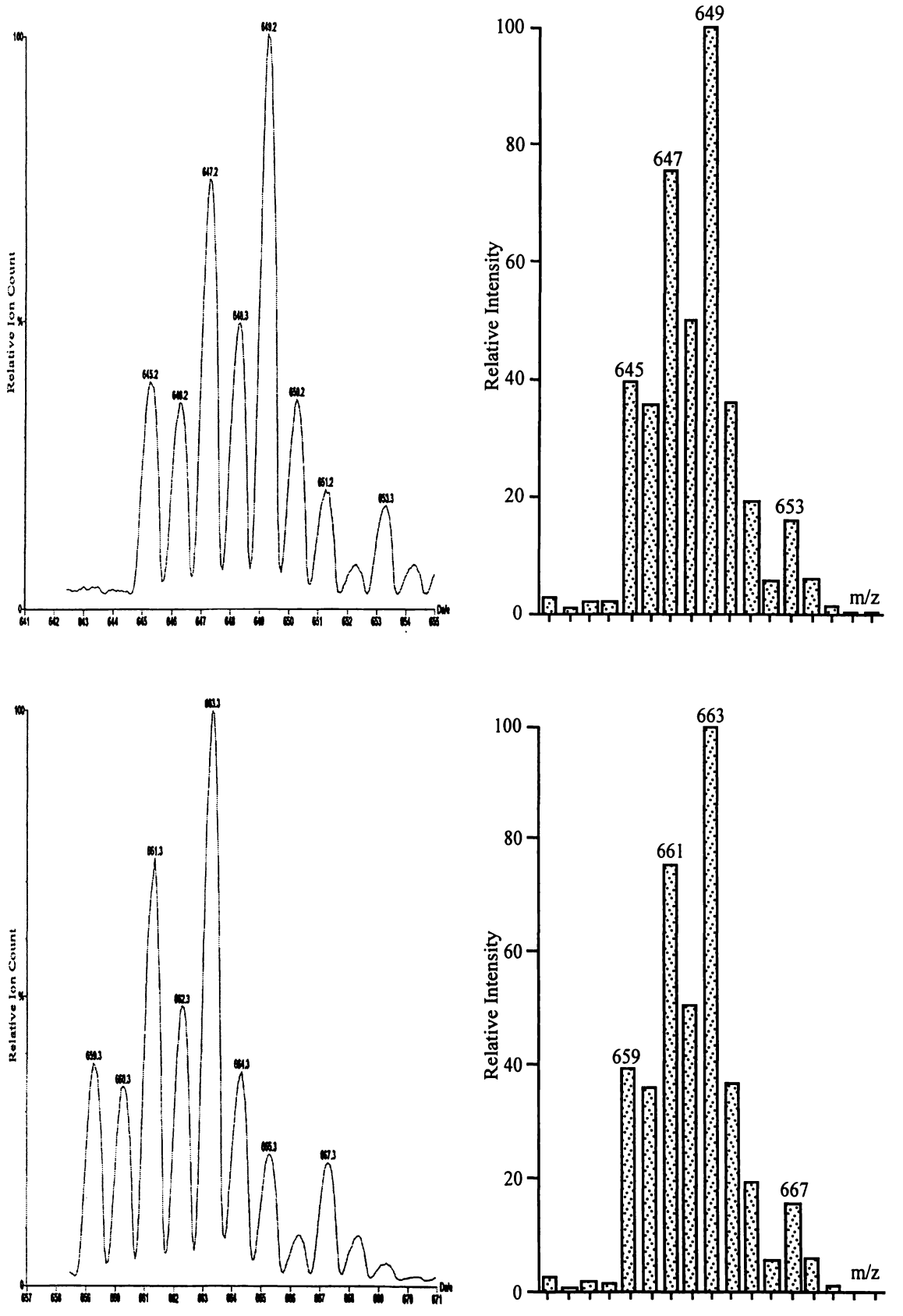


Figure 3.5 Comparison of high resolution ESMS spectra with calculated isotope patterns of $PcSn(OH)^+$ (top) and $PcSn(OCH_3)^+$ (bottom)

It is interesting to note that under ESMS conditions there is a qualitative difference in the labilities of the hydroxy groups between $TPPSn(OH)_2$ and $PcSn(OH)_2$. For

$TPPSn(OH)_2$ (Section 2.3.3) the $TPPSn(OCH_3)^+$ ion is the only species detected in methanol, $TPPSn(OH)^+$ is not seen. For $PcSn(OH)_2$ however, a variety of ions are detected. Not only are the $PcSn(OH)^+$ and $PcSn(OCH_3)^+$ ions seen, but at higher cone voltages $PcSn(OH)_2H^+$ and $PcSn^{\bullet+}$ ions are also seen (Figures 3.6, 3.7 and 3.8). The appearance of $PcSn^{\bullet+}$, a radical cation, while unusual is not without precedent. Radical cations are observed for metalloporphyrins under ESMS conditions [116, 117]. In this instance it is likely that the reduction of Sn from the +IV to the +II oxidation state would have occurred, followed by radical formation. Of more significance is that $PcSn(OH)^+$ and $PcSn(OH)_2H^+$ are seen. It is an indication that the hydroxy groups might be more securely bound to the Sn metal centre in the phthalocyanine than in the porphyrin. If this is the case then it would be anticipated that $PcSn(OH)_2$ might show less utility as a possible fingerprint reagent as it likely to be less reactive.

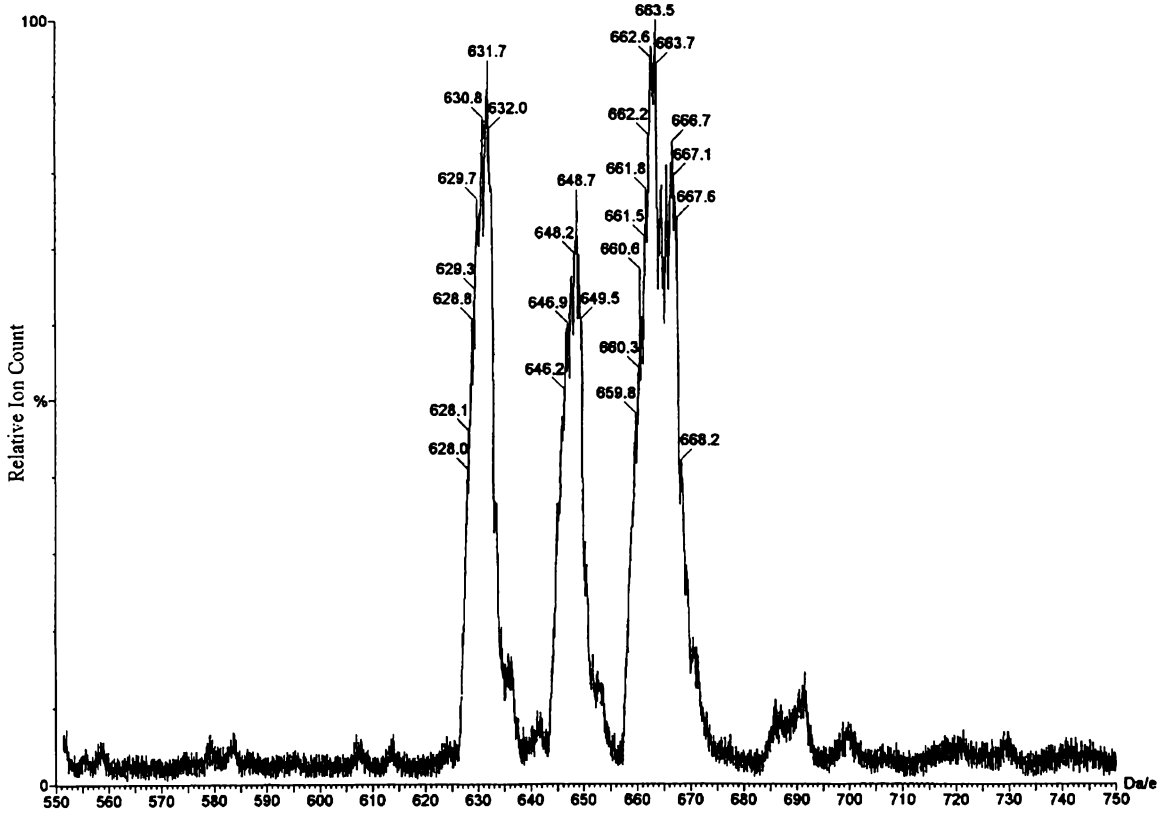


Figure 3.6 ESMS spectrum of $PcSn(OH)_2$ in methanol at a cone voltage of 110 V

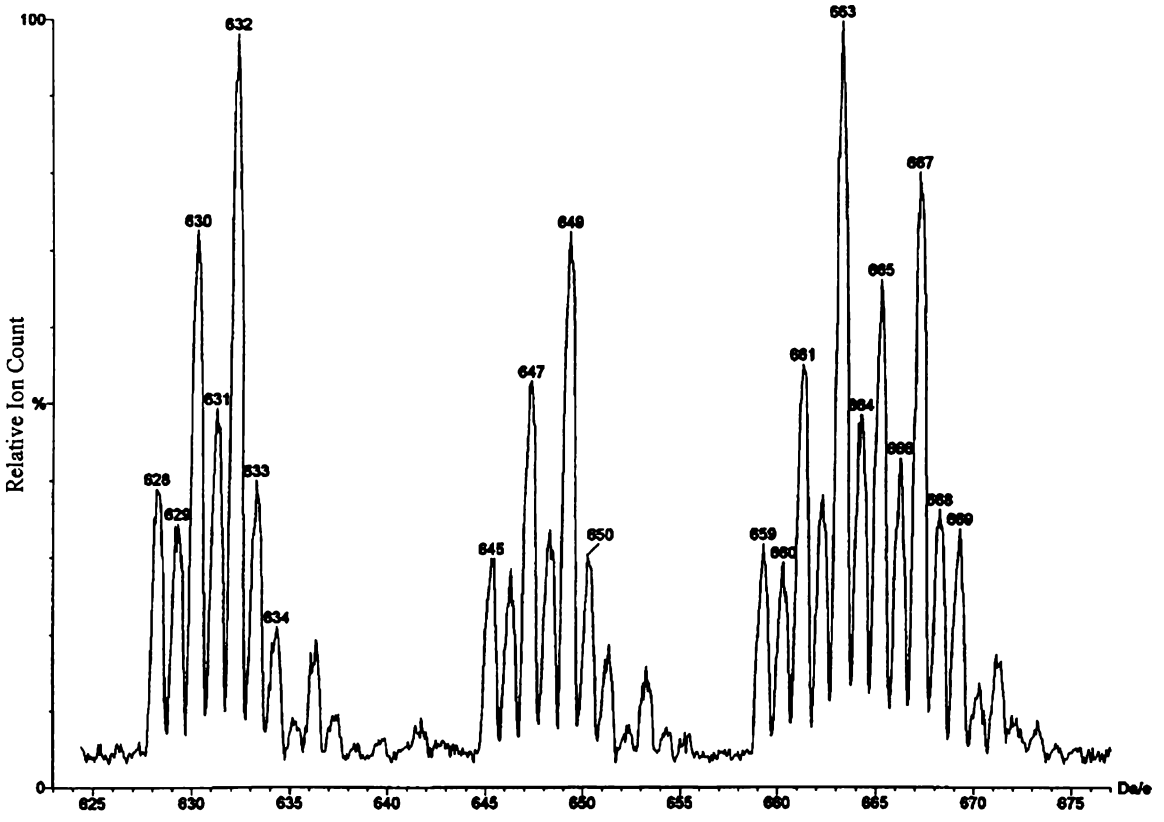


Figure 3.7 High resolution ESMS spectrum showing $PcSn^{\bullet+}$, $PcSn(OH)^+$, $PcSn(OCH_3)^+$, and $PcSn(OH)_2H^+$

Confirmation of the ions seen in Figure 3.7 is given by the calculated isotope patterns shown in Figure 3.8, except for $PcSn(OH)^+$ for which the calculated isotope pattern is already shown in Figure 3.6. The co-occurrence of $PcSn(OCH_3)^+$ and $PcSn(OH)_2H^+$ was elucidated by the calculation of the combined relative abundances of both ions, with the assumption that both ions were equally abundant. The calculated ratios are shown in Table 3.2.

Table 3.2 Calculated isotope ratios for $TPPSn(OCH_3)^+$ and $TPPSn(OH)_2H^+$[110]

m/z	$TPPSn(OCH_3)^+$	$TPPSn(OH)_2H^+$	Combined	Normalised
655	2.64		2.64	1.89
656	1.06		1.06	0.76
657	2.06		2.06	1.48
658	1.83		1.83	1.31
659	39.33	2.65	41.98	30.10
660	36.01	1.04	37.05	26.56
661	75.39	2.06	77.45	55.53
662	50.53	1.81	52.34	37.53
663	100.00	39.47	139.47	100.00
664	36.94	35.73	72.67	52.10
665	19.36	75.38	94.74	67.93
666	5.84	49.98	55.82	40.02
667	15.84	100.00	115.84	83.06
668	6.08	36.11	42.19	30.25
669	1.21	19.24	20.45	14.66
670	0.16	5.73	5.89	4.22
671	0.02	15.88	15.90	11.40
672		5.95	5.95	4.27
673		1.18	1.18	0.85
674		0.16	0.16	0.11
675		0.02	0.02	0.01

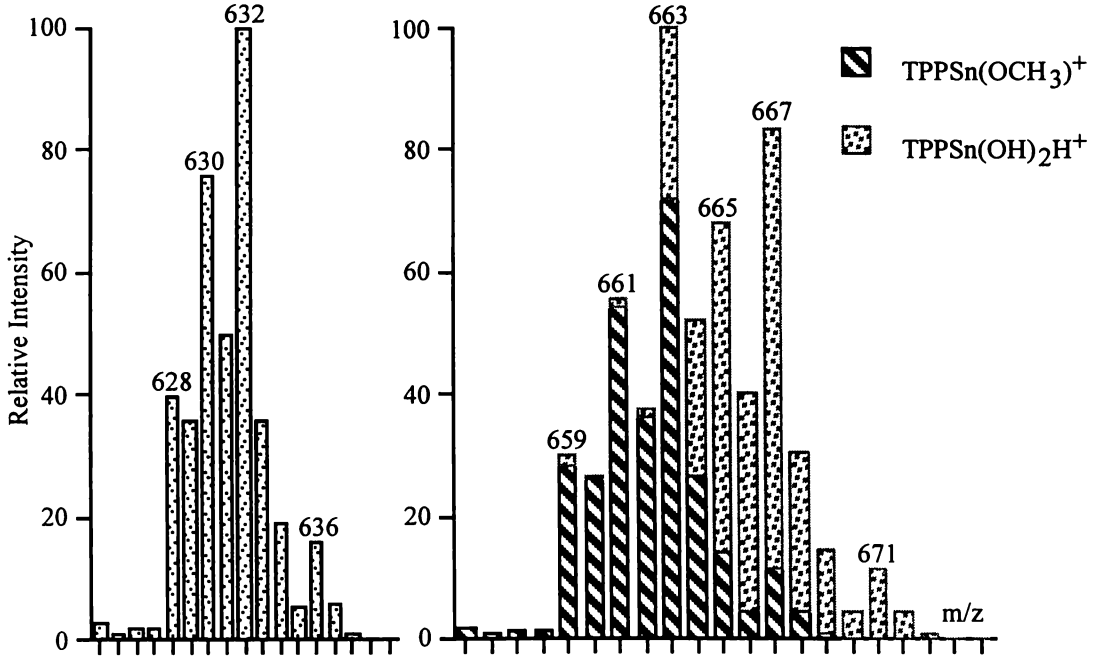


Figure 3.8 Calculated isotope patterns for $PcSn^{\bullet+}$ (left) and $PcSn(OCH_3)^+/PcSn(OH)_2H^+$ (right)

3.4 Fingerprint Screen of $PcSn(OH)_2$

3.4.1 Solubility of $PcSn(OH)_2$

Before undertaking a fingerprint screen it was necessary to determine what solvents $PcSn(OH)_2$ was soluble in. Test-tubes containing a small amount of $PcSn(OH)_2$ were prepared. To these was added approximately 1-2 mL of solvent, the solubility was gauged, the test-tubes were then sonicated for 30 seconds and the solubility again determined. The results obtained are shown in Table 3.3.

Table 3.3 Solubility of $PcSn(OH)_2$

Solvent	Solubility Upon Standing	Colour of Solution	Solubility After Sonication	Colour of Solution
Distilled water	X	-	X	-
Methanol	√	very pale blue	√	pale blue
Ethanol	√	very pale blue	√	pale blue
Acetonitrile	X	-	√	pale blue
Acetone[◊]	X	-	√	very pale blue
Tetrahydrofuran	X	-	X	-[†]
Chloroform	X	-	X	-[†]
Dichloromethane	X	-	√	pale blue
Toluene	X	-	X	-
Heptane	X	-	√	pale blue
Dimethylsulfoxide	√	pale blue	√	pale blue
Dimethylformamide	√	pale blue	√	pale blue[‡]
Ethyl acetate	√	very pale blue	√	pale blue[‡]
1-Chloronaphthalene	√	pale blue	√	pale blue

[†]note pale blue solutions after being left to stand overnight

[‡]solutions appeared to get darker but this may have been due to a very fine suspension giving an illusion of a darker solution

[◊]drum grade

None of the examined solvents solubilised $PcSn(OH)_2$ to any great extent. However as ethanol had been used successfully with $TPPSn(OH)_2$ it was selected as the solvent for use with $PcSn(OH)_2$.

3.4.2 Fingerprint Screen Experiments

A fingerprint screen was carried out in exactly the same manner as that described for $TPPSn(OH)_2$ in Section 2.5, with the addition of two further soaking times of 30 and 60

minutes. These longer times were included to take account of the low solubility of $PcSn(OH)_2$. A saturated solution of $PcSn(OH)_2$ in ethanol was used as the reagent solution, also to maximise the $PcSn(OH)_2$ available.

The following results were obtained. In ambient light no spots were visible on the drag or 2 minute samples whether they were air or oven-dried. Spots of a very pale blue colour were faintly apparent on the 10, 30, and 60 minute samples, both air and oven-dried. However they were difficult to see due to obliteration by undissolved $PcSn(OH)_2$. Hence the experiment was repeated, still using a saturated $PcSn(OH)_2$ ethanol solution, however this time no undissolved material was present in the soaking vessel.

Again in ambient light no spots were visible on the drag or 2 minute samples whether they were air or oven-dried. On the 10 minute samples very pale blue spots were faintly visible, with the oven-dried sample having marginally darker spots. For the 30 and 60 minute samples pale blue spots were more visible, with the oven-dried samples again being slightly darker, though there was little difference between the two treatment times. Samples were also examined under a UV lamp (Universal UV Lampe, CAMAG) at 254 nm, though it should be noted that this wavelength is fairly well removed from what is the weakest excitation wavelength of $PcSn(OH)_2$. Spots were visible as slightly darker regions on a dull, dark purple background, which were marginally more apparent on the oven dried samples. Spots were visible for all treatment times, though they were the least distinct for the drag samples. As spots had been weakly visible in ambient light and under UV illumination it was decided to see if this reagent would develop actual fingerprints.

3.5 Development of Fingerprints with $PcSn(OH)_2$

3.5.1 Initial Experiment

Fingerprints were collected on CopyRight 80 gsm white paper. They were soaked in a saturated ethanol solution of $PcSn(OH)_2$ for either 60 or 120 minutes, air-dried or oven-dried for 10 minutes at approximately 70°C, and then examined in ambient light. Very faint, partial ridge detail was seen on one 60 minute air-dried sample and three 120 minute air-dried samples, though it must be stated that in all instances the quality was extremely poor and clearly unsuitable for operational use.

The ability of $PcSn(OH)_2$ to successfully develop fingerprints was thought to be hindered by its low solubility. This was supported by the fingerprint screen (Section 3.4.2) requiring extended soaking times to give suitably resolved spots. Trying to increase the concentration of the reagent solution to improve the results obtained was deemed appropriate and some mixed solvent systems were investigated.

3.5.2 Mixed Solvent Systems for $PcSn(OH)_2$

Two mixed solvent systems were examined to determine whether the concentration of $PcSn(OH)_2$ in solution could be increased; percentages are by volume:

(i) dichloromethane with 10% methanol and

(ii) ethanol with 1% acetic acid.

Small amounts of $PcSn(OH)_2$ were added to vials containing 10 mL of the mixed solvents. Upon standing (i) was almost as blue as the reference ethanol solution and (ii) was paler than the reference ethanol solution. The vials were then sonicated for approximately 10 minutes, immediately subsequent to this, and after standing for 30 minutes, both solutions were darker than the reference ethanol solution. The solutions were then left to stand overnight. There was a noticeable colour change in both

solutions, (i) was now more green than blue and (ii) was slightly blue/green. There was little undissolved material for (i), whereas for (ii) settling of the undissolved material had occurred and the solution was as pale as the reference ethanol solution in terms of depth of colour.

With these results it was decided to undertake a further fingerprint screen experiment using the dichloromethane with 10% methanol mixed solvent system.

3.5.3 Further Fingerprint Screen

A fingerprint screen was carried out in exactly the same manner as that described in Section 2.5, using a saturated solution of $PcSn(OH)_2$ in dichloromethane with 10% methanol. Samples were then examined in ambient light and under UV illumination.

The results obtained are as follows. In ambient light the drag samples spots were not particularly obvious, on the 2 minute soak samples spots were faint but visible, and on the 10 minute soak samples spots were visible (Figure 3.9). The spots were a pale blue/green colour with no difference between air and oven-dried samples, though the oven-dried samples did have a slightly more coloured background. It should also be noted that the 2 minute soak samples had spots that were as dark, if not slightly darker, than those obtained with a 60 minute soak in the saturated ethanol solution. Under UV illumination at 254 nm the results obtained were almost identical to those obtained for the original fingerprint screen of $PcSn(OH)_2$ (Section 3.4.2). Glycine spots appeared as darker regions on a dull, dark purple background, with the spots being darker than those obtained in the original screen, that is increased resolution between the developed spots and the background.

These results indicated that the mixed solvent system was successful in delivering more $PcSn(OH)_2$, so it was decided to attempt to develop fingerprints using this system.

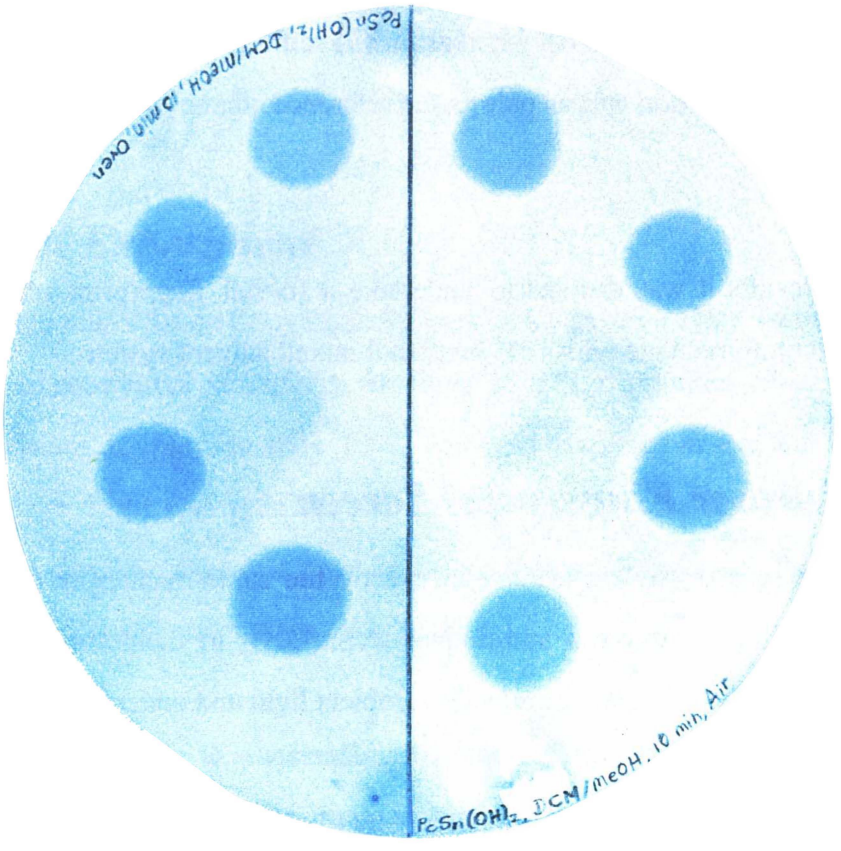


Figure 3.9 Glycine spots developed by a 10 minute soak in a saturated 90:10 CH_2Cl_2:MeOH $PcSn(OH)_2$ solution

3.5.4 Further Fingerprint Development Experiment

Fingerprints were collected on white paper. They were soaked in a saturated dichloromethane with 10% methanol solution of $PcSn(OH)_2$ for either 10 or 60 minutes, air-dried, and then examined in ambient light. No fingerprints were detected on any samples. It was thought that these negative results might be due to the solvent system solubilising the fingerprints and removing them from the paper. Chloroform, which is similar in nature to dichloromethane, has been documented to solubilise certain components of latent fingerprints [118]. Therefore it was decided to undertake an experiment to determine if the dichloromethane/methanol solvent system was having a similar effect.

3.5.5 Effect of Solvent System on Fingerprints

Fingerprints were collected on white paper. They were soaked in dichloromethane with 10% methanol for either 10 or 60 minutes and then left to air-dry, samples were then treated with iodine (Appendix I). No fingerprints were detected, apart from a control print to confirm that the iodine technique was working. The samples were then left for 6.5 hours to allow any residual iodine to diffuse out. Samples were then treated with ninhydrin (Appendix I). Ridge detail was developed on all samples.

These results indicated that the dichloromethane with 10% methanol solvent system was removing the non-water soluble fingerprint components, as iodine is considered to be a reagent that reacts with these types of components (Section 1.4.8). To confirm this regarding iodine a further experiment was undertaken.

Fingerprints were collected on white paper, from donors who had rubbed their thumbs on their faces, and then cut in half (Figure 2.9). One group of halves were soaked and gently agitated in distilled water for 10 minutes, air-dried, and then treated with iodine (Appendix I). The other group of halves were treated with iodine immediately. Both sets of halves developed prints with iodine, though the non-soaked prints were of marginally better quality. Samples were left overnight to allow the iodine to diffuse out of the prints. They were then treated with ninhydrin (Appendix I). The non-soaked halves were well developed by ninhydrin in all instances. In contrast the soaked halves were poorly developed, if developed at all by ninhydrin. These results were consistent with the notion that iodine develops latent fingerprints by interaction with the non-water soluble components.

These results confirmed that dichloromethane with 10% methanol was removing the non-water soluble components of fingerprints. So while this mixed solvent system was increasing the amount of $PcSn(OH)_2$ in solution it was simultaneously removing part of the fingerprint. The net consequence was no improvement in the performance of $PcSn(OH)_2$ as a fingerprint reagent in the mixed solvent system of dichloromethane with

10% methanol. After these results it was decided not to carry out any further fingerprint investigations with $PcSn(OH)_2$.

3.6 Mode of Action of $PcSn(OH)_2$

While very limited success was obtained with $PcSn(OH)_2$ in the development of fingerprints, a mode of action can still be described. It is likely that $PcSn(OH)_2$ reacts with both amino and fatty acids in a manner analogous to that of $TPPSn(OH)_2$. That is $PcSn(OH)_2$ should react according to the following:

$$PcSn(OH)_2 + 2RCOOH \rightarrow PcSn(OOCR)_2 + 2H_2O$$

with the amino and fatty acids probably bonded to the Sn metal centre though the oxygen of the carboxyl groups.

Reaction with amino acids was shown by the positive glycine fingerprint screen results obtained (Sections 3.4.2 and 3.5.3). Confirmation of reactivity towards fatty acids was procured by undertaking a similar experiment to that carried out with $TPPSn(OH)_2$. $PcSn(OH)_2$ and oleic acid (May & Baker, 99%, Figure 2.14) were magnetically stirred for 24 hours in dimethylformamide (BDH Laboratory Supplies, GPR grade), the reaction mixture was then examined by ESMS.

ESMS results were clearly indicative of a reaction between $PcSn(OH)_2$ and oleic acid. The spectrum in Figure 3.10 shows one major ion, $PcSn(O_2C_{18}H_{33})^+$ and in Figure 3.11 the high resolution spectrum is shown, along with the calculated isotope pattern. There is excellent agreement between the experimental and calculated data and hence confirmation of $PcSn(O_2C_{18}H_{33})^+$. These results are support for the notion that $PcSn(OH)_2$ has a mode of action that parallels $TPPSn(OH)_2$.

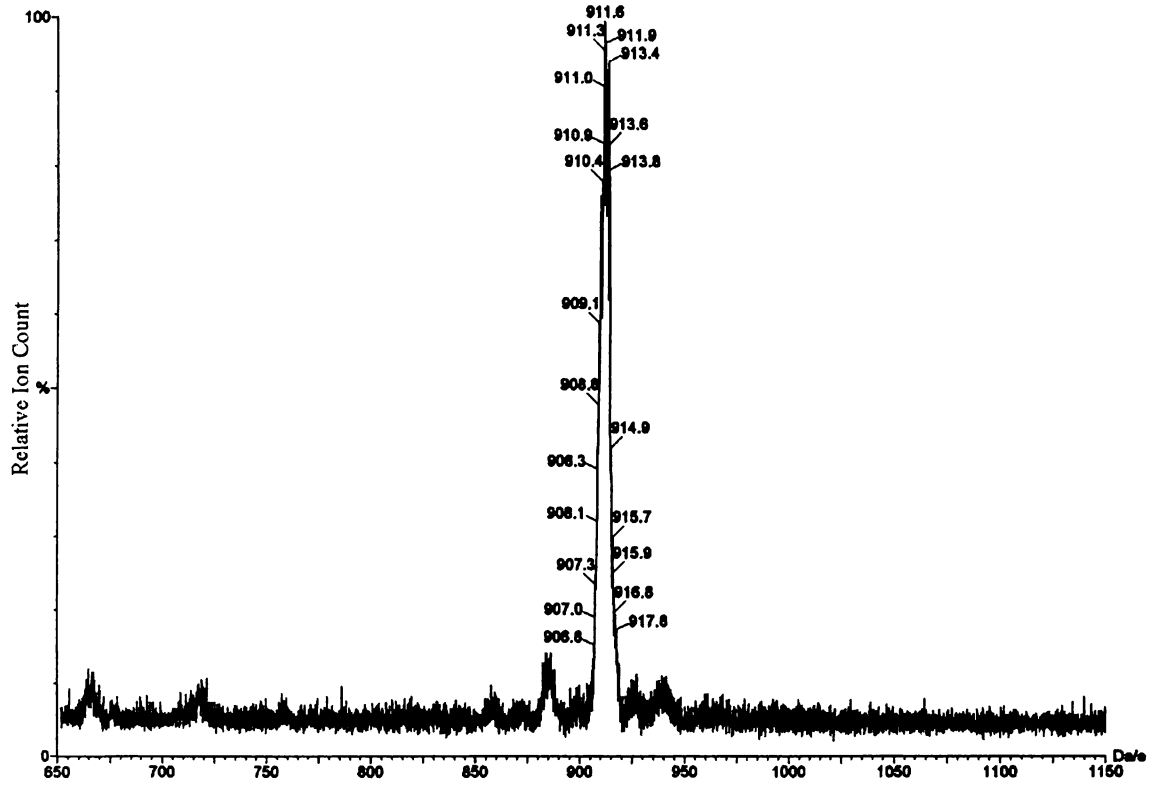


Figure 3.10 ESMS spectrum of $PcSn(OH)_2$ - oleic acid derivative in acetonitrile at a cone voltage of 90V

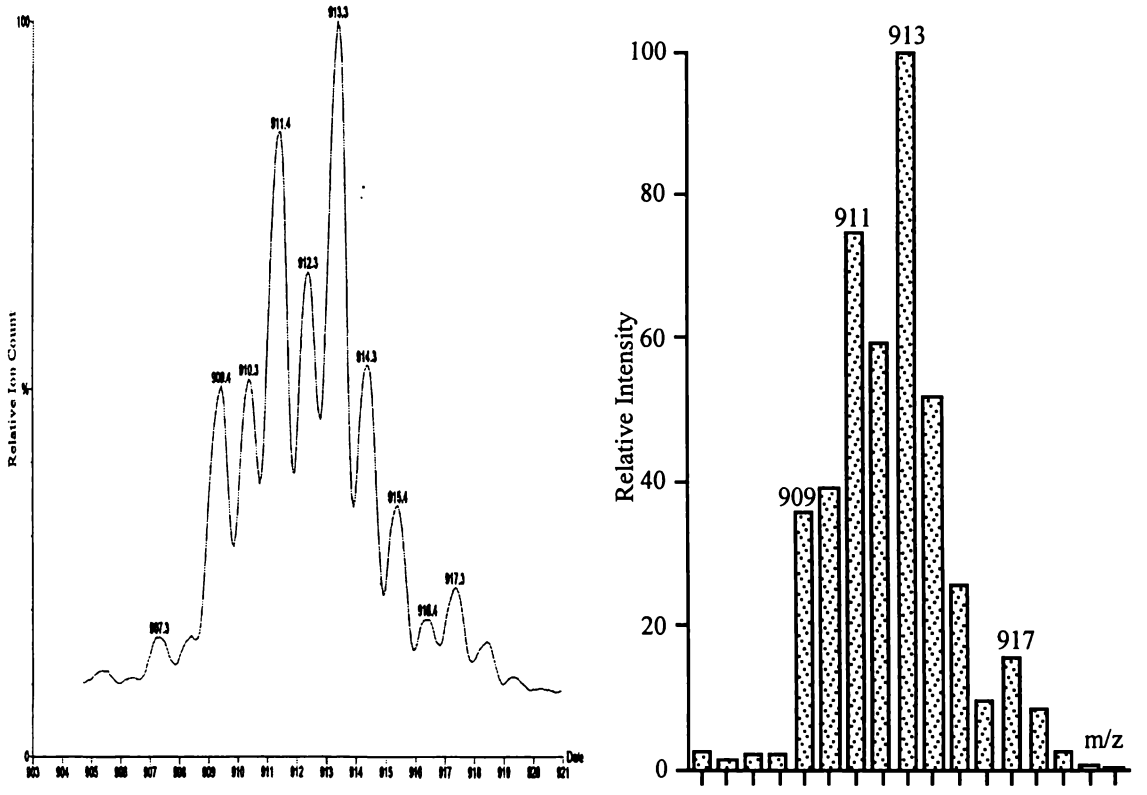


Figure 3.11 Comparison of high resolution ESMS spectrum of $PcSn(O_2C_{18}H_{33})^+$ with calculated isotope pattern of $PcSn(O_2C_{18}H_{33})^+$

3.7.1 Absorption Spectra of PcEuCl

UV-Vis spectra of PcEuCl were collected in 1-chloronaphthalene (~90%), ethanol and tetrahydrofuran. The 1-chloronaphthalene spectrum showed good agreement with the literature [82], the Q-band occurring at 672 nm. In ethanol a very similar spectrum was obtained, with the Q-band occurring at 668 nm. Sufficient PcEuCl was soluble in tetrahydrofuran to allow the calculation of the molar extinction coefficients and these are the results presented in Table 3.4.

Table 3.4 UV-Vis data for PcEuCl

Band	λ (nm)	ε (L mol^{-1} cm^{-1})
Q	667.2	11000 ± 1000
Satellite 1	601.4	3100 ± 300
Satellite 2	457.6	3200 ± 300
Soret	319.6	10000 ± 1000

3.7.2 Excitation and Emission Spectra of PcEuCl

Luminescence spectra of PcEuCl were collected in ethanol and methanol, and were essentially identical. It should be noted that for some spectra 5 nm excitation and emission slit widths were used.

PcEuCl exhibits two excitation maxima at approximately 247 and 348 nm (Figure 3.12). Excitation at either wavelength gave a strong, broad emission at approximately 435 nm along with a very weak emission at approximately 840 nm (Figure 3.13). Excitation at 348 nm gave the most intense emission. It should be noted that both excitation wavelengths coincide quite well with the 254 and 350 nm emissions of a UV lamp, a factor which may be of some practical use.

It is interesting to note that the detected luminescence of PcEuCl is not completely typical of that normally exhibited by lanthanide complexes. Generally lanthanide complexes, particularly those conjugated with organic ligands, under UV irradiation exhibit strong luminescence. The luminescence is generally sharp and for Eu^{3+} normally occurs in the red, specifically 579, 592, and 615 nm, with the latter emission being the

most intense. In this instance excitation does occur in the UV and the luminescence is strong, however in contrast the luminescence is rather broad and not in the red. These differences are probably an example of how the phthalocyanine macrocyclic ring dominates the characteristics exhibited, whereas the metal centre modifies the characteristics seen, which is consistent with the trends seen in absorption spectra of metallophthalocyanines. [39, 119]

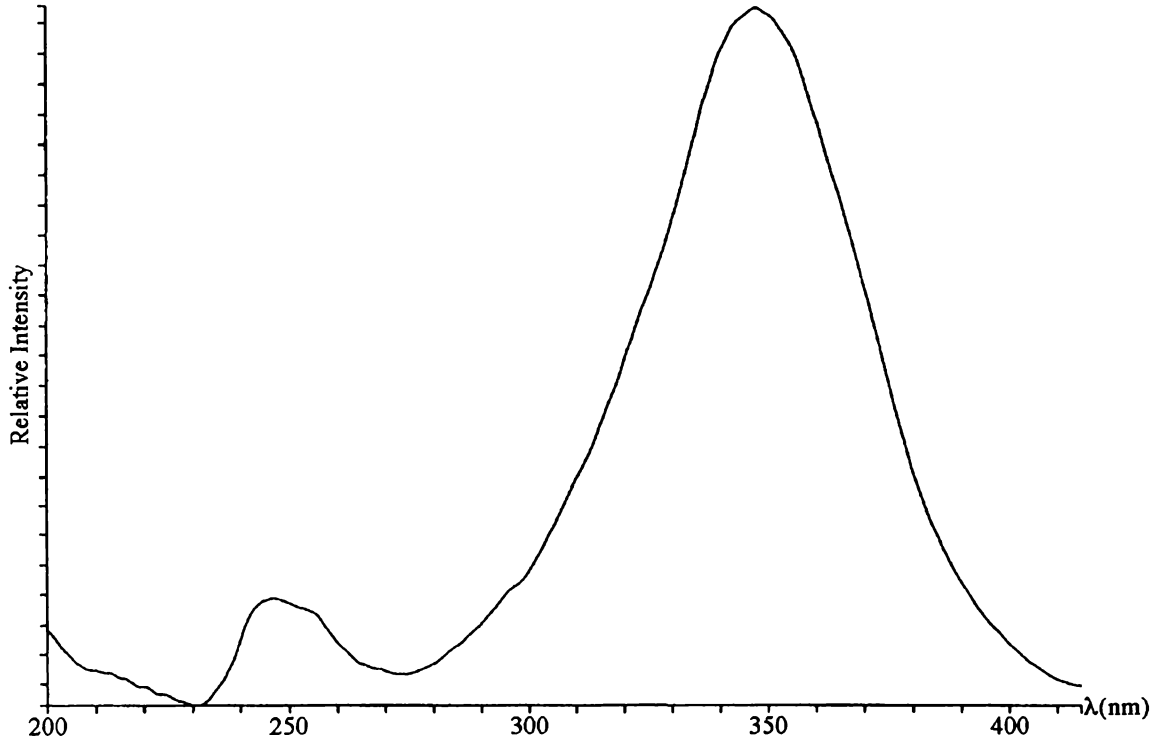


Figure 3.12 Excitation spectrum for the 435 nm emission of PcEuCl in methanol

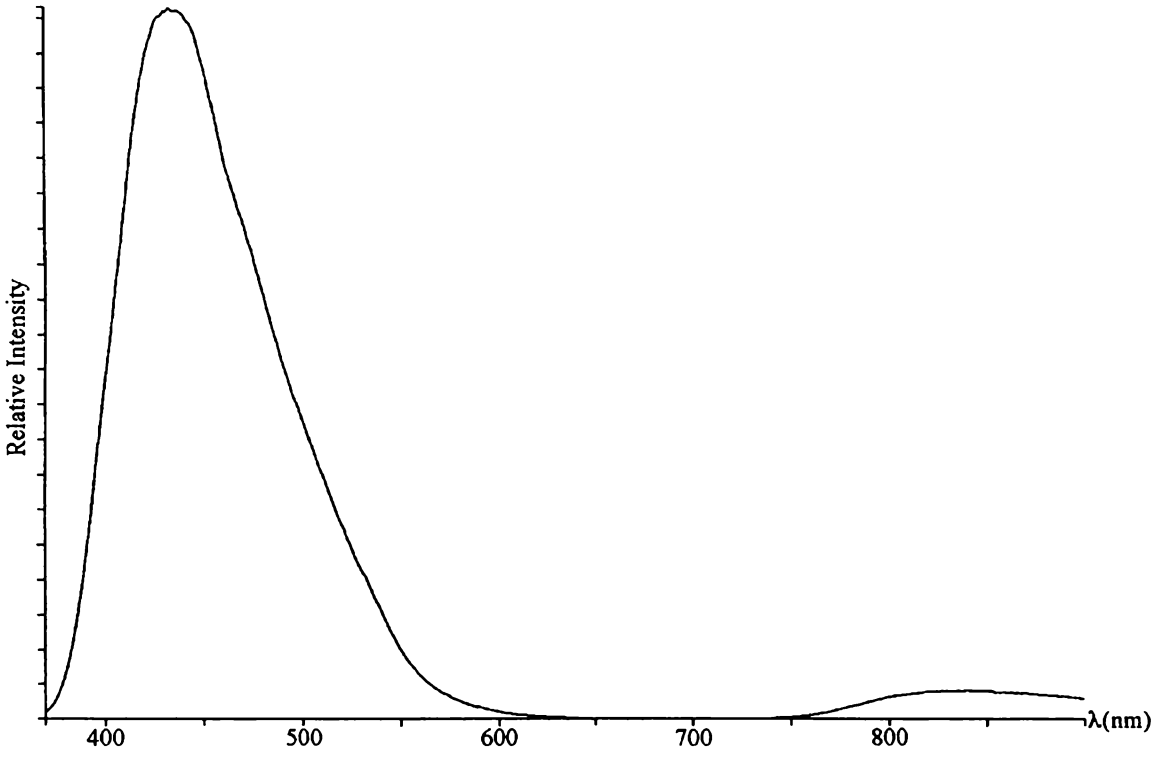


Figure 3.13 Emission spectrum which results from excitation of PcEuCl in methanol at 348 nm

Another attribute of Eu^{3+} is that the luminescence has a relatively long decay time, approximately 0.4 ms, which makes time-resolved imaging a possibility. The decay of PcEuCl luminescence was measured, and a plot of the decay curve (Figure 3.14) along with a linear decay plot (Figure 3.15) are shown. The linear decay plot is obtained through plotting ln(t) versus relative intensity, assuming exponential decay. The line has the following equation:

$$\ln I_t = -0.434t/\tau + \log I_o$$

where I_t = luminescence intensity at time t

t = delay time (ms)

τ = lifetime

I_o = luminescent intensity at time zero.

The gradient of the plot is $0.434/\tau$, which allows the calculation of the lifetime. The fitted lines in Figure 3.14 have a mean slope of 232.77 which gives $\tau = 0.0019$ ms, i.e. $\tau \approx 2$ μs, which translates to PcEuCl having a very short lifetime which is probably of limited practical use. [37, 81]

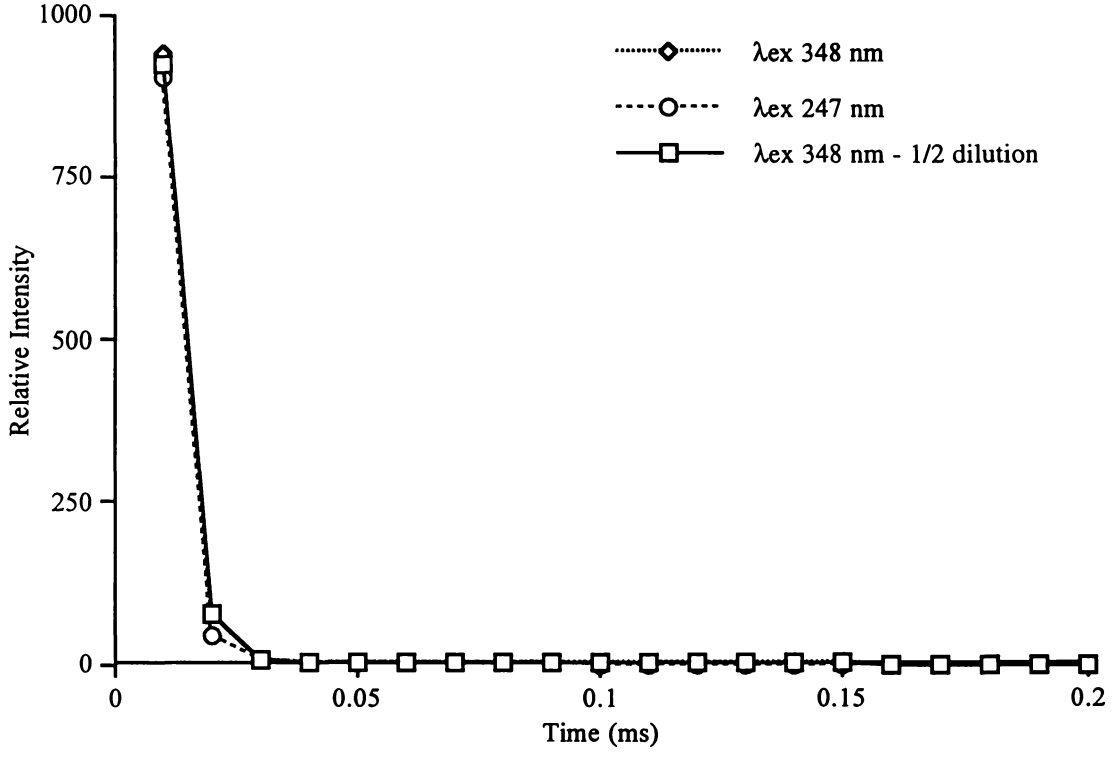


Figure 3.14 Decay plot of PcEuCl in methanol

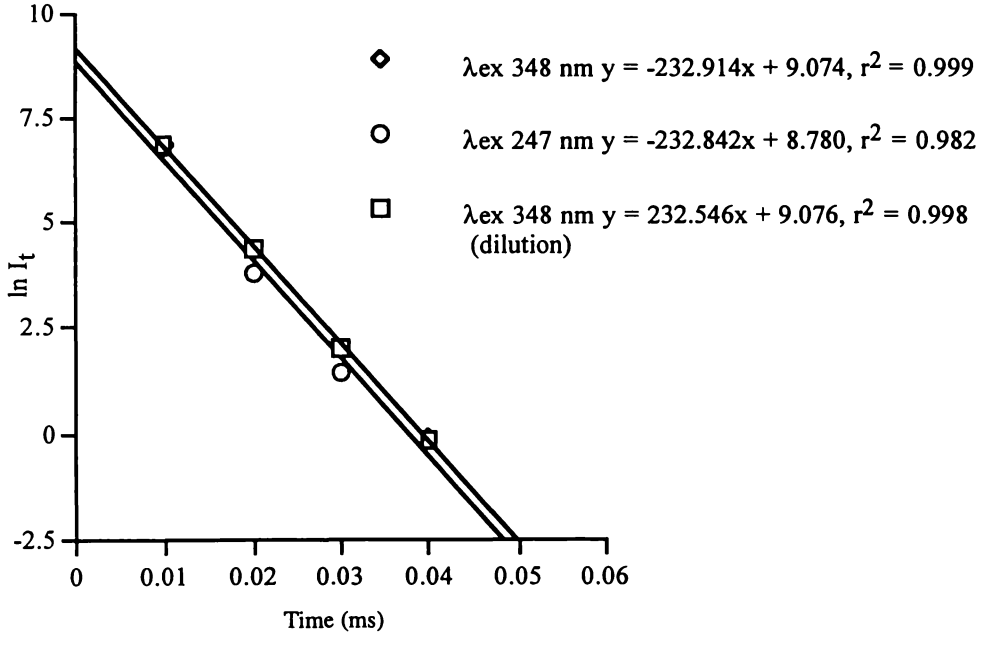


Figure 3.15 Linear plot of decay of PcEuCl in methanol

3.7.3 ESMS Of PcEuCl

PcEuCl was not found to be amenable to analysis by ESMS. A variety of solvents, methanol, methanol/water, acetonitrile, acetonitrile/water, and 1,2-dichloroethane, along with a range of cone voltages were examined. However no reliable spectra were obtained.

3.8 Fingerprint Screen of PcEuCl

3.8.1 Solubility of PcEuCl

As for $PcSn(OH)_2$, before undertaking a fingerprint screen with PcEuCl, it was necessary to determine what solvents PcEuCl was soluble in. Test-tubes containing a small amount of PcEuCl were prepared. To these was added approximately 1-2 mL of solvent, the solubility was gauged, the test-tubes were then sonicated for 1 minute and the solubility again determined. The results obtained are shown in Table 3.5.

Table 3.5 Solubility of PcEuCl

Solvent	Solubility Upon Standing	Colour of Solution	Solubility After Sonication	Colour of Solution
Distilled water	X	-	X	-
Methanol	√	very pale green	√	pale green
Ethanol	√	very pale green	√	very pale green
Acetonitrile	X	-	X	-
Acetone[◊]	√	very pale green	√	pale green
Tetrahydrofuran	√	pale green	√	darker green[†]
Chloroform	√	pale green	√	darker green[‡]
Dichloromethane	√	pale green	√	darker green[‡]
Toluene	√	very pale green	√	pale green
Heptane	X	-	X	-
Diethyl ether	X	-	X	-
Petroleum spirits	X	-	X	-
Ethyl acetate	√	very pale green	√	pale green

[†] quite a bright green

[‡] quite a dull green compared to that obtained in tetrahydrofuran

[◊] drum grade

3.8.2 Fingerprint Screen Experiment

A fingerprint screen was carried out in exactly the same manner as that described in Section 2.5. A saturated solution of PcEuCl in tetrahydrofuran was used as the reagent solution. This solvent was selected as it had exhibited one of the best results in the solubility tests. Chloroform and dichloromethane, while also exhibiting good solubility of PcEuCl, were avoided as previous work (Section 3.5.5) had indicated that these solvents were detrimental to certain fingerprint components.

The following results were obtained. In ambient light spots were very faintly apparent on the drag samples, with no obvious difference between the oven and air-dried samples. Very similar results were obtained for the 2 minute samples, with the spots marginally more apparent on the air-dried samples. No spots were discernible on the 10 minute samples. All samples were a very pale green colour, with no noticeable difference in colour with either soaking time or drying treatment.

Samples were then examined under a UV lamp. At 254 nm spots were absolutely obvious on all samples and pale purple/white in colour, with a faint background colouration. Oven-dried samples had spots that were slightly more luminescent than the air-dried samples, without a noticeable increase in the background luminescence. The 2 and 10 minutes samples appeared equivalent in intensity of luminescence, the drag samples were marginally less luminescent than the 2 and 10 minute samples. Examination under 350 nm gave very similar results, though the spots were of a duller appearance.

From the PcEuCl excitation spectrum (Figure 3.12) it might have been expected that the intensity of luminescence would be far greater for excitation at 350 nm. The lower intensity of emission seen is likely to be due to the relative intensity of the excitation wavelengths. For mercury the quantitative estimate of relative line strength indicates that the intensity of the 254 nm emission is approximately 40 times that of the 345 nm emission [120]. Therefore greater luminescence should be seen from a light source that has greater intensity of emission at 350 nm.

These results indicate that PcEuCl might develop latent fingerprints. However work with $ZnPc(SO_2Cl)_4$ (Section 4.10) had shown that detection of any developed glycine spots and/or fingerprints on white paper under UV illumination was at the most very limited, and more likely not possible. To determine if this was the case with PcEuCl another fingerprint screen experiment, as described in Section 2.5, was carried out on CopyRight 80 gsm white paper using a saturated tetrahydrofuran solution.

The results obtained are as follows. In ambient light no spots were visible on any combination. Under UV illumination at both 254 and 350 nm no spots were discernible on any combination either. At 350 nm all that was seen was the luminescence of the white paper, the treated paper was slightly duller. At 254 nm a similar result was obtained, though the difference between the treated and untreated white paper was more marked. This difference is probably related to the differing intensities of the two excitation wavelengths as earlier stated.

Unfortunately however this result indicated that the development of visible fingerprints, whether in ambient light or under UV illumination, by PcEuCl on white paper was unlikely. Not only is there strong background interference from the paper, but there is also a suggestion of a lack of specificity. The lack of specificity would not be unreasonable as paper has a large content of hydrated cellulose fibres which contain hydroxy groups [51], which Eu would also have an affinity for. These results indicated that more thorough investigation and further work are required if any use is to be made of the attributes that PcEuCl does exhibit, if it is to have a role in fingerprint development.

3.9 Mode of Action of PcEuCl

While only a preliminary investigation of PcEuCl was carried out, the results obtained, along with the literature regarding europium complexes, enables a mode of action to be proposed.

The bulk of lanthanide chemistry revolves around the +III oxidation state, with the bonding being predominantly ionic in character as a result of the large sizes of the lanthanide ions. Consequently the cations display a preference for *O*-donor ligands. Furthermore high coordination numbers are generally exhibited. Coordination numbers below 6 are only found with very bulky ligands, and coordination numbers of 7, 8, and 9 are typically characteristic. [119, 121, 122] From this it is possible to deduce that the positive glycine fingerprint screen results might be attributable to the glycine molecules attaching to the europium metal centres through the carboxyl groups, as opposed to through the nitrogen. Salts of acids such as oxalic, citric, and tartaric acid exhibit such behaviour, with the anions acting as chelating *O* ligands [121], and such lanthanide complexes are considered to be the most stable and common [122]. The reasoning behind carboxyl attachment in preference to attachment through lone pair donation from the nitrogen in glycine is the same as why *O*-donors are preferred to *N*-donors in lanthanide complexes. More negative character can be displayed, that is a negatively charged species can be formally generated which can participate in ionic bonding. Whether the chloride ion would remain attached or be displaced is a further question. Coordination by halide ions is considered to be rather weak [121] so speculation could be made either way.

To help confirm the suggested mode of action a fingerprint screen experiment, as described in Section 2.5, using a 1 % (v/v) oleic acid solution in chloroform, instead of a glycine solution, was carried out. The reagent solution was a saturated PcEuCl tetrahydrofuran solution. The following results were obtained.

No spots were visible in ambient light on any combination. Under UV illumination at 254 nm spots were clearly visible on the drag samples, though it was noted that the spots were not sharply defined, that is they had blurry edges. There was no significant difference between the air and oven-dried samples. No spots were visible on the 2 and 10 minute samples. Identical results were obtained under illumination at 350 nm, though the luminescence was much reduced. It should be noted that the inherent luminescence of oleic acid on filter paper was checked. Spots were barely luminescent and therefore eliminated as the reason for the positive results obtained. The negative results of the

longer treated samples were attributed to tetrahydrofuran solubilising oleic acid. This was checked and confirmed by a simple solubility test. The solubility of oleic acid in tetrahydrofuran also accounts for the blurriness detected with the drag samples.

This result gives credence to the reasoning that the mode of action of PcEuCl is through carboxyl groups attaching to the Eu metal centre. The result is also preliminary confirmation that PcEuCl exhibits reactivity towards non-water soluble fingerprint components, which is consistent with the proposed mode of action.

3.10 Conclusions and Recommendations

It has been shown that axially substituted metallophthalocyanines react with both water and non-water soluble latent fingerprint components. Both $PcSn(OH)_2$ and PcEuCl react with glycine. $PcSn(OH)_2$ gave developed glycine spots that were visible in ambient light as blue/green spots, and that were very weakly visible under UV illumination at 254 nm as dark spots on a dull, dark purple background. PcEuCl gave developed glycine spots that were not particularly visible in ambient light but were clearly visible under UV illumination at 254 and 350 nm. $PcSn(OH)_2$ and PcEuCl also showed reactivity towards oleic acid, a non-water soluble fingerprint component. However both compounds exhibited some limitations with regard to use as possible fingerprint reagents.

$PcSn(OH)_2$ had very low solubility in a range of common solvents which to a certain extent governed its inability to successfully develop fingerprints. The low solubility of metallophthalocyanines is well established [59, 82, 83], and it is plausible that this aspect could be improved upon. One possible approach is the examination of a wider range of solvents and mixed solvent systems that might allow a greater concentration of $PcSn(OH)_2$ to be exhibited. However such a study should take into account the effect on fingerprints as a solvent or solvent system that is detrimental to fingerprints is clearly of no practical use. Another perhaps more robust approach to improve solubility would be to synthesise derivatives of $PcSn(OH)_2$ that have peripheral

substituents on the macrocycle that aid solubility. Bulky groups such as *tert*-butyl improve solubility in organic solvents [83], and pyridyl and sulpho groups aid water solubility [86, 123], with tetrasubstituted metallophthalocyanines generally exhibiting higher solubilities than octasubstituted metallophthalocyanines. Either approach could sufficiently improve the solubility of $PcSn(OH)_2$ to enable the development of latent fingerprints.

PcEuCl underwent initial investigation and from this a small limitation was identified. While excellent luminescence properties were observed under an inexpensive light source, a UV lamp, which is an important practical consideration, this also provided a limitation. A substantial number of paper substrates contain optical brightners which result in the masking of any developed luminescence under UV illumination. However this result does not mean that PcEuCl merits no further investigation as a possible fingerprint reagent. A large number of substrates do not exhibit background luminescence under UV illumination. Further screens could be carried out to determine whether PcEuCl has any reactivity towards fingerprint components on such surfaces. Any positive results could then be followed up to determine if successful fingerprint development could be achieved and if so optimisation could be carried out.

CHAPTER FOUR

PERIPHERALLY SUBSTITUTED METALLOPHTHALOCYANINES

4.1 Introduction

In devising reagents of this class the aim is to have a functional group that is reactive towards one or more fingerprint components attached to the periphery of the macrocyclic ring of the metallophthalocyanine. If such a compound is bound to a fingerprint the central metal ion does not participate in the linkage. This allows variation of the metal centre which alters the colour and luminescence properties of the metallophthalocyanine and hence the colour and luminescence properties of the developed print. Such an approach brings forth the possibility of developing a suite of reagents whereby a reagent to be used can be selected based upon the colour and/or luminescence properties it exhibits, to maximise contrast between the developed print and the background. A choice such as this could be highly valuable considering the numerous types of backgrounds encountered in the detection of latent fingerprints.

The targeted metallophthalocyanine was copper(II) tetrachlorosulfonylphthalocyanine, $CuPc(SO_2Cl)_4$, (Figure 4.1). This particular compound was selected as the chlorosulfonyl functional group has been used with some success in the fingerprint reagent dansyl chloride (Section 1.5.2.2). Furthermore the mode of action with primary and secondary amines, groups which occur in amino acids, is well established [61], and reaction occurs according to the following.

Primary amine $RNH_2 + ArSO_2Cl \rightarrow ArSO_2\text{-}NHR$

Secondary amine $R_2NH + ArSO_2Cl \rightarrow ArSO_2\text{-}NR_2$

It follows that a chlorosulfonyl substituted metallophthalocyanine should react in an analogous manner, as follows.

Primary amine $MPc(SO_2Cl)_4 + RNH_2 \rightarrow MPc(SO_2\text{-}NHR)_4$

Secondary amine $MPc(SO_2Cl)_4 + R_2NH \rightarrow MPc(SO_2\text{-}NR_2)_4$

Copper was primarily selected as the central metal as a starting material was commercially available. If success was obtained with the copper compound as a fingerprint reagent then other metal analogues would be investigated, with the metals selected with the aim to change the colour and/or luminescent properties exhibited.

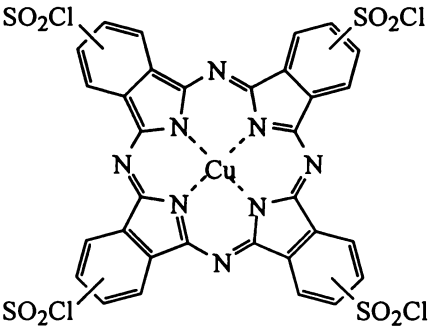


Figure 4.1 Structure of $CuPc(SO_2Cl)_4$

Please note that an oral presentation entitled "Phthalocyanines as Fingerprint Reagents - The Story Continues" was made at the 1998 Australia New Zealand Forensic Science Society (ANZFSS) Symposium in Adelaide, Australia, and was awarded the ANZFSS Medal for best presentation.

4.2 Synthesis

4.2.1 $CuPc(SO_2Cl)_4$

$CuPc(SO_2Cl)_4$ was synthesised according to the literature methods [123-125] as follows. Chlorosulfonic acid (5 mL, BDH Chemicals Ltd, 97%) was added dropwise to copper(II) phthalocyaninetetrasulfonic acid, tetrasodium salt (2 g, Aldrich Chemical Company, LR grade). While being continuously magnetically stirred, the reaction mixture was heated at approximately 75°C for 1 hour, the temperature was gradually increased over a period of 1 hour to approximately 140°C, and then held at this

temperature for 4 hours. The reaction mixture was cooled to 80°C, thionyl chloride (2.5 mL, Merck-Schuchardt, 99%) was added dropwise, and the reaction mixture stirred for a further 2 hours at 80°C. After the reaction mixture had cooled it was tipped onto crushed ice to isolate the product. The solid was collected by vacuum filtration, washed with distilled water and acetone, and then dried on a freeze dryer (FTS Systems Flexi-Dry™ μP Freeze Dryer) to give a dark, forest green crystalline powder, approximate yield 88.6%. Note that this compound was rather moisture sensitive and to avoid deterioration it was stored in a glass vial in a vacuum dessicator containing silica crystals.

4.2.2 Zinc Phthalocyanine

Zinc phthalocyanine (ZnPc), a precursor to $ZnPc(SO_2Cl)_4$, was synthesised with regard to the literature methods [115, 126] as follows. Zinc chloride (2.01 g, BDH Laboratory Supplies, AR grade) and 1,2 dicyanobenzene (7.56 g, Aldrich Chemical Company, 98%) were refluxed in 1-chloronaphthalene (50 mL, Aldrich Chemical Company, ~90%) for 7 hours. The reaction mixture was left to cool and the solid subsequently collected by vacuum filtration. The solid was washed with ethanol and then oven dried at 100°C to give a metallic blue crystalline powder, approximate yield 67.5%. Confirmation of ZnPc was obtained by comparison of experimental UV-Vis data with literature results [82].

4.2.3 Cobalt Phthalocyanine

Cobalt phthalocyanine (CoPc), a precursor to $CoPc(SO_2Cl)_4$, was synthesised with regard to the literature methods [115, 126] as follows. Cobalt chloride ($CoCl_2.6H_2O$, 2.40 g, Ajax Chemicals, AR grade) and 1,2 dicyanobenzene (5.23 g, Aldrich Chemical Company, 98%) were refluxed in 1-chloronaphthalene (25 mL, Aldrich Chemical Company, ~90%) for 6 hours. The reaction mixture was left to cool and the solid subsequently collected by vacuum filtration. The solid was washed with ether and then oven dried at 100°C to give a sparkling purple/blue crystalline powder, approximate yield 62.3%. Confirmation of CoPc was obtained by comparison of experimental UV-Vis data with literature results [82].

4.2.4 $ZnPc(SO_2Cl)_4$

Zinc was selected as a central metal ion as ZnPc has potentially desirable luminescence properties. It exhibits a good fluorescence quantum yield, Φ_F = 0.3 at 77K, and a reasonable phosphorescence quantum yield $\Phi_P = 1 \times 10^{-4}$ with a lifetime of 1100 μs (± 10%) at 77K, though no phosphorescence was detected at room temperature [95]. Peripherally substituted ZnPcs also show good luminescence properties [127], therefore $ZnPc(SO_2Cl)_4$ should also hopefully exhibit some luminescence.

The initial attempt at synthesising $ZnPc(SO_2Cl)_4$ was undertaken in a manner analogous to that used to obtain $CuPc(SO_2Cl)_4$ as Brinkley had stated in a patent [128] that the reaction:

$$MPc + XSO_3H + SOX_2 \rightarrow MPc(SO_2X)_4$$

applied for X = halogen and M = Co, Ni, Cu, Cr, Mg, and Zn, though M = Cu and X = Cl were preferred. Chlorosulfonic acid (10 mL, BDH Chemicals Ltd, 97%) was added dropwise to ZnPc (2.11 g). While being continuously magnetically stirred, the reaction mixture was heated at approximately 75°C for 1 hour, the temperature was gradually increased over a period of 1 hour to approximately 140°C, and then held at this temperature for 4 hours. During the four hours the reaction mixture looked green in colour and after four hours looked brown. The reaction mixture was cooled to 80°C, thionyl chloride (5 mL, Merck-Schuchardt, 99%) was added dropwise, and the reaction mixture stirred for a further 2 hours at 80°C. After the reaction mixture had cooled it was tipped onto crushed ice, to isolate the product. A khaki coloured material was collected by vacuum filtration, which indicated that the reaction had not been successful. It was thought that the earlier colour change noted during the chlorosulfonic acid stage of the reaction might have indicated that this reaction had proceeded more rapidly than that of $CuPc(SO_2Cl)_4$, noting that chlorosulfonic acid is an agent that can be employed to introduce the sulfonic acid group or the chlorosulfonyl group [129, 130].

While Brinkley [128] had included Zn as a central metal ion for $MPc(SO_2Cl)_4$, the process was only claimed for M = Cu, and only specific details regarding the Cu species were given. It was not apparent whether the Zn analogue had been prepared in this

manner, or whether it was just suggested as a likely metal alternative. The conditions for 'chlorosulfonation' are not particularly mild and in such instances the relative stabilities of metallophthalocyanines could be considered. The excellent stability of CuPc is documented, being stable at 900°C *in vacuo* [82]. ZnPc, while also a metallophthalocyanine exhibiting good stability, it can be reprecipitated unaltered from concentrated sulfuric acid [82], it may be sufficiently less stable so as to degrade under the reaction conditions to which it was exposed. It was thought that a similar approach with less forcing conditions might yield the desired product, and so a second attempt to synthesise $ZnPc(SO_2Cl)_4$ was undertaken.

Chlorosulfonic acid (5 mL, BDH Chemicals Ltd, 97%) was added dropwise to ZnPc (1.12 g). The reaction mixture was magnetically stirred and heated at approximately 80°C for 24 hours. Qualitative UV-Vis spectra were collected in chlorosulfonic acid for the reaction prior to commencement of heating, and during, heating. All spectra were similar and did not give any obvious indication of substitution or otherwise. This was not unexpected in that peripheral substitution to a metallophthalocyanine is not likely to have a large effect on the absorption spectra. Thionyl chloride (2 mL, Merck-Schuchardt, 99%) was then added dropwise and the reaction mixture stirred for another 2 hours at approximately 80°C. The reaction was cooled and then tipped onto crushed ice to isolate the product. The solid was collected by vacuum filtration and then dried on a freeze dryer, to give a bright, forest green crystalline powder, approximate yield 32.7%. Note that this compound was rather moisture sensitive and to avoid deterioration it was stored in a glass vial in a vacuum dessicator containing silica crystals.

4.2.5 $CoPc(SO_2Cl)_4$

Cobalt was selected as a central metal ion as there might be a shift in colour. CuPc and ZnPc have Q-bands that occur at similar wavelengths, 678 and 681 nm respectively in 1-chloronaphthalene [82], whereas CoPc is slightly removed, 672 nm in 1-chloronaphthalene [82]. There was also a possibility that a Co analogue might exhibit phosphorescence [95]. Such a compound would be desirable as there are few, if any documented phosphorescent fingerprint reagents. Like the Zn analogue, references to

the synthesis of $CoPc(SO_2Cl)_4$ with chlorosulfonic acid and thionyl chloride were made [125, 128]. Though again only specific details regarding Cu were given, it was difficult to ascertain whether the Co analogue had been actually prepared, or just suggested as a probable alternative. However as some success had been shown with this method for the Zn analogue a similar synthesis was undertaken to prepare $CoPc(SO_2Cl)_4$.

Chlorosulfonic acid (5 mL, BDH Chemicals Ltd, 97%) was added dropwise to CoPc (1.04 g). The reaction mixture was magnetically stirred for 24 hours at approximately 80°C. Thionyl chloride (2.5 mL, Merck-Schuchardt, 99%) was added dropwise and the stirring and heated continued for a further 2 hours. The reaction mixture was tipped onto crushed ice to isolate the product. The solid was collected by vacuum filtration, and then dried to give a dark purple crystalline powder, approximate yield 60.3%. This compound was stored in a glass vial in a vacuum dessicator to avoid any deterioration.

4.3 Characterisation of $CuPc(SO_2Cl)_4$

4.3.1 Absorption Spectra of $CuPc(SO_2Cl)_4$

UV-Vis spectra of $CuPc(SO_2Cl)_4$ were collected in ethanol and dimethylformamide (GPR grade). The spectra collected in ethanol showed varying degrees of aggregation with change in solution concentration, with aggregation of similar species being a known phenomena [91, 123, 131]. This is shown by progressive diminution of the Q-band and emergence of a broader and much less intense band at approximately 620 nm. Spectra collected in dimethylformamide showed no indication of aggregation and these are the results shown in Table 4.1.

Table 4.1 UV-Vis data for $CuPc(SO_2Cl)_4$

Band	λ (nm)	ε (L mol^{-1} cm^{-1})
Q	672.0	53000 ± 3000
Satellite	605.2	11500 ± 700
Soret†	~345.8	33000 ± 2000

†Note this particular band was seen as a broad shoulder-like band

4.3.2 Absorption Spectra of 2-Aminomethylpyridine Derivative of $CuPc(SO_2Cl)_4$

The nature of the $-SO_2Cl$ groups, that is the fact that they are highly reactive, made direct confirmation of $CuPc(SO_2Cl)_4$ slightly awkward. However UV-Vis data for copper(II) tetra(2-pyridylmethylaminosulfonyl)phthalocyanine was available, which enabled confirmation to be made. The derivative was prepared according to the literature [123]. $CuPc(SO_2Cl)_4$ was magnetically stirred with an excess of 2-aminomethylpyridine (Aldrich Chemical Company, 99%) for 2 hours at approximately 40°C, isolated, and then dried under vacuum at approximately 80°C. UV-Vis spectra were collected in dimethylformamide (GPR grade). The principal maximum was found to occur at a wavelength intermediate between those stated for the 3,10,17,24 and 4,11,18,25 copper(II) tetra(2-pyridylmethylaminosulfonylphthalocyanine) isomers. Correspondence with Sigma-Aldrich [132] regarding the initial starting material, copper(II) phthalocyaninetetrasulfonic acid tetrasodium salt, however stated that the material was a mixture of isomers, which explains the position of the maximum obtained.

4.3.3 Excitation and Emission Spectra of $CuPc(SO_2Cl)_4$

Luminescence spectra of $CuPc(SO_2Cl)_4$ were collected in ethanol. They showed that $CuPc(SO_2Cl)_4$ did not have any inherent luminescence. In terms of fingerprint visualisation this translates to the compound, if successful in developing fingerprints, giving prints that are visible in ambient light only. That is, excitation with a light source will not render the prints luminescent.

4.4 Fingerprint Screen of $CuPc(SO_2Cl)_4$

4.4.1 Solubility of $CuPc(SO_2Cl)_4$

Before undertaking a fingerprint screen it was necessary to determine what solvents $CuPc(SO_2Cl)_4$ was soluble in. Test-tubes containing a small amount of $CuPc(SO_2Cl)_4$

were prepared. To these was added approximately 1-2 mL of solvent. The results obtained are shown in Table 4.2.

Table 4.2 Solubility of $CuPc(SO_2Cl)_4$

Solvent	Solubility	Colour of Resulting Solution
Distilled Water	√	bright green
Methanol	√	bright green
Ethanol	√	lighter green
Acetonitrile	X	-
Ethyl Acetate	X	-
Acetone†	√	pale green
Tetrahydrofuran	X	-
Dichloromethane	X	-
Chloroform	X	-
Toluene	X	-
Petroleum Spirits	X	-
Heptane	X	-

†drum grade

While ethanol did not exhibit the highest solubility of $CuPc(SO_2Cl)_4$, it had been successfully used with $TPPSn(OH)_2$, so it was selected as the solvent for use with $CuPc(SO_2Cl)_4$. Note that ethanol could be replaced by methanol if a solution with a higher concentration was required.

4.4.2 Fingerprint Screen Experiment

A fingerprint screen was carried out in exactly the same manner as that described for $TPPSn(OH)_2$ in Section 2.5, using a 1.6×10^{-4} M solution of $CuPc(SO_2Cl)_4$ in ethanol.

Success was had, with green spots being visible on all combinations. Spots were least visible on the drag samples and most visible on the 10 minute samples, with no obvious distinction between the air and oven-dried samples. The most exciting result however was the partial fingerprint noticed on the edge of the 10 minute air-dried sample (Figure 4.2). This clearly indicated that $CuPc(SO_2Cl)_4$ could develop actual fingerprints and that further experiments should be undertaken

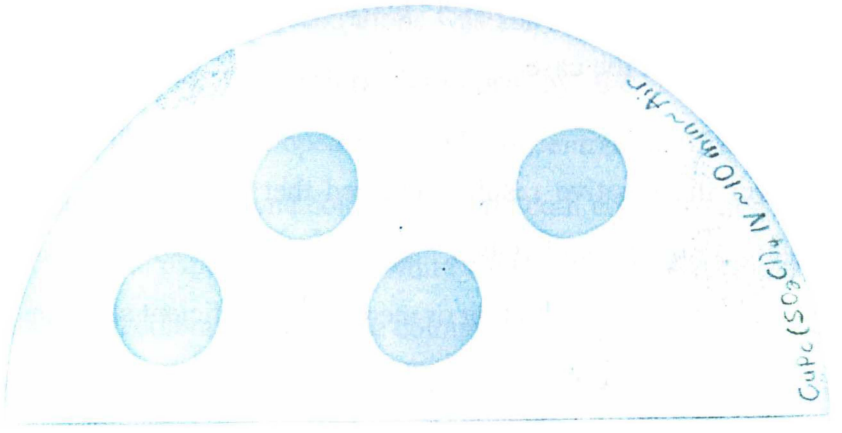


Figure 4.2 Glycine spots developed by a 10 minute soak in a 1.6 x 10^{-4} M $CuPc(SO_2Cl)_4$ ethanol solution; note that the fingerprint is still visible approximately 20 months after development

4.5 Development of Fingerprints with $CuPc(SO_2Cl)_4$

4.5.1 Initial Experiment

Fingerprints from a variety of donors were collected on CopyRight 80 gsm white paper, soaked in a 1.6 x 10^{-4} M $CuPc(SO_2Cl)_4$ ethanol solution for 10 minutes, and then air-dried. This regime was selected as it had inadvertently shown success in the fingerprint screen.

Examination of the samples in ambient light gave good results, with many samples having partial or faint ridge detail visible. However an inconsistency was noticed. All of the successfully developed samples, except one, were those that were initially treated, whereas all of the latter treated samples, except one, showed no ridge detail at all.

It was thought that this result was due to their not being sufficient $CuPc(SO_2Cl)_4$ in the reagent solution, that is the reagent solution had been exhausted by the number of samples that had been treated. This idea was consistent with the fact that the latter treated samples were of a far paler colour than those treated earlier. An alternative idea for the inconsistent results was that the reagent solution was degrading rapidly upon

exposure to the air, as -SO_2Cl groups are known to be highly reactive. Obviously it was hoped that this was not the case.

Overall however the positive results indicated that optimisation of the development conditions should now be undertaken. Such experiments should also consider whether the reagent solution was deteriorating or merely of insufficient strength.

4.5.2 Concentration Optimisation

In all comparisons, fingerprints from a variety of donors were collected on white paper, cut in half, treated, and then 'rejoined' for examination in ambient light (Figure 2.11). The initial comparison was made using a 1.6 x 10^{-4} M (1X) solution against a 1.6 x 10^{-3} M (10X) solution, using six different soaking times:

(i) a drag
(ii) 2 minute soak
(iii) 5 minute soak
(iv) 10 minute soak
(v) 20 minute soak
(vi) 30 minute soak.

All samples were left to air dry and the results obtained are shown in Table 4.3.

Table 4.3 Results obtained from the concentration comparison of 1.6 x 10^{-4} M (1X, left-hand columns) against 1.6 x 10^{-3} M (10X, right-hand columns) for $CuPc(SO_2Cl)_4$, key below

Time	Drag		2 min		5 min		10 min		20 min		30 min	
1	X	√	√F	√	√F	√	√F	√	√F	√	√VF	√
2	X	X	X	X	√VF	√VF	√VF	√F	X	X	X	X
3	X	√	√F	√	√F	√	√	√B	√F	√	√F	√F
4	X	√	√F	√	√VF	√	√VF	√VF	X	X	X	X
5	X	√F	X	√F	X	√VF	√VF	√	X	√VF	√	√B

Key: X = no developed print
√ = full or partial developed print
V = very
F = faint
B = better

From these results it can be seen that for all soaking times the 10X concentration had a superior performance. This was particularly evident for the short and intermediate soaking times. With a drag treatment, no fingerprints were developed by the 1X concentration. However as the length of soaking time increased, the quality and number of fingerprints developed by the 1X concentration also increased. For the long soaking

times the performance of both concentrations was starting to become more equivalent, however the quality of the fingerprints developed by the 10X concentration still exceeded the quality of those developed by the 1X concentration. It was also noted that there was a difference in background colouration with both concentration and time. The 1X concentration left the paper essentially white whereas the 10X concentration left the paper a very pale green colour. Though the colour of the background increased for both concentrations with treatment time, i.e. the 30 minutes samples had a darker background than the drag samples.

From these results it was decided to undertake a comparison with a greater concentration. A 1.6×10^{-3} M (10X) was compared to a 1.6×10^{-2} M (100X) solution, using the same six treatment times as used previously. The results obtained are shown in Table 4.4.

Table 4.4 Results obtained from the concentration comparison of 1.6×10^{-3} M (10X, left-hand columns) against 1.6×10^{-2} M (100X, right-hand columns) for $CuPc(SO_2Cl)_4$, key below

Time	Drag		2 min		5 min		10 min		20 min		30 min	
1	√F	X	X	X	√VF	X	√	X	√	X	√	X
2	√	√F	√	X	√F	X	X	X	√F	X	√	X
3	X	X	√	X	√	X	√F	X	√F	X	√	X
4	√	√	X	X	√	X	√	X	√VF	X	√	X
5	√F	X	X	X	√	X	X	X	√	X	√	X

Key: X = no developed print
√ = full or partial developed print
V = very
F = faint

From these results it can be clearly seen that the 10X concentration was far superior to the 100X concentration, at all treatment times. The 100X concentration was far too strong with it over-developing all the samples, that is the samples were so darkly stained that no fingerprints were actually discernible, except for the short treatment of a drag. It should be noted that the 100X concentration was saturated and that the true concentration would have been lower than 1.6×10^{-2} M, but for comparison purposes was used as is.

From these results it was decided to undertake a comparison with a concentration still greater than 10X, but less concentrated than the 100X solution. A 1.6 x 10^{-3} M (10X) solution was compared to a 3.2 x 10^{-3} M (20X) solution, using the same six treatment times previously used. The results obtained are shown in Table 4.5.

Table 4.5 Results obtained from the concentration comparison of 1.6 x 10^{-3} M (10X, left-hand columns) against 3.2 x 10^{-3} M (20X, right-hand columns) for $CuPc(SO_2Cl)_4$, key below

Time	Drag		2 min		5 min		10 min		20 min		30 min	
1	√	√F	√=	√=	X	X	√=	√=	X	X	√	X
2	√B	√	√	X	√	X	√	√F	X	X	X	X
3	√VF	X	√=	√=	√	X	√	√F	√B	√	√	√VF
4	X	X	√VF	X	√B	√	X	X	√	√F	X	X
5	√	X	√	√F	√=	√=	√	X	X	X	X	X

Key: X = no developed print
√ = full or partial developed print
V = very
F = faint
B = better quality, i.e. better contrast between print and background
'=' = equivalent prints

These results show that there was not a large difference in performance of the 10X and 20X concentrations. Overall however the 10X concentration gave marginally better resolution between the developed fingerprint and the background. This was due to the 20X concentration giving a darker background than the 10X concentration.

From these results it was decided to undertake a comparison with a lower concentrated solution. An 8.3 x 10^{-3} M (5X) solution was compared to a 1.6 x 10^{-3} M (10X) solution, again using the same six treatment times previously used. The results obtained are shown in Table 4.6.

Table 4.6 Results obtained from the concentration comparison of 8.3 x 10^{-3} M (5X, left-hand columns) against 1.6 x 10^{-3} M (10X, right-hand columns) for $CuPc(SO_2Cl)_4$, key below

Time	Drag		2 min		5 min		10 min		20 min		30 min	
1	√VF	√F	√	√B	√	√B	√=	√=	√	√B^j	√=	√=
2	√F	√	√	√B	√VF	√	X	X	√=	√=	√	√B
3	√F	√VF	X	√	√	√B	X	X	√=	√=	√=	√=
4	X	√	√	√B	X	√	√=	√=	√=	√=	√	√B^j
5	X	X	√F	√	√	√B	√=	√=	√VF	√	√=	√=

Key: X = no developed print
√ = full or partial developed print
V = very
F = faint
B = better quality, i.e. better contrast between print and background
'=' = equivalent prints
j = just/marginally (qualification of quality)

Like the previous comparison these results show there was little difference in performance between the 5X and 10X concentrations. At the longer soaking times there was almost no discrimination between the two concentrations. However at the shorter soaking times the 10X concentration exhibited slightly better resolution between developed fingerprints and the background. As shorter treatment times are favoured operationally conclude that the 10X concentration, 1.6 x 10^{-3} M, is the optimum concentration to use. Though this could be reconsidered if it was found that longer soaking times were better than shorter soaking times for the development of fingerprints. The resolution between the developed print and the background could perhaps be further improved by a washing step, though operationally it would be advantageous to avoid this.

4.5.3 Soaking Time Optimisation

An experiment was undertaken to determine which length of soaking time gave the best quality fingerprints. In the comparisons, fingerprints from a variety of donors were collected on white paper, cut in half, treated, and then 'rejoined' for examination in ambient light (Figure 2.11). A drag was compared to a 2 minute soak, and a 2 minute soak was compared to a 5 minute soak, using a 1.6 x 10^{-3} M solution. As shorter treatment times are more favoured operationally, no soaking times greater than 5 minutes were investigated. The results obtained are shown in Table 4.7.

Table 4.7 Results obtained for soaking time comparison for $CuPc(SO_2Cl)_4$, key below

Sample	Drag versus 2 min		2 min versus 5 min	
1	√=	√=	√	√B
2	√†	√†	√B	√
3	√=	√=	√=	√=
4	√=	√=	√	√F
5	√=	√=	√=	√=

Key: √ = full or partial developed print
B = better quality print, i.e. better contrast between print and background
'=' = equivalent prints
† = very partial print, difficult to distinguish any difference

From these results it can be seen that there was no noticeable gain in either the quality or the quantity of fingerprints developed by a longer treatment time. It should be stated

that as soaking time increases so does both the colour of the developed print and the background (Figure 4.3). Though it should be noted that the resolution between the print and the background remains relatively constant. As minimal background coloration is desirable the shortest treatment time is more favourable. Though low background colour could conceivably also be achieved by a longer treatment time followed by a rinse. However this would add an extra step in the development process. Operationally a quick method with only one step is more favourable. Therefore recommend that a drag through a 1.6 x 10^{-3} M solution is the optimum treatment time.

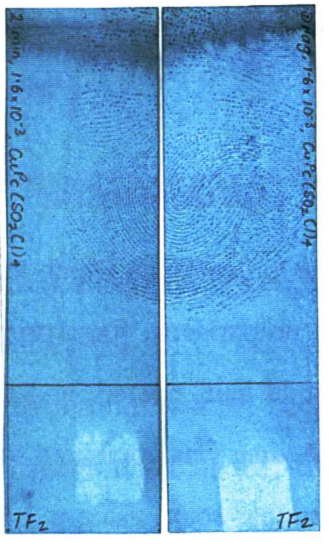


Figure 4.3 A fingerprint treated with both a drag (right-hand side) and a 2 minute soak (left-hand side) in a 1.6 x 10^{-3} M $CuPc(SO_2Cl)_4$ ethanol solution; note approximately 1 year since print developed

4.5.4 Drying Optimisation

An experiment was undertaken to determine whether oven-drying would improve the quality of developed fingerprints. For the comparison, fingerprints from a variety of donors were collected on white paper, cut in half, treated with by a drag in a 1.6 x 10^{-3} M solution, either air-dried or oven dried at approximately 70°C for 10 minutes, and then 'rejoined' for examination in ambient light (Figure 2.11). The results obtained are shown in Table 4.8.

Table 4.8 Results obtained for the air-drying/oven-drying comparison for $CuPc(SO_2Cl)_4$, key below

Treatment	1	2	3	4	5	6
Air	√	√=	√†	√=	√B^j	√
Oven	√B^j	√=	√†	√=	√	√B^j

Key: √ = full or partial developed print
B = better quality print, i.e. better contrast between print and background
'=' = equivalent prints ;
j = just/marginally (qualification of quality)
† = very partial print, difficult to distinguish any difference

These results show that there was marginal improvement in the quality of developed fingerprints if they are heat treated. The oven-dried prints seemed to be slightly darker in colour compared to the air-dried prints, without an increase in background colour. However the improvement in resolution did not appear sufficient to justify the inclusion of a heating step. Especially if like ninhydrin treated items, the fingerprints can improve in contrast given a longer lag time between treatment and examination. The prints from this experiment were examined approximately 1 year later and there was no noticeable difference between the air and oven-dried prints (Figure 4.4). Therefore recommend that air-drying is sufficient to produce good quality fingerprints.

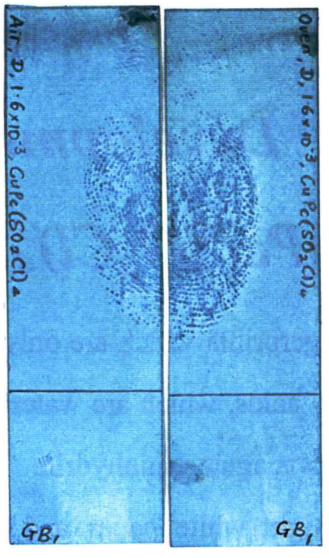


Figure 4.4 A fingerprint treated by a drag in a 1.6 x 10^{-3} M $CuPc(SO_2Cl)_4$ solution with air (left-hand side) and oven-drying (right hand side); note approximately 1 year since print developed

Hence it is concluded that the best conditions for developing fingerprints with $CuPc(SO_2Cl)_4$ can be considered to be a drag in a 1.6 x 10^{-3} M ethanol solution, followed

by air-drying (Figure 4.5). No further inconsistent results were obtained during the optimisation experiments, as compared to the initial fingerprint experiment conducted (Section 4.5.1). While no specific experiment was undertaken it could be suggested that this was due to non-exhaustion of reagent solutions as stronger concentrations were used. However it would still be recommended that certain precautions, such as using dry solvents, are taken to minimise any deterioration of the reagent solution that might occur. Further, as with any reagent, if control prints are not satisfactorily developed then the reagent solution should be discarded.

Figure 4.5 A fingerprint developed by a drag in a 1.6 x 10^{-3} M $CuPc(SO_2Cl)_4$ ethanol solution, followed by air-drying; note approximately 1 year since print developed

Having determined the optimum conditions for developing fingerprints with $CuPc(SO_2Cl)_4$, it was now possible to investigate how this reagent compared with currently used reagents.

4.6 Fingerprint Development Comparison Between $CuPc(SO_2Cl)_4$ and Ninhydrin

As $CuPc(SO_2Cl)_4$ develops fingerprints which are only visible in ambient light, and is likely to be reacting with amino acids, which are water soluble fingerprint components, then the appropriate comparison is against ninhydrin. Fingerprints were collected from a variety of donors on CopyRight white paper and Croxley 100% recycled manilla envelope paper, cut in half, treated with either $CuPc(SO_2Cl)_4$ or ninhydrin (Appendix I), and then 'rejoined' for examination in ambient light (Figure 2.11). A summary of the results obtained is shown in Table 4.9.

with ninhydrin developed fingerprints, or it may have some use on substrates where the application of heat is undesirable. Alternatively a lowering of the background coloration may be obtained, either by a rinsing step, or by a different solvent system. Such an improvement would be highly desirable and if achieved, further comparisons would be required.

4.7 Order of Treatment with Respect to Ninhydrin

As always, the primary goal is to develop the maximum number of fingerprints of the highest quality possible. To achieve this strategies are generally employed involving the application of more than one fingerprint technique in a certain sequential order. Therefore if $CuPc(SO_2Cl)_4$ is to have any use as a fingerprint reagent it is necessary to determine where its use may be appropriate and if it impedes the use of other reagents. As the suggestions for use were in situations where contrast with ninhydrin might be low or where heat was undesirable it was decided to see whether $CuPc(SO_2Cl)_4$ could be used prior to ninhydrin without affecting the performance of ninhydrin.

Fingerprints from a variety of donors were collected on white paper, treated with a drag in a 1.6×10^{-3} M $CuPc(SO_2Cl)_4$ ethanol solution, examined in ambient light, then treated with ninhydrin (Appendix I) and again examined in ambient light.

It was found that $CuPc(SO_2Cl)_4$ and ninhydrin are completely compatible reagents when used in this order. In all instances ninhydrin performed successfully after treatment with $CuPc(SO_2Cl)_4$, with developed prints appearing a darker purple than those ordinarily developed, this being due to the developed prints now being a combination of green plus purple (Figure 4.7).

Figure 4.7 A fingerprint developed by a drag in 1.6 x 10-3 M $CuPc(SO_2Cl)_4$ ethanol solution, followed by ninhydrin; note approximately 1 year since print developed

4.8 Characterisation of $ZnPc(SO_2Cl)_4$

4.8.1 Absorption Spectra of $ZnPc(SO_2Cl)_4$

UV-Vis spectra of $ZnPc(SO_2Cl)_4$ were collected in ethanol and dimethylformamide. The results presented in Table 4.10 are those collected in dimethylformamide. $ZnPc(SO_2Cl)_4$ has a very similar absorption spectrum to $CuPc(SO_2Cl)_4$, thought it was noted that $ZnPc(SO_2Cl)_4$ did not show signs of aggregation in ethanol. $ZnPc(SO_2Cl)_4$ also exhibited a more intense Q-band.

Table 4.10 UV-Vis data for $ZnPc(SO_2Cl)_4$

Band	λ (nm)	ε (L mol^{-1} cm^{-1})
Q	670.8	190000 ± 10000
Satellite	604.6	34000 ± 2000
Soret	345.4	61000 ± 3000

4.8.2 Absorption Spectra of Glycine Derivative of $ZnPc(SO_2Cl)_4$

As for $CuPc(SO_2Cl)_4$, the highly reactive nature of $-SO_2Cl$ groups makes direct confirmation of $ZnPc(SO_2Cl)_4$ slightly awkward. However UV-Vis data for zinc(II)

tetra(N-carboxymethylsulfonamide)phthalocyanine was available. The derivative was prepared in a manner similar to the literature [86]. $ZnPc(SO_2Cl)_4$ was dissolved in ethanol, magnetically stirred with an excess of glycine for 2 hours at approximately 40°C, isolated, and then dried. UV-Vis spectra were collected in dimethylformamide (GPR grade) and good agreement was shown with the literature.

4.8.3 Excitation and Emission Spectra of $ZnPc(SO_2Cl)_4$

Luminescence spectra of $ZnPc(SO_2Cl)_4$ were collected in ethanol. $ZnPc(SO_2Cl)_4$ exhibits three excitation maxima that occur at approximately 227, 238, and 296 nm (Figure 4.8). The 227 and 238 nm maxima were of large intensity, while the 296 nm maximum was broader and of lower relative intensity.

Excitation at 227 and 238 nm gave very similar emission spectra. There was one major emission at approximately 355 nm of high intensity, along with a very weak emission at approximately 602 nm and a weak emission at approximately 676 nm (Figure 4.9).

Excitation at 296 nm was slightly different to that obtained by excitation at the other two wavelengths. One major emission was still evident, it was of lower intensity, which was not unexpected, it had also shifted slightly to approximately 368 nm. The very weak emission at 602 nm was not present, the weak emission at 675 nm was present, and there was the appearance of another weak emission at approximately 720 nm.

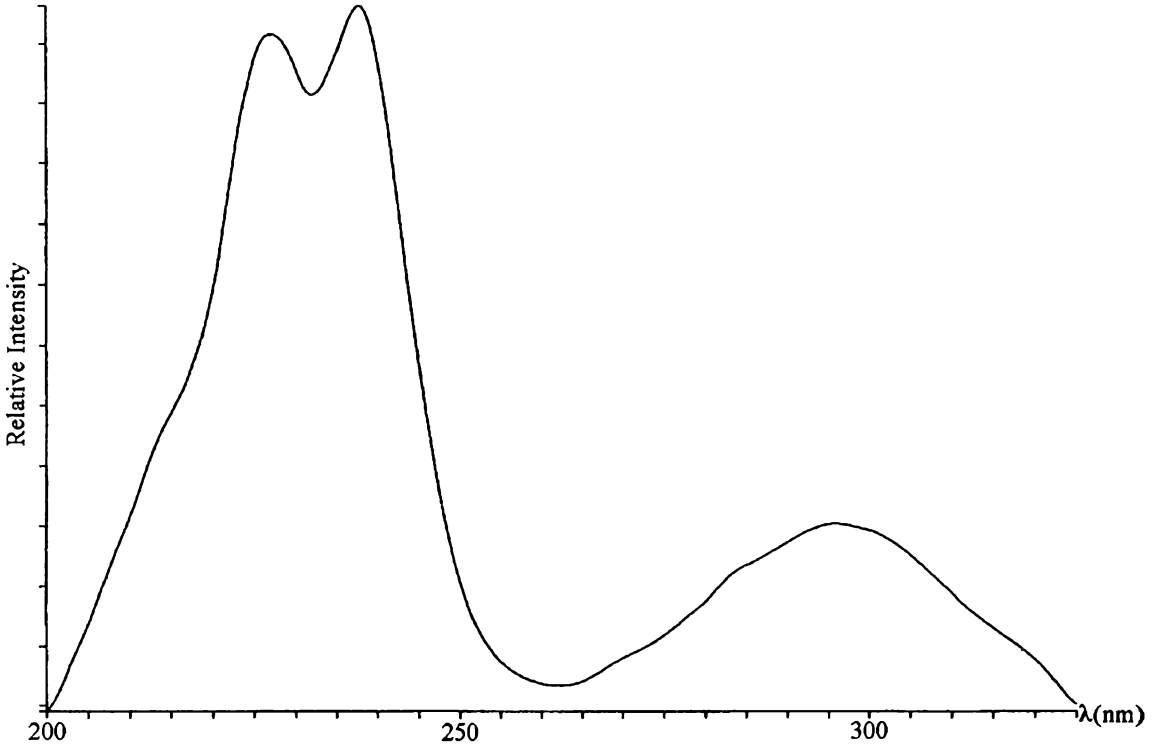


Figure 4.8 Excitation spectrum for the 355 nm emission of $ZnPc(SO_2Cl)_4$ in ethanol

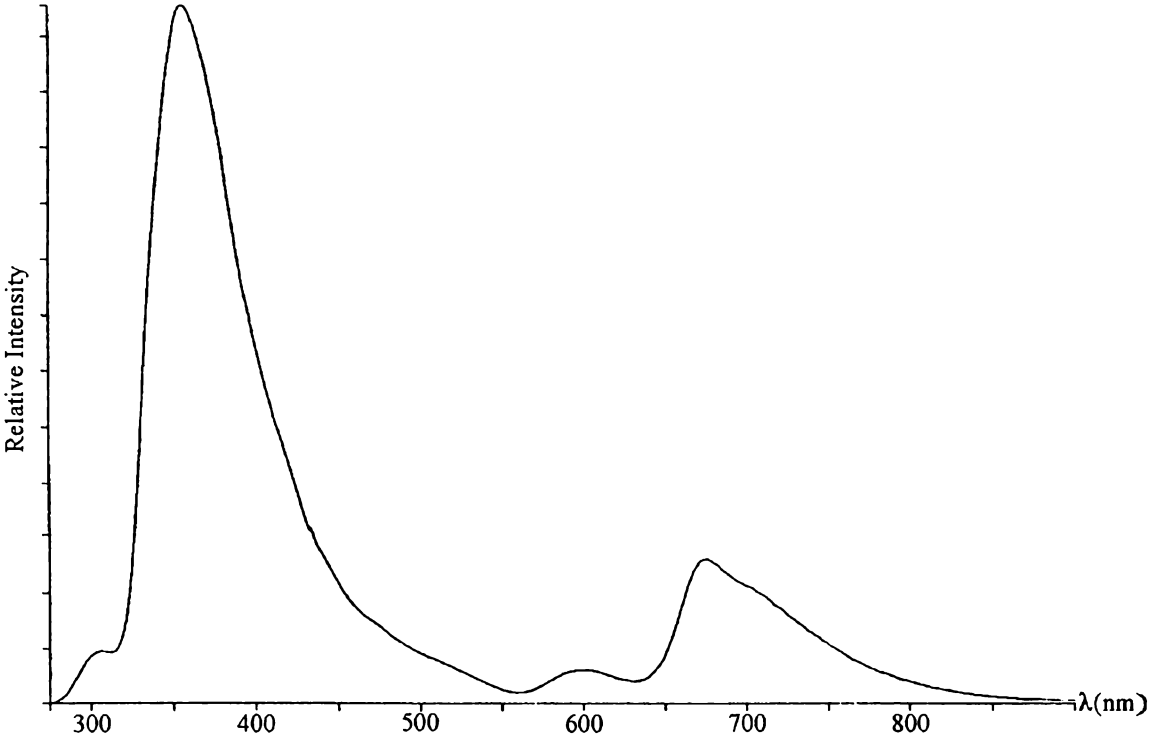


Figure 4.9 Emission spectrum which results from excitation of $ZnPc(SO_2Cl)_4$ in ethanol at 238 nm

4.9 Fingerprint Screen of $ZnPc(SO_2Cl)_4$

4.9.1 Solubility of $ZnPc(SO_2Cl)_4$

Before undertaking a fingerprint screen it was necessary to determine what solvents $ZnPc(SO_2Cl)_4$ was soluble in. Test-tubes containing a small amount of $ZnPc(SO_2Cl)_4$ were prepared. To these was added approximately 1-2 mL of solvent. The results obtained are shown in Table 4.11.

Table 4.11 Solubility of $ZnPc(SO_2Cl)_4$

Solvent	Solubility	Colour of Resulting Solution
Distilled water	X	-
Methanol	√	pale blue
Ethanol	√	blue
Acetonitrile	√	very pale blue
Acetone†	√	pale blue
Tetrahydrofuran	√	blue
Chloroform	√	very pale blue
Dichloromethane	√	very pale blue
Toluene	√	very pale blue
Heptane	X	-
Freon	X	-
Ethyl acetate	X	-
Petroleum spirits	X	-

† drum grade

The highest solubility was exhibited in ethanol and this solvent was selected for use with $ZnPc(SO_2Cl)_4$. It was noted that there was a noticeable difference in solubility between $ZnPc(SO_2Cl)_4$ and $CuPc(SO_2Cl)_4$. This difference is likely to be attributable to the change from Cu to Zn, as unlike the consistency of many other physical properties of metallophthalocyanines, solubilities vary greatly with coordinated metal centre [89]. There is also a possibility that the material may contain components with non-complete substitution, that is $ZnPc(SO_2Cl)_x$, where x = 1, 2, or 3. Though confirmation for $ZnPc(SO_2Cl)_4$ was obtained spectroscopically, a small amount of non-fully substituted material may well have been masked. While this would not be ideal, for the purposes of gauging reactivity and possible suitability as a fingerprint reagent, the $ZnPc(SO_2Cl)_4$ was used as is.

4.9.2 Fingerprint Screen Experiment

A fingerprint screen was carried out in exactly the same manner as that described in Section 2.5, using a saturated solution of $ZnPc(SO_2Cl)_4$ in ethanol. The solution was saturated as a concentration of 1.6×10^{-3} M had been selected, this being the optimal concentration found for $CuPc(SO_2Cl)_4$. However as stated in the previous section the solubility characteristics for the Zn analogue were different. The following results were obtained.

In ambient light clear spots were visible on all combinations, with the best contrast seen for the 10 minute soak samples, with an example shown in Figure 4.10. Oven-dried samples seemed to be slightly more developed than the air-dried samples. That is, the spots appeared slightly darker and slightly different in colour, without a noticeable increase in background colour.

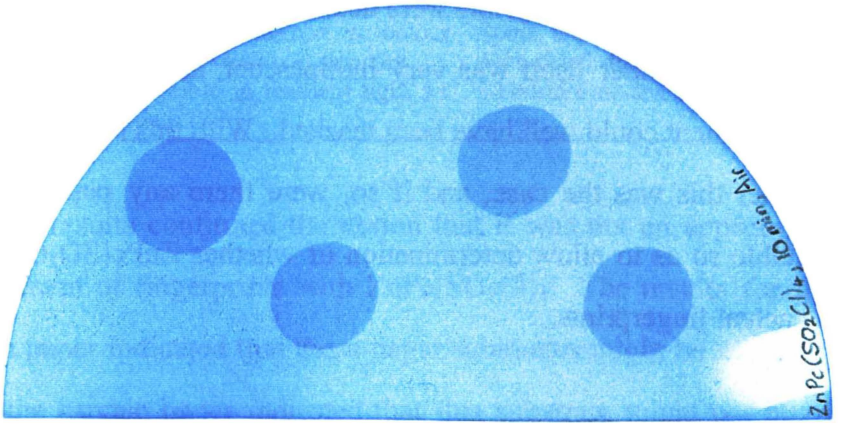


Figure 4.10 Glycine spots developed by a 10 minute soak, in a saturated $ZnPc(SO_2Cl)_4$ ethanol solution, followed by air-drying,

Samples were then examined under a UV lamp at 254 nm, as this wavelength was not to far removed from the most intense excitation wavelength of 238 nm. Spots were clearly luminescent for all combinations, though there was a marked difference in exhibited luminescence with treatment. For the air-dried samples there was a noticeable difference in luminescence with treatment time. The drag sample was the most faint, while the 2 and 10 minute samples were of similar intensity, with the 10 minute sample being slightly more intense. The oven-dried samples were clearly more luminescent than the

air-dried samples and furthermore it was difficult to distinguish any difference in luminescence with treatment time. That is the drag, 2, and 10 minute samples showed equivalent intensity of luminescence. The results of the fingerprint screen were clearly positive so it was appropriate to determine whether $ZnPc(SO_2Cl)_4$ would develop actual fingerprints.

4.10 Development of Fingerprints with $ZnPc(SO_2Cl)_4$

4.10.1 Initial Experiment

Fingerprints were collected on CopyRight 80 gsm white paper, from a variety of donors, soaked in a saturated $ZnPc(SO_2Cl)_4$ ethanol solution for 10 minutes, oven-dried at approximately 70°C for 10 minutes, and then examined in ambient light and under UV illumination at 254 nm. No fingerprints were detected. It was however noted that under UV illumination white paper itself was very luminescent, and it was thought that any fingerprint development could well have been masked. With this result it was decided to determine whether this was the case, and if so, were there any paper substrates that were more suitable so as to allow determination of whether $ZnPc(SO_2Cl)_4$ was capable of developing actual fingerprints.

4.10.2 Fingerprint Screen of a Variety of Papers

A number of papers were examined under UV illumination at 254 nm. Of those examined the following showed little or no luminescence: Pacesetter 80 gsm pink paper, Croxley Bargain Pad paper, and 1998 ANZFSS Symposium Pad paper. Fingerprint screen experiments, as described in Section 2.5, were undertaken on these papers, along with white paper. Note that unlike on filter paper, the glycine solution did not soak into these papers. To overcome this the spots were smeared in with a finger, left to air-dry on a flat surface until spots looked unlikely to 'run', and then hung up to finish air-drying. It was also noted that glycine spots were faintly visible on the bargain paper,

and appeared pale yellow in colour. Papers were treated with a saturated $ZnPc(SO_2Cl)_4$ ethanol solution. A summary of the results obtained for the different papers is shown in Table 4.12.

Table 4.12 Summary of fingerprint screen results obtained for a selection of paper substrates

Paper	Ambient light	UV illumination
White	Oven-dried samples were darker in colour than the air-dried samples No spots were visible on any combination, though it was possible that there were very faintly coloured regions on the 2 and 10 minute oven-dried samples	No spots were visible on any combination The paper was also highly luminescent
Bargain	Oven-dried samples were darker in colour than the air-dried samples Spots were visible on all combinations, however it was thought that the spots had not actually improved in resolution subsequent to treatment, recalling that untreated glycine spots were visible on this paper	No spots were visible on any combination The paper also had a very dull appearance
Pink	There was a slight difference in colour between the air and oven-dried samples Spots were possibly visible on the drag and 10 minute air-dried samples, and the 2 minute oven-dried sample	Spots were faintly visible on all the oven-dried samples, along with the air-dried drag sample
Symposium	There was a slight difference in colour between the air and oven-dried samples No spots were visible in ambient light for any combination	Spots were faintly visible on the air and oven-dried drag samples, and also on the 10 minute air-dried sample

The white paper results confirmed the notion that it was not an appropriate substrate for the development of fingerprints with $ZnPc(SO_2Cl)_4$. The results for the pink and the symposium paper indicated that these paper substrates might be of some use, while the results for the bargain paper indicated that this substrate might not be of use. The slightly inconsistent results obtained were attributed to the nature of the experiment. That is the smearing of the glycine drops to encourage absorption could have caused inconsistencies in the applied spots. From these results it was decided to undertake a further experiment to try and develop fingerprints on paper. The substrates chosen were pink, symposium, bargain, and filter paper. The bargain paper was included to confirm that it was not a suitable substrate. Filter paper was included as a substrate as it was the only paper that had given clear, luminescent spots under UV illumination.

4.10.3 Further Fingerprint Development Experiment

Fingerprints from a variety of donors were collected on pink, symposium, bargain and filter paper, soaked for 30 minutes in a saturated $ZnPc(SO_2Cl)_4$ ethanol solution, and then oven-dried for 10 minutes at approximately 70°C. Samples were examined in ambient light and under UV illumination at 254 nm. The results obtained are shown in Table 4.13.

Table 4.13 Results obtained for fingerprint development with a saturated $ZnPc(SO_2Cl)_4$ ethanol solution on a variety of papers, key below

Paper	Pink	Symposium	Bargain	Filter
Ambient light	√ X X X X	? X X X X	√ X X X X	X X X X X
UV	X √ X X X	X X X X X	X X X X X	√ √ ? ? X

Key: √ = fingerprint ridges definitely visible
? = fingerprint ridges possibly visible
X = no fingerprint ridges visible

From these results it was evident that the paper substrate was having an affect on the detection of developed fingerprints. As the main interest in $ZnPc(SO_2Cl)_4$ was as a possible luminescent fingerprint reagent it was decided to undertake some optimisation experiments on filter paper, to gain some idea of its effectiveness in developing fingerprints. It was noted that this substrate is unlikely to be frequently encountered in the detection of crime, though it may possibly possess some similarity to substrates such as wallpaper.

4.10.4 Optimisation of $ZnPc(SO_2Cl)_4$ on Filter Paper

It was decided to first examine the length of treatment time. In all experiments fingerprints were collected from a variety of donors on Whatman No. 1 filter paper, prepared as in Figure 2.11, treated with a saturated $ZnPc(SO_2Cl)_4$ ethanol solution, oven-dried at approximately 70°C, then examined in ambient light and under UV illumination at 254 nm. The first two experiments undertaken compared a 30 minute soak with a 15 minute soak, and a 30 minute soak with a 60 minutes soak. The results obtained are shown in Table 4.14.

Table 4.14 Results from soak time comparison of 30 minutes (left-hand columns) against 15 minutes (right-hand columns), samples 1-10, and 30 minutes(left-hand columns) against 60 minutes (right-hand columns), samples 11-20, key below

30&15 Samples	1		2		3		4		5	
AL	X	X	X	X	X	X	X	X	X	X
UV	X	√F	√	√VF	√F	X	X	X	X	X
30&15 Samples	**6**		**7**		**8**		**9**		**10**	
AL	√VF	√VF	X	X	√	X	√	X	√	√F
UV	√	√B	X	√	X	X	√†	√†	√	√B
30&60 Samples	**11**		**12**		**13**		**14**		**15**	
AL	√	√B	√	√B	X	X	√F	√F	√	√B
UV	√=	√=	√=	√=	X	X	√B	√	√=	√=
30&60 Samples	**16**		**17**		**18**		**19**		**20**	
AL	√VF	√VF	X	X	√VF	X	√=	√=	X	X
UV	√VF	X	X	X	√F	X	√=	√=	X	X

Key AL = ambient light
X = no developed print
√ = full or partial developed print
V = very
F = faint
B = better quality, i.e. better contrast between print and background
'=' = equivalent prints
† this print probably contaminated

These results are explained more fully as follows. First of all it was noticed that there was a definite difference in colour of samples with treatment time, the 60 minute samples were darkest in colour and the 15 minute samples were lightest in colour. Under UV illumination this was far more noticeable, with a large increase in luminescence with treatment time. This gave reduced contrast between the fingerprint and the background. Though it was noted that this trend might have been exacerbated by the substrate. On non-absorbent papers there may be less of a background problem, as was the case for $TPPSn(OH)_2$. Also noted was that at times the developed prints were blurry in nature, that is not very distinct. Overall however, the 15 minute soak gave the better results under UV illumination while the 30 and 60 minute soaks gave the better results in ambient light. As interest in $ZnPc(SO_2Cl)_4$ was mainly as a possible luminescent fingerprint reagent the 15 minute soak was deemed the most appropriate treatment time of those examined.

As a 15 minute soak would not be favoured operationally, a further experiment was undertaken, comparing a 15 minutes soak to a 7.5 minute soak. The initial attempt gave

the following results. Of 10 samples, one 15 minute sample had a very faint print that was visible in ambient light, this result was consistent with those obtained in the previous experiment. Under UV illumination better results were obtained, 4 of the 10 samples for both treatment times showed detectable prints. However blurriness of the developed prints was again noted. This experiment was repeated and very similar results were obtained. There was a slightly higher rate of detection under UV illumination for both treatment times, 6 out of the 10 samples showed detectable prints, but again the developed prints were blurry. At this point it was decided to not undertake any further optimisation experiments with $ZnPc(SO_2Cl)_4$. Filter paper, while very useful for fingerprint screen experiments, was less useful for fingerprint development work.

It was concluded that $ZnPc(SO_2Cl)_4$ showed some utility as a fingerprint reagent. However further work on better suited substrates would be necessary to determine the optimum conditions for fingerprint development. It was also noted that examination with a light source closer to the excitation wavelengths of $ZnPc(SO_2Cl)_4$ should yield better results than those obtained with the UV lamp

4.11 Characterisation of $CoPc(SO_2Cl)_4$

4.11.1 Absorption Spectra of $CoPc(SO_2Cl)_4$

UV-Vis spectra of $CoPc(SO_2Cl)_4$ were collected in dimethylformamide (GPR grade) and the results obtained are shown in Table 4.15. The spectra were very similar in nature to those observed for $CuPc(SO_2Cl)_4$ and $ZnPc(SO_2Cl)_4$. It was interesting to note that a similar trend was seen for the position of the Q-band for the $MPc(SO_2Cl)_4$ species, as is seen for the MPcs. That is the Cu and Zn analogues have Q-bands that occur at similar wavelengths, 672 and 670.8 nm respectively, while the Q-band of the Co analogue is slightly removed, occurring at 663.0 nm.

Table 4.15 UV-Vis data for $CoPc(SO_2Cl)_4$

Band	λ (nm)	ε (L mol^{-1} cm^{-1})
Q	663.0	116000 ± 6000
Satellite 1	601.0	32000 ± 2000
Soret	331.0	59000 ± 3000
Satellite 2	290.4	53000 ± 3000

4.11.2 Excitation and Emission Spectra of $CoPc(SO_2Cl)_4$

Luminescence spectra of $CoPc(SO_2Cl)_4$ were collected in ethanol. They showed that $CoPc(SO_2Cl)_4$ did not exhibit any significant luminescence. In terms of a fingerprint reagent this translates to the compound developing fingerprints that would only be visible in ambient light, they would not be rendered luminescent by excitation with a light source.

4.12 Fingerprint Screen of $CoPc(SO_2Cl)_4$

4.12.1 Solubility of $CoPc(SO_2Cl)_4$

As with all other compounds, before undertaking a fingerprint screen it was necessary to determine what solvents $CoPc(SO_2Cl)_4$ was soluble in. Test-tubes containing a small amount of $CoPc(SO_2Cl)_4$ were prepared. To these was added approximately 1-2 mL of solvent. The results obtained are shown in Table 4.16.

Table 4.16 Solubility of $CoPc(SO_2Cl)_4$

Solvent	Solubility	Colour of Resulting Solution
Distilled water	X	-
Methanol	√	bright blue
Ethanol	√	bright blue
Acetonitrile	√	pale blue
Acetone†	√	bright aqua (blue/green)
Tetrahydrofuran	√	bright blue
Chloroform	√	very pale green
Dichloromethane	√	very pale blue
Toluene	√	very pale blue
Hexane	X	-
Freon	X	-
Ethyl acetate	√	very pale blue
Petroleum spirits	X	-
Diethyl ether	X	-

† drum grade

Good solubility was exhibited in a number of solvents. However it should be noted that this compound is highly likely to be a mixture of components, i.e. $CoPc(SO_2Cl)_x$ where x = 1, 2, 3, or 4. The synthesis had produced a material that was of a similar colour to that of the parent compound. This was in contrast to the Cu and Zn analogues, where both solids had changed colour from starting materials that were blue to products that were dark green. While no fundamental work could be carried out with such a material, it was decided that an indication of likely reactivity towards glycine could be gained, but that no other experiments would be undertaken.

4.12.2 Fingerprint Screen Experiment

A fingerprint screen experiment was carried out in exactly the same manner as that described in Section 2.5, using a saturated $CoPc(SO_2Cl)_x$ ethanol solution. The following results were obtained. Spots were visible on all samples, with spots darker in colour with increased soaking time. There was little difference between air and oven-dried samples, an example is shown in Figure 4.11. This experiment confirmed that $CoPc(SO_2Cl)_x$ had reactivity towards glycine.

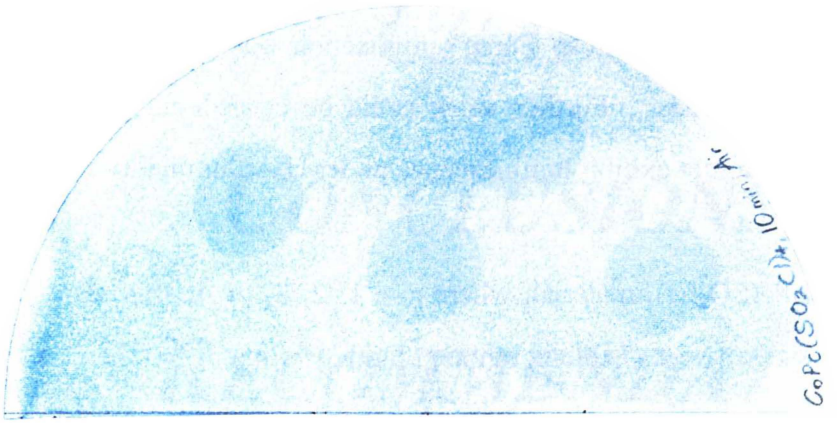


Figure 4.11 Glycine spots developed by a 10 minute soak in a saturated $CoPc(SO_2Cl)_x$ ethanol solution

4.13 Conclusions and Recommendations

It has been shown that peripherally substituted metallophthalocyanines, $MPc(SO_2Cl)_4$, where M = Cu, Zn, and Co, react with water soluble fingerprint components. All gave positive glycine fingerprint screen results, with the mode of action likely to be reaction between the chlorosulfonyl groups of the metallophthalocyanine and the amine groups of amino acids.

$CuPc(SO_2Cl)_4$ gave glycine spots that were visible in ambient light. It was also found to develop latent fingerprints. The optimum conditions were found to be a drag in 1.6 x 10^{-3} M $CuPc(SO_2Cl)_4$ ethanol solution followed by air-drying. This reagent was compared to ninhydrin, and ninhydrin was found to be the superior reagent to use on the papers examined. However this does not preclude all operational use of $CuPc(SO_2Cl)_4$ as a fingerprint reagent. It may have application on surfaces where ninhydrin exhibits poor contrast. $CuPc(SO_2Cl)_4$ was also found not to interfere with the performance of ninhydrin. That is, $CuPc(SO_2Cl)_4$ can be used prior to ninhydrin without effect on the subsequent performance of ninhydrin. This sequential treatment gave ninhydrin developed prints that had a darker purple colour than that which is normally observed for ninhydrin treatment alone.

$ZnPc(SO_2Cl)_4$ developed glycine spots and latent fingerprints that were visible in ambient light and under UV illumination at 254 nm. However the nature of the

luminescent properties of $ZnPc(SO_2Cl)_4$ hampered optimisation on routinely encountered paper substrates. Some optimisation was attempted on filter paper but it was of limited success. Further work could be carried out with this compound on substrates that do not exhibit luminescence under UV illumination.

A mixed $CoPc(SO_2Cl)_x$ material, where x = 1, 2, 3, or 4, was also shown to develop glycine spots that were visible in ambient light.

Overall these results showed that developing a suite of fingerprint reagents with a range of colour and luminescence properties is clearly possible. A variety of metal analogues of $MPc(SO_2Cl)_4$ could be synthesised, characterised and investigated to give a range of reagents with different colour and luminescence properties. Though it is noted that the chlorosulfonic acid/thionyl chloride synthetic strategy does require further work. It may be that an alternative synthetic strategy, one that involves substitution of the $-SO_3^-$ functional group, prior to formation of the metallophthalocyanine [133-135], may show more utility than the approach employed in this thesis.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

As originally stated the aim of this research was to investigate the potential of porphyrins and phthalocyanines as reagents for the development of latent fingerprints. This was undertaken by synthesising, characterising, and appraising a number of representative compounds.

$TPPSn(OH)_2$, an example of an axially substituted metalloporphyrin was investigated. It was found that this compound was able to develop latent fingerprints by reacting with both water and non-water soluble fingerprint components. Optimum conditions for the use of this reagent were determined to be:

(i) a 2 minute soak in a 2.5×10^{-3} M ethanol solution or

(ii) a 20 second soak in a 5×10^{-3} M ethanol solution.

Fingerprint development is rapid, and requires no application of heat. Viewing of the prints does require a light source, such as a Polilight®, with several excitation wavelengths available, these being 330, 405, 435, 518, and 550 nm. Specific suggestions were made for the use of this reagent. As there is reactivity towards non-water soluble fingerprint components, $TPPSn(OH)_2$ may be a useful treatment prior to the application of PD, in the detection of fingerprints that have been exposed to water. As this method requires no heating, another application is the development of fingerprints on thermal papers. Finally this reagent meets some of those suggestions that were put forward at the International Symposium on Fingerprint Detection and Identification (Section 1.8, [96]). While no sensitivity studies were undertaken, this reagent does react with both amino acids and lipids, without heating, to give a fluorescent print, that can be used on paper. Furthermore with modification to the periphery of the porphyrin macrocycle,

derivatives of $TPPSn(OH)_2$ may be prepared that might show more suitability as fingerprint reagents.

$PcSn(OH)_2$ and PcEuCl, examples of axially substituted metallophthalocyanines, were examined. It was found that $PcSn(OH)_2$, like $TPPSn(OH)_2$, had reactivity towards water and non-water soluble fingerprint components. However the ability of this compound to develop latent fingerprints was largely hampered by the low solubility it exhibited in a number of common solvents. The performance of this compound may be improved by peripheral substitution to the phthalocyanine macrocyclic ring by functional groups that aid solubility. PcEuCl was also found to have reactivity towards water and non-water soluble fingerprint components. However this reagent has excitation wavelengths that coincide with those of optical brighteners which are found in a number of papers. However the excellent luminescence qualities of this compound suggest that further work could be carried out to determine whether this compound would show utility on surfaces that do not contain optical brighteners, such as brown cardboard.

Lastly, $CuPc(SO_2Cl)_4$, along with the Zn and Co analogues, which are examples of peripherally substituted metallophthalocyanines, were examined. It should be noted here that while peripherally substituted metalloporphyrins were not investigated, there is every expectation that such compounds should also show an ability to develop latent fingerprints. It was found that $CuPc(SO_2Cl)_4$ was able to develop latent fingerprints by reacting with water soluble fingerprint components. Optimum conditions for the use of this reagent were determined to be a drag in a 1.6×10^{-3} M ethanol solution. Fingerprint development is rapid, and no heating is required. Fingerprints are visible in ambient light and are green/blue in colour. This reagent was compared to ninhydrin, which was found to be the superior reagent to use on the paper substrates examined. However $CuPc(SO_2Cl)_4$ may still have application on surfaces where the contrast obtained by treatment with ninhydrin is poor. The Zn analogue also showed reactivity towards both glycine and fingerprints. This analogue also had luminescent properties with excitation wavelengths occurring at 227, 238, and 296 nm, and emission at 355 nm. However optimisation of $ZnPc(SO_2Cl)_4$ was largely hampered on commonly encountered papers

by the luminescence of optical brighteners that such papers usually contain. Further work could be carried out on surfaces which do not contain such compounds. A Co analogue of mixed substitution was also found to have reactivity towards glycine. This work showed that it could be possible to develop a suite of fingerprint reagents that have different colour and luminescence properties. This would allow matching of a reagent to the surface encountered enabling the contrast between the developed print and the background to be maximised, which would be highly desirable. Other metal analogues of the form $MPc(SO_2Cl)_4$ could be examined, or a similar approach utilising a different functional group could be investigated.

Thus the overall conclusion reached is that porphyrins and phthalocyanines are not only capable of developing latent fingerprints, but actually show utility and promise in this area. While porphyrins and phthalocyanines, as a consequence of their unique characteristics, are used in a vast number of applications, this is the first time they have been put forward for application in the field of forensic science. This thesis showcases this novel approach for the development of new fingerprint reagents. Considering the almost unlimited possibilities in synthesising these compounds this avenue of research clearly merits further investigation.

APPENDIX I

FINGERPRINT

DEVELOPMENT TECHNIQUES

The following protocols were adhered to for the preparation and use of the PD, ninhydrin/$ZnCl_2$, DFO, and iodine techniques. Note that the methodology presented for the first three techniques is a summarised version of that which is contained in [13]. The methodology for iodine followed that viewed at the Hamilton Fingerprint Section of the NZ Police.

I.I Physical Developer

Preparation Of Solutions

Glassware

All glassware used must be scrupulously clean. All internal glass surfaces should be wiped with paper under cold, running tap water and then rinsed with distilled water three times. After use all glassware should be washed with running tap water and dried with a cloth or tissue paper. Difficult stains may be cleaned with detergent, but not abrasive cleaning agents, followed by rinsing with running tap water for 10 minutes.

Maleic Acid Solution

Place distilled water (1 L) in a clean glass beaker (2 L). While stirring with a magnetic stirrer add maleic acid (25 g). Stir until all solid dissolved. A colourless solution will be produced that will keep indefinitely.

Stock Detergent Solution

Place distilled water (1 L) in a clean glass beaker (2 L). While stirring with a magnetic stirrer add n-dodecylamine acetate† (4 g) followed by Synperonic N (4 g). Stir for at least 30 minutes. A slightly cloudy, colourless stock detergent solution will be produced that will keep indefinitely.

Note that the stock detergent solution used for experimental work in this thesis was not prepared by the author, it was obtained from the Hamilton Fingerprint Section of the NZ Police.

Working Solution

Place distilled water (50 mL) in a clean glass beaker (100 mL). While stirring with a magnetic stirrer add silver nitrate (10 g) and stir for 1 minute. Put this solution aside in the dark. Place distilled water (900 mL) in a clean glass beaker (2 L). While stirring with a magnetic stirrer add as quickly as possible and in the following order, ferric nitrate (30 g), ammonium ferrous sulphate (80 g), and citric acid (20 g). Stir until all solid has dissolved and for a further 5 minutes. Add stock detergent solution (40 mL) and stir for 2 minutes and then add silver nitrate solution previously prepared and stir for a further 2 minutes. A working solution will be produced which may vary from pale yellow to dark brown. It will keep for several weeks at room temperature in the dark.

Treatment Of Articles

Again all glassware used must be scrupulously clean. Lay out 5 glass dishes as in Figure I.I. In the first put maleic acid, in the second, working solution, and in the last three put distilled water. All should be filled to a depth of at least 2 cm.

Figure I.I Order of glass dishes for treatment of articles with PD

†Note the manual states n-dodecylamine acetate, however it is probable that n-dodecylammonium acetate was actually meant

Immerse item in maleic acid solution for 10 minutes or until no more bubbles are seen coming from the paper, whichever is the longer. Transfer item to working solution and gently rock dish. Fingerprints should appear as dark grey images. Remove item when background appears significantly darker or after 20 minutes if no fingerprints have started to develop, longer if the working solution is not fresh. Place item in first dish of distilled water, wash item for 5 minutes rocking dish occasionally. Transfer item to second dish of distilled water, wash item for 5 minutes rocking dish occasionally. Transfer item to last dish of distilled water and again wash item for 5 minutes rocking dish occasionally. Finally wash item for 10 minutes in gently running, cold tap water then allow item to dry at room temperature.

I.II Ninhydrin - Zinc Chloride

Preparation Of Solutions

Concentrated Solution

Place acetic acid (50 mL) in a clean, dry, glass beaker (250 mL). Add ninhydrin (25 g) and stir with a magnetic stirrer, a slurry will be produced. Add ethanol (100 mL) and stir until a clear, yellow solution is produced that will keep at least 3 months at room temperature.

Working Solution

Place concentrated solution (30 mL) in a clean, dry, glass beaker (2 L). Stir with a magnetic stirrer and add 1,1,2-trichlorofluoroethane (1 L) until a clear, pale yellow working solution is produced that will keep indefinitely at room temperature.

Zinc Toning Solution

Place ethanol (50 mL) in a clean, dry, glass beaker (500 mL). While stirring with a magnetic stirrer add 2-propanol (10 mL) and then acetic acid (10 mL). Add zinc chloride (6 g) and stir until all solid has dissolved. Add 1,1,2-trichlorotrifluoroethane (200 mL)

and stir for 5 minutes. A colourless zinc toning solution will be produced that will keep for several months.

Treatment Of Articles

Pour working solution into a clean, dry, shallow dish to an approximate depth of 1 cm. Draw item through working solution using tweezers or immerse for a maximum of 5 seconds. Allow item to dry completely on clean cardboard and then heat in an oven at 80°C and 65% relative humidity for 4 minutes. Examine in ambient light.

Place zinc toning solution in a spraying vessel and lightly spray ninhydrin treated item, but do not visibly wet the surface. Heat item in an oven at 80°C and 65% relative humidity for 4 minutes. Examine under an appropriate light source. The absorption spectrum of zinc toned ninhydrin fingerprints is broad, rising to a maximum between 480 - 490 nm. The fingerprints emit over a wide wavelength range with maxima occuring at approximately 545, 565, and 595 nm. For further details see [36].

I.III DFO

Preparation Of Solution

Working Solution

Place DFO (0.25 g) in a clean, dry, glass beaker (100 mL). Add methanol (30 mL) and stir with a magnetic stirrer, a slurry will be produced. Add acetic acid (20 mL) and stir until a clear, yellow solution is produced. Transfer this solution to a large, clean, dry, glass beaker (2 L). Add 1,1,2-trichlorotrifluoroethane (1 L) and stir with a magnetic stirrer for 2 minutes. A clear, pale yellow working solution will be produced that will keep for at least one month at room temperature, longer if stored in a refrigerator.

Treatment Of Articles

Pour working solution into a clean, dry, shallow dish to an approximate depth of 1 cm. Draw item through working solution using tweezers or immerse for a maximum of 5 seconds. Let item dry completely on thick, clean, dry tissue and then heat article in an oven at 100°C for 20 minutes. Examine under an appropriate light source. The absorption spectrum of DFO is broad, rising to a maximum at approximately 568 nm with smaller peaks at 470 and 525 nm. DFO emits over a wide wavelength range with the maximum occuring at approximately 578 nm. For further details see [36].

I.IV Iodine

Preparation Of Iodine Vapour Wand

A 100 mL separating funnel was prepared as follows. A layer of glass wool was inserted into the separating funnel, then silica crystals, then a layer of glass wool, then iodine crystals, then a layer of glass wool, then silica crystals, and then a final layer of glass wool. A representation is shown in Figure I.II.

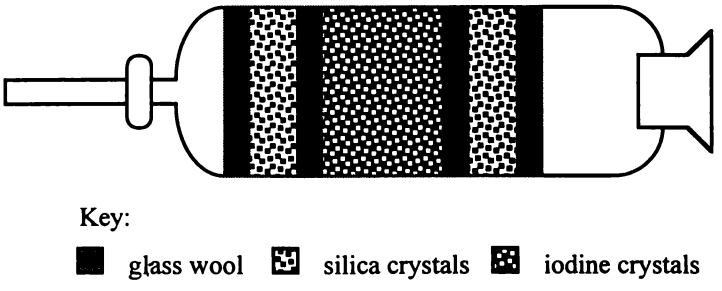


Figure I.II Representation of iodine vapour wand

Treatment Of Articles

Articles are wafted with iodine vapours. These are produced by fitting the iodine vapour wand with a rubber hose that is connected to an air source. When the tap is open, the stopper is off, and air is flowing through, vapours will be emitted at the stopper end. Wafting is continued until prints are seen to develop. Prints fade quite rapidly but can be treated with an α-napthoflavone fixing solution to make permanent.

However for the experimental work carried out for this thesis, the fixing step was unnecessary.

APPENDIX II

INSTRUCTIONS AND SURVEY FORM FOR TPPSN(OH)$_2$

A copy of the instructions[†] and survey form (both slightly modified to accommodate smaller margins of thesis) that was sent out, with a covering letter and $TPPSn(OH)_2$ sample, are shown. They were sent to the following agencies or individuals:

- Auckland, Hamilton and Wellington Fingerprint Sections of the NZ Police
- Dr Joseph Almog, Director, Division of Identification and Forensic Science, Israel Police
- Senior Sergeant Ross Bauer, Scientific Section, Queensland Police Service
- Mr Juan Cabrera[‡], Crime Scene Unit, Fort Lauderdale Police Department, Florida, USA
- Mr Terry Kent, Fingerprint Research Group, Scientific Research and Development Branch, Home Office, UK
- Dr Chris Lennard, Director, Scientific Forensic Services, Australian Federal Police
- Detective Senior Sergeant Leigh Purday, Fingerprint Specialist Support, Branch Forensic Services Group, NSW Police Service

Two survey forms were returned and these replies are shown. Some feedback with a third party via email has been received, and this is also shown.

[†]The latter version of the instructions include the 330 nm excitation option, however it was omitted in the earlier version

[‡]A latter version of the survey form was sent in this instance and is also included

Instructions for the Use of $TPPSn(OH)_2$

Treatment of Articles

1 Pour solution into a shallow dish to a depth of approximately 1 cm.

2 Soak item in solution for required time (2 minutes or 20 seconds, depending on the solution used).

3 Allow item to air-dry.

4 View item under Polilight® (or similar) with the Polilight® set at the following wavelengths: 550 nm, 518 nm, 435 nm, 405 or 330 nm (see below) using appropriate goggles.

Note: 330, 405, and 435 nm are best for thermal papers as no prints are visible if 518 or 550 nm is used.

Wavelengths correspond approximately as follows:
550: Filter Select on 555, "10 Scale" on 2
518: Filter Select on 530, "10 Scale" on 4
435: Filter Select on 450, "10 Scale" on 5
405: Filter Select on 415, "10 Scale" on 3
330: Filter Select on 350, "10 Scale" on 7

Preparation of Solutions

"2 Minute Soak Solution"

1 Weigh out **0.19 g** of **$TPPSn(OH)_2$** into a glass beaker containing a magnetic stirrer bar.

2 Measure out **100 mL** of **Ethanol**.

3 Add the ethanol to the $TPPSn(OH)_2$ and stir vigorously until all the solid has dissolved.

4 The solution can now be transferred to a glass bottle with a lid. The solution will keep indefinitely. The appearance of small amounts of solid in the solution does not affect the performance of the solution, indeed a small volume of ethanol can be added to redissolve any solid if desired.

Note: If a magnetic stirrer is not available then the solution can be made up directly in a glass bottle which can be shaken to dissolve the solid.

"20 Second Soak Solution"

(Note identical preparation as for 2 minute soak solution except for the change in the amount of $TPPSn(OH)_2$ weighed out)

1 Weigh out **0.38 g** of **$TPPSn(OH)_2$** into a glass beaker containing a magnetic stirrer bar.

2 Measure out **100 mL** of **Ethanol**.

3 Add the ethanol to the $TPPSn(OH)_2$ and stir vigorously until all the solid has dissolved.

4 The solution can now be transferred to a glass bottle with a lid. The solution will keep indefinitely. The appearance of small amounts of solid in the solution does not affect the performance of the solution, a small volume of ethanol can be added to redissolve any solid if desired.

Note: If a magnetic stirrer is not available then the solution can be made up directly in a glass bottle which can be shaken to dissolve the solid.

$TPPSn(OH)_2$ Survey

Please fill out and return to: Karen Murphy
Chemistry Department
University of Waikato
Private Bag 3105
Hamilton
NEW ZEALAND

1. **Did you find $TPPSn(OH)_2$ solutions easy to prepare?**

.....

2. **Did you find it easy to process items with $TPPSn(OH)_2$?**

.....

3. **Were the instructions provided suitable?**

.....

4. **How did you find the performance of $TPPSn(OH)_2$?**

.....

.....

.....

5. **How would you rate it compared to Ninhydrin, Ninhydrin/$ZnCl_2$, DFO, and/or Physical Developer?**

.....

6. **Did you find it to perform better or worse on any particular type of surface? e.g. fax paper, eft-pos receipts**

.....

.....

7. **Would you use $TPPSn(OH)_2$ if it was available?**

.....

8. **Any other comments...**

.....

.....

.....

$TPPSn(OH)_2$ Survey: Reply 1

1. **Did you find $TPPSn(OH)_2$ solutions easy to prepare?**

Yes

2. **Did you find it easy to process items with $TPPSn(OH)_2$?**

Yes

3. **Were the instructions provided suitable?**

*Mostly**

4. **How did you find the performance of $TPPSn(OH)_2$?**

Results under 150 watt Polilight - Nil

Results under 300 watt Polilight - Slight fluorescence only on very shiny paper

5. **How would you rate it compared to Ninhydrin, Ninhydrin/$ZnCl_2$, DFO, and/or Physical Developer?**

Ninhydrin found easier - obvious visual prints on comparative test papers

6. **Did you find it to perform better or worse on any particular type of surface? e.g. fax paper, eft-pos receipts**

Couldn't get it to work at all on flat surfaced paper

7. **Would you use $TPPSn(OH)_2$ if it was available?**

Not at this stage

8. **Any other comments...**

Whilst the results we achieved were poor, the discoloration of the treated paper was also seen as undesirable

*Other communication was had with this individual as the person had some trouble in getting this method to work.

$TPPSn(OH)_2$ Survey: Reply 2

1. **Did you find $TPPSn(OH)_2$ solutions easy to prepare?**

..... Yes.....

2. **Did you find it easy to process items with $TPPSn(OH)_2$?**

..... Yes.....

3. **Were the instructions provided suitable?**

..... Yes.....

4. **How did you find the performance of $TPPSn(OH)_2$?**

..... $TPPSn(OH)_2$ did not perform very well on fresh fingerprints. Most of the .....

.....fingerprints that did not develop with ridge detail but as "spots".....

.....

5. **How would you rate it compared to Ninhydrin, Ninhydrin/$ZnCl_2$, DFO, and/or Physical Developer?**

..... PD gave better results than $TPPSn(OH)_2$. Old PD was not compared.....

..... because it would not be used in actual case work.....

.....

6. **Did you find it to perform better or worse on any particular type of surface? e.g. fax paper, eft-pos receipts**

..... The performance on fax paper was not as good as on white paper (non-thermal).....

.....

7. **Would you use $TPPSn(OH)_2$ if it was available?**

..... No. There are better choices to use.....

8. **Any other comments...**

..... The comparison to DFO/Ninhydrin on thermal paper is not suitable in our .....

..... eyes. We compared $TPPSn(OH)_2$ to DMAC which develops fingerprints.....

..... on thermal paper without harming the paper. DMAC was superior to.....

..... $TPPSn(OH)_2$ on thermal paper .....

..... Overall the study was very interesting and important in its attempt to find.....

..... alternative and better fingerprint development methods.....

$TPPSn(OH)_2$ Survey (Version II)

Please fill out and return to: Karen Murphy
Chemistry Department
University of Waikato
Private Bag 3105
Hamilton
NEW ZEALAND

1. **Did you find $TPPSn(OH)_2$ solutions easy to prepare?**

.....

2. **Did you find it easy to process items with $TPPSn(OH)_2$?**

.....

3. **Were the instructions provided suitable?**

.....

4. **Did you try both the "2-minute" and "20-second" soak solutions?**

.....

5. **If so, did you have any preference? Why?**

.....

.....

6. **How did you find the performance of $TPPSn(OH)_2$?**

.....

.....

7. **Did you find it to perform better or worse on any particular substrate, e.g. thermal paper?**

.....

.....

8. **How did it compare to other reagents?**

.....

.....

9. **Would you use $TPPSn(OH)_2$ if it was available?**

.....

10. **Any other comments...**

.....

.....

.....

.....

Email Received Regarding Performance of $TPPSn(OH)_2$

"I want to thank you and Karen Murphy for the trial shipment of the working solution.

I would have contacted Ms. Murphy however I don't have her email address. Unfortunately our first test was on a very difficult item, US currency (a US one dollar bill). I subjected it to Ninhydrin, then Maleic Acid pre-wash with Physical Developer. The Ninhydrin developed a few unidentifiable ridges. No ridges were observed with the Physical Developer. I then subjected the dollar bill to $TPPSn(OH)_2$. Unfortunately no latents of value were observed. Do you think the Maleic Acid pre-wash had an unexpected adverse effect on the $TPPSn(OH)_2$ reagent?

We will continue to experiment with the reagent and will send you feedback on the outcome.

We would be interested in some additional samples of the compound if at all possible."

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