Faculty of Science and Engineering

Department of Chemistry

Natural Products as Novel Reagents for the Detection of Latent Fingermarks

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Of

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DECLARATION

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

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ABSTRACT

The ability to detect latent fingermarks on porous surfaces, such as paper-based documents, is extremely important in resolving criminal cases. Detection methods that target amino acids present in latent fingermark deposits have achieved widespread use. This is due to the chemical stability of this component of the eccrine secretion and the binding of the amino acids to paper fibres, thus resulting in a good representation of the fingermark. This dissertation presents studies into the ability of naphthoquinones to develop latent fingermarks on paper surfaces. These compounds represent a completely new class of fingermark detection reagents.

Lawsone (2-hydroxy-1,4-naphthoquinone), the compound thought to be responsible for the staining properties of henna, was found to react with latent fingermarks on porous surfaces to yield purple/brown images that exhibit photoluminescence without further treatment. Luminescence spectrophotometry of developed amino acid spots on paper indicated that lawsone is reacting with the amino acids in latent fingermarks. A mechanism for this reaction is postulated.

Lawsone is a naphthoquinone, a group of compounds, which are known for reacting with amino acids to produce highly coloured compounds. On this basis, the following naphthoquinones were selected for subsequent investigations: 1,4-dihydroxy-2-naphthoic acid; 1,2-naphthoquinone-4-sulfonate; 2-methoxy-1,4-naphthoquinone; and 2-methyl-1,4-naphthoquinone. All of the tested compounds yielded brown visible impressions, which also exhibited luminescence. Luminescence spectrophotometry revealed differences in photoluminescence characteristics for fingermarks developed with the different naphthoquinones with excitation over the range 530-590 nm. Luminescence

spectrophotometry of developed amino acid spots on paper confirmed that the naphthoquinones were reacting with amino acids in the latent fingermarks.

Fingermarks and amino acids on cellulose thin layer chromatography plates were developed with lawsone and the additional selected naphthoquinones listed above. These specimens were then subjected to analysis by synchrotron infrared microscopy. Despite significant issues with the presence of a cellulose background signal, it was possible to successfully obtain spectra of the reaction products *in-situ*. In an attempt to rationalise these results, lawsone was reacted with glycine in solution to afford a mixture of two coloured compounds. These compounds were characterised and one was tentatively identified as 2-amino-1,4-naphthoquinone or a structural isomer thereof. A structure could not be assigned to the second compound, however nuclear magnetic resonance spectroscopy indicated a symmetrical dimer, containing two lawsone moieties.

Some preliminary results are presented for a screening of additional natural product based colour forming compounds as latent fingermark detection reagents. Two compounds, juglone and alizarin, were found to successfully develop latent fingermarks on paper. In addition a systematic retrosynthetic approach was taken to explore the potential of a natural dye precursor as a fingermark reagent. Finally, a serendipitous discovery of the skin dyeing properties of 6-N,N-dimethylaminofulvene, provided an additional route to a possible class of fingermark detection compounds unrelated to ninhydrin. These preliminary studies indicate potential strategies for further research towards novel latent fingermark reagents for porous surfaces.

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PUBLICATIONS

Peer reviewed publications that have arisen from the research presented in this thesis:

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R. Jelly, E.L.T. Patton, S.W. Lewis, C. Lennard, K.F. Lim. **The detection of latent fingermarks on porous surfaces using amino acid sensitive reagents: A review**, *Analytica Chimica Acta*, (2009), Vol 652 (1-2), p. 128-142.*

* Awarded the Kagi-Alexander HDR prize for 2009 (Curtin University of Technology) for the most outstanding paper published by a higher degree research student in a peer-reviewed journal.

R. Jelly, S.W. Lewis, C. Lennard, K.F. Lim, J. Almog. **Substituted naphthoquinones as novel amino acid sensitive reagents for the development of latent fingermarks on paper surfaces,** *Talanta*, (2010), Vol 82, p. 1717-1724.

R. Jelly, S.W. Lewis, K.F. Lim, A. Payne, W. van Bronswijk. **Synchrotron IR Studies of Fingermarks on Microscopically Rough Surfaces Developed with Novel Natural Product Based Reagents,** *in-preparation, Applied Spectroscopy (for submission in December 2010).*

PRESENTATIONS

Selected aspects of the work contained within this thesis were also presented at the following conferences:

- 6th International Fingerprint Research Group Meeting (IFRG), Canberra, March 2007.
- International Symposium on Advances in Fingerprint Identification, Patiala, India, February 2008.
- 93rd International Educational Conference for the International Association for Identification (IAI), Louisville, Kentucky, USA, August 2008.
- Australian and New Zealand Forensic Science Society (ANZFSS) International Symposium on the Forensic Sciences, Melbourne, Victoria, October 2008.
- 7th International Fingerprint Research Group Meeting (IFRG), Lausanne, Switzerland, June 2009.
- 5th International Conference on Advanced Vibrational Spectroscopy (incorporating the 8th Australian Conference on Vibrational Spectroscopy) (ICAVS5), Melbourne, July 2009.
- RACI Research and Development Topics, Gold Coast, December 2009.
- Australian and New Zealand Forensic Science Society (ANZFSS) 20th International Symposium on the Forensic Sciences, Sydney, September 2010.

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CHAPTER 1: INTRODUCTION

Portions of this chapter have been published in the journal *Analytica Chimica Acta*:

R. Jelly, E.L.T. Patton, S.W. Lewis, C. Lennard, K.F. Lim. The detection of latent fingermarks on porous surfaces using amino acid sensitive reagents: A review, *Analytica Chimica Acta,* (2009), Vol 652 (1-2), p. 128-142.

1.1 INTRODUCTION

A central tenet of forensic science is the exchange principle first proposed by Edmond Locard, which is often summarised to "every contact leaves a trace" [1]. Every time there is contact between persons, objects and locales there is an exchange of physical information. This is vitally important in crime scene investigation as it enables investigators to establish links between the scene, victims and the perpetrators [1]. The impressions left by the friction ridge skin on the palmar surfaces of the hands, most often referred to as fingermarks or fingerprints, not only demonstrate contact but are also sufficiently unique to enable personal identification [2-4]. The most common form of these is latent (hidden) fingermarks, and successful recovery from a surface or object relies upon their detection. To this end, a range of physical and chemical methods have been developed for the visualisation of latent fingermarks [2-4]. These methods target differences between the latent fingermark and the substrate upon which it is deposited, and are based either on physical attraction or a chemical reaction [2-4].

Paper-based evidence such as documents, wrapping material and containers, are frequently encountered in criminal investigations. The most widely used methods for detecting latent fingermarks on porous surfaces relies upon the detection of the amino acids present in natural skin secretions [5-7]. When deposited on paper substrates, the amino acids are believed to bind tightly to the cellulose (provided that moisture levels are not excessive), preserving an impression of the friction ridge patterns [4]. These impressions can have longevity, with impressions over 40 years of age being successfully visualised [4]. The first amino acid sensitive reagent to be used for the detection of latent fingermarks was ninhydrin, which gives visible purple prints [8]. Since its introduction, there has been significant research into more sensitive treatments, which have resulted in a range of techniques used routinely by law enforcement for fingermark detection [9-11]. The detection of latent fingermarks on paper

surfaces using these techniques can be considered as the trace detection of amino acids where the spatial distribution of the amino acids within (upon) the substrate needs to be retained.

This thesis describes a program of research into novel amino acid sensitive reagents for the detection of latent fingermarks on paper surfaces. The primary focus was inspired by natural products, which react to form coloured compounds.

1.2 LATENT FINGERMARKS AND THEIR FORENSIC SIGNIFICANCE

1.2.1 FRICTION RIDGE SKIN AND FINGERMARKS

Skin, or the cutaneous membrane, in combination with a variety of accessory structures (hair, nails and glands), forms the integumentary system, which is the largest organ system in the human body [12]. Skin has two major components, the epidermis and the dermis (Figure 1.1) [2].

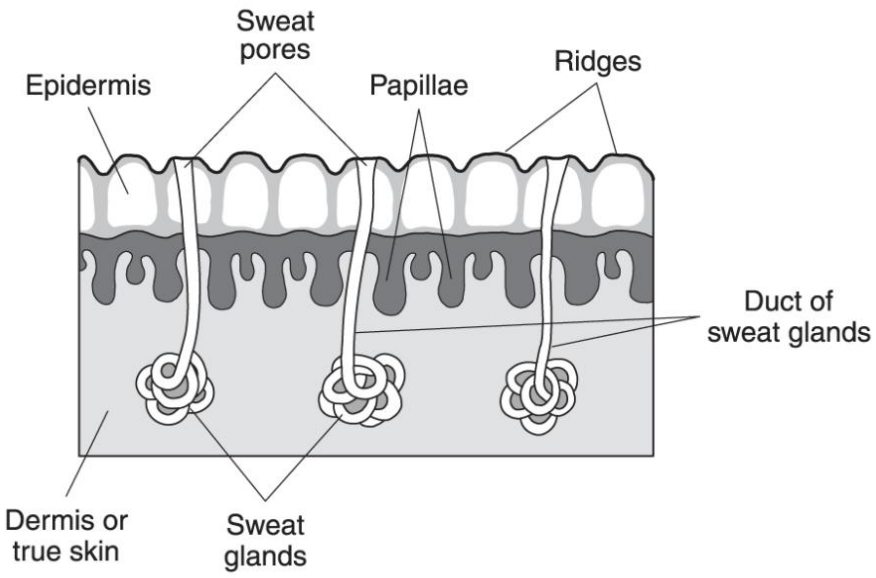


Figure 1.1: Cross-section of the friction ridge skin [13], used with permission.

The epidermis provides protection for the body from mechanical injury and from micro-organisms. Depending on location, the epidermis can range in thickness from 0.08 mm to 0.5 mm, with thicker skin being found on the gripping surfaces of the hands and the soles of the feet. Beneath the epidermis is the underlying connective tissue of the dermis, within which can be found blood vessels, sensory neurons, and the various accessory structures such as hair follicles and sweat glands, which project through the epidermis to the surface of the skin [12, 14].

The grasping surfaces of the skin covering the fingers, palms and soles of the feet are covered in ridges and furrows, with sweat pores located along the top of the ridges. The ridges and furrows, which form characteristic patterns, develop at an early stage of gestation and have their basis in the underlying dermis [4]. Superficial damage to the epidermis will not affect them, with the patterns re-appearing on recovery. The patterns have longevity, lasting throughout the life of the individual, with only deep scar tissue potentially obscuring them. The exact shape and form of the patterns observed are controlled by both genetic and physical variables *in utero*, although the mechanism of their formation is not well understood [4].

The first use of friction ridge skin impressions for identification has been a matter of some conjecture. There are indications that fingermark impressions in wax, clay and ink were used for signing legal documents in ancient Rome and the Far East. Early anatomical studies were carried out by Nehemiah Grew (1684) and Marcello Malphigi (1686), with the first major work in this area being carried out by Johan-Evangelist Purkinje, who published a study on fingermark patterns, including a classification system in 1823 [15]. This study went by unremarked in the early stages of the introduction of fingermarks for criminal investigations. This was probably due to the limited circulation of the report and the fact that it was written in Latin [15].

The advent of the modern use of friction ridge skin impressions for law enforcement and criminal investigations was during the period 1870 to 1900. The exact course of events is complicated, and there was significant acrimony between some of the early pioneers [15-20]. By the mid-twentieth century the use of fingerprints in criminal investigations was firmly established within law enforcement around the world.

Initially, fingerprint impressions were examined as a potential method for identifying habitual criminals after arrest; however, in one of the earliest papers on the subject, Henry Faulds suggested the potential for their use in criminal investigations [21]. It was subsequent years after this, in 1892 that the first recorded use of a fingermark at a crime scene occurred. The evidence enabled the conviction of Francesca Rojas in Argentina for the murder of her children [15, 17]. Since that time, fingermarks detected at crime scenes and on items of physical evidence have become one of the most useful tools for law enforcement in the investigation of crimes.

Friction ridge skin impressions can be classified into two main groups, visible and latent. Visible marks occur due to the presence of a coloured contaminant on the skin (such as blood, oil or ink) giving a positive visible impression, or a coloured substance on the substrate that can be removed when touched (such as a layer of soot or dust), to leave a negative visible impression [4, 22]. Occasionally, a visible print will be made by impression in a soft material such as clay or putty [4, 22]. Latent, essentially invisible, friction ridge impressions are formed by the transfer of skin secretions and non-visible surface contaminants to the substrate [2, 4, 22]. This is the most common type of fingermark evidence found at crime scenes [2, 4]. It is also the most problematic as latent fingermarks require some form of development to enable them to be detected and recorded.

1.2.2 Chemical Composition of Latent Fingermarks

The successful detection of latent fingermarks relies heavily upon the chemistry of the latent fingermark residue [23]. On deposition, the fingermark can be considered to be a mixture of natural secretions – an emulsion of waxes, oils and aqueous components – and surface contaminants present on the skin surface [14, 23]. With time, the chemical nature of the latent deposit will change due to evaporation of volatile components, bacterial action and oxidation [23]. The rate of change will be dependent upon the initial chemical composition of the residue and environmental conditions. This aging process can have a significant effect upon the successful development of a latent fingermark. Despite these issues, most fingermark detection techniques have been developed from a knowledge of the components of human skin secretions, without regard to the potential for the aging of the print [23]. However, researchers have recently begun to examine the aging process and how it can influence fingermark development [24].

The glands responsible for the skin secretions are found within the dermis and fall into three kinds: eccrine, sebaceous and apocrine (Table 1.1) [12]. For the purpose of latent fingermark detection, the most important are the eccrine and sebaceous glands [4, 14, 23]. Eccrine glands are the only glands on the palms of the hands, and thus contribute the major aqueous component of a latent fingermark. In addition, the hands are commonly contaminated with sebaceous secretions due to activities such as touching the face and combing the hair. Latent deposits are made up of varying combinations of secretions from these two types of glands, and while one type of secretion may predominate, there can be no purely eccrine or purely sebaceous deposit [4, 14, 23]. The composition of these secretions has been reviewed from the forensic detection standpoint and is summarised in Table 1.2 [4, 14, 23].

Table 1.1: Human skin secretory glands [12].

Types of Glands	Secretion types	Body Distribution	Role of Gland
Sebaceous	Sebum (lipids)	Typically localised to regions containing hair follicles	Inhibits the growth of bacteria, lubricates and protects the keratin of the hair shaft and conditions the surrounding skin
Sweat (sudiferous) glands			
Eccrine (merocrine)	Sweat (aqueous)	Entire body, highly concentrated on the palms of the hands and soles of the feet	Cooling the surface of the skin to reduce body temperature, excretion of water, electrolytes and metabolites, protection from environmental hazards
Apocrine	Sweat (aqueous)	Associated with hair follicles around the auxiliary regions. In particular, the armpits, groin and chest.	Scent glands (pheromones)

Table 1.2: Summary of main constituents of eccrine and sebaceous skin secretions [4, 14, 23].

Secretion	Constituents	
	Organic	**Inorganic**
Eccrine	Amino acids	Water (>98%)
	Proteins	Chloride
	Urea	Metal ions (Na^+, K^+, Ca^{2+})
	Uric acid	Sulfate
	Lactic acid	Phosphate
	Sugars	Hydrogen carbonate
	Creatinine	Ammonia
	Choline	
Sebaceous	Glycerides	
	Fatty acids	
	Wax esters	
	Squalene	
	Sterol esters	
	Sterols	

The presence of amino acids in human sweat has been widely reported in the biomedical literature (e.g. [5-7, 25-28]), with a wide range of amino acids being identified in human sweat (Table 1.3) [5]. It is known that the exact profile of amino acids present, and at what concentration, will depend upon the individual and a variety of other factors including general health, diet, gender and age [14]. This means that whenever a new method for the detection of latent fingermarks on paper surfaces is under consideration, non-specific amino acid sensitive reagents are likely to have greater applicability [4].

Table 1.3: Major amino acids found in a single wet thumb print [5].

Amino acid	Amount (μmol)
Serine	0.106
Glycine	0.071
Ornithine	0.034
Alanine	0.029
Aspartic acid	0.023
Threonine	0.018
Histidine	0.018
Valine	0.013
Proline	0.011
Leucine	0.011

1.2.3 Amino Acids and The Detection of Latent Fingermarks on Porous Surfaces

The amino acid component of skin secretions, and of environmental contaminants, is extremely important when seeking latent fingermarks on paper substrates. The amino acids, when transferred to the surface of a paper substrate, will bind strongly with minimal migration provided that the surface is not wet or exposed to very high humidity (Figure 1.2) [4]. Latent fingermarks formed in this way can be extremely long lived. Prior to the introduction of ninhydrin, paper documents were considered to be extremely challenging for latent fingermark detection, with limited techniques being applied (typically limited to the use of conventional fingermark powders). It would not be an understatement that the introduction of ninhydrin revolutionised this aspect of latent fingermark detection.

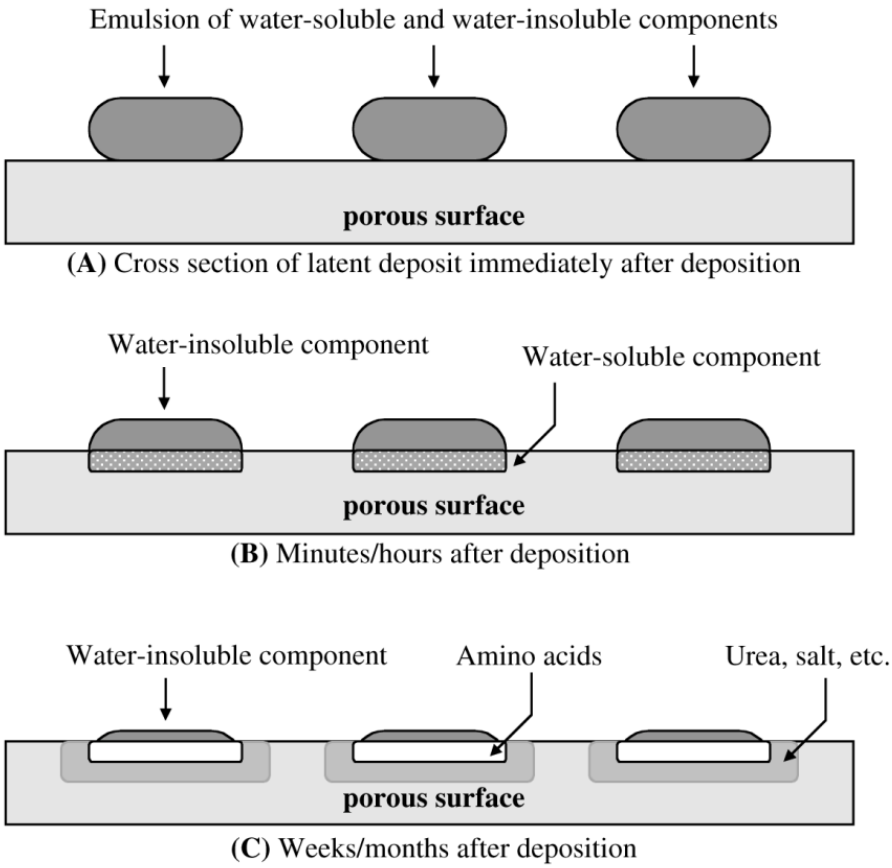


Figure 1.2: Cross section of latent fingermark on a porous substrate (e.g. paper) at various time periods after deposition [13], used by permission.

The use of amino acid sensitive reagents is one stage in the sequential approach taken by law enforcement in the examination of porous materials such as paper or cardboard, an example of which is presented in Figure 1.3 [13, 29]. The first step is a non-destructive visual examination of the evidence. If the evidence is wet, treatment with amino acid reagents is not appropriate as the amino acid component of any latent fingermarks present will have been diffused or washed away [4].

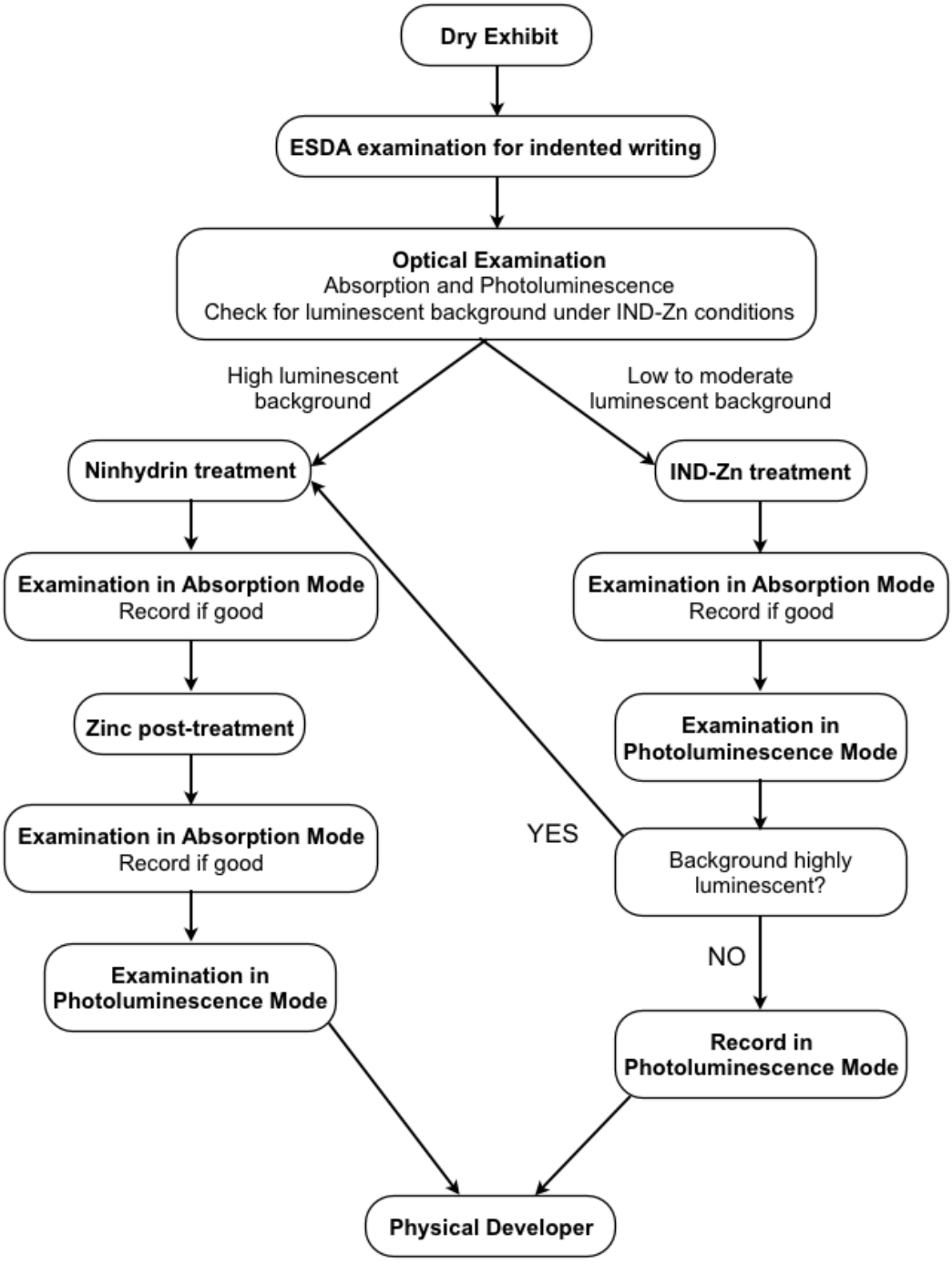


Figure 1.3: Typical development sequence for latent fingermark detection on dry porous surfaces, modified from reference [13]. ESDA: Electrostatic Detection Apparatus, IND-Zn: 1,2-Indanedione-zinc.

An important consideration for a fingerprint reagent or treatment is its place in the fingerprint development sequence. Items of evidence may require subsequent analysis for deoxyribonucleic acid (DNA) or be subjected to document examination. Any proposed new procedure requires testing for its compatibility with other forensic tests [23]. It is also well established that the sequential use of specific amino acid sensitive treatments will result in the detection of a greater number of fingermarks [29].

Treatment of paper-based documents involves dipping or spraying the item of interest with a solution of a reagent capable of targeting the presence of amino acid residues. The success of these reagents is highly dependent on the operational conditions employed. Amino acid sensitive reagents require a suitable medium not only for dissolution, but as a means of transport onto or into the surface of the item of interest, and must be performed under suitable reaction conditions [23]. This is generally in the form of specific formulations and heating methods. A more detailed discussion of this notion is outlined in Chapter 2.

1.3 AMINO ACID SENSITIVE FINGERMARK DETECTION REAGENTS

1.3.1 NINHYDRIN

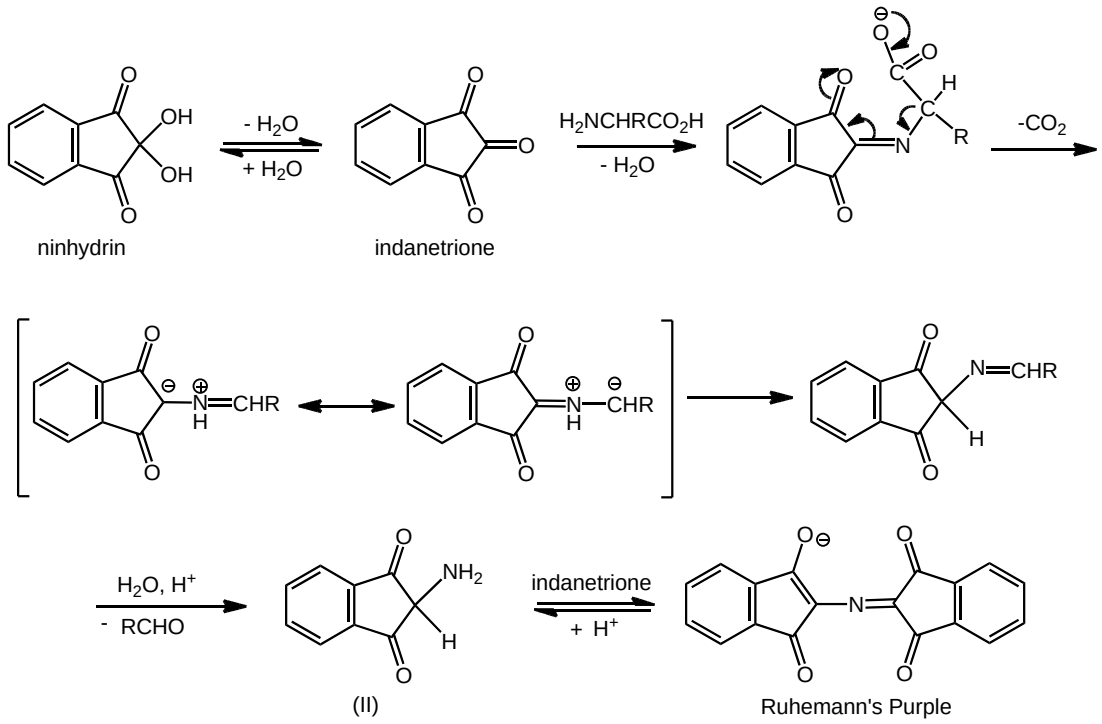
Ninhydrin has been recognised as the predominant reagent for the visualisation of latent fingermarks on porous surfaces to aid criminal investigations for many years [30-33]. On reaction with amino acids, ninhydrin (2,2-dihydroxy-1,3-indanedione) forms a non-fluorescent purple product. The reagent was first synthesised and discovered to react with amino acids in 1910 by Siegfried Ruhemann. A colour change was observed after the reagent contacted his skin, with the formation of a purple compound that was subsequently named "Ruhemann's purple" [30, 31]. In 1954, Oden and von Hofsten, while using

ninhydrin to detect amino acids separated on thin layer chromatography (TLC) plates, discovered that it could be used as a means to detect latent fingermarks on porous substrates (Figure 1.4) [8].

Figure 1.4: Latent fingermarks on a paper surface developed with ninhydrin (photo: Steven Morris, WA Police).

Historically ninhydrin has been the most extensively used and researched amino acid visualisation reagent for latent fingermarks [34]. Initial debates in relation to the types of amino acids responsible for this purple formation are well documented. Some indicated the involvement of all amino acids [3], whereas others reported that only alpha amino acids were reactive in this way [3]. Collective opinions suggested the likelihood that the purple colour was the same, irrespective of the amino acid [3]. This was after indications that only a fragment of the amino acid (the nitrogen of the amino group) is featured in the structure of Ruhemann's purple [3, 35].

The accepted general mechanism for the ninhydrin reaction was proposed by Friedman and Williams in 1974 [36] and was confirmed, with slight modifications, by Grigg and colleagues with the use of x-ray crystallographic studies [31, 37]. The most documented proposal involves a Strekker degradation where reduction of the central carbonyl on indanetrione forms 2-amino-1,3-indanedione (II in Scheme 1.1) by means of a resonance stabilised azomethine ylide. The 2-amino-1,3-indanedione can then react with another indanetrione molecule to form the stable 1,3-dipole Ruhemann's purple [3, 37-39].



Scheme 1.1: The reaction mechanism of ninhydrin with amino acids to form Ruhemann's purple [3, 31, 34, 36].

Despite ninhydrin's operational success with respect to developing latent fingermarks on porous surfaces, several limitations became apparent with time. Of particular importance was the lack of contrast observed with weaker fingermarks, which was resolved with the introduction of a secondary metal salt treatment [3] and the use of lasers and alternate light sources [40].

Previous studies involving the separation of amino acids by thin layer chromatography using ninhydrin as a visualising agent had utilised an additional treatment with particular metal salts which resulted in a colour change to red or orange [3]. Fingermark chemists in turn, looked to this as a means to overcome the aforementioned contrast issues, in particular with coloured substrates, and also found evidence of improved stability of the coloured product [3].

In 1982, Herod and Menzel not only found that fingermarks underwent a colour change with a post-treatment using zinc chloride but also observed intense fluorescence when viewed under an argon laser [40]. Photoluminescence characteristics are valuable in analytical chemistry due to increased sensitivity, resulting in improved detection limits. When applied to fingermark chemistry, this allows for excellent contrast, offering fingermarks with intense luminescence and minimal background interference (Figure 1.5) [1, 3, 41].

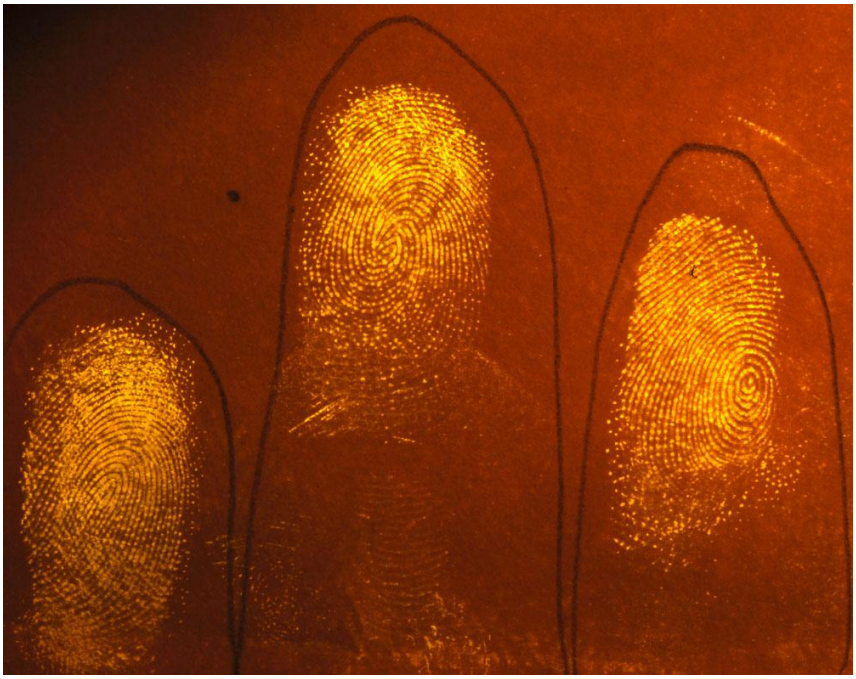
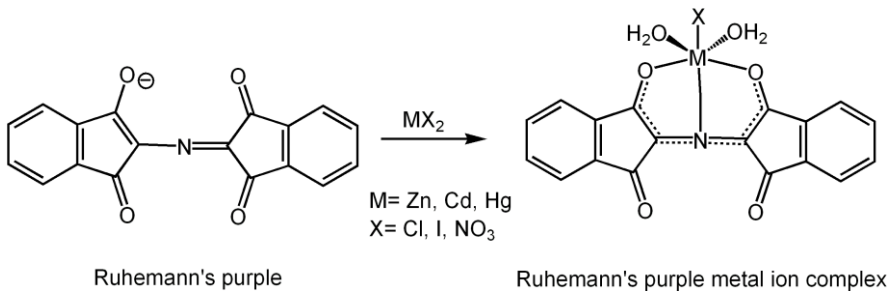


Figure 1.5: Latent fingermarks on a paper surface developed with 1,2-indanedione. Images were taken with a Nikon D300 SLR (60 mm focal length, ISO 200): excitation using a Polilight PL 500 at 505nm and viewed through a KV 550 filter, (shutter speed 0.5 s, aperture f11).

Kobus and co-workers discovered significant improvements could be achieved by cooling the exhibit with liquid nitrogen (77K) to observe luminescence when excited using illumination from a filtered Xenon arc lamp [42]. Ruhemann's purple forms a coordination complex with the metal salt changing the colour of the compound (Scheme 1.2). The colour change observed is due to energy transitions of electrons in the *d*-orbitals of the transition metal. The increased rigidity of the π system in the Ruhemann's purple-metal ion complex is thought to cause the observed luminescence.



Scheme 1.2: The reaction of Ruhemann's purple with metals salts to form a coordination complex [43, 44].

The luminescence characteristics observed for the Ruhemann's purple–metal ion complex is dependent on the type of metal salt used; for example, when using zinc chloride as the post-treatment, the excitation maximum is 495 nm, with an emission maximum of 540 nm [13].

Ruhemann's purple is known to be an active chelating agent that readily forms coordination complexes with certain metal ions [44]. The accepted structure of the Ruhemann's purple metal complex (scheme 1.2) was first determined by Lennard and colleagues with the use of x-ray diffraction [44]. This was verified via single crystal x-ray diffraction studies conducted by Davies and co-workers [43, 45].

As previously mentioned, the production of luminescence is further enhanced when cooled with liquid nitrogen. This provides a similar outcome to the use of an argon laser and serves as an alternative to law enforcement agencies that lack funding or access to an argon laser [42].

A large body of work has been carried out into determining the optimal developmental conditions for ninhydrin [2, 4, 46-49]. The formation of Ruhemann's purple has a slow reaction rate, which can be accelerated with the application of heat however; heat application has not generally been recommended as ninhydrin may react with particular additives incorporated in the paper. The application of heat will speed up both the desired and undesired processes, resulting in a degradation of contrast and a potential destruction of any fingermark evidence. As this undesired side-reaction is considered to be slower than the reaction that takes place with amino acids, it is preferable to monitor the reaction without the application of heat to ensure that any developed marks are recorded immediately and before background staining becomes problematic [13].

In addition, temperature, acidity (pH) and humidity, must be controlled to ensure optimal production of the coloured product. A typical formulation consists of a final concentration of approximately 0.5% w/v ninhydrin, with fingermark development allowed to progress at room temperature over a 24–48 hour period in an environment with 50–80 % relative humidity [2, 44].

1.3.2 NINHYDRIN ANALOGUES

The discovery of ninhydrin as an effective fingermark detection reagent prompted further investigations into ninhydrin analogues [3, 31]. This was based on the awareness that Ruhemann's finding were serendipitous, not on the basis of chemical knowledge and theoretical design [3]. It was recognised that the issues with contrast and visualisation could not be solely overcome by simple modification of the ninhydrin formulation and working conditions. This prompted researchers to investigate various molecules that possessed similar structural features to ninhydrin, that were responsible for the formation of Ruhemann's purple [3, 31].

In 1982, Almog and colleagues were the first to use this approach as a means to improve visualisation properties with respect to fingermark detection. In principle, the inclusion of electron donating and/or electron accepting substituents alters the electronic properties of the conjugated system, to produce variations in colour and/or photoluminescence. The general consensus was to develop specifically coloured complexes that could be applied to aid visualisation on a variety of backgrounds – in particular, backgrounds notorious for being problematic with conventional ninhydrin treatment [3, 31]. Many ninhydrin analogues were synthesised and have been studied, some of which are shown in Figure 1.6 [31, 32, 50].

Some of these analogues (Figure 1.6) showed promise, with both improvements in visualisation and variation in colour and luminescence [3]. The most prominent ninhydrin analogues, which surpassed initial expectations, were 1,8-diazafluorene-9-one (DFO) and 1,2-indanedione. These were of particular interest because they produce both colour and intense luminescence on reaction with the amino acids in latent fingermarks, without further treatment and without the need for cooling.

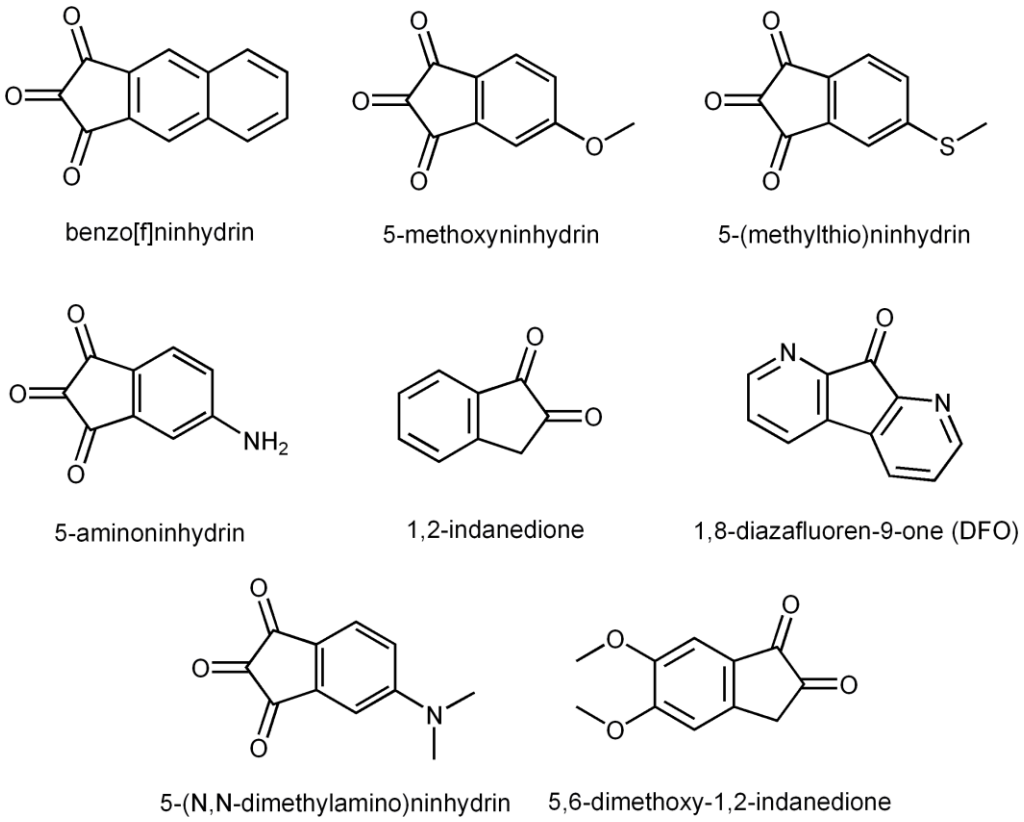
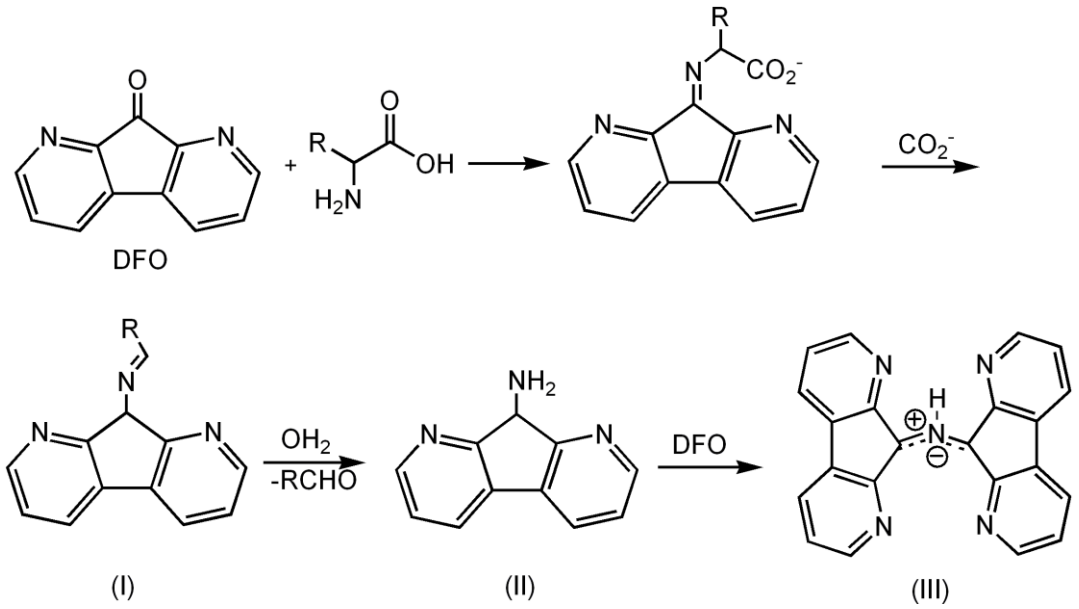


Figure 1.6: Structures for a number of ninhydrin analogues.

1.3.2.1 1,8-DIAZAFLUOREN-9-ONE (DFO)

DFO was first synthesized by Druey and Schmidt in 1950 [51] and was later established as a fingermark reagent by Grigg and Pounds in 1990 [52, 53]. Although not a direct analogue of ninhydrin, DFO is thought to react in a similar manner with amino acids, and therefore is often categorized as a relative of ninhydrin. DFO non-specifically reacts with amino acids to produce a red product with luminescence characteristics (λ_{ex} 430-580nm, λ_{em} 560-620nm [4]). Isolation and identification of the reaction product confirmed that the reaction of DFO with amino acids forms an imine intermediate (I), which undergoes decarboxylation and hydrolysis, forming an aromatic amine (II). This amine further reacts with DFO resulting in the formation of the red product (III) (Scheme 1.3) [52, 54-56].



Scheme1.3: Reaction mechanism of DFO [52, 54-56].

Unlike ninhydrin, heat is essential for the reaction to proceed, and can be applied with the use of an oven (20 min at 100 °C [4]), or by direct heat/ironing press (10 s at 180 °C [53]). However, it is important to note, that prolonged heat, high temperatures and humidity may have a detrimental effect on developed fingermarks [57, 58]. Observation of DFO treated fingermarks in the luminescence mode presents greater contrast than ninhydrin, with the added advantage of this being achieved without secondary treatment or a reduction in temperature [2, 52, 53, 55, 59]. However, ninhydrin developed fingermarks still display greater contrast in the absorbance mode [53, 55, 60].

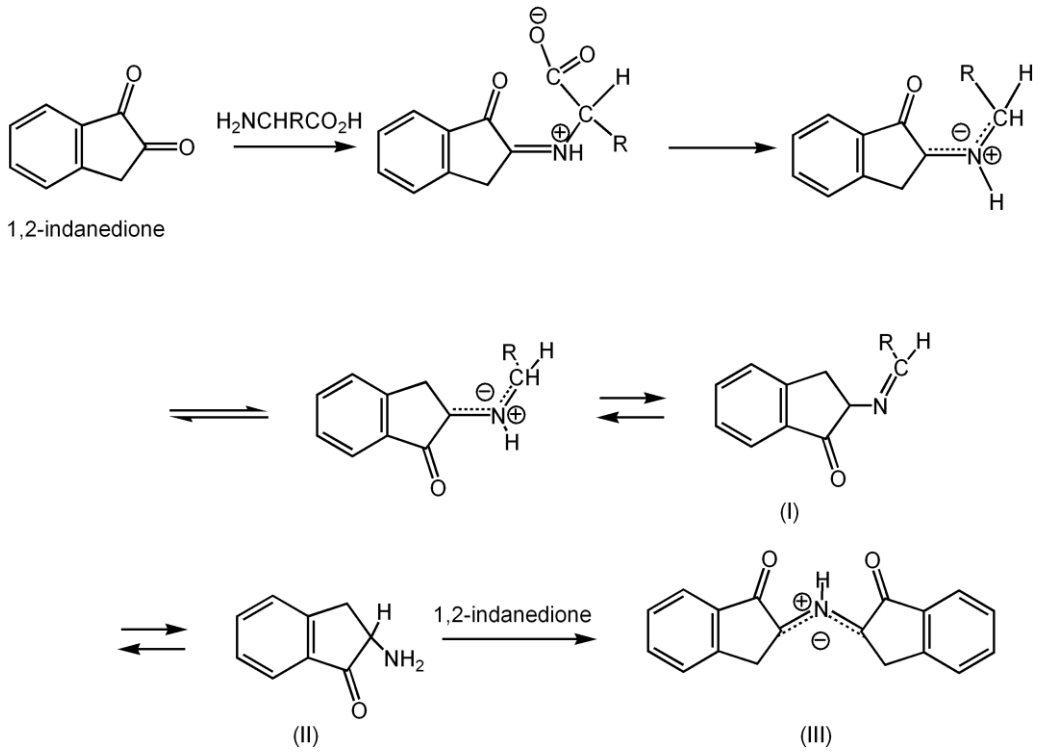
It is highly recommended that DFO development is followed by treatment with ninhydrin, in particular, where interference may arise due to luminescent paper substrates [31, 58, 61, 62]. The use of sequential treatments for latent fingermark development, ultimately allows for the greatest sensitivity (as detailed in section 2.3 of this chapter). The routine procedure for paper-based documents is therefore DFO treatment followed by ninhydrin development.

Fingermarks undetected with the use of DFO may, in some cases, be detected with the use of ninhydrin.

In a similar manner to ninhydrin, improvements in detection limits using DFO have been investigated by several research groups, but to no avail. Unlike the use of metal salts, which enhance ninhydrin development, investigations into the use of metal salts for DFO found no significant improvement in luminescence. However, as with ninhydrin, this treatment produced a variation in colour, indicating that these metal salts form a complex with the resulting reaction product [54]. Under routine procedure, the use of metal salts with DFO is not commonly employed due to the opinion that it provides no added advantage, particularly as ninhydrin development follows in sequence [13].

1.3.2.2 1,2-INDANEDIONE

In 1997, Joullie and co-workers were first to report on the ability of 1,2-indanedione to react with the amino acids present in latent fingermarks [32, 63]. Since this initial discovery, significant research has been conducted into the use and potential of 1,2-indanedione as a fingermark reagent. In a similar manner to DFO, 1,2-indanedione reacts with amino acids producing a pale pink colour with intense luminescence characteristics at room temperature (Figure 1.5) [10, 32, 63, 64]. Studies into the mechanism involved have suggested the formation of an imine (I) [39, 64, 65], followed by a decarboxylation and Strekker degradation to produce 2-amino-1-indanone (II) [39]. This then has the ability to react further with excess 1,2-indanedione to produce the coloured and luminescent product (III) (Scheme 1.4). Although proposed, this particular species has yet to be isolated or confirmed as being produced when reacting with amino acids on paper surfaces.



Scheme 1.4: Proposed reaction mechanism of 1,2-indanedione with amino acids [39].

Following in a similar path to both ninhydrin and DFO, investigations into the use of metal salts with 1,2-indanedione were carried out. However unlike DFO, results demonstrated a valuable increase in sensitivity with respect to both luminescence intensity [32, 60, 63, 66] and colour contrast [60]. As a result, the inclusion of zinc chloride in the working solution is highly recommended. Not only has it been found to increase sensitivity, it also assists in the longevity of developed fingermarks which has thus far been a continual concern for forensic examiners.

Early studies reported on the decomposition of both colour and luminescence fingermarks within a few days after treatment with 1,2-indanedione alone. However, Gardner and Hewlett found that the inclusion of zinc chloride was not responsible for the increase in time before degradation and suggested that photolysis of the product was the cause of degradation [67]. Therefore, the presence of zinc chloride only appears to slow down the rate of degradation.

This is due to the greater strength in colour and luminescence produced in comparison to 1,2-indanedione alone and does not play a part in affecting the rate of this degradation process.

Whilst these fingermark reagents, ninhydrin, DFO and 1,2-indanedione, all work effectively on their own, the question remains as to which of these reagents surpasses the others. The general consensus is that there is no one specific reagent, which surpasses the rest. It is generally agreed that in the absorbance mode, ninhydrin exceeds both 1,2-indanedione and DFO as it provides greater contrast. Whereas, in the luminescence mode, DFO and 1,2-indanedione exhibit greater sensitivity than ninhydrin with metal salt post-treatment [60, 66]. However, there is some conjecture into which of these two reagents, DFO or 1,2-indanedione, is superior [10, 32, 60, 62, 66-70].

The definition of superiority when applied to fingermark development tends to be two-fold: (i) whether a reagent develops a greater number of identifiable fingermarks; and (ii) whether these fingermarks appear enhanced in both absorbance and luminescence modes. An extensive literature search has found conflicting evidence for the advantageous use of DFO verses 1,2-indanedione, to the point where an opinion based on demonstrated superiority cannot be specifically reached [62, 66-70]. These discrepancies could result from differences in the local environment, the substrates used, and any minor adjustments in the reagent formulation. Evidence shows that a reagent that works best under one set of conditions (formulation, environmental factors, substrates and light source etc.) may not be the best reagent under another set of conditions (e.g. different country, different climate, different substrates). On this basis, there cannot be one global optimum for any of the discussed fingermark treatments.

1.3.3 Alternative Amino Acid Sensitive Reagents

With a greater understanding of fingermark chemistry and the introduction of lasers and other forensic light sources, research into chemical alternatives to ninhydrin and its analogues for amino acid detection have also been explored. Reagents that demonstrated the greatest prospects were fluorescamine, o-phthaladehyde and NBD-chloride (7-chloro-4-nitrobenzo-2-oxa-1,3-diazole) [22, 71]. However, these reagents have not come into operational use due to disadvantages compared to ninhydrin and its analogues. Fluorescamine and o-phthaldehyde react with amino acids to form products that are luminescent under ultraviolet (UV) light and thus their application is limited due to interference from the UV elicited photoluminescence from the optical brighteners present in many paper substrates [22]. The products of the reaction of NBD-chloride with amino acids exhibit luminescence when excited in the visible region. However, NBD-chloride lacks specificity as it reacts with other unidentified components present in some paper substrates leading to background luminescence and reduced contrast [22]. In addition, NBD-chloride only gives products that are visible when viewed in the luminescence mode with a suitable light source [22].

1.3.4 Reagents Based on Natural Products

Prior to 2004, research into non-specific amino acid targeting reagents primarily focused on ninhydrin and related compounds. An alternative research path developed with the discovery of genipin [11, 72], which, unlike other reagents, was not synthesised as a ninhydrin analogue. It is important to note, that the essence of investigating alternative reagents is not to replace existing reagents, but to increase the number and variety of these reagents available to forensic investigators. There are always new challenges arising with fingermark detection, for instance, the recent transition from paper to polymer bank notes

or the increased number of thermally printed receipts. Along with this, some of the most difficulties associated with fingermark detection may not necessarily correspond to the substrate itself, but the inclusion of highly coloured labels and brighteners for advertising and packaging. Therefore, it is vitally important that examiners have access to a broad range of options for fingermark development, which can be selected depending on the nature of the surface, the age and condition of the fingermark and potential background interference. One avenue to combat this is to offer a range of complementary fingermark reagents that differ in colour and luminescence, ultimately increasing the limits of detection and the probability of successful detection and identification for fingerprint examiners.

1.3.4.1 GENIPIN

Genipin (Figure 1.7) was first suggested as a reagent for the detection of latent fingermarks on paper by Almog and co-workers [72].

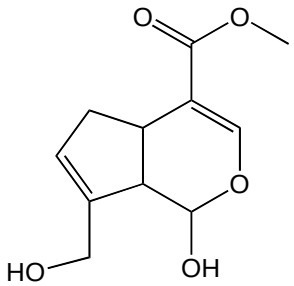


Figure 1.7: Structure of genipin.

Genipin is derived from a number of different varieties of the Gardenia. Genipin has not just become a molecule of interest in the forensic community, but a number of other scientific research areas are investigating characteristics of this natural plant extract. The food industry has paid particular attention to genipin as a natural colorant, with growing concerns over the health and safety of

synthetic dyes [73, 74]. The biomedical research community has shown an interest in genipin's cross-linking ability [75-77], a prerequisite for a number of different medical applications [78-83].

Genipin's ability to stain the skin was first described in the scientific literature by Djerassi and co-workers who stated "genipin itself is colourless, but if brought to the skin, it rapidly produces an indelible bluish/violet colour," and later established genipin's ability to rapidly react with amino acids [72, 84, 85]. The paper also described historical reports of how the native Americans in the 18th century bathed in the clear juice of the fruit when tired and as a means of painting their skin, implying the use of genipin not only as a therapeutic remedy but also as a form of adornment [84]. When subsequently evaluated for use as a stain for amino acids on TLC plates, the genipin products were found to have higher molar absorptivities than Ruhemann's purple and were also more stable [84, 85]. These observations led Almog and co-workers to investigate genipin as a fingermark reagent, and it was found that it could develop marks that were both coloured and luminescent [11, 72, 86].

Genipin may provide operational advantages over existing fingermark reagents. Its wavelength of maximum emission as reported by Almog and co-workers is at the red end of the spectrum (greater than 600 nm), leading to the potential for improved signal-to-noise as it is shifted away from the background fluorescence commonly displayed by paper substrates. In addition, evidence of low cytotoxicity indicates that the occupational hazards associated with using genipin are significantly reduced compared to other fingermark development reagents. However, the promise of genipin as a fingermark reagent does not rely solely on these advantages alone, but the variation in both colour and luminescence, which could have implications in terms of being a complementary method for use by fingerprint examiners.

One key area of research is focussing on determining the reaction mechanism and the resulting chromophore and/or fluorophore, which has yet to be verified. Investigations have looked at the reaction of genipin with simple compounds containing primary amines, which in turn indicate the formation of heterocyclic amines. These amines appear to further associate to form cross-linking networks, containing short chain dimer, trimer and tetramer bridges [75, 77, 87, 88]. Additionally, the reaction of genipin with amino acids has been reported to produce more than one coloured compound [72, 89]. Touyama and colleagues reported the presence of one yellow and 9 brownish-red pigments (A-I), which were proposed to be precursors of the blue product(s) [90, 91].

It appeared that the blue product(s) was formed through oxygen radical-induced polymerisation and dehydrogenation of a mixture of intermediary pigments as depicted in Figure 1.8 [90, 91]. Alternatively, Fujikawa proposed that a monomeric adduct, genipocyanin, was formed from genipin reacting with glycine, which further cross-linked to proteins (R in Figure 1.9b) [88]. Although structural similarities are present between compounds in Figure 1.9 and Touyama's postulations featured in Figure 1.8, significant conformational variations exist, exemplifying the difficulty in deducing the exact mechanism involved.

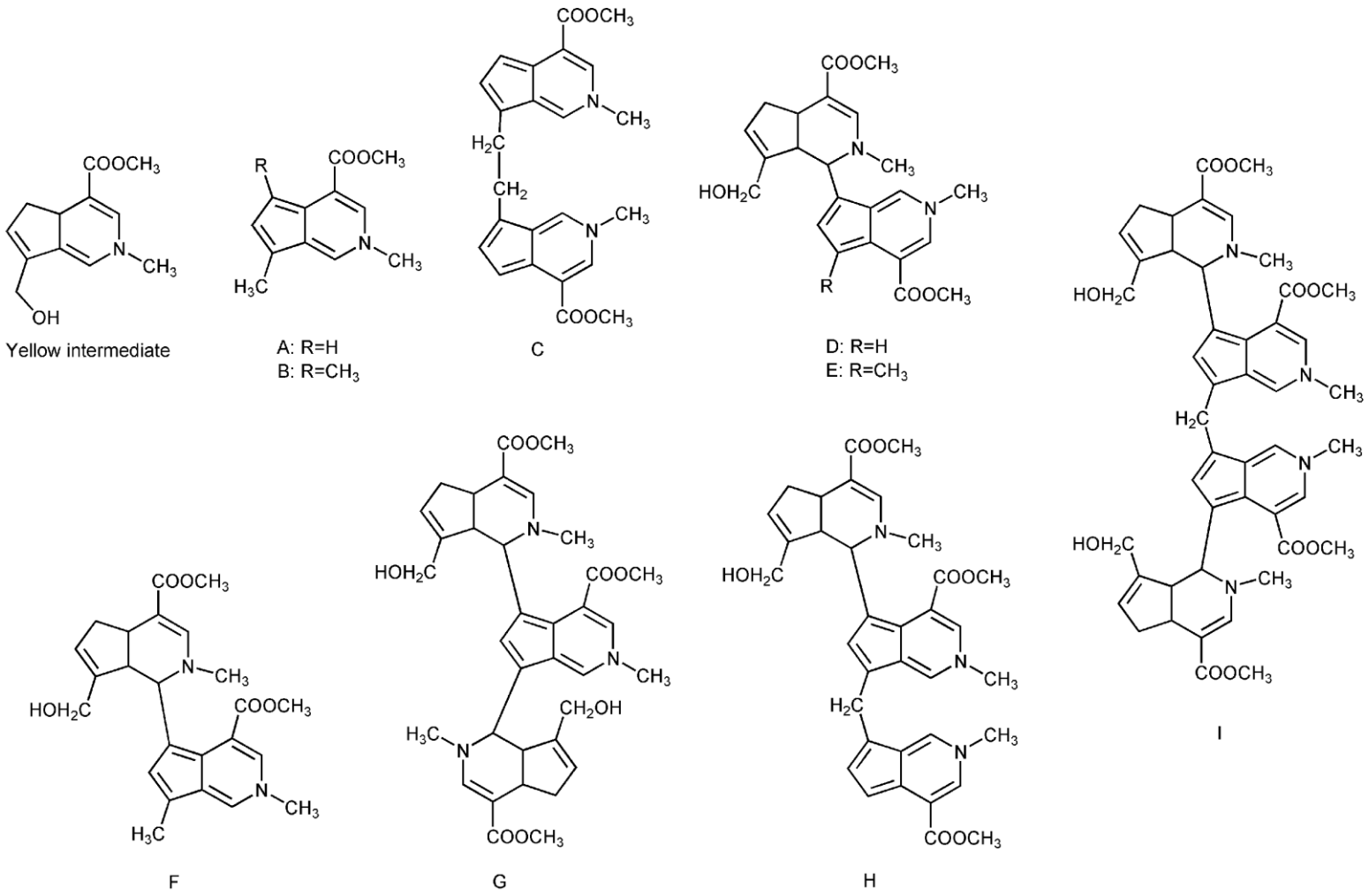


Figure 1.8: Proposed structures of the yellow and brownish-red (A-I) intermediates [90,91].

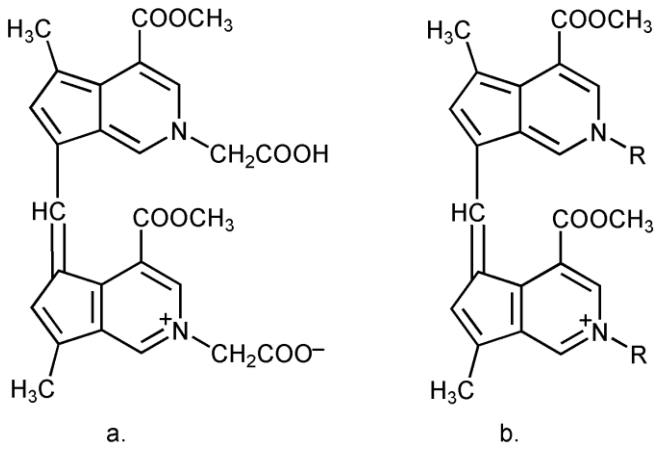


Figure 1.9: Proposed structures of: (a) Genipocyanin; (b) a dimer from genipin and a primary amine [88].

These investigations by Fujikawa and Touyama were carried out in solution phase, which may not give a true representation of the mechanism involved when fingermarks are developed on paper substrates. Studies conducted in a solvent medium, whilst perhaps more simple, allows for the amino acid units and fingermark reagents to mix freely, permitting the formation of products not necessarily representative of the products in an adsorbed/restrictive environment. This lack of mobility means that oligomeric products derived from multiple amino acid units are highly unlikely. Unpublished work by Fazendin provides LCMS evidence for the formation of products involving more than 8 amino-acid-genipin units when the reaction is conducted in solution [92]. The exact nature of the reaction occurring between genipin and latent fingermark deposits on paper substrate, including the number of products formed in the reaction, is yet to be established.

1.4 AIMS AND OVERVIEW

The fundamental purpose of this dissertation is to investigate a novel class of fingermark detection reagents and to instigate new approaches for the continual development and growth of fingermark detection chemistry.

The primary focus was inspired by natural products, which react to form coloured compounds. Specifically henna has been used as a natural skin and hair dye. The active dying ingredient in henna, lawsone has structural similarities to current fingermark reagents. This suggests that further investigations are warranted into lawsone and subsequently other naphthoquinones for their ability to detect latent fingermarks on paper surfaces.

Preliminary investigations into the use of lawsone and related naphthoquinones as reagents for the development of latent fingermarks on porous surfaces are described in Chapters 3 and 4. Additionally, these chapters will cover preliminary procedures outlining the equipment and developmental requirements for enhancement via the use of these reagents. These naphthoquinones will subsequently be evaluated on their potential as fingermark detection reagents. Luminescence spectrophotometry of developed amino acid spots on paper will be used to confirm whether the naphthoquinones are reacting with amino acids in the latent fingermark. Further discussion in Chapter 4 will focus on comparisons between the selected naphthoquinones and lawsone.

Investigation of the reaction of selected naphthoquinones with latent fingermarks and amino acids via synchrotron ATR-FTIR is described in Chapter 5. Synthetic studies were used in conjunction with Synchrotron ATR-FTIR to rationalise and clarify the results obtained. Furthermore, this chapter discusses the difficulties associated with identification procedures of this nature.

Chapter 6 examines three approaches towards identifying novel fingermark reagents. These include investigating additional natural product based colour forming compounds, applying a retrosynthetic approach and a serendipitous discovery.

CHAPTER 2: EXPERIMENTAL CONSIDERATIONS

2.1 INTRODUCTION

Treatment of evidence with an amino acid sensitive reagent involves dipping or spraying the item of interest with a solution of the reagent, often followed by heating [2-4]. The developed article is examined and photographed under normal light conditions, and if appropriate using photoluminescence. The exact photographic conditions used are dependent upon the reagent used and the mode in which the image is captured. Photography of photoluminescence requires the use of an excitation light source combined with a suitable filter, and generally results in images being recorded through longer exposure times.

A wide range of formulations has been proposed for the more established reagents such as ninhydrin, 1,8-diazafluoren-9-one (DFO) and 1,2-indanedione [2-4]. These formulations have generally been developed on the basis of observation and experience, although cost, health, safety and operational simplicity are contributing factors [2-4, 22]. There have been wide divergences of opinion in the literature as to optimal reagent formulations and subsequent reaction conditions [13, 53, 55, 58, 61, 69, 93]. As previously mentioned in Chapter 1, these variations are in all likelihood due to the local environment and the chances of a global optimum for these reagents would be highly unlikely. This is understandable considering the role temperature and humidity can play in the performance of certain reagents [3].

It is evident that a consideration of operational requirements is vitally important when evaluating fingermark detection reagents. Therefore, it is important to consider established development procedures when devising a systematic approach for the evaluation of potentially novel fingermark reagents. This chapter outlines the experimental procedures implemented throughout the course of this work.

2.2 LATENT FINGERMARKS

Bramble and Brennan in 2001 suggested that a key unresolved problem in latent fingermark detection was the lack of a systematic testing regime for establishing the effectiveness of new detection methods [23]. Researchers generally use the "split print" approach, where a single fingermark is divided into two parts, which can then be treated separately with different conditions or reagents (Figure 2.1).

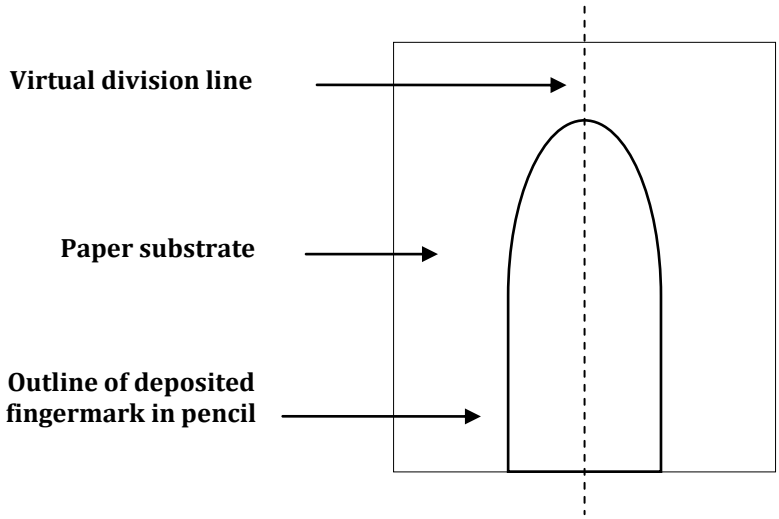


Figure 2.1: Schematic diagram of the "split print" approach.

The British Home Office Scientific Development Branch (HOSDB) uses an approach, whereby a series of latent fingermarks are deposited on the surface of interest without touching anything between depositions. This is to give a depletion series of prints, with diminishing quantities of material deposited with each subsequent impression [62]. This allows estimation of the sensitivity of the treatment. A similar approach has also been used by Roux and co-workers [54, 60, 66, 70, 94]. Ramotowski and colleagues used the examination of naturally handled envelopes to determine the operational usefulness of 1,2-indanedione [95].

Schwarz and co-workers reported the retrofitting of an ink jet printer to print amino acids onto paper to produce standard patterns of amino acids of known concentration [96]. While this is useful as a research tool and potentially for quality assurance purposes, there is still the issue that the printer cannot reproduce the other components of a latent fingermark that may affect development.

With consideration of the approaches outlined above, investigations will be carried out with the use of latent fingermarks from several donors and a range of amino acid solutions deposited on paper and cellulose TLC plate surfaces. The majority of the investigations in this dissertation will utilise the "split print" approach.

2.3 REAGENT FORMULATION

Bramble and Brennan have summarised the key requirements of any successful fingermark visualisation reagent as being: (1) a suitable medium for the reagent; (2) a method of transport for the reagent onto or into the surface of the item of interest; and (3) provision of suitable reaction conditions [23]. Amino acid sensitive reagents are typically dissolved in a carrier solvent along with additional components such as polar solvent modifiers, acetic acid and metal salts.

An ideal carrier solvent is required to be volatile enough to evaporate quickly, non-toxic, non-flammable and non-polar, so as to avoid the running of inks on treated documents. These requirements led to the widespread use of 1,1,2-trichloro-1,2,2-trifluoroethane (also known as CFC 113, Freon 113, Fluorisol and Arklone P) as a carrier solvent for ninhydrin and its analogues [3, 4]. However, due to its action as an ozone-depleting chemical, this solvent is no

longer available for law enforcement use and alternatives such as the Freon replacement 1-methoxynonafluorobutane (HFE-7100) have been introduced [3, 4]. Petroleum ether and other hydrocarbons have also been used as carrier solvents, but these are highly flammable and thus, not always able to be used under normal operational conditions [4]. In addition to the carrier solvent, small amounts of a more polar solvent such ethyl acetate, dichloromethane, ethanol or methanol may be required to ensure that the reagents remain in solution. Depending upon the reagent, formulations may also contain other components such as acetic acid (to modify pH) and metal salts (to improve development).

These guidelines need to be considered for subsequent formulation investigations to ensure that any information obtained is generally applicable to the broader forensic community. As a result, HFE-7100 and petroleum spirit 60-80 °C were investigated as potential carrier solvents along with the addition of small amounts of various polar solvents to ensure that the reagents remained in solution.

2.4 APPLICATION OF HEAT

Most reagents generally require the application of heat to develop the latent fingermark. As is the case with formulation composition, there has been a wide variety of heating regimes proposed. This heat can be applied through the use of an oven [60], domestic iron [57] or laundry press [60]. Depending on the reagent, a certain level of humidity may also be required; for example, humidity improves the development obtained with ninhydrin and genipin [3, 11, 97]. In some cases, such as DFO, humidity can be detrimental to successful development [3, 4, 58].

For some reagents, it has been proposed that the heating step can be omitted, although this leads to extended development times [3, 4]. Furthermore, Brown and colleagues have found that the application of heat alone has the ability to develop latent fingermarks on porous surfaces [98]. The suggested mechanism is via preferential charring of the paper substrate where latent fingermark deposits exist. Although this is only achieved with excessive heat, it does emphasise the need for the careful use of controls and blanks to ensure an accurate assessment of performance. For the purposes of this dissertation, it is important to accurately evaluate the development effect of heat; therefore, all three methods — oven, domestic iron and laundry press — will be investigated as a means of developing latent fingermarks after reagent application.

2.5 VISUALISATION AND RECORDING

Developed latent fingermarks need to be examined and recorded photographically for subsequent fingermark identification. The exact recording conditions used will depend upon the reagent that has been used to develop the fingermark. While the fingermark may be visible to the naked eye under natural light, it can generally be enhanced by use of the light absorbing characteristics of the developed print. For example, Ruhemann's purple, the reaction product of ninhydrin with amino acids, has a strong absorption band at approximately 560 nm. Ninhydrin developed fingermarks are thus best observed by illuminating with white light while viewing through a green-yellow band pass filter (Figure 2.2).

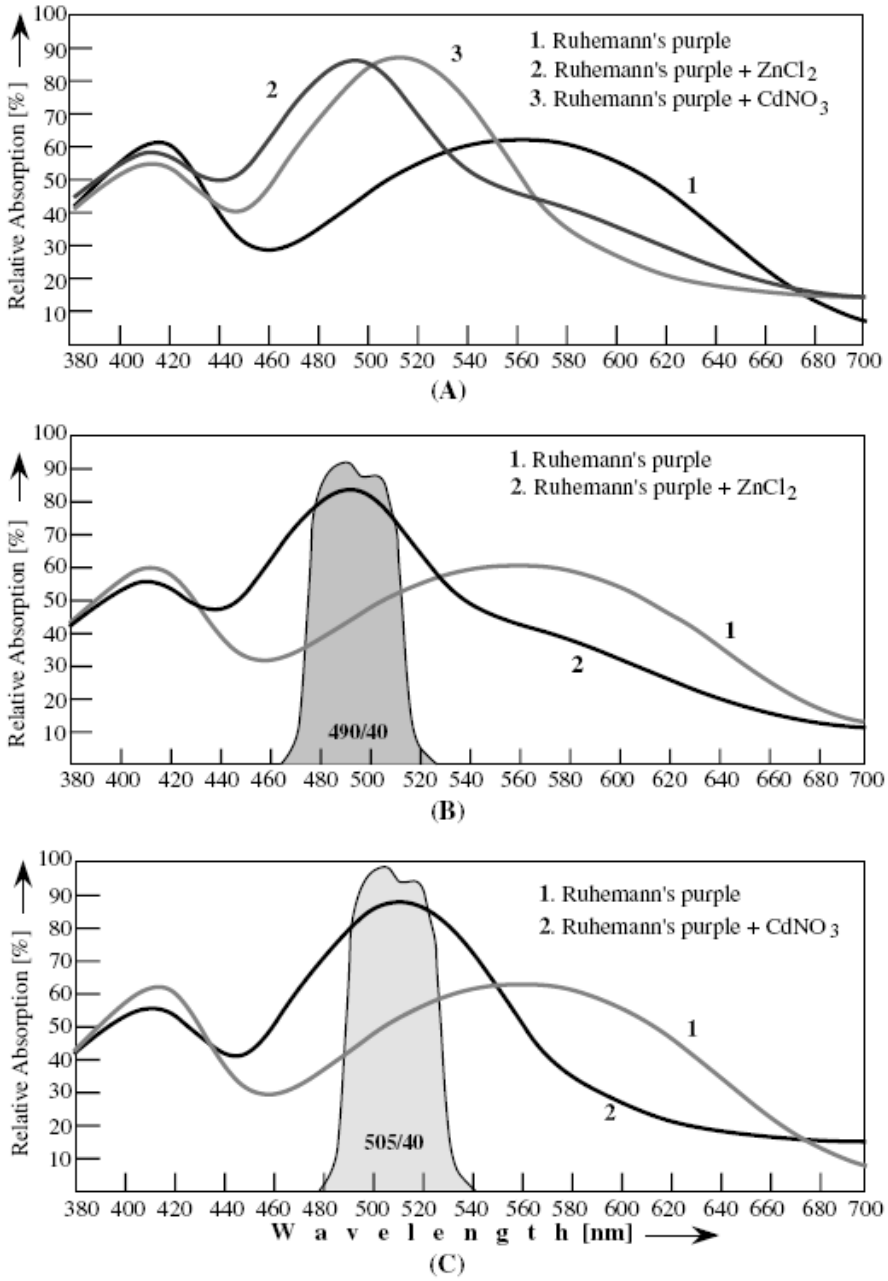


Figure 2.2: (a) Relative absorption spectrum of Ruhemann's purple and its complexes with zinc and cadmium (b) recommended band-pass filters for observation in the absorption mode for the zinc complex, and (c) recommended band-pass filters for observation in the absorption mode for the cadmium complex [4], used by permission ($CdNO_3$ (sic) = $Cd(NO_3)_2$).

An important advance for the detection of fingermarks using amino acid sensitive reagents was the advent of photoluminescence methods. It was found that ninhydrin developed fingermarks, when post-treated with a zinc (II) or cadmium (II) metal salt and cooled with liquid nitrogen, exhibited photoluminescence which could be used to significantly enhance detection sensitivity and contrast [42]. Since these early studies, the main focus of amino acid reagent research has been on developing reagents that exhibit good colour and superior photoluminescence, without the need for additional treatments (such as metal salt treatment and/or cooling) [9]. Photoluminescence is observed by illuminating the developed fingermark with monochromatic light from a filtered light source (or laser) and viewing through an appropriate barrier filter (Figure 2.3 and Table 2.1), with low ambient light required for optimal results.

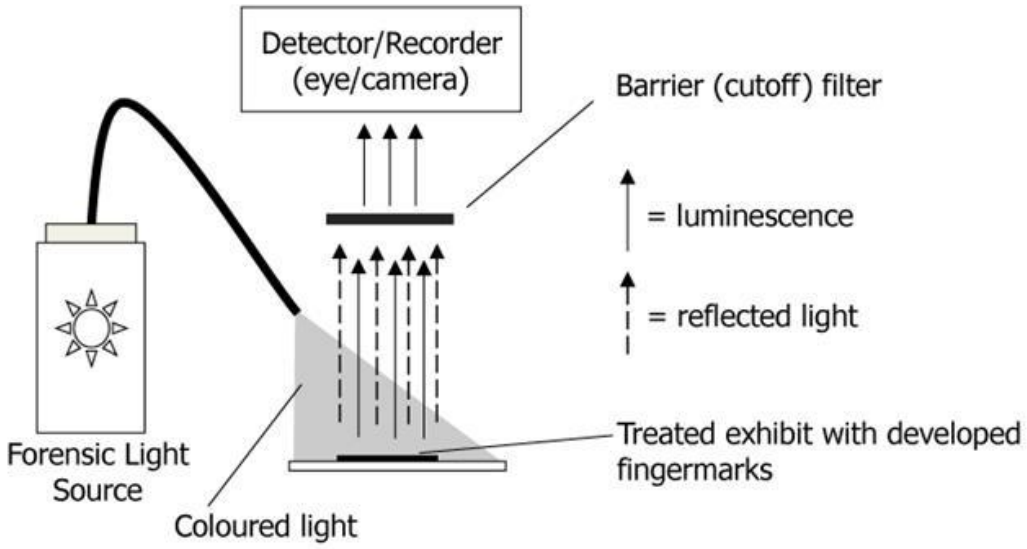


Figure 2.3: Schematic of the photoluminescence set-up for observing/recording treated latent fingermarks.

Table 2.1: Conditions for observing the photoluminescence emission from latent fingermarks treated with amino acid reagents [13].

Reagent	Excitation Band (Polilight PL 500)	Viewing and recording conditions (goggles and camera barrier filters)
Ninhydrin post-treated with zinc chloride	490 nm	Orange goggles, band-pass IF565 or long pass KV550/OG 550
1,8-Diazaflouren-9-one (DFO)	505 nm	Orange goggles, band-pass IF565 or long pass KV550/OG 550
	530 nm	Red goggles, long pass OG590/IF590
	555 nm	Red goggles, band-pass IF600 or IF610
1,2-Indanedione–zinc (IND-Zn)	505 nm	Orange goggles, band-pass IF565 or long pass KV550/OG550
	530 nm	Red goggles, long pass OG590/IF590
Genipin	555 nm	Red goggles, band-pass IF600 or IF610
Lawsone	590 nm	Red goggles, Wratten NA29

The application of lasers to the detection of untreated latent fingermarks was first proposed by Dalrymple *et al.* in 1977 [99]. It was found in operational use that very few latent fingermarks exhibited native photoluminescence, however this became the starting point for the investigation of various detection techniques based on fingermark luminescence. Lasers subsequently became increasingly employed in combination with latent fingermark development reagents that induce luminescence. Herod and Menzel found that the 488 nm line of the argon laser was ideal for exciting the Ruhemann's purple-zinc complex (λ_{ex} 485 nm) [40].

While lasers are powerful light sources for exciting treated latent fingermarks, earlier models suffered from high cost and a lack of portability. Kobus and co-workers demonstrated the suitability of a xenon arc lamp fitted with a range of filters as a light source for exciting treated latent fingermarks [42, 71]. Since then, a wide range of non-laser light sources, collectively referred to as forensic light sources, have become commercially available and are extensively used in forensic investigations [3, 100]. Such light sources are generally more versatile than lasers due to the wide range of wavelength bands that are available (compared to the limited number of laser lines that are typically available with laser-based systems).

More recently, chemical imaging systems have been investigated for the visualization of treated latent fingermarks [94, 101]. While this approach can provide significant advantages for weak marks and those on highly luminescent backgrounds, it is a very specialised technique that is generally not available to operational laboratories for routine use.

This research employs various forensic light sources for examining developed fingermarks in the luminescence mode. This will include the use of a Polilight forensic light source, the Poliview system, a laser, and photoluminescence characteristics will be measured with the use of a fluorescence spectrophotometer.

2.6 EXPERIMENTAL

The general experimental procedures followed throughout the course of this thesis are described below. Additional methodologies for specific investigations are further outlined in the relevant chapters.

2.6.1 CHEMICALS AND REAGENTS

The following naphthoquinones and related compounds were obtained from Sigma Aldrich (Australia) and were used as supplied without any further refinement: 1,4-dihydroxy-2-naphthoic acid; 1,2-naphthoquinone-4-sulfonate; 2-methoxy-1,4-naphthoquinone; 2-methyl-1,4-naphthoquinone; and 2-hydroxy-1,4-naphthoquinone (lawsone).

Ethyl acetate (Univar Analytical, Australia), petroleum spirit 60-80 °C (Univar, Australia) and methyl nonafluoroisobutyl ether (HFE-7100) (NovecTM, Australia) were analytical grade unless otherwise stated and were used as supplied without further purification. All working solutions were prepared fresh on a daily basis.

The lawsone and naphthoquinone working solutions were prepared by dissolving 0.05 g lawsone/naphthoquinone in 10 mL of ethyl acetate and the solution further diluted with 40 mL HFE-7100 or petroleum spirits 60-80 °C. This optimised formulation was the result of the investigations detailed in Chapter 3.

2.6.2 Sample Preparation and Treatment

Latent fingermarks were collected on filter paper (Whatman No 1, England) or cellulose thin-layer chromatography plates (TLC plates) (Merck, Germany) from a number of different donors. Donors were requested not to wash their hands immediately before collecting impressions and the fingers were not "charged" with additional secretions (for example, by rubbing against the forehead). However, the donors were asked to rub their hands together just prior to touching the paper surface (to ensure a uniform coating of natural secretions across the fingertip surfaces). Samples were developed within two days of deposition.

Solutions of amino acids in water [glycine (Chem-Supply, Australia), lysine, serine and proline (Sigma, Australia)], all at 900 μg/mL, were dispensed (5 μL) onto filter paper and TLC plates and allowed to air dry before subsequent lawsone and naphthoquinone treatment.

Fresh fingermark deposits and amino acid spots were dipped in the lawsone/naphthoquinone working solution and allowed to dry. Subsequently, the samples were heated for colour and luminescence development. Heat was provided via an oven (All-lab Scientific, Australia) at 150 °C for 1 hour.

Investigations requiring comparative analysis were conducted via the "split print" approach. As previously mentioned, this is where a single fingermark was divided into two or more parts, and was subsequently treated separately with different conditions and reagents.

2.7 INSTRUMENTATION

2.7.1 PHOTOLUMINESCENCE SPECTROSCOPY AND ILLUMINATION

Treated latent fingermarks were viewed at λ_{ex} 555 nm using a Polilight® PL 500 forensic light source (Rofin, Australia), with observation using a 600 nm cut-off barrier filter. Photoluminescence was investigated using a Cary Eclipse fluorescence spectrophotometer with a fibre optic probe attachment and Cary Eclipse scan application software version 1.1 (Varian, Mulgrave, Australia). Fluorescence emission spectra for lawsone and naphthoquinone analogues were collected using excitation wavelengths of 530, 555 and 590 nm, with each spectral output totalling an average of 10 scans, with excitation and emission slit widths of 5 nm.[1]

2.7.2 IMAGING CONDITIONS

Developed fingermarks were captured using either the Poliview system or via a digital camera, as specified below. Displayed images throughout this dissertation will indicate the conditions used.

2.7.2.1 POLIVIEW SYSTEM

A Poliview imaging system (Rofin, Australia) was used to capture and record developed fingermarks, with illumination at λ_{ex} 590 nm and visualisation through a 650 nm band pass interference filter with a 1 s exposure time.

[1] Selected fluorescence spectra were collected by Anusha Menon from Curtin University under my direct supervision.

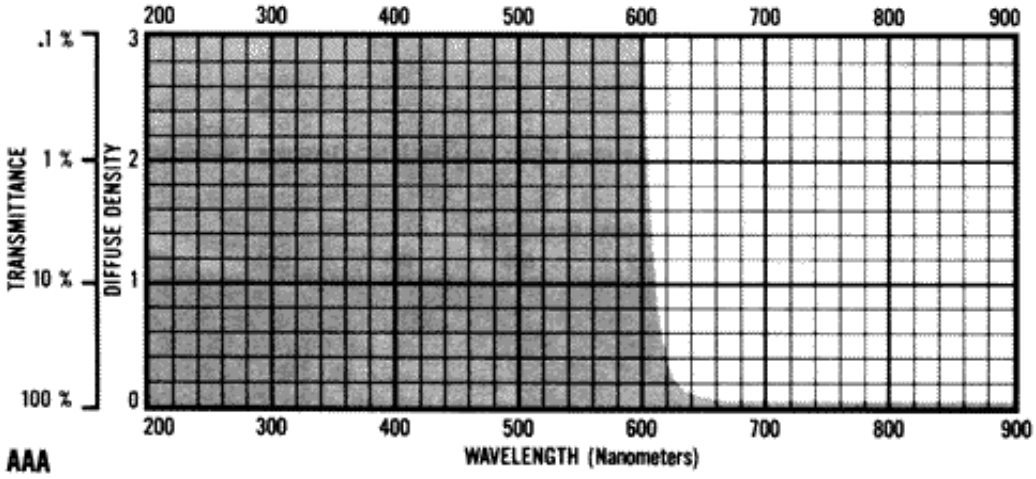
2.7.2.2 NIKON D300

Samples were photographed in both the absorbance (white-light) mode and the photoluminescence mode using a Nikon D300 digital camera mounted on a Firenze Mini Repro camera stand. The camera settings for the majority of photographs are as shown in Table 2.2. Illumination in the absorbance mode was achieved using incandescent light bulbs with no camera filter attachments. Illumination in the luminescence mode was achieved using a Rofin Polilight® PL500 (Rofin, Australia), with an excitation wavelength of 590 nm and a Wratten NA29 filter (equivalent to a long-pass barrier filter), unless otherwise indicated. Images were captured to a desktop computer using Nikon Camera Control Pro Version 2.0.0 and adjusted for brightness and contrast using Adobe Photoshop CS4 Version 9.0.

Table 2.2: Photographic conditions for absorbance and luminescence mode photographs, unless otherwise stated.

	Absorbance mode	**Luminescence mode**
Focal Length (mm)	85	85
Exposure Mode	Manual	Manual
Shutter Speed (s)	1/30	15
Aperture	f/11	f/11
Sensitivity	ISO 200	ISO 200
Barrier Filter	none	Wratten NA29 filter*

*Equivalent to long-pass barrier filter with 50% transmission at 620 nm as seen in Figure 2.4 [102].



29 Deep Red Tricolor. For color separation and tricolor printing work. Tricolor projection (tungsten) with No. 47 and 61.

WAVELENGTH	PERCENT TRANSMITTANCE
400	--
10	--
20	--
30	--
40	--
50	--
60	--
70	--
80	--
90	--
500	--
10	--
20	--
30	--
40	--
50	--
60	--
70	--
80	--
90	--
600	--
10	10.5
20	45.0
30	73.5
40	84.2
50	87.8
60	89.2
70	89.8
80	90.3
90	90.4
700	90.5

Figure 2.4: Characteristics of a Wratten NA29 filter [102].

CHAPTER 3: LAWSONE AS A NOVEL REAGENT FOR THE DETECTION OF LATENT FINGERMARKS ON PAPER SURFACES

Portions of this chapter have been published in the journal *Chemical Communications*:

R. Jelly, S.W. Lewis, C. Lennard, K.F. Lim, J. Almog. Lawsone: A Novel Reagent for the Detection of Latent Fingermarks on Paper Surfaces, *Chemical Communications*, (2008), Vol 30, p. 3513-3515.

3.1 INTRODUCTION

As previously outlined in Chapter 1, genipin was the first reported natural product used as a means to develop latent fingermarks on paper substrates [72]. Genipin's use as a fabric and skin dye prompted an investigation of the potential of other natural products, which could be used in the same fashion as fingermark reagents. Henna, which is cultivated from the leaves of the *Lawsonia inermis*, a member of the *Lythraceae* family, is well known as a source of natural dyes. In a similar manner to genipin, henna has been used as a skin and hair dye for millennia with reports of its use dating back to 1,400 BC [103]. Indigenous cultures use henna as part of religious, social and ritualistic traditions, the most prominently recognised being mehndi decorations. This tradition consists of intricate designs drawn in henna as a temporary form of body art and is applied to brides before their wedding ceremonies (Figure 3.1) [103].

(a)  (b) 

Figure 3.1 Use of henna as a temporary tattoo (a) henna paste applied to skin (b) resulting orange/brown staining of skin (photos: Simon Lewis).

Evidence suggests that along with its use as a form of adornment, in ancient times henna was used for medicinal purposes [104]. Based on these historical applications, more recent reports have described its use as an effective antitumor, antifungal, antiparasitic, antimycotic, antiviral and antimicrobial agent [105-108]. With growing concerns over the increasing resistant strains of micro-organisms to the currently available and synthesised antibiotics, the diverse antimicrobial activity associated with henna, could be vitally important as a future alternative to combat these antibiotic-resistant strains [107].

A further potential application is described by Dev and colleagues, who report on the ability of henna to inhibit the growth of micro-organisms that are involved in burn wound infections. Initially, before the discovery of these remedial and staining properties, henna was simply used as a means to cool the body in climates of extremely high temperatures [103]. Therefore, the use of henna could be effective in the treatment of burns, not only by providing a cooling effect, but also preventing infection [108]. Furthermore, henna has been described as a prophylactic, indicating its potential use as an anti-inflammatory, in particular the treatment against skin inflammation [109]. These biomedical applications affiliated with henna, when investigated further, are credited due to the presence of specific naphthoquinones [110], which are discussed in more detail in Chapter 4.

In addition to these health and staining properties, researchers have also investigated henna extracts as environmentally friendly corrosion inhibitors [111-113], which directly relates to henna's historical use as a means to rustproof the hulls of ships [103].

Lawsone (2-hydroxy-1,4-naphthoquinone, Figure 3.2), is the compound thought to be responsible for the staining properties of henna [103].

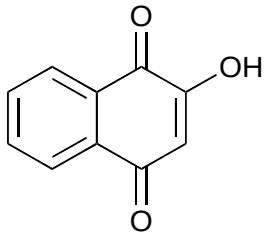


Figure 3.2: Chemical structure of lawsone.

Lawsone was first isolated by Tommasi and appears to behave as an acid levelling, non-metallised acid dye when applied to wool and nylon [114]. Further investigation found a number of reports detailing the reaction of 1,4-naphthoquinone derivatives with amines and amino acids [115-120], which will be further discussed in Chapter 4 and 5. In light of this evidence, lawsone was selected to be investigated for its ability to develop latent fingermarks on paper surfaces.

This chapter investigates the potential of lawsone as a novel reagent for the detection of latent fingermarks.

3.2 EXPERIMENTAL

3.2.1 CHEMICALS AND PROCEDURES

A number of materials and methods used throughout this chapter have previously been described in Chapter 2.

The following chemicals were used for formulation investigation studies: dichloromethane (Mallingkrodt Chemicals, Australia); ethyl acetate (Univar Analytical, Australia); absolute ethanol (CSR Chemicals, Australia); glacial acetic

acid (CSR Chemicals, Australia); petroleum spirit 60-80 °C (Univar, Australia); and methyl nonafluoroisobutyl ether (HFE-7100) (NovecTM, Australia). These were all analytical grade and used as supplied, without further purification. All solutions were prepared fresh as required.

Lawsone treated fingermarks were subsequently heated for colour and luminescence development, via three methods: (i) heating in an oven (All-lab Scientific, Australia) at 150 °C for 1 hour; (ii) direct heat from a laundry press (Elna, Australia) at ~160 °C for 10 s; and (iii) from a commercial hand-held steam iron (Mistral, Australia) at ~160 °C for 30 s.

Zinc chloride (analytical grade, BDH, Australia) was prepared by dissolving 0.4 g in 10 mL of absolute ethanol (analytical grade, CSR Chemicals, Australia). 0.5 mL of this solution was added to 25 mL of the lawsone working solution.

1 mL aliquots of citric acid (Univar, Australia) in ethanolic solution (0.1 M) were applied at various stages in the treatment process as a means of investigating potential reaction enhancers.

3.2.2 ILLUMINATION

Lawsone treated fingermarks were illuminated with the use of a Polilight forensic light source (details as previously outlined in Chapter 2). However, alternatively, selected fingermarks were also illuminated with the use of a 9 W CW 532 nm diode-pumped solid-state laser (Millennia KMC100; Spectra-Physics, USA) fitted with a fibre optic coupler (model 316-015 Fingerprint Detection System; Spectra-Physics, USA).

3.3 RESULTS AND DISCUSSION

3.3.1 INITIAL INVESTIGATIONS

Latent fingermarks deposited onto filter paper were subsequently dipped into ethanolic lawsone solution, which consisted of approximately 0.2% w/v. Samples were allowed to air dry before heating in an oven at 150 °C for 1 hour. This heat development resulted in a visible impression that was brown/purple in colour (Figure 3.3). This is believed to be the first ever fingermark developed with the use of lawsone. Initial observations showed a slight discolouration of the background surface compared to the experimental blank (which consisted of a heated latent fingermark without lawsone treatment). Luminescence was observed with an excitation of 555 nm via a forensic light source and a suitable barrier filter.

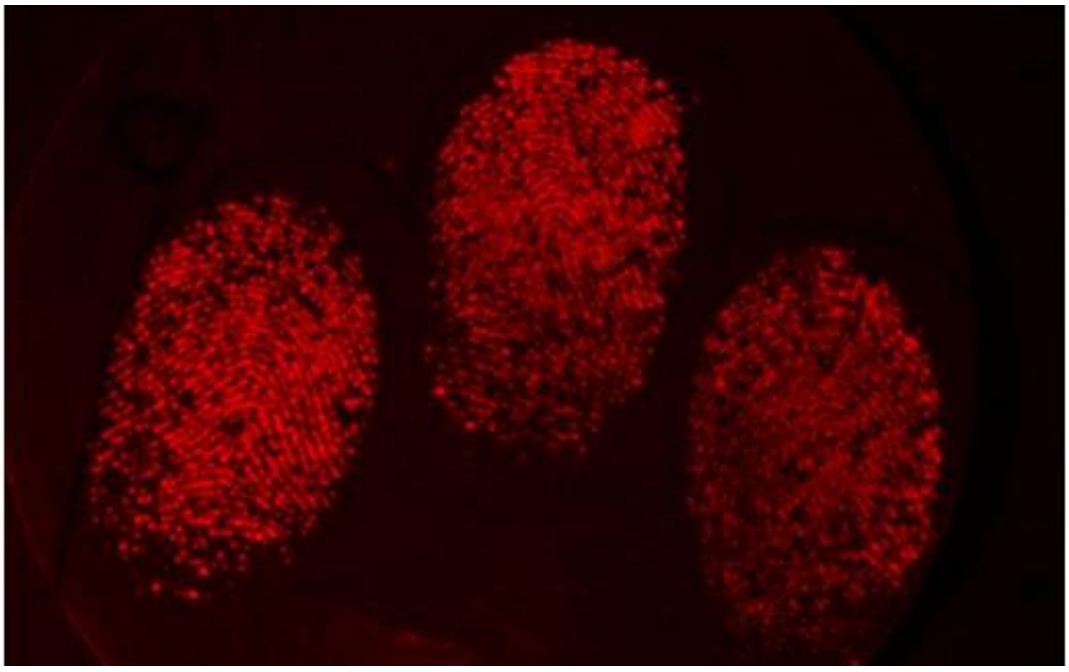
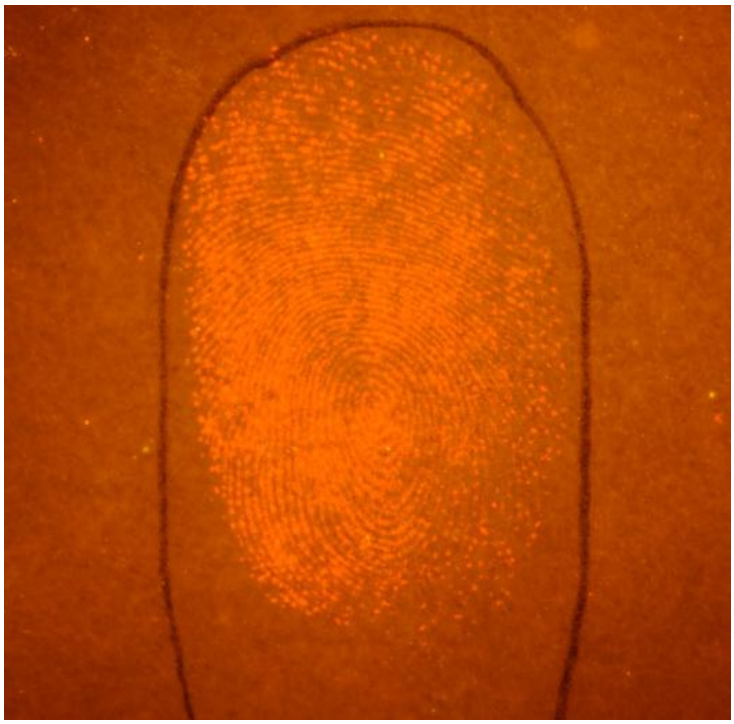


Figure 3.3: Lawsone treated fingermarks captured using a Pentax K10 digital SLR, 50 mm focal length, ISO 100 under Photoluminescence mode (excitation with a Polilight PL 500 at 590 nm and viewed through a Wratten NA29 filter, shutter speed 6.0 s, aperture f2.8).

During the course of this investigation, the opportunity arose whilst visiting Minneapolis-Saint Paul (USA), to access a 532 nm diode laser (Millennia KMC100; Spectra-Physics, USA) at the Minnesota Bureau of Criminal Apprehension. Developed fingermarks were recorded in order to investigate the use of an alternative excitation source other than the Polilight. Fingermarks were captured with excitation via the laser and with either a Wratten NA29 filter or a KV 550 filter (Figure 3.4). The use of the Wratten NA29 filter appeared to produce greater contrast; however, both filters were able to produce significant ridge detail and therefore, could be considered that any cut-off filter between 550-620 nm could be used for this application.

Visualisation with the laser, although not at the optimum wavelength, produced useful luminescence (Figure 3.4). This is due to the more powerful input of 9 W, resulting in a greater output or observed luminescence emission, which can be viewed with an orange filter (KV 550). This is significant as these filters are more widely available than the Wratten NA29 filter, which is needed when using illumination at 590 nm with the Polilight. Excitation at 530 nm with the Polilight does not produce the luminescence emission required for effective visualisation in a like manner. Nevertheless, as previously mentioned in Chapter 2, the cost, size and lack of versatility in available excitation wavelengths renders the use of lasers to be generally impractical for routine use in operational laboratories.

(a)



(b)

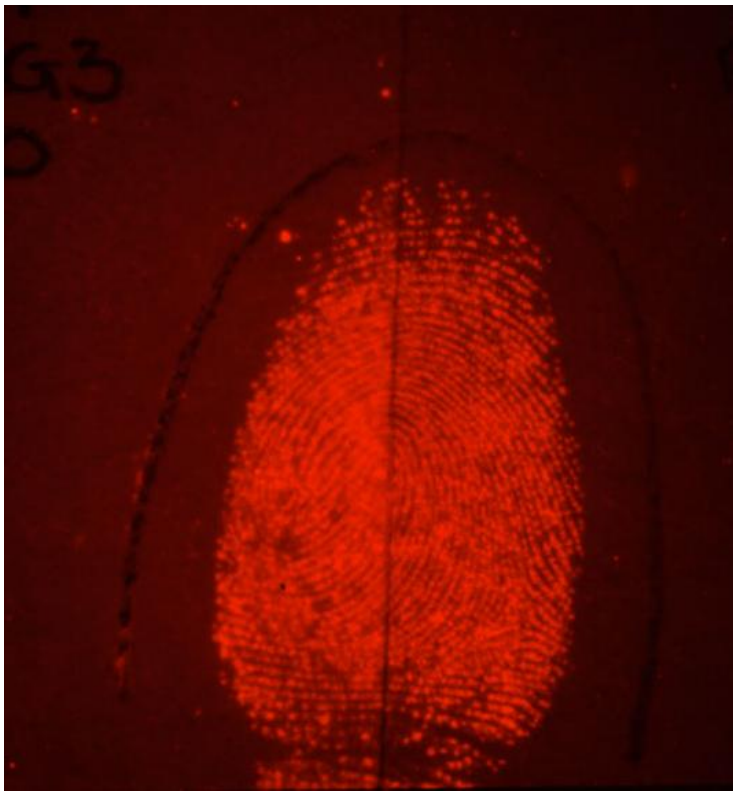


Figure 3.4: Lawsone treated fingermarks captured using a Nikon D2X digital SLR (60 mm focal length, ISO 100) in the photoluminescence mode with excitation using a 532 nm diode laser and viewed through: a) a KV 550 filter, (shutter speed 1/3 s, aperture f9); and b) a Wratten NA29 filter (shutter speed 18 s, aperture f9).

3.3.2 METHOD DEVELOPMENT

3.3.2.1 LAWSONE CONCENTRATION

Reagent concentration can have a significant impact on the performance of a latent fingermark visualisation method. The concentration of lawsone tested ranged from 0.1 mg/mL to 2 mg/mL. The higher concentration levels resulted in significantly saturated solutions, with the dissolution of lawsone being unsuccessful at 2 mg/mL.

One key aspect to consider was lawsone's natural dyeing ability. If present in significant amounts, this could have the potential of staining the paper surface itself, which was found to be evident at the higher concentrations. Therefore, it was important to find a compromise where the concentration of lawsone was sufficient to react with all the potential analytes, but with negligible effect on the substrate. From the observations undertaken, a 1 mg/mL concentration was selected as being the most suitable (Figure 3.5).

(a)

(b)

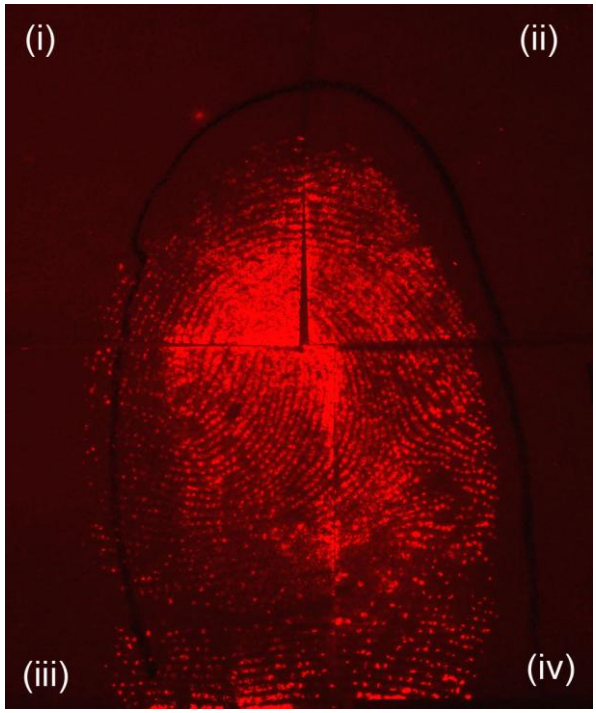


Figure 3.5: Fingermark treated with working solutions containing various concentration of lawsone (i) 0.2 mg/mL; (ii) 0.1 mg/mL: iii) 0.5 mg/mL and (iv) 1 mg/mL;. Images were captured using a Pentax K10 digital SLR, 50 mm focal length, ISO 100 in a) absorbance mode (shutter speed 1/125 s, aperture f/2.8) and in b) photoluminescence mode (excitation with a Polilight PL 500 at 590 nm and viewed through a Wratten NA29 filter (shutter speed 6.0 s, aperture f2.8).

3.3.2.2 CARRIER SOLVENT

The development of latent fingermarks on paper requires treatment with a solution of the reagent, either by immersion or spraying [2-4]. This is often followed by heating either directly using a laundry press or clothes iron, or indirectly through the use of an oven [2-4]. A wide range of formulations for amino acid sensitive reagents have been proposed, with the carrier solvent being a key component. The ideal solvent should evaporate rapidly, be non-toxic, non-flammable and non-polar so as to avoid any running of ink on treated documents [3, 4]. This last requirement is a significant issue with lawsone as it is not readily soluble in non-polar solvents such as HFE-7100 (1-methoxynonafluorobutane), which is a non-flammable, non-toxic CFC replacement that is widely used as a carrier solvent for fingermark reagents [3, 4].

Initially, ethyl acetate was used as a co-solvent to dissolve the lawsone prior to mixing with HFE-7100 (Table 3.1; formulation D). At this stage, no additional components such as acetic acid and zinc salts, which have been shown to provide for improved development with other reagents [40, 54, 60], were employed. To investigate this, a number of lawsone formulations were evaluated for their performance in developing latent fingermarks. The formulations selected here, outlined in Table 3.1, were based upon existing formulations used with ninhydrin, DFO and 1,2-indanedione [13]. Formulations A to C were compared directly against formulation D by using the "split print" approach, as detailed in Chapter 2.

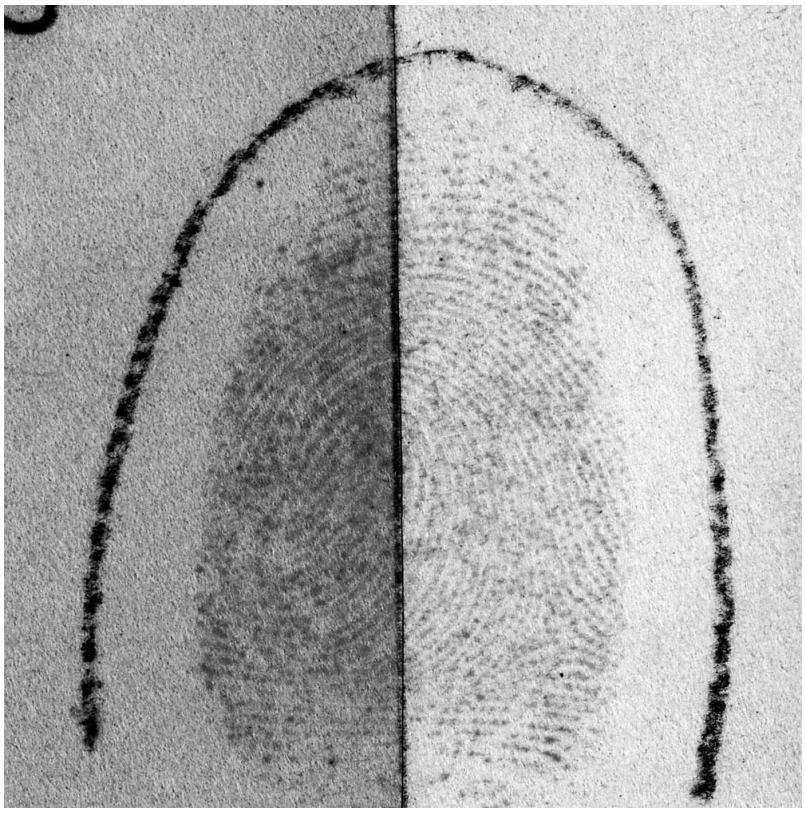
Table 3.1: Preparation of the various lawsone working solutions investigated [13].

Formulation A	Formulation B	Formulation C	Formulation D
0.1 g Lawsone	0.1 g Lawsone	0.1 g Lawsone	0.1 g Lawsone
3mL Dichloromethane	3 mL Dichloromethane	8.5 mL Absolute ethanol	20 mL Ethyl acetate
6.4mL Absolute ethanol	6 mL Ethyl acetate	0.7 mL Ethyl acetate	80 mL HFE-7100
0.6 mL Acetic acid	1 mL Acetic acid	0.8 mL Acetic acid	
90 mL HFE-7100	0.1 mL Zinc chloride solution	90 mL HFE-7100	
	90 mL HFE-7100		

It was found that formulations C and D consistently performed better than formulations A and B in terms of both colour and luminescence. In addition, formulations A and B were extremely unstable, with lawsone precipitating from the solution almost immediately. It is important to note that decisions concerning the most appropriate method of visualisation must not just rely upon the intensity of colour and/or photoluminescence produced, but also on the clarity of the enhanced fingermark, with particular reference to ridge detail. While formulations C and D were similar in terms of colour and luminescence, closer examination of the developed fingermarks showed a slight loss of fine ridge detail for formulation C (Figure 3.6). The reasons for this, at this stage, are unclear; however, the inclusion of ethanol could be associated with the apparent migration of the resulting product from the surface.

Therefore, on the basis of simplicity and performance, working solutions of lawsone were subsequently prepared by dissolution of the reagent in ethyl acetate and subsequent dilution with HFE-7100 to produce a working solution.

(a)



(b)

Figure 3.6: Effect of different lawsone formulations using the "split print" approach: a) formulation C (left half) vs. formulation D (right half) under white light; and b) formulation C (left half) vs. formulation D (right half) captured under photoluminescence conditions as described in Chapter2, Table 2.2.

3.3.2.3 Citric Acid Treatment

The art of henna on the skin of individuals has been reported to be improved by the application of lemon juice [103]. Anecdotally when lemon juice is applied to the skin, either by incorporation into the henna paste or applied as a post-treatment, it results in a darker colouration of the tattoo itself [103]. This indicates that the inclusion of acid may assist in the enhancement of lawsone as a fingermark reagent and therefore, attempts were made to investigate this further.

Citric acid was used as a substitute for lemon juice, as the impurities and the aqueous environment would be detrimental in terms of fingermark detection on porous substrates. The inclusion of the acid (0.1 M in ethanolic solution) was applied at three various stages in the treatment process: (i) prior to heating; (ii) after heating; and (iii) by addition in the lawsone formulation.

It was found that the addition of acid (prior to heating or in the lawsone formulation) provided no enhanced colouration of the fingermark itself; however, when applied after heating, it improved contrast by eliminating the background discolouration. However, it was also found that, not only did it remove the staining effect of lawsone from the surface, it also removed the fingermark itself to some degree, as the luminescence intensity was reduced considerably, as depicted in Figure 3.7.

This would suggest that either the reaction product and excess lawsone is being removed from the substrate, or the inclusion of citric acid in ethanol is interacting with the reaction product in a manner that is quenching the observed luminescence. In order to determine whether the citric acid or ethanol is responsible for this observation, a simple solvent wash post development was

applied. Both ethanol and ethyl acetate were investigated. However, results were found to have no effect on the sample as observed previously with the inclusion of citric acid. This indicates that the ethanol is not solely responsible for the observed contrast improvement in the absorbance mode, and this must result from the involvement of citric acid in some way.

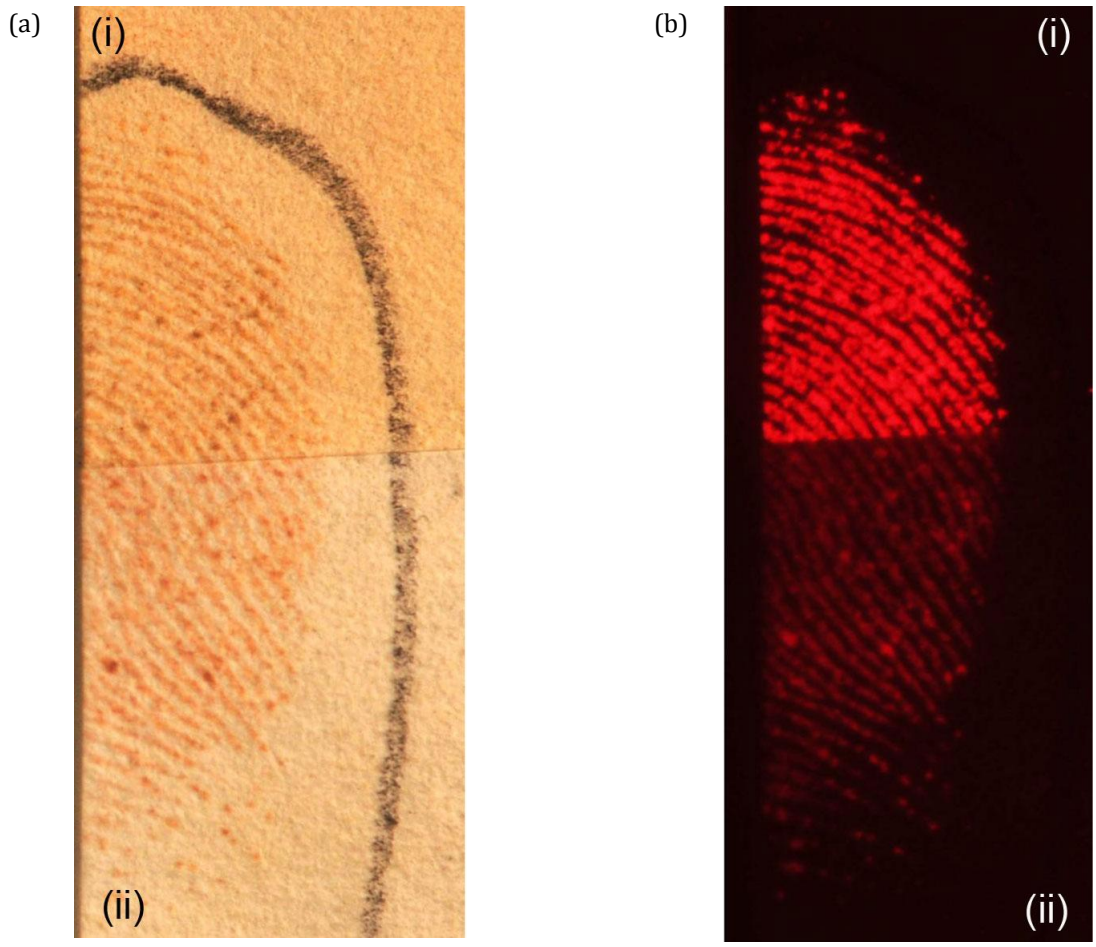


Figure 3.7: Comparison of fingermarks treated with i) no citric acid treatment and ii) post-heat treatment with acid. Images were taken with a Nikon D300 SLR (60 mm focal length, ISO 200): a) in absorbance mode (shutter speed 1/160 s and aperture f5); and b) in photoluminescence mode, excitation with a Polilight PL 500 at 590nm and viewed through a Wratten NA29 filter (shutter speed 20 s, aperture f10).

3.3.2.4 Addition of Metal Salts

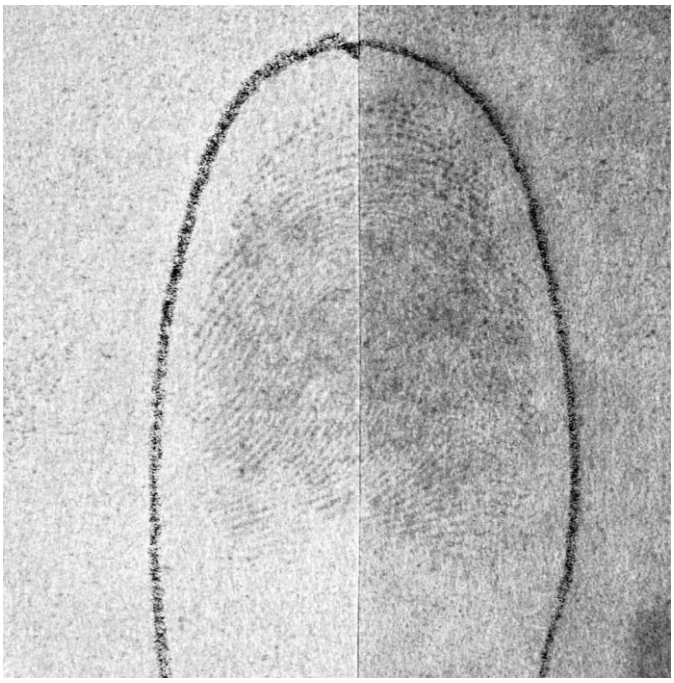
Various post-treatments have been found to be instrumental in improving fingermark detection on paper substrates [40, 42]. In particular, investigations into the use of metal salts as a means to improve latent fingermark development have been well documented and supported by various institutions and law enforcement agencies [13, 32, 60, 63, 66]. In addition, textile chemistry widely uses mordants (metal salts) to permanently set dyes onto fibres by strengthening the affinity between them. In addition, some mordants also have the ability of altering the hue of particular dyes [121].

A range of metal salts was investigated for their ability to enhance the performance of lawsone in developing latent fingermarks. This was both as part of the formulation and post-treatment. No significant improvement with respect to fingermark development on filter paper was noted for any metal salt, either as part of the formulation or as a post-treatment. However, the presence of zinc chloride led to the enhancement of fingermarks on cellulose TLC plates with both colour and luminescence. The reasons for this phenomenon are unclear and require further investigation. It may be the result of substrate particle size, presence of binding agents or an effect of the aluminium backing on the TLC plates. Nevertheless, at this stage in the investigations, the inclusion of metal salts from an operational context would not provide any additional value for fingermark detection protocols and further studies into the use of metal salts were abandoned.

3.3.2.5 HEAT TREATMENT

In our initial study into lawsone, an oven was used to heat the treated fingermarks. This led to development times of approximately an hour when heated at 150 °C. Temperatures exceeding this were found to provide no further enhancement in development. Attempts to use a laundry press led to inconsistent development due to the lack of time available for heating, with a safety switch preventing direct heat for longer than 10 s. Increasing the development time could be achieved by opening and reclosing the press; however as an alternative, a clothes iron was substituted as the direct heat option. This was found to successfully develop fingermarks in a much shorter period of time compared to the oven (30–60 s). In addition, the developed marks exhibited a deeper colour. However, along with development of the prints, there was an increase in background colouration of the paper substrate (Figure 3.8) and reduction in luminescence. Therefore, despite the longer development time required, the oven was used for all subsequent treatments.

(a)



(b)

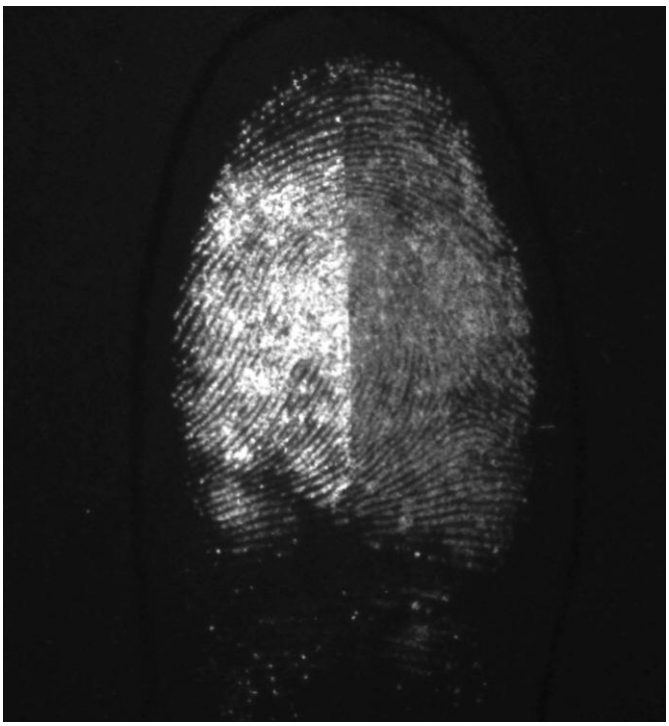


Figure 3.8: Effect of heating process on development of latent fingermarks with lawsone: a) oven (left half) vs. iron (right half) under white light; and b) oven (left half) vs. iron (right half) captured under photoluminescence conditions. conditions as described in Chapter 2, Table 2.2.

3.3.2.6 Issues with Solubility and Performance

Due to the novel nature of lawsone as a potential fingermark reagent, additional research into the formulation had to be performed before comparative studies against current reagents could commence. It has been found that lawsone has a tendency, over a short period of time, to "drop out" of solution, and it appears that the rate is influenced by seasonal changes, i.e. temperature and humidity. Along with this, throughout this research, the performance of lawsone appeared to reduce, even with fresh working solutions. Results have been extremely promising with 'fresh' lawsone, to a point where, even prior to heating the sample, an orange outline of the fingermark becomes visible. However, after the vial of solid lawsone was unsealed and left in storage for some time, fingermark development using associated lawsone working solutions appeared to diminish (Figure 3.9).

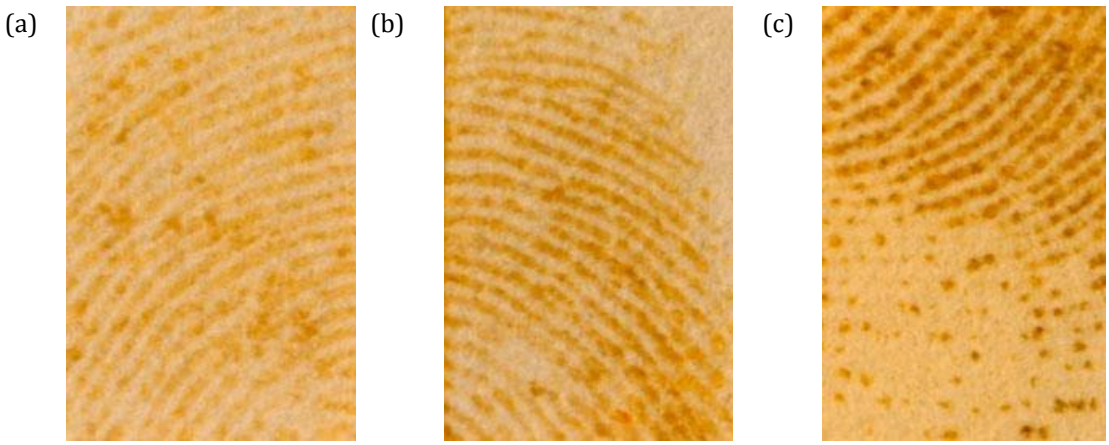


Figure 3.9: Effect of ageing of the vial of solid lawsone: a) lawsone opened ~ 5 years; b) lawsone opened ~2 years; and c) freshly opened lawsone. Solutions prepared and samples treated contemporaneously for direct comparison. Images captured with a Nikon D300 SLR (60 mm focal length, ISO 200) under white light (shutter speed 1/20 s, aperture f11). [2]

[2] Samples developed and photographed by Anusha Menon from Curtin University under my direct supervision.

3.3.3 REACTION CHARACTERISTICS

As with established fingermark reagents for paper substrates, the basis of visualisation depends on the fingermark reagent reacting with amino acid residues present in the deposit to produce a visible and/or luminescent product [3]. As outlined in Chapter 1, the amino acids adhere to the cellulose fibres and do not tend to diffuse through the paper under normal environmental conditions. Reaction with the amino acids produces a visible outcome that is representative of the manner in which the fingermark was deposited and consequently produces admissible contact evidence.

In order to verify that lawsone was reacting with the amino acid content of the latent fingermarks, solutions of amino acids in water (lysine, serine, glycine, and proline; all at 900 mg/mL) were dispensed onto filter paper and allowed to air dry before subsequent lawsone treatment. In common with the developed latent fingermarks, the amino acid spots, with the exception of proline, developed as purple/brown stains that were also photoluminescent. Excitation spectra, recorded an optimal excitation wavelength of 590 nm, and therefore was the wavelength selected from subsequent emission spectra (Figure 3.10).

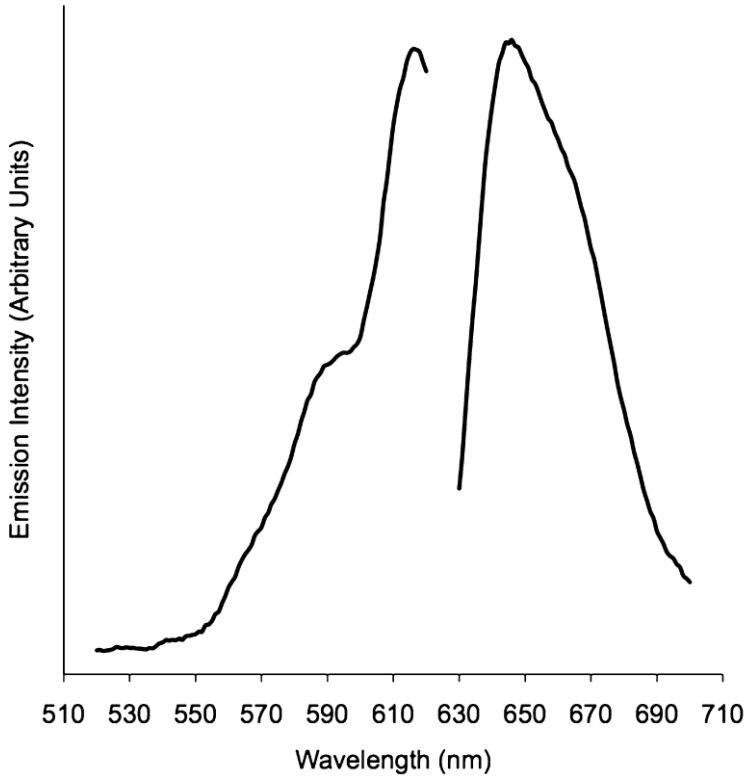


Figure 3.10: Excitation spectra for lawsone treated glycine spot (λem 645 nm) compared with emission spectra for same spot (λex 590 nm). Spectra have been normalised.

Luminescence spectra of the developed amino acid spots and a developed latent fingermark are presented in Figure 3.11. It is clear from the profiles that the photoluminescence characteristics of the reaction product between primary amino acids and lawsone are similar to that in lawsone developed fingermarks, thus suggesting that the lawsone reagent is non-specifically targeting primary amino acids in the latent fingermark deposit. This is a key requirement of latent fingermark reagents, as the amino acid content of natural secretions is highly variable from one individual to another [23].

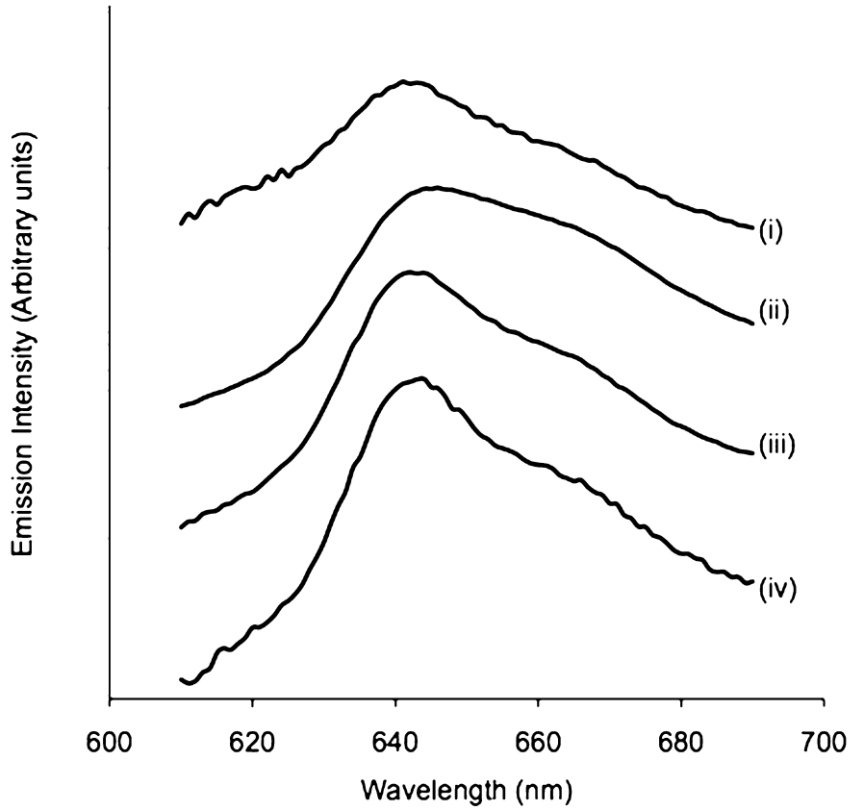
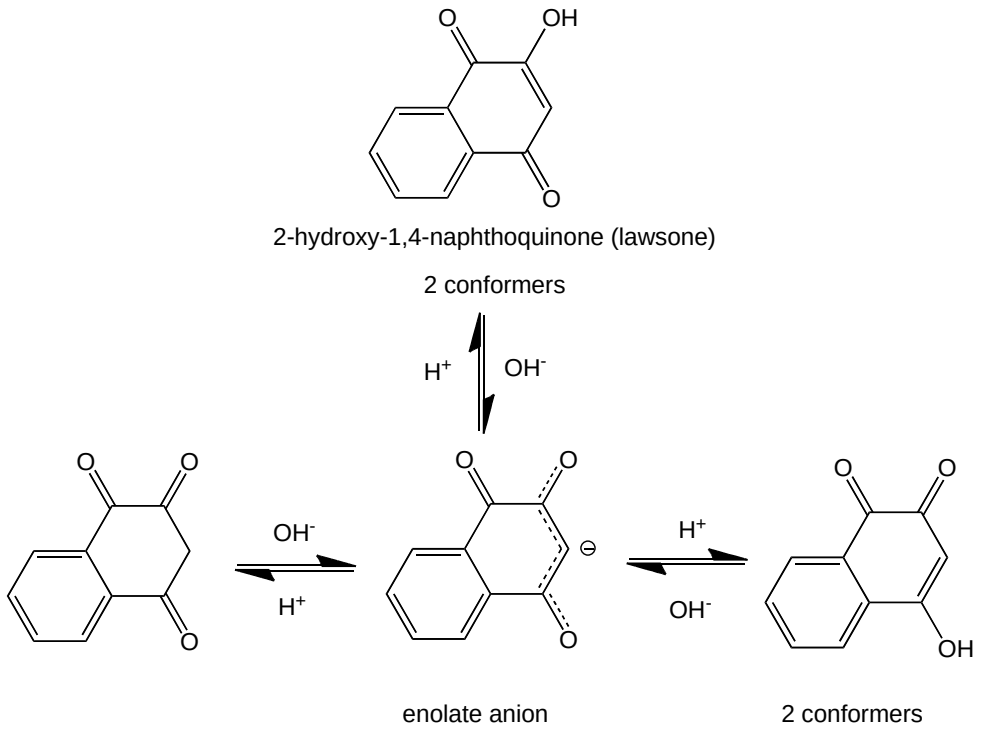


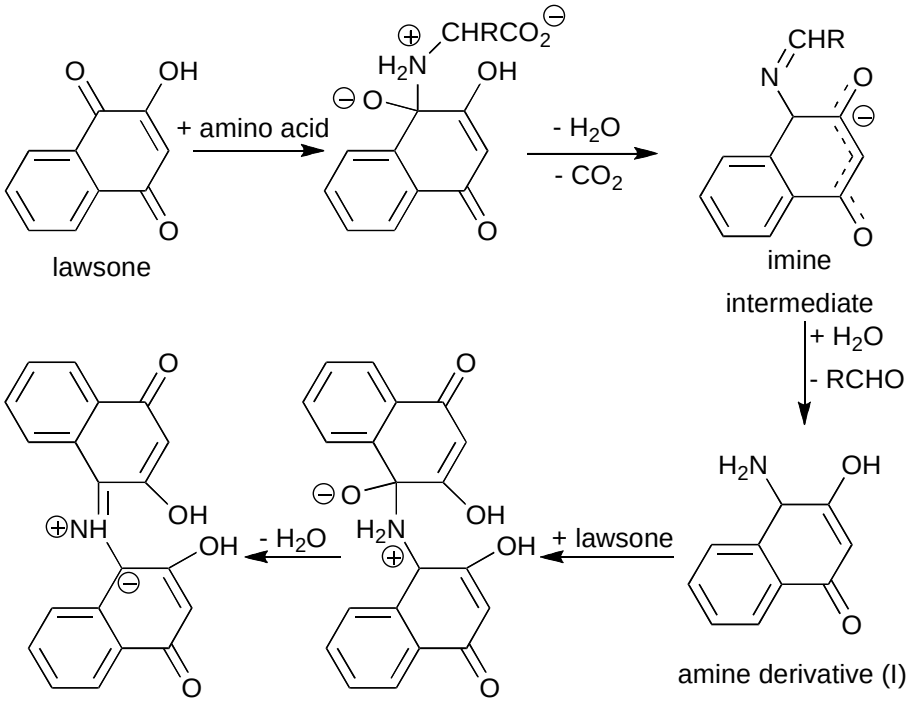
Figure 3.11: Luminescence characteristics of lawsone treated amino acids and latent fingermark (λ_{ex} 590nm): (i) lysine; (ii) glycine; (iii) serine; (iv) fingermark. Spectra have been normalised and offset to illustrate similarities and differences in shape and maxima.

Predictions of the resulting products obtained from established fingermark reagents have come under some conjecture in recent times, and it is possible that any solution phase analysis aimed at deducing the resulting products may not truly represent the chemistry involved on the surface itself. Spyroudis has previously reported on the reaction of lawsone with amino acids [120]. Along with this, we have used computational chemistry and knowledge of reaction pathways to postulate a structure for the product from the reaction of lawsone with amino acids.



Scheme 3.1: Lawsone and isomers.

Lawsone can undergo keto–enol tautomerisation as shown in Scheme 3.1. Spartan '0414 ab initio calculations using HF/6 31G* theory indicates that the carbon at the 1-position is the most electron deficient carbonyl carbon of all 6 species/conformers. It is postulated that this carbon is most likely to react with an incoming nucleophile. Based on the known reactivity of hydroxyquinones [120] and ninhydrin [122, 123], we postulate that lawsone undergoes a Strekker degradation at the 1-position to form the amine intermediate (I), as shown in Scheme 3.2. The Strekker degradation requires a primary amino acid, which would explain why proline does not form the fluorescent product. The amine (I) is a nucleophilic base that can react with a second lawsone molecule to reduce the ketone at the 10-position, thus forming the final product, in which the negative charge can be delocalised over the extended π-system.



Scheme 3.2: Proposed reaction pathway and product for the reaction of lawsone with primary amino acids.

In order to verify this postulation, confirmatory *in-situ* analysis was investigated and performed, which is further discussed in Chapter 5.

3.4 CONCLUSION

In conclusion, lawsone has shown promise as a potential amino acid reagent with the ability of developing latent fingermarks on paper surfaces. This compound represents the first in a completely new class of fingermark detection reagents. Lawsone is a naphthoquinone, a group of compounds that are well known for their reactions with amino acids [124-128]. Naphthoquinones therefore, represent a class of compounds of significant interest as potential fingermark detection reagents.

CHAPTER 4: SUBSTITUTED NAPHTHOQUINONES AS NOVEL AMINO ACID REAGENTS

Portions of this chapter have been published in the journal *Talanta*:

R. Jelly, S.W. Lewis, C. Lennard, K.F. Lim, J. Almog. Substituted naphthoquinones as novel amino acid sensitive reagents for the development of latent fingermarks on paper surfaces, *Talanta*, (2010), Vol 82, p. 1717-1724.

4.1 INTRODUCTION

As indicated in the previous chapter naphthoquinones have been of interest to science as a result of their diverse applications across a broad range of fields. More specifically, this class of compounds have been fundamental in the progression and advancement of research in various areas of biological and chemical sciences [120, 129].

Naphthoquinones have found a number of key applications in the biomedical community [120], in particular, 2-methyl-1,4-naphthoquinone derivatives are the backbone of vitamin K groups, where the variations occur via the addition of aliphatic side chains at the 3-position [130, 131]. Vitamin K is a dietary requirement and plays a vital role in the coagulation of blood [130, 131]. Along with this, strong evidence has identified naphthoquinones as potential antitumor and antibacterial agents, by inducing growth inhibitory effects [110, 132-135]. Other avenues have demonstrated the influence of naphthoquinones in the pharmaceutical industry, with the development of these compounds helping combat parasitic diseases in the form of antimalarial drugs [136]. The general consensus in the biomedical community, with respect to naphthoquinone derivatives, is that they are important inhibitory agents. This view also applies in the agricultural community. Foote and co-workers describe the use of various naphthoquinones as plant fungicides by inhibiting spore germination, which has led to the commercial use of 2,3-dichloro-1,4-naphthoquinone for this purpose [137].

The significant contribution of these compounds, accounting for their potential diverse applicability, is specifically related to the inclusion of the quinone moiety [120, 129]. Quinones are well known for their ability to act as oxidising or dehydrogenating agents [129], and therefore are suitable for a variety of applications in synthetic and organic chemistry. Along with this, quinone

derivatives such as naphthoquinones and anthraquinones are sources of natural and synthetic dyestuffs. As a result, they have application in areas requiring colorant technology such as the cosmetic and textile industries [138, 139].

Furthermore, in relation to chemical applications, naphthoquinone based compounds have found use in the areas of electrochemistry and analytical chemistry. Raoof and co-workers were able to adsorb 1,2-naphthoquinone-4-sulfonic acid sodium onto a gold electrode to detect cysteamine in an aqueous solution [140]. This correlates with detection-based methods used for forensic and analytical applications. As an example, 1,2-naphthoquinone-4-sulfonate can be used as a derivatising agent to detect amphetamine and methamphetamine in biological samples [141-146]. This is based on the ability of 1,2-naphthoquinone-4-sulfonate to associate with the compound of interest in a manner that can be affiliated with electrochemical or spectrophotometric detection, which can be more difficult to achieve with just the compound of interest [141-146].

The application of naphthoquinones as chromogenic reagents has been shown to be highly diverse, with extensive reports available that relate to their ability to react with amino groups [115-120, 147-153]. 1,2-Naphthoquinone-4-sulfonate has been employed for the determination of amino acids through the formation of highly coloured compounds [124-128]. Rees and colleagues specifically studied the use of 1,2-naphthoquinone to form a purple/brown compound on reaction with cysteine, and also noted that the reaction was believed to target the amino group of the amino acid [154]. This provides strong evidence to suggest the importance of naphthoquinones for the detection and colorimetric analysis of primary amines or associated compounds and, in turn, their potential use for detecting latent fingermarks on porous surfaces. Therefore, this chapter reports on the investigations of some potential

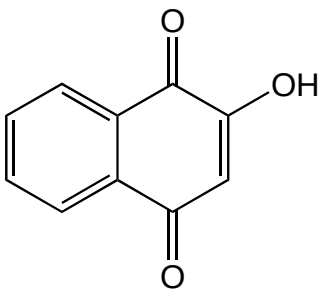
naphthoquinones and their ability to react with amino acids and latent fingermarks on porous surfaces.

4.2 EXPERIMENTAL

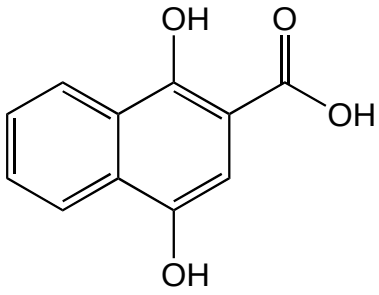
The work presented in this chapter was conducted via the use of chemicals and procedures outlined in Chapter 2.

4.3 RESULTS AND DISCUSSION

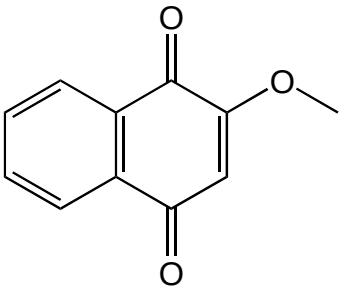
Building on our initial studies with lawsone described in the previous chapter, a number of structurally related compounds (Figure 4.1) were selected for investigation as to their ability to develop latent fingermarks on paper surfaces. This approach is very similar to that taken with ninhydrin and its analogues, with the added advantage that naphthoquinones are readily commercially available, thus obviating the necessity for synthesis which is typically the case with ninhydrin analogues [3, 31]. There is a wide range of naphthoquinones available and the compounds to be tested in this study were selected on the basis of their availability and the low hazard they represented to health. In addition to their potential to act as fingermark detection reagents, any variations in performance may provide information as to the nature of the reaction of naphthoquinones with amino acids.



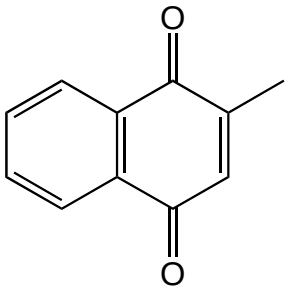
lawsone



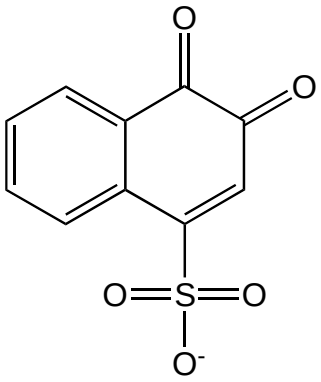
1,4-dihydroxy-2-naphthoic acid



2-methoxy-1,4-naphthoquinone



2-methyl-1,4-naphthoquinone



1,2-naphthoquinone-4-sulfonate

Figure 4.1: Selected naphthoquinones.

4.3.1 Development of Latent Fingermarks by Selected Naphthoquinones

On the basis of simplicity and performance, working solutions of naphthoquinones were prepared in the same manner as for lawsone, by dissolution of the reagent in ethyl acetate and subsequent dilution with HFE-7100 to produce a working solution.

4.3.1.1 INITIAL INVESTIGATIONS

Four compounds with naphthoquinone structures were tested for their ability to visualise latent fingermarks on filter paper: 1,4-dihydroxy-2-naphthoic acid; 1,2-naphthoquinone-4-sulfonate; 2-methoxy-1,4-naphthoquinone; and 2-methyl-1,4-naphthoquinone (Figure 4.1). The naphthoquinones were prepared at the concentration of 1 mg/mL (0.1% w/v). A non-treated fingermark was also heated as a control in order to ensure that results were not due to the development of the mark by heat alone [98].

All of the tested naphthoquinones gave brown visible prints that exhibited photoluminescence when illuminated at 555 nm using the forensic light source and viewed through red goggles, or when illuminated at 590 nm and viewed using the Poliview system (Figures 4.2 and 4.3). The control latent fingermark on filter paper, which was heated but not treated with naphthoquinone, showed no significant development under the heating conditions used in this experiment. Initial observations revealed some variation in colour and luminescence intensity for the naphthoquinone treated fingermarks. Latent fingermarks developed with 1,4-dihydroxy-2-naphthoic acid consistently showed stronger colour but weaker luminescence intensity, while those developed with 2-naphthoquinone-4-sulfonate were less coloured but exhibited stronger photoluminescence emission (Figure 4.2).

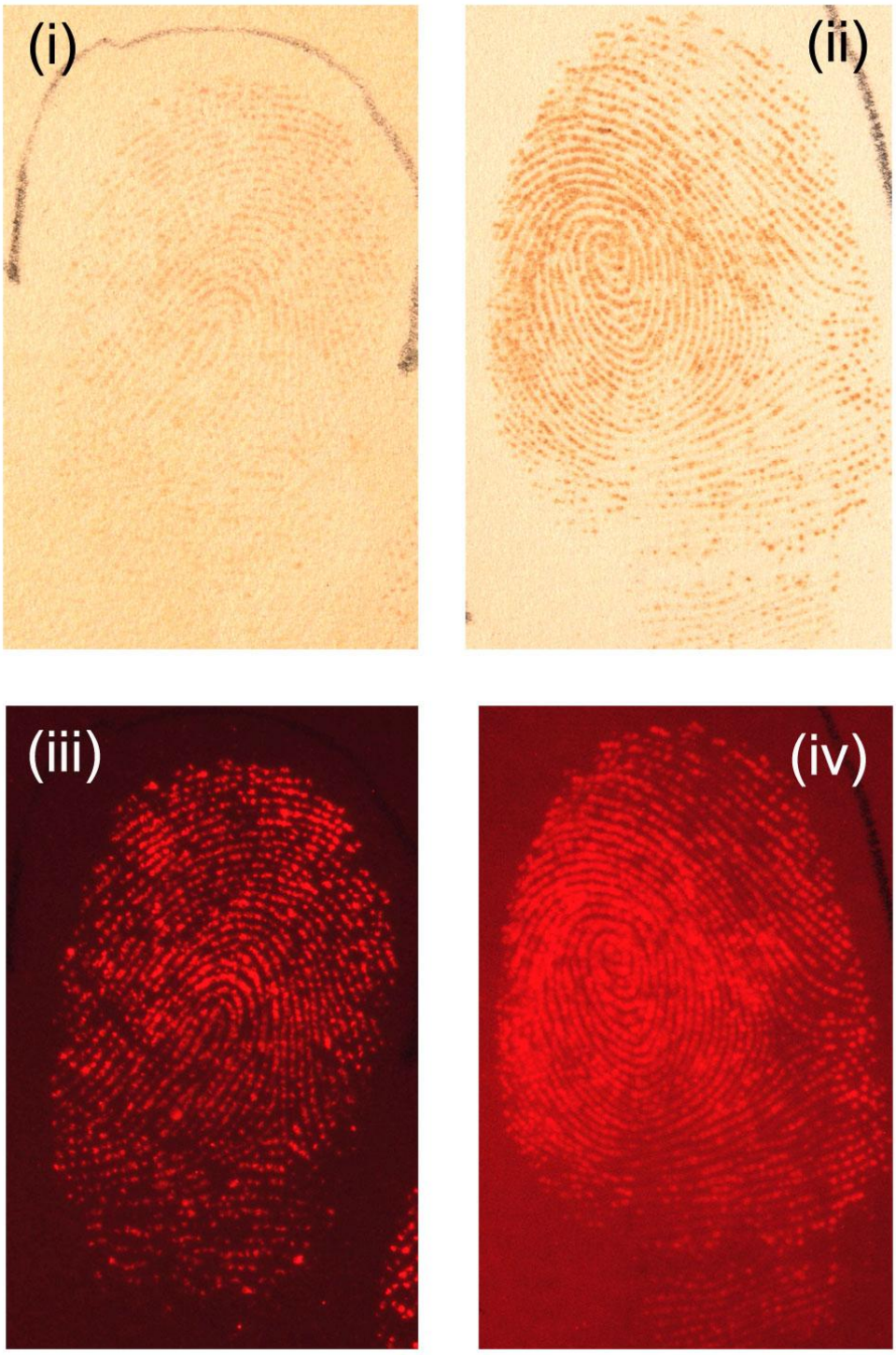


Figure 4.2: Latent fingermarks developed with naphthoquinones (i) lawsone and (ii) 1,4-dihydroxy-2-naphthoic acid under white light (iii) lawsone and 1,4-dihydroxy-2-naphthoic acid in the photoluminescence mode. Images were taken with a Nikon D300 SLR, 60 mm focal length, ISO 200, white light (shutter speed 1/40 s, aperture f10), and photoluminescence mode, excitation with a Polilight PL 500 at 590nm and viewed through a Wratten NA29 filter (shutter speed 15 s, aperture f10). Images enhanced for brightness and contrast using Adobe Photoshop CS4 Version 9.0.

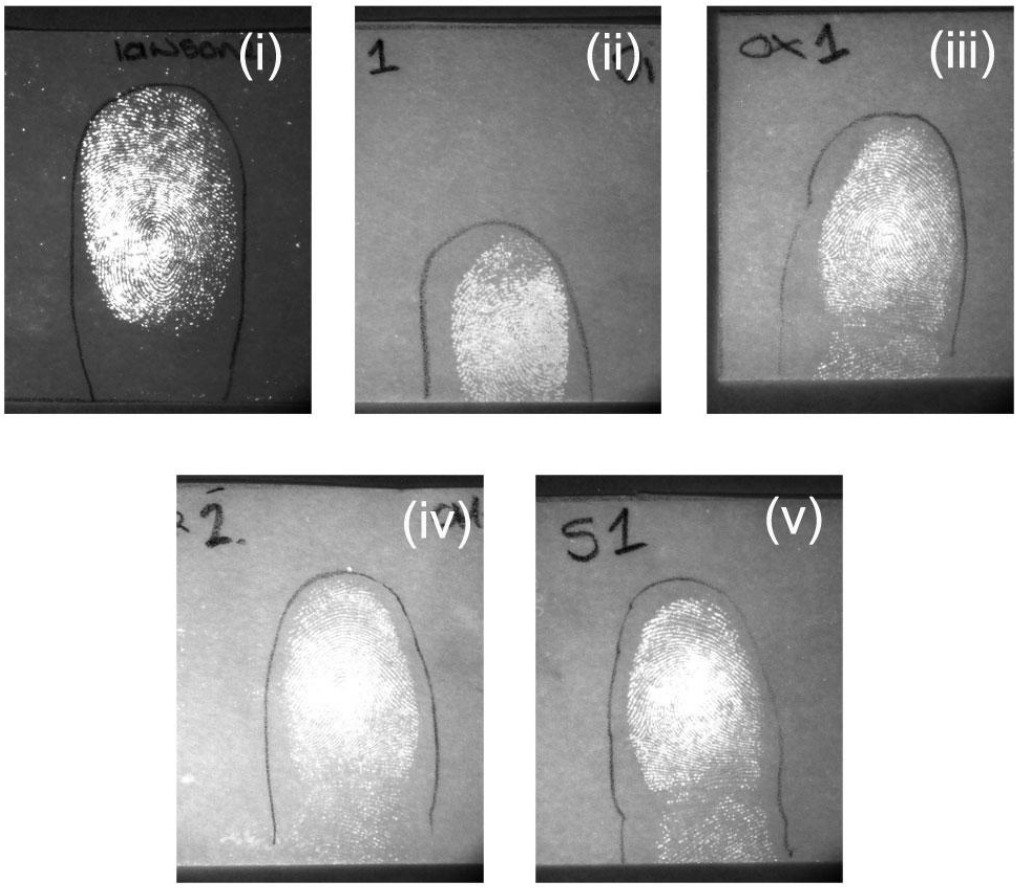


Figure 4.3: Latent fingermarks developed with naphthoquinones photoluminescence from naphthoquinone treated latent fingermarks, acquired using a Poliview digital imaging system (Rofin, Australia) with excitation at 590 nm, viewed through a 650 nm interference filter with a 1 s exposure time: (i) lawsone; (ii) 1,4-dihydroxy-2-naphthoic acid; iii) 2-methoxy-1,4-naphthoquinone; (iv) 2-methyl-1,4-naphthoquinone; (v) 1,2-naphthoquinone-4-sulfonate.

4.3.1.2 LUMINESCENCE CHARACTERISTICS

Marked differences in luminescence characteristics for fingermarks developed with the different naphthoquinones were observed when samples were examined under different wavelength bands from the Polilight over the range λ_{ex} 530–590 nm. To examine this in more detail, luminescence spectra from the developed fingermarks were obtained using a fluorescence spectrophotometer fitted with a fibre-optic probe. Excitation was carried out at 530, 555 and 590 nm. These wavelengths were selected as they corresponded to wavelengths available on the Polilight forensic light source.

Examination of the spectra revealed variations between lawsone and the other naphthoquinones selected (Figure 4.4a). The optimal response for the substituted naphthoquinones occurred at λ_{ex} 530 nm, in comparison to lawsone at λ_{ex} 590 nm. Additionally, different luminescence emission characteristics for some of the individual naphthoquinones became apparent once spectra had been normalised for comparison purposes. 1,4-Dihydroxy-2-naphthoic acid produced weaker luminescence characteristics, creating slightly noisy spectra; however, the product from this compound appears to have a slight shift in emission towards the red. In comparison, 1,2-naphthoquinone-4-sulfonate, although producing similar spectra to 2-methoxy-1,4-naphthoquinone and 2-methyl-1,4-naphthoquinone, showed greater luminescence emission, which corresponded to our visual observations (Figure 4.4b).

Operationally, these variations would not be a significant issue as all of the developed prints were clearly visible using the combination of excitation at 555 nm and observation through red goggles (corresponding to a red long-pass filter). Emission spectra for latent fingermarks developed with each individual naphthoquinone, with excitation at the optimum wavelength based on emission intensity, are presented in Figure 4.4.

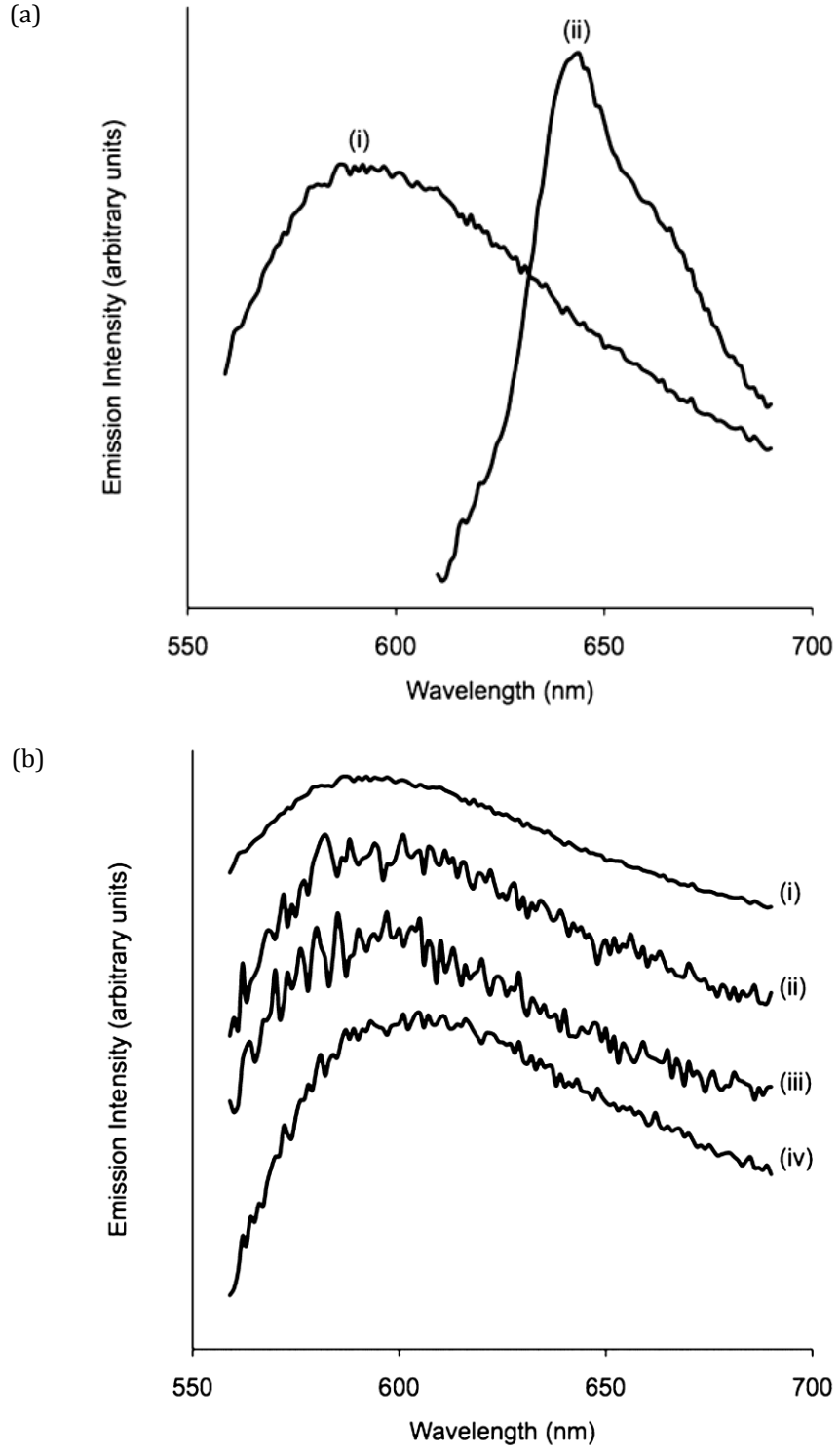


Figure 4.4: Luminescence emission spectra for fingermarks developed with naphthoquinones. Spectra have been normalised and offset to illustrate similarities and differences in the shape and maxima. a): (i) 1,2-naphthoquinone-4-sulfonate (λ_{ex} 530 nm) and (ii) Lawsone (λ_{ex} 590 nm); b) selected naphthoquinones (λ_{ex} 530 nm); (i) 1,2-naphthoquinone-4-sulfonate; (ii) 2-methoxy-1,4-naphthoquinone; (iii) 2-methyl-1,4-naphthoquinone; (iv) 1,4-dihydroxy-2-naphthoic acid.

In the previous chapter, we confirmed that lawsone was reacting with amino acids in the latent fingermark by treating dried amino acid spots on paper. A similar approach was taken with the substituted naphthoquinone compounds in this study. Initially, lysine was selected for this investigation due to an enhanced response with lawsone in comparison to other amino acids (refer to Chapter 3, Figure 3.11). For each compound, solutions of lysine in water (900 μg/mL) were dispensed (5 μL) onto filter paper and the deposited spots allowed to air dry before development. In a similar fashion to the developed latent fingermarks, the treated amino acid spots were visible as brown stains that were also photoluminescent. Fluorescence spectra of the developed amino acid spots were measured at the optimum excitation wavelengths for each naphthoquinone as described above.

The signal obtained was found to be quite weak, indicating that the sensitivity of these selected naphthoquinones is reduced in comparison to lawsone. Along with this, the potential role of the additional amino group present in lysine could influence the resulting product in a way that does not correlate with the characteristics observed with developed fingermarks. Therefore, this investigation was replicated with the use of glycine and serine. These amino acids were selected on the basis of their simplicity and their relatively high concentration in latent fingermark residues [5]. Amino acid concentrations were increased to approximately 9000 μg/mL (0.1 M) to ensure a high signal intensity.

As seen in Figure 4.5, different luminescence characteristics were observed between the two amino acids. This may be due to the presence of the side chain influencing the resulting luminescent species, thus altering the observed luminescence characteristics. The spectra obtained from the serine product correlated closely with that obtained from treated fingermarks, in contrast to the product formed from the glycine reaction. Although difficult to ascertain due

to the complex chemical nature of fingermark residues, it could be speculated that this similarity may be a result of higher levels of serine in fingermark deposits compared to glycine [5]. As was observed in an earlier chapter, the luminescence spectra are very similar between the developed latent fingermarks and the amino acids, thus indicating that the reagents are targeting the amino acids in the latent fingermark deposit.

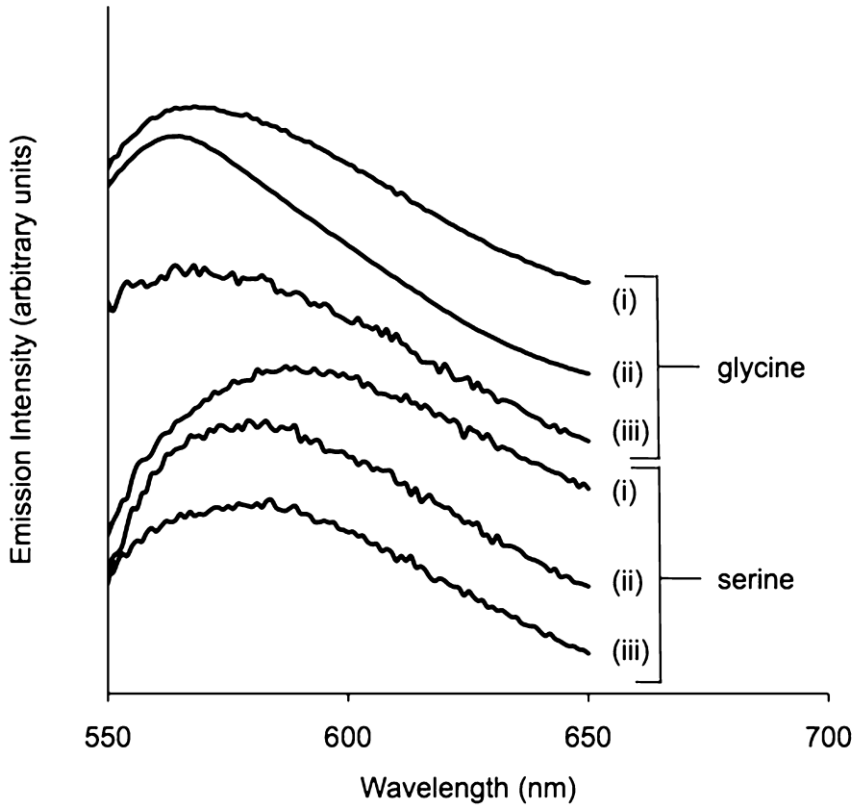


Figure 4.5: Luminescence characteristics of naphthoquinone treated amino acids. Glycine and serine amino acid spots treated with naphthoquinones (λ_{ex} 530 nm), for both amino acids (i) 2-methoxy-1,4-naphthoquinone; (ii) 2-methyl-1,4-naphthoquinone; (iii) 1,2-naphthoquinone-4-sulfonate. Spectra have been normalised and offset to illustrate similarities and differences in shape and maxima.

4.3.2 Evaluation of Naphthoquinones as a Class of Fingermark Detection Reagents

This study has shown that naphthoquinones have significant potential as reagents for the detection of latent fingermarks on paper and other porous surfaces. Lawsone shows the most promise as a potential fingermark visualisation reagent in comparison to the other naphthoquinones tested. However, this investigation may be biased towards lawsone as the formulations and procedural conditions have not been optimised for these other naphthoquinones. Therefore, the results obtained can generally be considered as an initial screening test only.

The variations observed in terms of colour and luminescence, as outlined in Section 3.1, may indicate the formation of alternative products resulting from the reaction with amino acids. This may provide some insight into the reaction mechanisms involved and the effects of the differences in structure associated with each naphthoquinone. Figure 4.6 provides an overview of some indications into the structural differences, which could account for the variation in performance. This information may play an important role in predicting other naphthoquinones that may surpass the current performance of the naphthoquinones investigated in the current study. One avenue, highlighted in red in Figure 4.6, is the inclusion of one or more moieties in position(s) 6, 7 and/or 8 of the naphthoquinone. The inclusion of such substituents, may result in a significant change in both colour and luminescence, without significantly influencing the nucleophilic reactive site. If the electronic properties of a conjugated system are altered in a manner that allows increased colour and luminescence, then an improved outcome can be achieved with respect to the use of naphthoquinones as fingermark detection reagents.

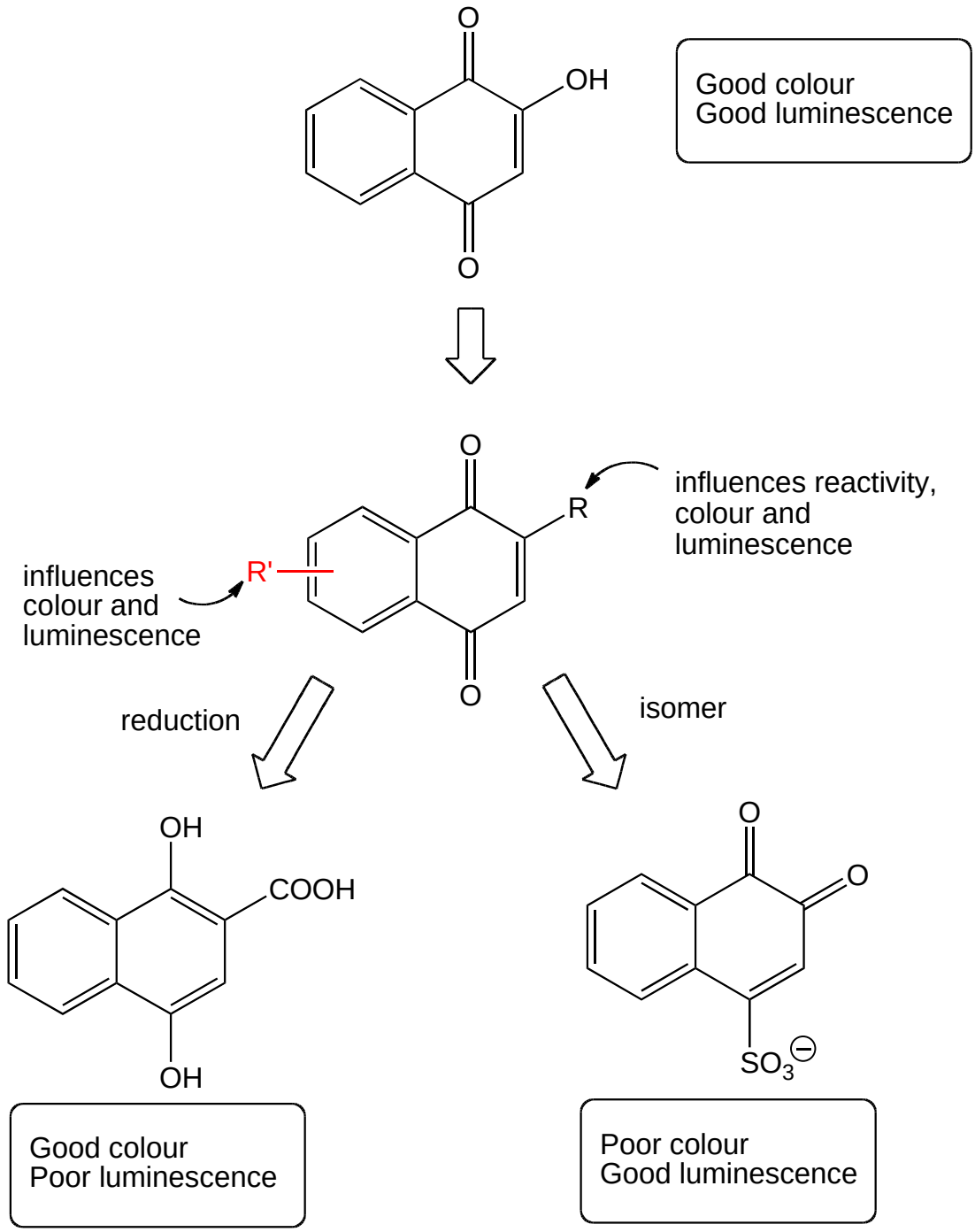


Figure 4.6: Outline of lawsone and derivatives thereof, indicating performance on reaction with latent fingermarks and potential influences affecting reactivity.

The results can be used for the selection of naphthoquinones to be targeted for further optimisation of development and observation conditions. 1,4-Dihydroxy-2-naphthoic acid and 1,2-naphthoquione-4-sulfonate are the most likely candidates for further in-depth method development on an individual basis to evaluate their potential as fingermark reagents. These reagents stood out as being significantly different in terms of colour and luminescence. Relating back to the previous paragraph, these variations could be understood based on the fact that 1,4-dihydroxy-2-naphthoic acid is a hydroquinone and 1,2-naphthoquione-4-sulfonate, an orthoquinone. These compounds, which can be considered derivatives of lawsone, differ more considerably in structure than the remaining naphthoquinones tested (i.e. 2-methoxy-1,4-naphthoquinone and 2-methoxy-1,4-naphthoquinone). Optimisation has the potential to significantly enhance their utility. For instance, 1,2-naphthoquinone-4-sulfonate exists as a salt and, as such, its solubility in the working formulation is quite poor, which may have a detrimental effect on its ability to develop latent fingermarks.

4.4 CONCLUSION

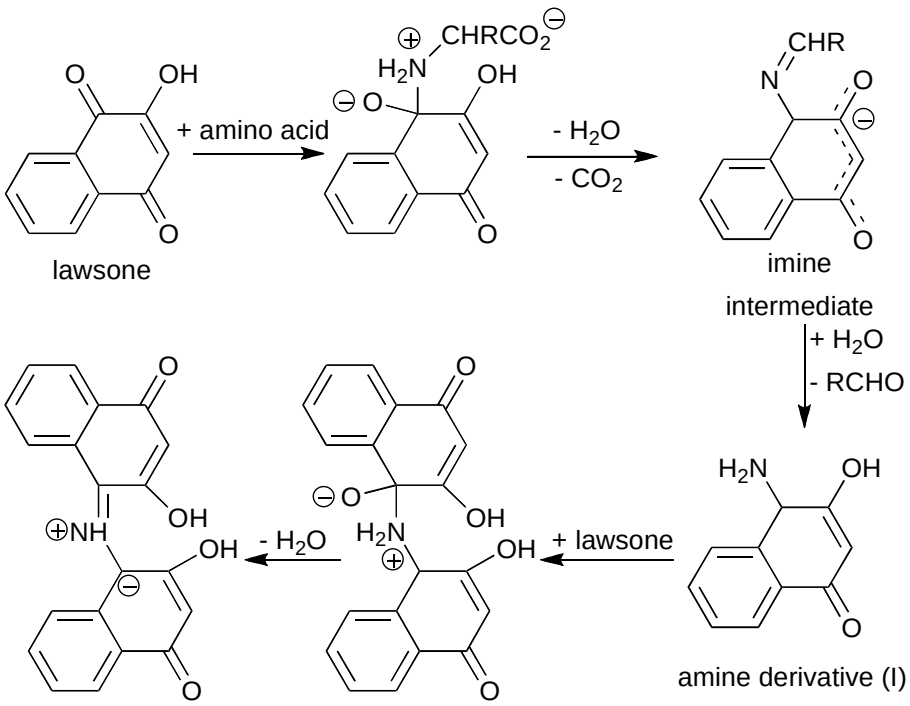
In conclusion, this study has shown the potential of naphthoquinones as effective reagents for the detection of latent fingermarks on paper, with lawsone, 1,2-naphthoquinone-4-sulfonate and 1,4-dihydroxy-2-naphthoic acid showing the most promise as dual reagents (i.e. strong development colour and luminescence).

CHAPTER 5: ANALYSIS OF FINGERMARKS AND AMINO ACID SPOTS ON MICROSCOPICALLY ROUGH SURFACES USING SYNCHROTRON ATR-FTIR

5.1 INTRODUCTION

A key issue with the development of amino acid sensitive fingermark detection reagents is the ability to determine the relevant reaction mechanisms of these with amino acids and latent fingermarks on paper substrates. These provide the framework to enable rational approaches for the optimisation of the conditions for fingerprint detection, as well as for the design of improved reagents.

As mentioned in Chapter 3, in regards to lawsone, we postulated the formation of a dimeric product (Scheme 5.1) in a similar manner as ninhydrin with amines and amino acids however, this pathway is speculative and unconfirmed.



Scheme 5.1: Reaction mechanism of lawsone with amino acids to produce a dimeric product.

As described in Chapter 4 further naphthoquinones were discovered to be able to produce visible and photoluminescent fingermarks on paper surfaces. The photoluminescence characteristics of these additional compounds were found to be different to those of lawsone, indicating the possibility of alternative reaction products with amino acids.

The first step of any mechanistic study involves examining the reaction products *in-situ*, which requires suitable surface analysis techniques in order to obtain results that are applicable to fingermark detection on porous surfaces. Studies in a solvent medium, whilst perhaps more simple, allow the amino acid units and fingermark reagents to mix freely, permitting the formation of products that are not necessarily representative of the products formed in an adsorbed/restrictive environment. However, solution studies may provide ancillary information that can assist in the interpretation of surface analysis data.

Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy is a well-established method for studying surfaces that have a relatively shallow depth of penetration (~1-2 μm). However, even this shallow depth of penetration cannot totally eliminate contributions to the spectra from the substrate, as the thickness of the adsorbed layers maybe significantly less than a micron. Furthermore, as porous surfaces are topographically rough, and hence deposits on them are non-uniformly dispersed, high spatial resolution microscopy methods are required. The modest spatial resolution (~30-50 μm) and sensitivity of conventional FTIR microscopy is not optimal for such work and a brighter more highly focussed source is required.

In a synchrotron, electrons are accelerated to velocities close to the speed of light, in effect creating a relativistic electron beam. As this beam is passed through a magnetic field, the electrons deviate in direction, releasing electromagnetic radiation, also called synchrotron light. Slower electron beams will emit electromagnetic radiation of longer wavelengths; the use of a relativistic electron beam produces radiation, which has wavelengths that are short enough to be spectroscopically useful, and is also of extremely high intensity. This light is channelled down beamlines to experimental workstations where it is available for research applications (Figure 5.1). Synchrotron radiation ATR-FTIR characteristically gives a high signal-to-noise ratio at the highest spatial resolution compared to conventional ATR-FTIR. This results from the highly collimated, polarised and intense synchrotron radiation based light. These are the primary factors associated with synchrotron light, making it unique and — more importantly — permitting innovative applications unable to be achieved via conventional methods [155-157].

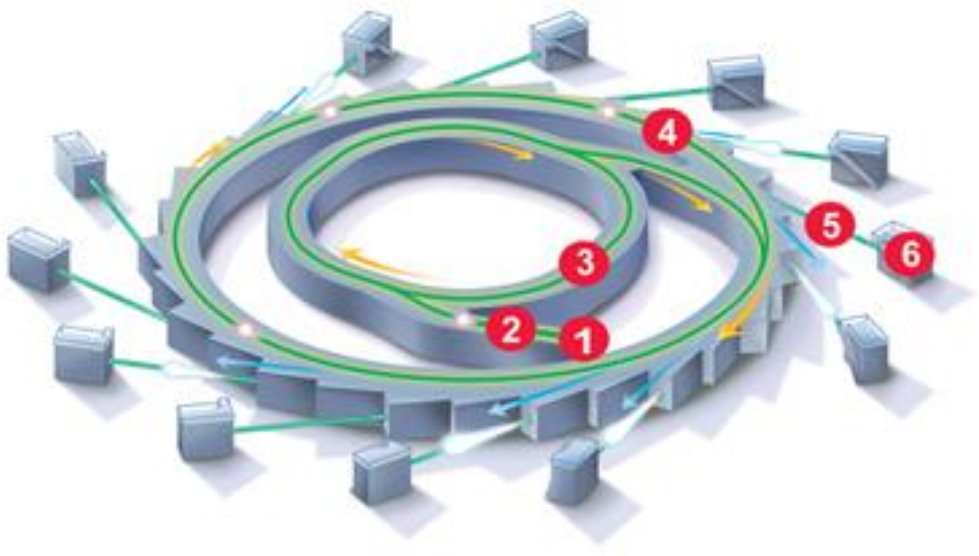


Figure 5.1: Schematic diagram of a Synchrotron: 1) electron gun; 2) linear accelerator (Linac); 3) booster ring; 4) storage ring; 5) beamline; and 6) end station [158].

The use of synchrotron radiation has been shown to be highly beneficial in a number of research fields. Kempson and co-workers provide a detailed discussion into the applications of synchrotron radiation with respect to forensic trace evidence [155]. Furthermore, a large emphasis was placed on the capabilities of synchrotron light for improving the limits of detection, which is highly applicable for trace evidence analysis. Like any aspect of analytical chemistry, this is an avenue that has the potential of significantly improving sensitivity. As such, this would result in the ability to analyse smaller sample sizes or zone in on areas of interest within a sample to an extent that is unable to be achieved with conventional approaches [155].

This chapter investigates the use of synchrotron radiation ATR-FTIR microscopy for the *in-situ* analysis of fingermarks and amino acids on a cellulose substrate that have been reacted with lawsone and other naphthoquinones. In order to rationalise the spectral results obtained, further spectroscopic studies were carried out on the products of reactions between amino acids and lawsone.

5.2 EXPERIMENTAL

5.2.1 IN-SITU SOLID STATE INVESTIGATION

5.2.1.1 CHEMICALS AND PROCEDURES

This investigation was conducted with use of chemicals and procedures described in Chapter 2.

5.2.1.2 Synchrotron ATR-FTIR Microscopy

Spectra of developed samples and blanks were obtained at the Australian Synchrotron using a Bruker Vertex70 FTIR spectrometer, a Hyperion microscope with a ×32 germanium ATR objective, and a computer system running Opus 6.5 software. Typically, 128 scans were accumulated at 4 cm^{-1} resolution using a 20 × 20 μm microscope aperture and a 5 μm mapping grid.

5.2.2 Solution Studies

5.2.2.1 Reaction of Lawsone and Glycine

Glycine (0.75 g, 10 mmol) was added to lawsone (3.5 g, 20 mmol) in ethanol (20 mL) and heated under reflux and monitored by TLC. When all the lawsone was consumed (48 hours), the reaction was allowed to cool to room temperature, and the resulting solid was filtered and washed with water. The solid was subjected to silica gel column chromatography. Elution with petroleum spirits: ethyl acetate (8:2) gave two distinctive fractions, red and yellow in colour, which were subsequently collected and attempted to be characterised by ATR-FTIR, gas chromatography-mass spectrometry (GCMS, ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Refer to Appendices 1-3 for spectra.

Yellow Product: This was identified as 2-amino-1,4-naphthoquinone based upon comparison with NMR, MS and FTIR reference spectra [159, 160].

Red Product: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (^{1}H, br. s), 8.16 (2H, dd, *J* = 7.2, 1.1 Hz), 8.13 (2H, dd, *J* = 7.2, 1.1 Hz), 7.89 (2H, ddd, *J* = 7.5, 7.5, 1.4 Hz), 7.71 (2H, ddd, *J* = 7.5, 7.5, 1.4 Hz), 4.65 (^{1}H, q, *J* = 6.7 Hz), 1.31 (3H, d, *J* = 6.7 Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.3, 179.1, 137.5, 134.9, 133.0, 132.8, 130.2, 126.6, 126.3, 120.6, 24.7, 21.9 ppm. Infrared spectrum (ATR) 3375 cm^{-1}, 2925 cm^{-1}, 1600 cm^{-1}.

5.2.2.2 Nuclear Magnetic Resonance (NMR)

NMR spectra were collected on a Varian 400 MHz and Ultrashield Bruker 400 MHz spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz respectively.

5.2.2.3 Gas Chromatography Mass Spectrometry (GCMS)

The red and yellow products were diluted to 500 ppm with dichloromethane. The samples were analysed by gas chromatography (Agilent 7890) interfaced with a mass selective detector (MSD) (Agilent 5975C inert XL EI/CI MSD with a triple-axis detector). A 1 µL aliquot was introduced into the splitless injector by means of an Agilent 7683B autosampler. The gas chromatograph was fitted with a 30 m × 0.25 mm ID × 0.25 µm (df) BP20 SGE column. The initial GC oven temperature was 40 °C, held for 1 min, then ramped at 10 °C/min to 260 °C for 25 mins. The injector was operated at 280 °C. Helium was used as the carrier gas at a constant pressure of 9 psi (1.2 mL/min). MSD conditions were: ionisation energy 70 eV, source temperature 230 °C, and electron multiplier voltage 2600 V.

5.2.2.4 Attenuated Total Reflectance-Fourier Transform Infrared Radiation (ATR-FTIR)

Infrared spectra of the potential products were collected with the use of a Perkin Elmer Spectrometer 100, fitted with a universal diamond ATR accessory, and using a computer running Spectrum 6.3.2 software. Typically, 4 scans were accumulated at 4 cm^{-1} resolution.

5.3 RESULTS AND DISCUSSION

5.3.1 Preliminary In-Situ Solid State Investigation

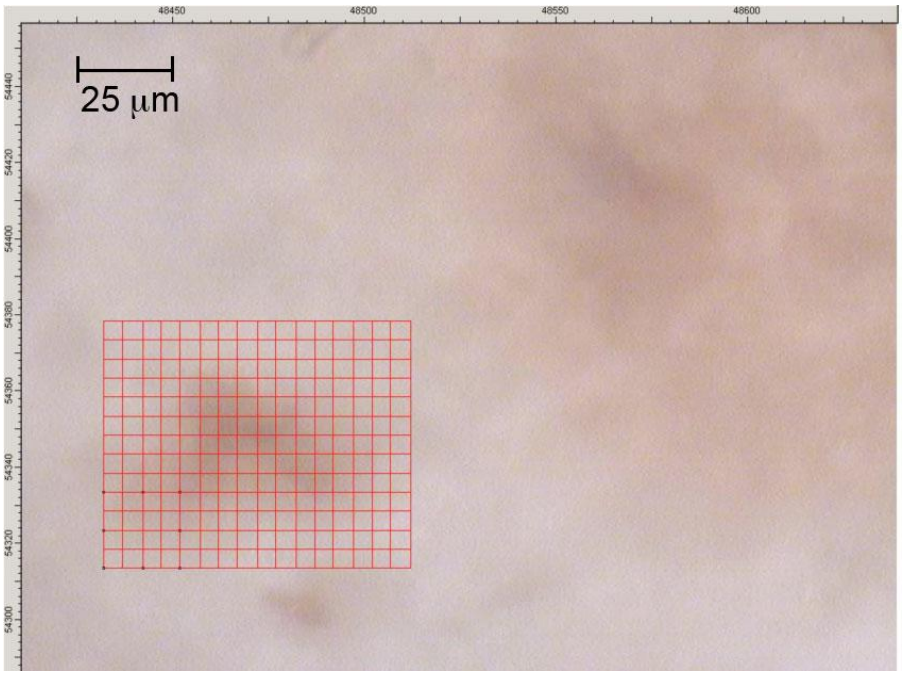
Attempts to obtain in-situ infrared spectra with conventional ATR-FTIR microscopy were unsuccessful. Spectra of lawsone treated fingermarks on filter paper were dominated by cellulose and subtraction to obtain the spectra of the developed fingermarks was unsatisfactory. The amino acids present within the latent fingermark, which are targeted by the development reagents, and their subsequent reaction products, have been found to be present at very low levels (~900 µg/mL in human perspiration) [23]. This, coupled with the roughness of the filter paper surface and the likelihood that the majority of the fingermark components will have diffused into the paper (rather than remaining on the surface), could account for the absence of the anticipated spectra at the 30-50 µm spatial resolution achievable with the conventional FTIR system employed.

5.3.2 SYNCHROTRON ATR-FTIR MICROSCOPY

The focus turned to the use of synchrotron ATR-FTIR microscopy, which has a higher spatial resolution (5 μm) and a greater intensity that significantly enhances signal-to-noise ratios, and hence substantially increases sensitivity.

Preliminary studies utilising synchrotron ATR-FTIR microscopy focused on reducing the effect of the substrate on the resulting spectra. It was found that cellulose TLC plates provided more consistent results than filter paper. Due to the spherical particle shape and greater uniformity of the cellulose used as a coating on TLC plates, it is a more compacted and more uniform substrate than fibrous filter paper, which has a rougher, more abrasive texture. Nevertheless, despite their greater homogeneity, at the microscopic level cellulose TLC plates are still relatively “rough” (Figure 5.2) as the particles are about 50 μm in diameter. It must be remembered that the area of the ATR objective tip is approximately 100 μm in diameter and that this will bridge a number of cellulose fibres or particles. Hence, the ability to locate areas of interest by generating grid maps is not realisable as "spectra" may be generated from voids, partial voids, sloping surfaces and occasionally a surface parallel to the ATR objective tip. Even in obvious areas of interest it can be difficult to obtain spectra of potential product/intermediate/reagents as they may be under or between fibres or in cavities. This leads to inconsistent spectra and makes grid maps difficult to assess. Therefore, a “hit or miss” single point mapping strategy, using a 5 μm grid with a 20 × 20 μm aperture, was adopted, with individual spectra being assessed. Using a 20 × 20 μm aperture and a germanium ATR element (refractive index 4) gives an approximately 5 × 5 μm measurement area, hence the use of a 5 μm grid to obtain full coverage of the area of interest (Figure 5.2a). A scattered series of single point measurements of selected areas of interest were also used (Figure 5.2b). As the aim of the measurements was to obtain spectra of adsorbed products and/or reactants, and not their disposition, both strategies were considered reasonable.

(a)



(b)

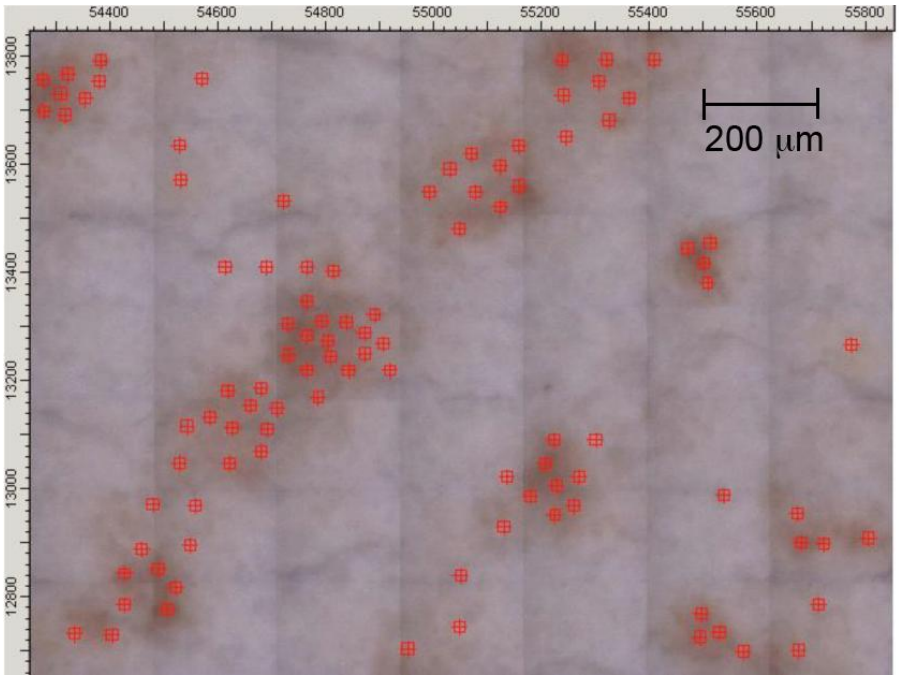


Figure 5.2: Microscope images of a TLC sample, a) TLC plate, depicting the use of a 5 µm grid for data acquisition and dark areas of interest, b) TLC plate, showing the use of a scattered 5 µm measurement points and dark areas of interest.

Although many of the spectra obtained were totally dominated by cellulose, some were clearly a combination of cellulose and other materials. Of the latter, some showed little or no contribution from cellulose (Figure 5.3), presumably due to the product or reactant deposit lying near parallel to the ATR objective surface and/or being of sufficient thickness to eliminate or significantly reduce the cellulose contribution.

Spectra other than of cellulose were found only on the lawsone treated fingermark and the lysine TLC plate samples. No spectra other than that of pure cellulose were obtained from either the lawsone reagent or lysine on TLC plates, which suggests that these spectra are of reaction products. It is unlikely that the spectra are of lawsone or an amino acid, as spectra of lawsone or lysine were not observed on the lawsone, lysine and lawsone treated fingermark and lysine samples. Conventional ATR-FTIR spectra of lawsone and lysine confirmed this (Figure 5.4). Refer to Appendix 1.1-1.4 for synchrotron ATR-FTIR spectra associated with the other naphthoquinones.

Comparison of the lawsone treated fingermark and lawsone treated lysine shows the spectra to be near identical, suggesting the formation of the same or similar products. There is clearly some contribution from cellulose ($\sim$1000 cm^{-1}) to the lawsone treated lysine spectrum, but little or no contribution to the lawsone treated fingermark spectrum.

The presence of an aliphatic alkyl group ($\sim$2900 cm^{-1}) is significant as our initially proposed product does not account for the inclusion of such a group, irrespective of which amino acid is used. It suggests that the aliphatic chain of the amino acid remains as part of the product, or the aliphatic alkyl group is associated with a by-product. The appearance of a strong C=O stretch at $\sim$1650 cm^{-1} suggests that a quinone fragment has been retained.

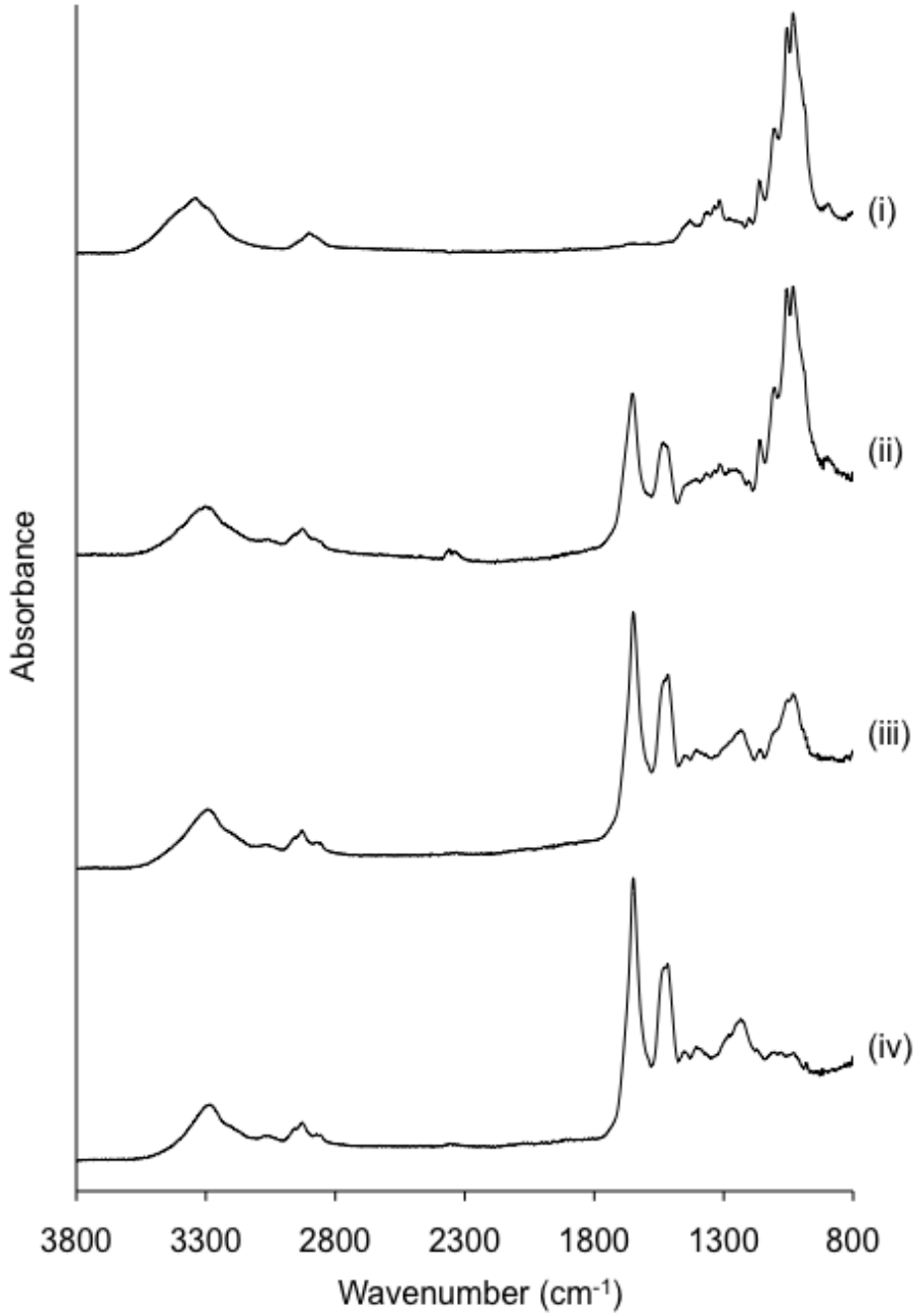


Figure 5.3: Synchrotron ATR-FTIR spectra of lawsone treated fingermarks on TLC plates showing the influence of cellulose: (i) TLC cellulose blank; (ii) Lawsone treated fingermark on TLC with significant cellulose interference; (iii) Lawsone treated fingermark on TLC with limited cellulose interference and; (iv) Lawsone treated fingermark on TLC with minimal cellulose interference. Spectra have been normalised and offset to illustrate similarities and differences.

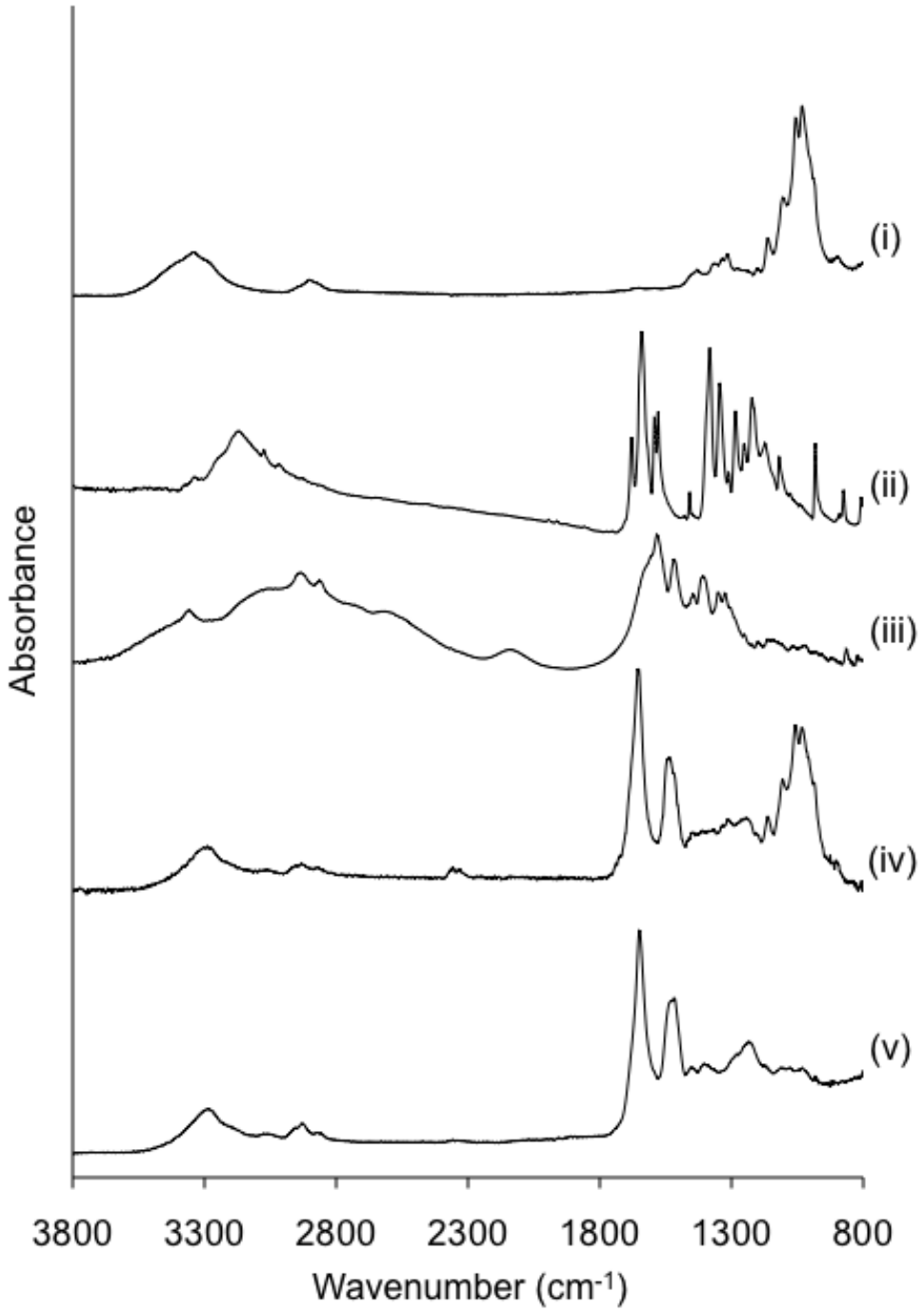


Figure 5.4: Synchrotron ATR-FTIR spectra of: (i) TLC cellulose; (iv) lawsone treated lysine spot on TLC; (v) lawsone treated fingermark on TLC; and conventional ATR-FTIR spectra of bulk samples of: (ii) lawsone; and (iii) lysine. Spectra have been normalised and offset to illustrate similarities and differences.

Spectra of the various naphthoquinones were found to be quite similar, with the exceptions of 1,4-dihydroxy-2-naphtoic acid and 2-methyl-1,4-naphthoquinone, for which we were unable to obtain spectra that differed considerably from cellulose. Nevertheless, the spectra obtained for 2-methoxy-1,4-naphthoquinone, 1,2-naphthoquinone-4-sulfonate and lawsone showed similarities in the region of ~2850-2950 cm^{-1} and also two sharp bands at ~1650 cm^{-1} and ~1510 cm^{-1} (Figure 5.5). However, there appears to be slight differences in the region between ~1450 cm^{-1} to ~1150 cm^{-1}. This indicates that there could be alternative products formed with the reaction of amino acids with selected naphthoquinones. This observation complements the fluorescence studies outlined in the previous chapter, depicting marked differences in photoluminescence characteristics between lawsone and the other naphthoquinones, also suggesting alternative products.

Whilst the use of synchrotron ATR-FTIR has provided us with valuable information on the products associated with the reaction of amino acids/fingermarks and naphthoquinones, it is not conclusive in terms of suggesting the structure of the product (or products) with any confidence. Attempts to extract the product from its cellulose substrate were unsuccessful; hence, attempts were made to synthesise the product, or at least a similar product, in solution. This avenue of investigation may not specifically identify the surface reaction product; however, it may give an insight into its possible structure.

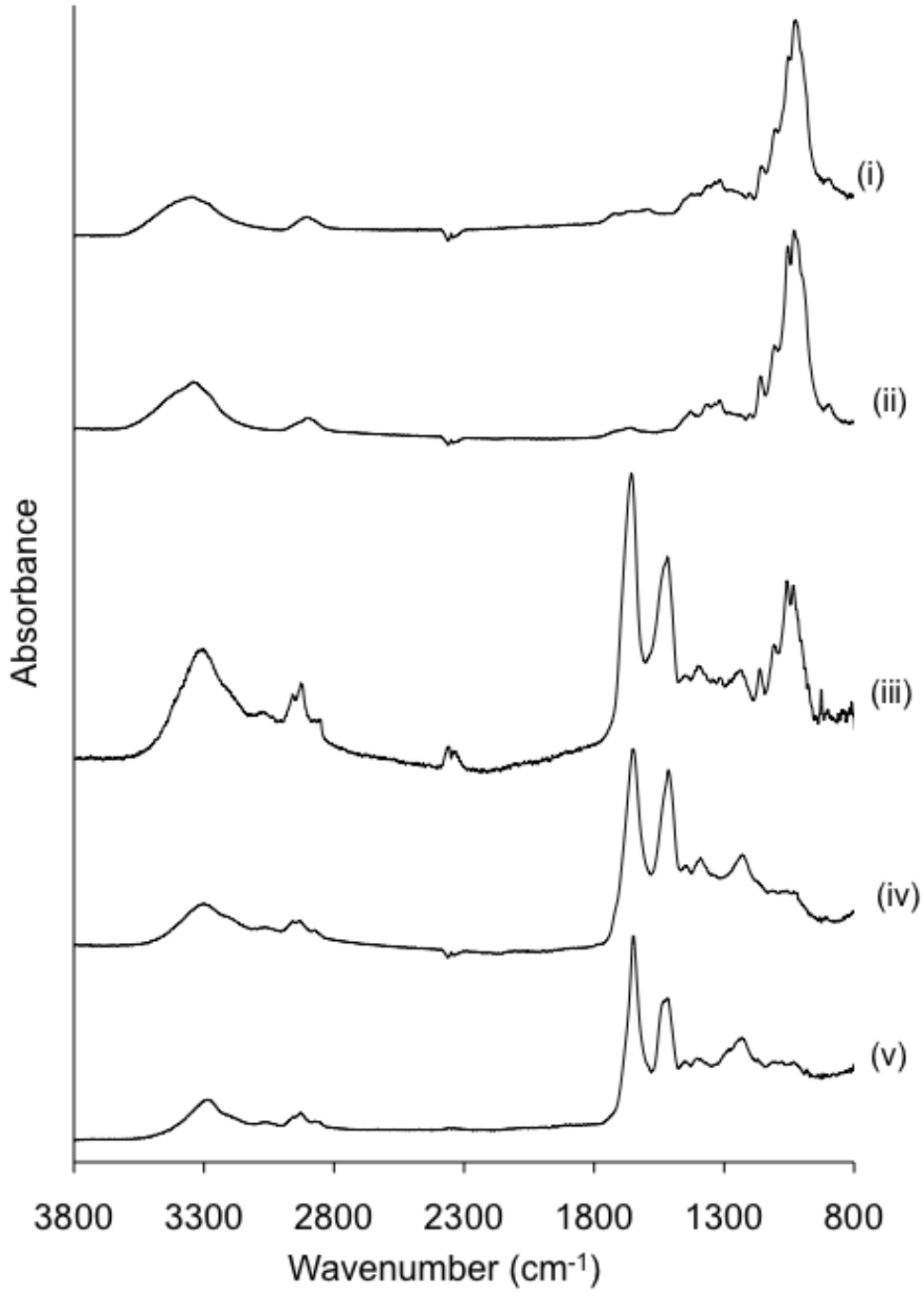


Figure 5.5: Synchrotron ATR-FTIR spectra of naphthoquinone treated fingermarks on TLC plates: (i) 1,4-Dihydroxy-2-naphthoic acid; (ii) 2-Methyl-1,4-naphthoquinone; (iii) 2-Methoxy-1,4-naphthoquinone; (iv) 1,2-Naphthoquinone-4-sulfonate; and (v) 2-Hydroxy-1,4-naphthoquinone (lawsone). Spectra have been normalised and offset to illustrate similarities and differences.

5.3.3 SYNTHETIC STUDIES

Lysine was selected as the amino acid of interest for investigation on the synchrotron ATR-FTIR and this was based on it producing the strongest depth of colour and luminescence. However, as it contains two amino groups, there is the potential for either one or both to react in some way. Glycine is a much less complex amino acid and it is one of the most common found in latent fingermarks [5]. Hence, it was chosen as a model compound for the solution reactions.

Reacting lawsone with glycine in ethanol gave a purple crude product, similarly observed with lawsone treated latent fingermarks. Purification of this material by silica gel chromatography gave two distinct fractions, one red and one yellow. These were subjected to GCMS, NMR and FTIR analyses. Due to the limited quantities associated with these fractions, it was difficult to obtain well defined spectra in some instances.

The infrared spectra of the red and yellow fractions are more resolved than those obtained for the adsorbed species. Nevertheless, the spectra possessed similarities in the areas of interest but with shifts in the vibrational frequencies, which may be associated with matrix effects. See Appendix 1.5-1.8 for ATR-FTIR spectra of lawsone, glycine, yellow and red products.

A library search from the GCMS results obtained from the yellow fraction (peak at R_t 35 mins) showed a mass spectrum consistent with 2-amino-1,4-naphthoquinone, which was further supported by ^1H NMR. No suitable MS data relating to the red fraction could be obtained. Refer to Appendix 3 for MS data of yellow product.

At this stage, the red product has yet to be determined, however, useful insights were obtained from the ^{1}H NMR spectrum. The aromatic signals between δ 8.32 ppm and δ 7.71 ppm appeared to double the integration when compared to the aliphatic CH signal, suggesting a symmetrical molecule containing 2 lawsone fragments. In the aliphatic region of the molecule, there appears to be a CH attached to an electronegative atom (δ 4.65 ppm) coupled to a methyl group (δ 1.31 ppm) and the vinylic hydrogen of lawsone is absent. The ^{13}C NMR spectrum shows 12 signals, further suggesting a symmetrical structure from 2 lawsone fragments. The structural information provided by NMR spectroscopy for the red product is summarised in Figure 5.6. While the evidence for a dimer is consistent with ninhydrin, at present it is not possible to explain the existence of the methyl group (δ 1.31 ppm) as there is no obvious precursor functionality in the reactants to account for this observation.

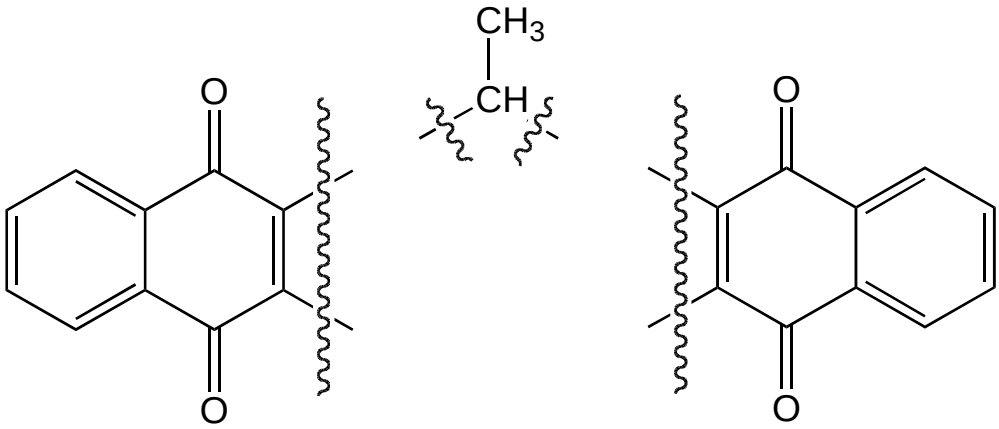


Figure 5.6: Summary of structural information obtained from NMR data of the red product.

5.4 CONCLUSION

Synchrotron ATR-FTIR microscopy has demonstrated significant potential in respect to performing an *in-situ* study into the reaction of lawsone and other naphthoquinones with amino acids and latent fingermarks microscopically rough cellulose surfaces. These preliminary results demonstrate an ability to detect trace components linked to the reaction product of naphthoquinones with amino acids using synchrotron light, which could not be achieved via conventional methods. This form of investigation may provide a platform to gain a better understanding of any surface-based reactions, specifically the *in-situ* analysis of cellulose-based substrates. The results can be further rationalised with the use of synthetic based investigations.

CHAPTER 6: ALTERNATIVE STRATEGIES TOWARDS NEW FINGERMARK REAGENTS

6.1 INTRODUCTION

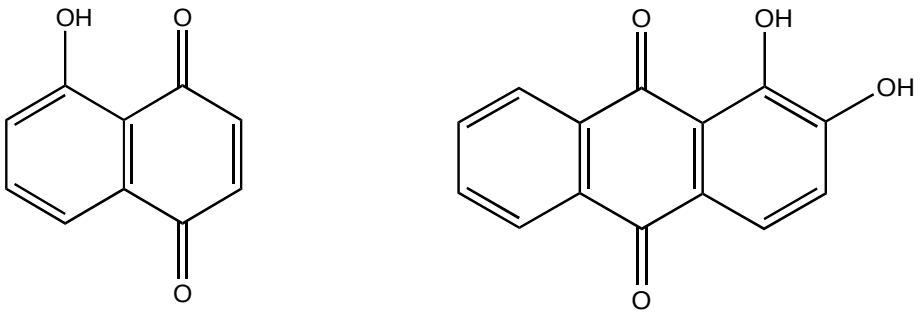
Over the last three decades the most common approach to developing new amino acids sensitive latent fingermark detection techniques has focused on ninhydrin and related compounds [3, 4, 31]. In this thesis it has been shown that an alternative approach based upon traditional natural product colour-forming compounds first suggested by Almog and co-workers [11, 72] is a viable route to new fingermark detection reagents. This chapter reports some initial results extending this approach to include additional natural product derived compounds. A systematic retrosynthetic approach was taken to explore the potential of a natural dye precursor as a fingermark reagent. Finally, a serendipitous discovery of the skin dyeing properties of a non-related compound provided an additional route to a possible non-ninhydrin related class of fingermark detection compounds. These preliminary studies indicate potential strategies for further research towards novel latent fingermark reagents for porous surfaces.

6.1.1 COLOUR-FORMING COMPOUNDS

Natural dyes were the primary source of dyes until Perkin's discovery of Mauveine in 1856 [161]. This instigated a decline in the use of natural dyes, which were replaced by synthetic compounds. Natural dyes required the application of mordants to ensure satisfactory fastness of the dye. Along with this, synthetic dyes had a number of advantages in performance over natural dyes, including an ease of production [161]. However, of late, the interest in the commercial use of natural dyes has grown, due to the strict environmental standards, which have been enforced in response to concerns relating to toxicity and allergic reactions associated with synthetic dyes [162, 163]. Furthermore, natural dyes exhibit enhanced biodegradability and, therefore, an increased compatibility with the environment [162]. Thus, there is a wide range of

naturally derived colour forming compounds that may be potentially useful for fingermark detection. In effect, using the same terminology as medicinal chemistry, there is the possibility of a significant number of "lead" compounds suitable for investigation

In this study the two key compounds of interest are alizarin and juglone (Figure 6.1). Juglone (5-hydroxy-1,4-naphthoquinone) is a naphthoquinone derivative; however, unlike its isomer, lawsone, which is sourced from henna, juglone is the characteristic compound in the *Juglandaceae* family, in particular in the leaves, husk, wood and roots of walnut varieties [164, 165]. Juglone, like the majority of naphthoquinones, is an allelochemical responsible for the allopathic characteristics exhibited in walnut trees [164, 166]. Allelopathy is a biological process that produces certain biochemicals, which influence the growth and survival of surrounding plant species and can consist of either beneficial or negative effects. Juglone causes growth-inhibiting effects on neighbouring plants and, as a result of this phenomenon, has applications as a herbicide [164, 166]. Furthermore, juglone is known to react with keratin proteins present in the skin to form sclerojuglonic compounds that are red/brown in colour [167].



Juglone Alizarin

Figure 6.1: Structures of selected natural product based dyes.

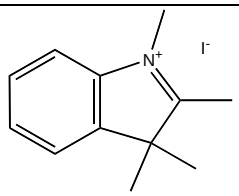
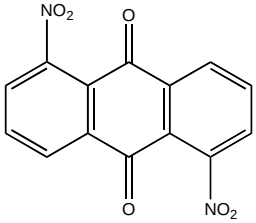
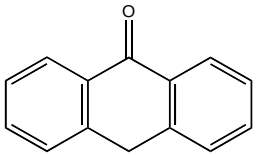
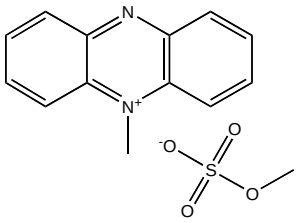
Alizarin is an anthraquinone, which are compounds well known for their dyeing qualities [139]. Anthraquinones are very versatile, having the ability to produce shades from pink to blue-violet [139, 168]. This results from derivatives associated with anthraquinones containing electron donating substituents that can be introduced into one or more of the 8 available positions [139]. Alizarin (1,2-dihydroxyanthraquinone) can be extracted from various varieties of madder plants [161, 163] and is used as a red dye. Madder has been cultivated as a source of dye throughout history, with reports of its use dating back to 1500 BC [163]. Interestingly, madder dyed cloth was discovered in the tomb of the Pharaoh Tutankhamen [163].

Alizarin was identified as the source of the red colorant in madder in 1826 and as a result of this, alizarin was synthetically reproduced in 1868 and at half the cost of the natural product, which consequently eliminated the need for the cultivation of madder [163]. Along with this, alizarin has the ability to form deeply coloured metal complexes with fluorescent properties. This has found applications in analytical chemistry by acting as a chelating agent for the spectroscopic determination of metals [169]. This in turn relates to the ability of Ruhemann's purple to form a metal complex on reaction with metal salts [3]. In addition, alizarin possesses structural similarities to lawsone, providing evidence to suggest its potential as a fingermark detection reagent.

As well as these primary lead compounds, a variety of additional miscellaneous compounds (Table 6.1) were also selected for investigation for their potential as fingermark detection compounds. They were selected either as a result of being a natural dye analogue, being themselves a dye, or based on a prediction of the potential reactivity of the species. Nevertheless, it was important to ensure the inclusion of particular moieties and ring structures that would have the potential of reacting with amino acids to produce a coloured and/or

luminescent product. This was in the hope of discovering new fingermark reagents that possess different characteristics than for current techniques.

Table 6.1: Miscellaneous compounds selected as potential fingermark reagents, showing structure and the basis for their selection.

Name	Structure	Basis for selection
1,2,3,3-tetramethyl-3H-indolium iodide		Highly reactive indolium ion, structural similarities to known fingermark reagents and considered a known dye [170].
1,5-dinitroanthraquinone		Related to natural dyes, with the inclusion of nitro components to potentially increase reactivity.
Anthrone		Has potential movement of electrons through the system and a skeletal structure for some dyes.
Phenazine methosulfate		Highly reactive, specifically the potential movement of electrons through the system and has dyeing properties [170].

6.1.2 RETROSYNTHETIC APPROACH

Discovering novel reagents for fingermark detection may not necessarily be the most challenging element for researchers, as the associated *in-situ* characterisation of the reaction product, and thus determination of the mode of action can be extremely difficult. As outlined in Chapter 5, even with numerous avenues of investigation and various instrumental methods available, a definitive outcome is not assured. An approach addressing this issue is to produce a specific dye on reaction with the amino acid residues in the latent fingermark. This approach can be related to the common method of retrosynthesis, which is widely utilised in medicinal and synthetic chemistry, however has yet to be applied in the area of fingermark research.

The concept being, if a suitable precursor is applied as the fingermark reagent, reaction with the amino acids would create a known dye substance with the required desirable properties of colour and photoluminescence. There are a huge variety of dyes that are commercially available, thus there is a very large pool of potential lead compounds that can be investigated in this manner. Although this particular direction of investigation would still require the use of characterisation methods, it may provide confidence in the results obtained by anticipating what the potential product should be. This is a better outcome than having a product that has been typically categorised as "an unknown" and furthermore, would provide an avenue for a direct comparison against the known dye.

In a similar manner to madder and henna, the blue dye indigo has been sourced from a number of plant sources of the *Indigofera* varieties and is the chemical dye extracted from woad. Woad has been used in antiquity for painting the bodies of warriors as they believed it to give magical powers as its antiseptic abilities prevented infection of any wounds received during battle [171]. The

precursor of indigo is indoxyl which, when exposed to air oxidises to yield indigo. Initially, indoxyl was selected for investigation; however, this compound was unavailable at the time the study was undertaken. The more readily available isatin (1H-indol-2,3-dione) can form in a potential side reaction with the oxidation of indoxyl and it is a known oxidation product of indigo [171, 172]. Maugard and co-workers showed the identification of a number of indigo precursors from the leaves of woad [171]. These were all very similar in structure and, as the conformation of the structures varied, so in turn did the pigments produced (Figure 6.2).

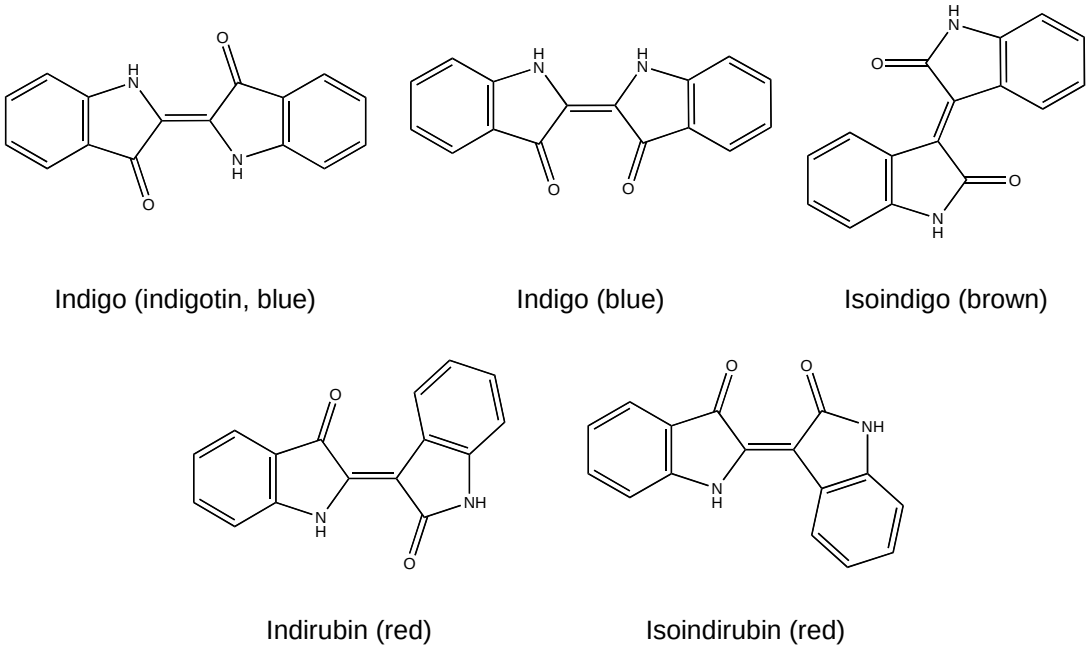


Figure 6.2: Identification of indigo precursors found in woad leaves [171].

Isatin has structural similarities to known fingermark reagents, such as ninhydrin and 1,2-indanedione, therefore suggesting potential application in relation to fingermark detection. In a parallel investigation carried out contemporaneously with the research outlined in this chapter, Chan and co-workers reported on the ability of isatin to produce latent fingermarks on porous surfaces [173]. Analogues of isatin also have the potential to act as

fingermark reagents, for example the presence of the nitro moiety in 5-nitroisatin could increase the reactivity of isatin on reaction with amino acids.

6.1.3 SERENDIPITY

As previously mentioned in Chapter 1, the accidental discovery of the staining properties of ninhydrin by Ruhemann [30, 31] and subsequent application as a fingermark reagent came about serendipitously as described by Oden and colleagues [8]. In a similar fashion, Djerassi and co-workers observations on genipin [72, 84] led to the successful application of this compound to fingermark detection by Almog and co-workers [11, 72].

The synthesis of 6-N,N-dimethylaminofulvene (Figure 6.3) is currently part of a recently introduced undergraduate laboratory at Curtin University [174]. It was noted by the developer of the laboratory exercise that care was required in handling the compound, as it had a tendency to cause a persistent pink stain on contact with skin [174]. These skin staining properties would suggest, in a similar fashion to genipin [11, 72] and lawsone (see Chapter 3), that this completely synthetic compound has the potential to act as a fingermark detection reagent. It also has the added advantage of being soluble in non-polar solvents, which is a desirable attribute for latent fingermark development to preserve any written document-based evidence.

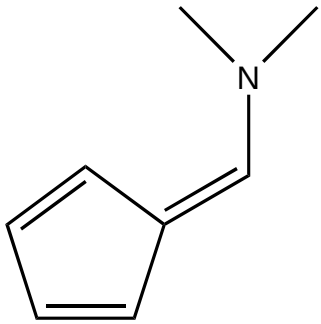


Figure 6.3: Structure of 6-N,N-dimethylaminofulvene.

6.2 EXPERIMENTAL

6.2.1 REAGENTS AND PROCEDURES

The following natural dyes and miscellaneous compounds were obtained from Sigma Aldrich (Australia) and were used as supplied, without further purification: 1,2-dihydroxyanthraquinone (alizarin); 5-hydroxy-1,4-naphthoquinone (juglone); 1H-indol-2,3-dione (isatin); 5-nitroindole-2,3-dione (5-nitroisatin); 1,2,3,3-tetramethyl-3H-indolium iodide; 1,5-dinitroanthraquinone; anthrone; and phenazine methosulfate. 6-N,N-Dimethylaminofulvene was donated by A. Payne, Curtin University.

Ethyl acetate (Univar Analytical, Australia), absolute ethanol (CSR Chemicals, Australia), petroleum spirit 60-80 °C (Univar, Australia) and methyl nonafluoroisobutyl ether (HFE-7100) (NovecTM, Australia) were analytical grade, unless otherwise stated and were used as supplied, without further purification. All working solutions were prepared fresh on a daily basis.

The alizarin and juglone working solutions were prepared by dissolving 0.05 g of the reagent in 5 ml of ethyl acetate, and this was further diluted with 40 mL

of HFE-7100. Alizarin and juglone treated fingermarks were subsequently heated in either an oven (All-lab Scientific, Australia) for 1 hour at 150 °C or with the use of a domestic iron (Mistral, Australia) at the highest heat setting for ~30 s.

A 6-N,N-dimethylaminofulvene working solution was prepared with a concentration of 0.1 mg/mL (0.1% w/v). The two non-polar solvents tested were petroleum spirit 60-80 °C and HFE-7100. The treated samples were subsequently heated in either an oven at 80 °C for 30 mins or via direct heat from a laundry press at ~160 °C for 10 s.

1H-Indol-2,3-dione (isatin), 5-nitroindole-2,3-dione (5-nitroisatin), 1,2,3,3-tetramethyl-3H-indolium iodide, 1,5-dinitroanthraquinone, anthrone, and phenazine methosulfate solutions were prepared by dissolving 0.02 g of reagent in 20 mL absolute ethanol. Treated fingermarks were subsequently heated for possible colour and luminescence development in an oven at 160 °C with monitoring every 15 mins until 1.5 hours was reached.

6.2.2 Sample Preparation and Treatment

Sample preparation and treatment of latent fingermarks were carried out as described in Chapter 2.

6.2.3 PHOTOGRAPHIC CONDITIONS

Selected samples were photographed in both the absorbance (white-light) and photoluminescence modes, using a Nikon D300 digital camera mounted on a Firenze Mini Repro camera stand. The camera settings for each photograph are outlined in the figure captions throughout this chapter. Illumination in the absorbance mode was achieved using incandescent light bulbs with no camera barrier filter. Illumination in the luminescence mode was achieved using a Rofin Polilight® PL500 (Rofin Australia), with an excitation wavelength of 490-505 nm and a KV 550 barrier filter. Images were captured to a desktop computer using Nikon Camera Control Pro Version 2.0.0 and adjusted for brightness and contrast using Adobe Photoshop CS4 Version 9.0.

6.3 RESULTS AND DISCUSSION

6.3.1 COLOUR-FORMING COMPOUNDS; ALIZARIN AND JUGLONE

6.3.1.1 ALIZARIN AND JUGLONE

Due to the similarity in structures of both alizarin and juglone to lawsone, investigations into these two reagents were conducted using formulations and procedures previously investigated in Chapter 3. Juglone appeared to behave in a similar way to lawsone, with complete solubility in the working solution. However, alizarin showed poor solubility in both ethyl acetate and HFE-7100. A viscous opaque solution was observed with alizarin in ethyl acetate. Furthermore, the addition of HFE-7100 to the alizarin stock solution resulted in the rapid formation of a precipitate, suggesting that the amount of dissolved alizarin remaining in solution would be quite limited. Despite these issues, an attempt was made to develop latent fingermarks on filter paper. Samples were dipped in the alizarin working solution and allowed to dry before subsequent

heat treatment. No visible prints were apparent under white light. However, clearly visible luminescent fingermarks were observed when illuminated with the Polilight at λ_{ex} 505 nm and viewed with orange goggles (equivalent to a 550 nm long-pass filter), as shown in Figure 6.4. The samples heated with the iron, appeared to have greater observed luminescence and ridge detail than the samples heated via the oven.

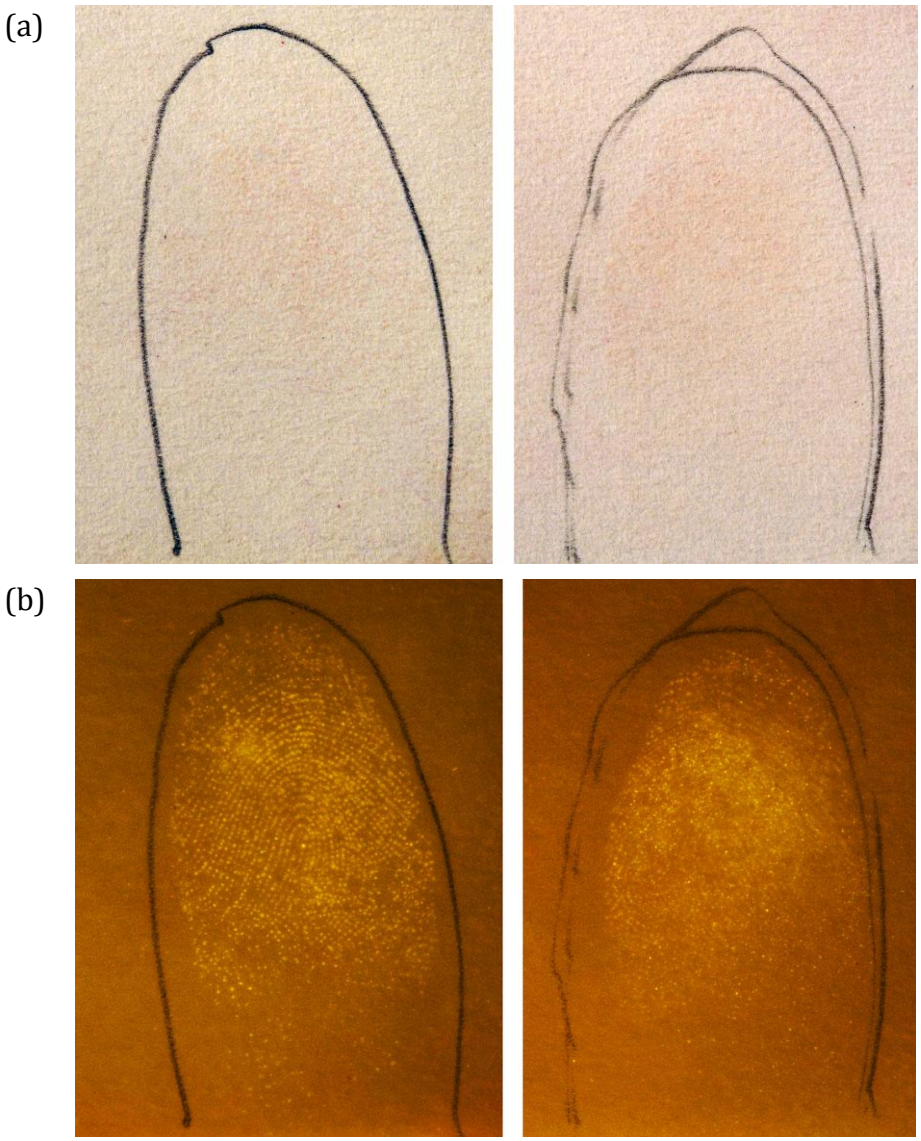


Figure 6.4: Alizarin treated latent fingermarks. Images were taken with a Nikon D300 SLR (60 mm focal length, ISO 200) under: a) white light (shutter speed 1/15 s, aperture f11); and b) photoluminescence mode with excitation using a Polilight PL 500 at 505 nm and viewed through a KV 550 filter (shutter speed 1 s, aperture f11). Left impression was heated via an iron, in comparison to the right impression, which was heated with the use of an oven.

An attempt was made to measure the luminescence spectrum for the developed fingermarks (Figure 6.5), which has a broad emission band centred on 565 nm.

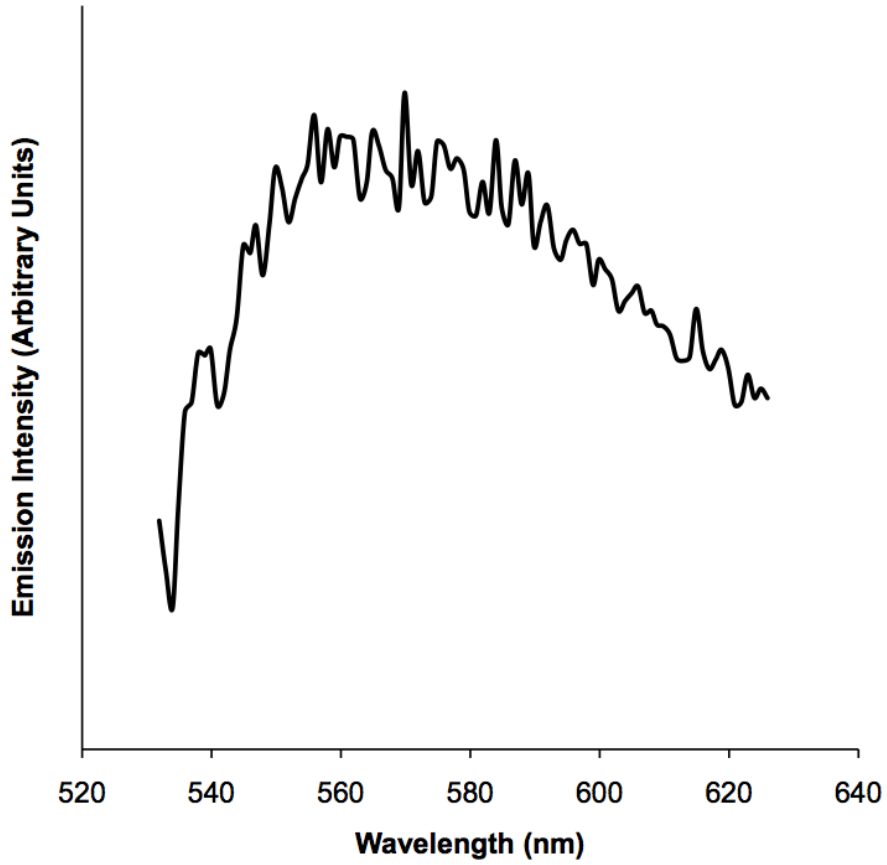


Figure 6.5: Emission spectrum of an alizarin treated fingermark (λ_{ex} 505 nm). Spectrum has been normalised.

This observed luminescence correlates closely with existing fingermark reagents such as 1,2-indanedione, and therefore this reagent may not provide any additional benefits for fingermark detection. Nevertheless, as previously mentioned, anthraquinones have the ability to produce a variety of colours and shades [139, 168], and therefore further formulation investigations may result in the observation of a coloured reaction product in addition to the observed luminescence. Alizarin may also be a stepping-stone to discovering an analogue capable of providing characteristics complementary to existing reagents.

Like the other naphthoquinones evaluated, juglone treated fingermarks showed indications of the formation of a brown reaction product; however, juglone did not appear to be as sensitive as the other naphthoquinones. As seen in Figure 6.6, ridge detail only appears around the edges of the fingermark and the luminescence observed is very weak. Nevertheless, this warrants further research with respect to the notion of naphthoquinones as potential fingermark detection reagents.

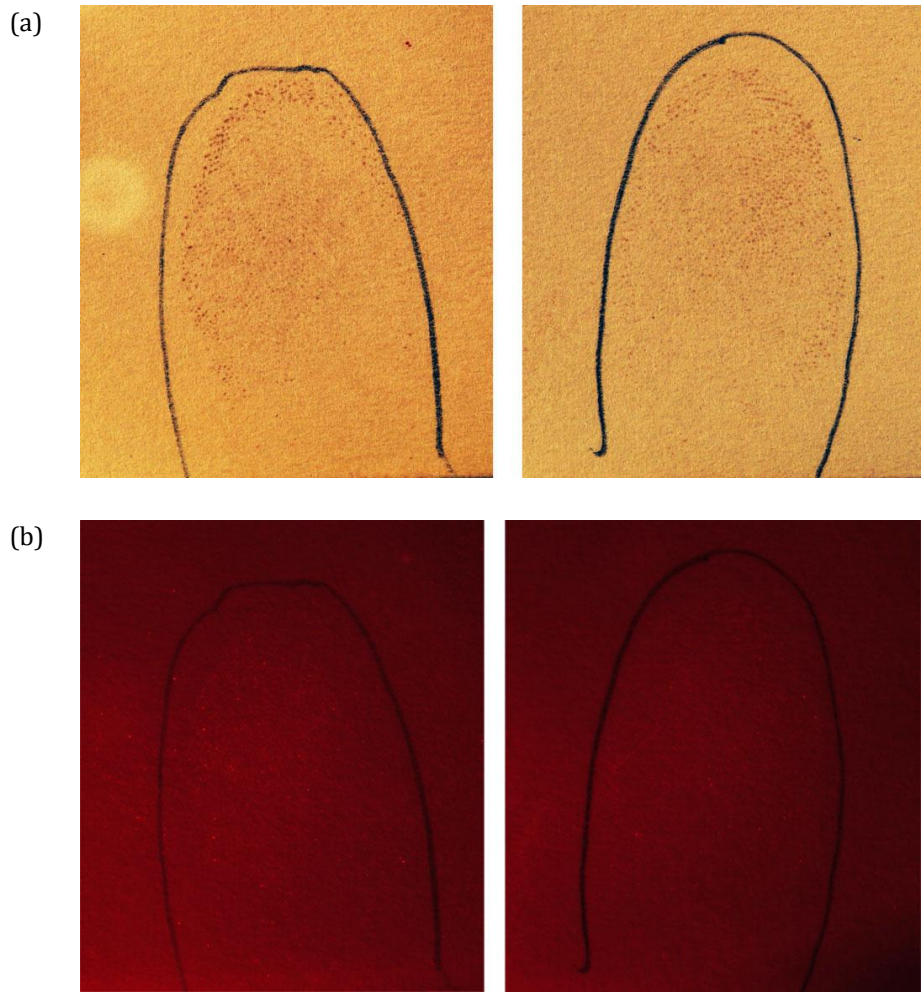


Figure 6.6: Juglone treated latent fingermarks. Images were taken with a Nikon D300 SLR (60 mm focal length, ISO 200): a) absorbance mode (shutter speed 1/30 s and aperture f11); b) photoluminescence mode with excitation using a Polilight PL 500 at 505nm and viewed through a KV 550 filter, (shutter speed 10 s, aperture f6.3). Left impression was heated with the use of an iron, in comparison to the right impression, which was heated via an oven.

6.3.1.2 MISCELLANEOUS COMPOUNDS

The miscellaneous compounds listed in Table 6.1 were screened for their potential to develop latent fingermarks on paper surfaces. Some were selected in relation to being a natural dye analogue, such as 1,5-dinitroanthraquinone, which contains nitro moieties that may improve reactivity if a reaction occurs with amino acids. Other selections were based on compounds not only possessing dyeing qualities, but structures that were believed to be capable of reacting with amino acids.

To screen these reagents on their ability to react with latent fingermarks, a simple solvent system was used in conjugation with heat applied via an oven at 160 °C and results monitored every 15 mins until 1.5 hours was reached. Dissolution of the reagents in ethanolic solution was quite poor and this was a common characteristic of all these compounds to some degree.

Results were found to show no ridge development for any of the reagents, with the exception of phenazine methosulfate, which gave partial development, however insufficient to record or gain defined ridge detail. 1,2,3,3-Tetramethyl-3H-indolium iodide did not produce any visible fingermarks, however treated filter paper produced a highly pink colouration with intense luminescence characteristics visible at λ_{ex} 505 nm and viewed with orange goggles (equivalent to a 550 nm long-pass filter). This background coloration and luminescence was not observed with the use of cellulose TLC plates. The reason for this observed variation between the different substrates has yet to be determined.

Further research in this area should investigate appropriate working solutions to allow for a more thorough assessment of these compounds. Furthermore, amino acid based studies could be undertaken to determine if any coloured or luminescent products are formed.

6.3.2 Retrosynthetic Approach; Isatin and 5-nitroisatin

Latent fingermarks on filter paper were treated with solutions of isatin and 5-nitroisatin followed by a heat treatment. No development of any description was observed despite length and type of heat treatment (oven or direct). Chan and co-workers, who were carrying out parallel studies on isatin reported that fingermarks developed using an acetone/dioxane working solution with an adjusted pH only showed photoluminescence but no apparent colour [173]. Taken with the results of this investigation it would appear that indigo, or analogue thereof, is not being formed in any appreciable quantity. Further research is required to investigate a more suitable precursor molecule with a greater potential for the formation of a known dye, however this is outside the scope of the current study.

6.3.3 Serendipity; 6-N,N-Dimethylaminofulvene

An initial trial with a sample of 6-N,N-dimethylaminofulvene was successful in developing a latent fingermark on filter paper, as a pink/brown impression, which exhibited luminescence when illuminated at 505 nm and viewed through orange goggles (equivalent to a 550 nm long-pass filter) (Figure 6.7). Development required heating in an oven for 5 mins at 90 °C.

(a) (b)

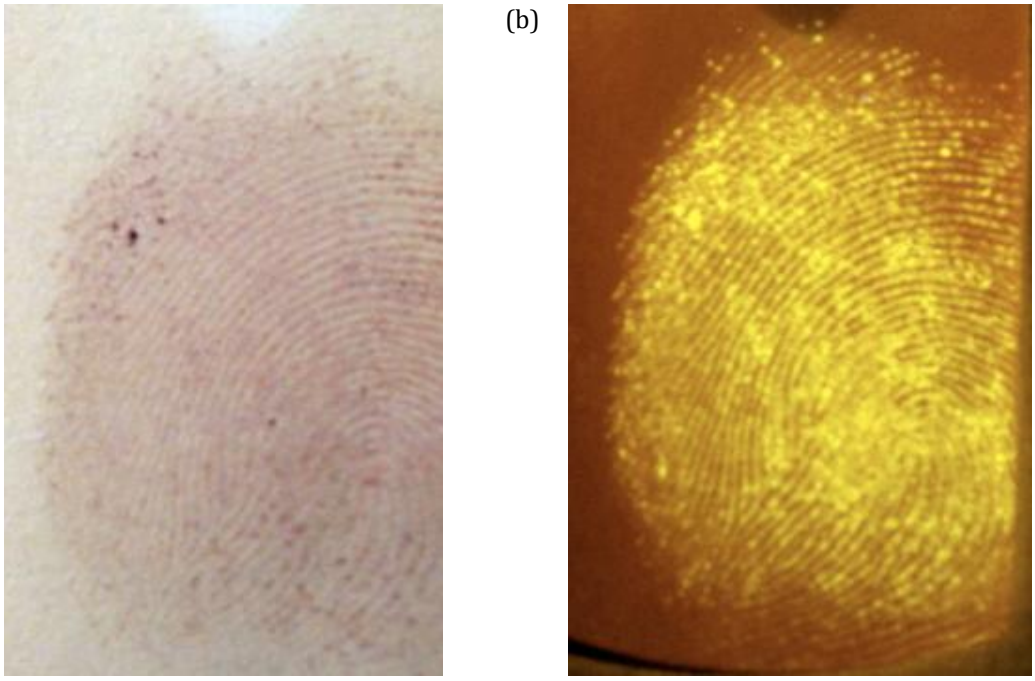
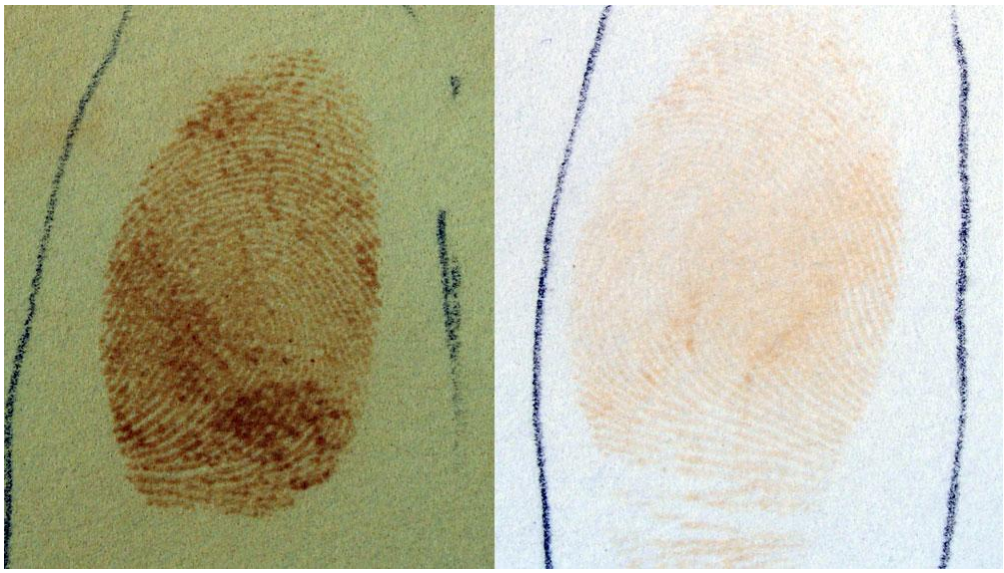


Figure 6.7: First recorded 6-N,N-dimethylaminofulvene treated latent fingermark. Images were taken with a Nikon D100 SLR (85 mm focal length, ISO 200): a) absorbance mode (shutter speed 1/30 s and aperture f9); b) photoluminescence mode with excitation using a Polilight PL 500 at 505 nm and viewed through a KV 550 filter, (shutter speed 1 s, aperture f9).

On the basis of this initial successful result, further investigations into this compound were initiated. 6-N,N-Dimethylaminofulvene treated fingermarks were heated in both a laundry press and an oven. Results showed a significant variation in performance with the slight formation of ridge characteristics and luminescence on selected samples. Furthermore, there was a slight darkening of the background itself, which in turn affected the contrast. After storage in the dark for approximately 6 months, all samples appeared to have visible fingermarks; however, these had no obvious luminescence characteristics, apart from one sample, indicating that the luminescence appears to diminish over time. Interestingly, one sample looked different from the rest, with no background discolouration and distinct photoluminescence as demonstrated in Figure 6.8.

(a)



(b)

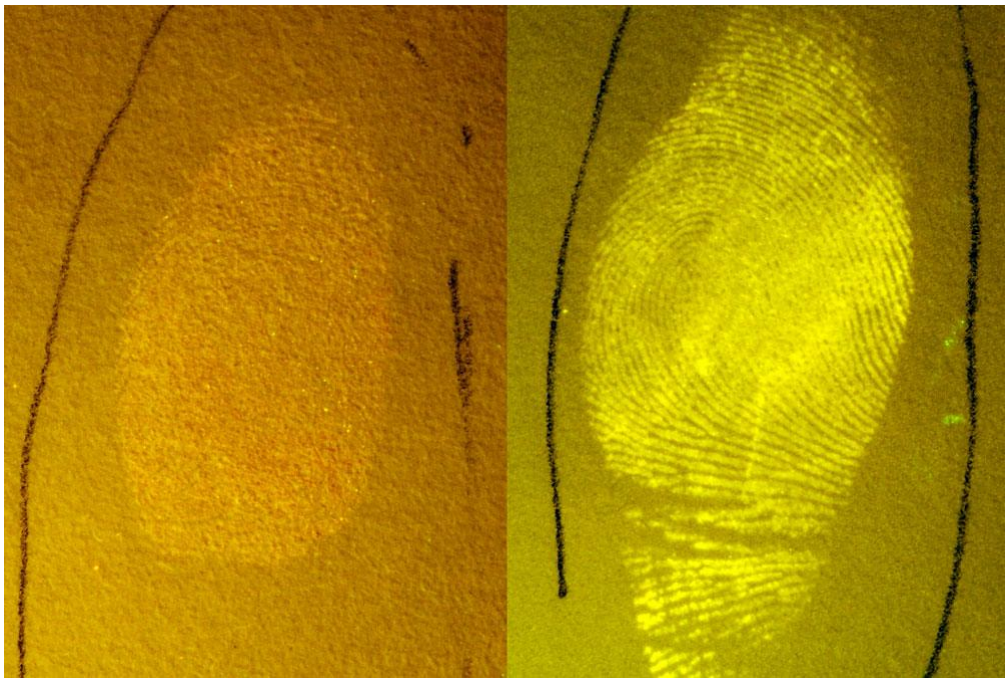


Figure 6.8: 6-N,N-dimethylaminofulvene treated latent fingermark. Images were taken with a Nikon D300 SLR (60 mm focal length, ISO 200): a) absorbance mode (shutter speed 1/15 s and aperture f11); b) photoluminescence mode with excitation using a Polilight PL 500 at 505 nm and viewed through a KV 550 filter, (shutter speed 2.5 s, aperture f11). Comparison of the left impression vs. right impression shows variation in both colour and luminescence intensity.

A luminescence spectrum was obtained for this fingermark revealing a very broad emission band with a maximum emission between 550 and 610 nm (Figure 6.9).

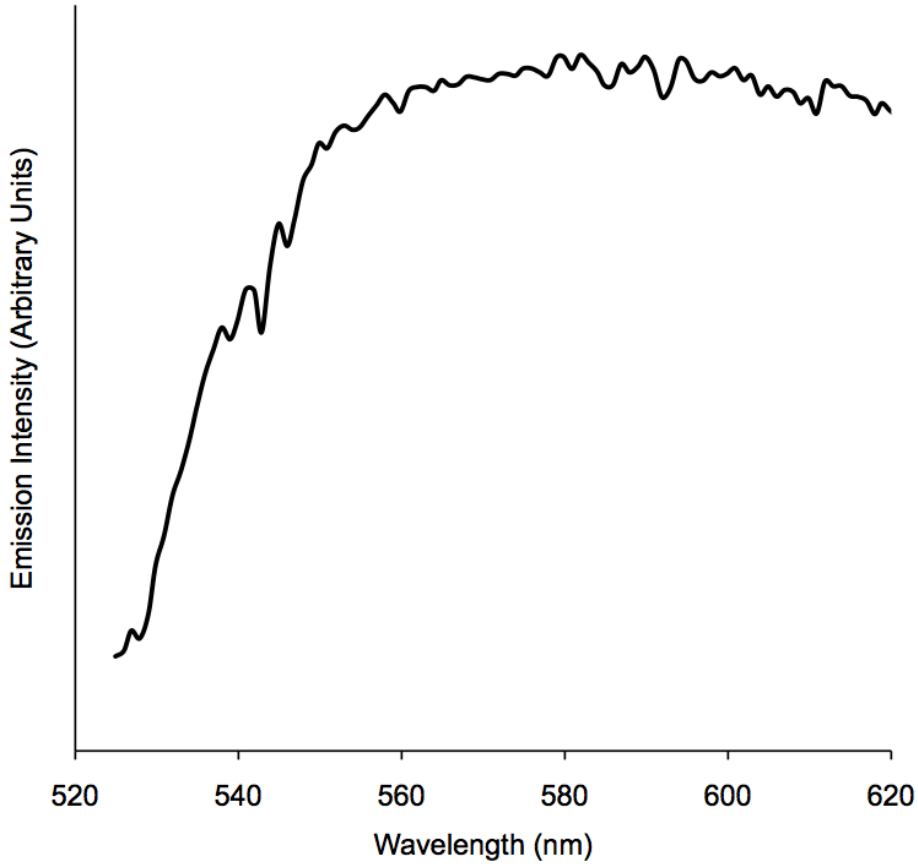


Figure 6.9: Emission spectrum of a 6-N,N-dimethylaminofulvene treated fingermark (λ_{ex} 505 nm). Spectrum has been normalised.

Attempts to replicate this result were conducted, looking into heating samples via the oven and laundry press, and also not applying heat to the samples, but to no avail. Results still exhibited background discolouration, in particular on the non-heated samples left for a period of time. Petroleum Spirits and HFE-7100 were evaluated and was found to produce similar results and therefore, the solvent was considered not to be a factor in the observed variations. At this stage, these results are yet to be fully understood and further research is required to investigate this further.

Due to the variability in performance, and investigation into the nature of the reaction between 6-N,N-dimethylaminofulvene and latent fingermark residues could not be carried out within the time constraints of the project. In particular no examination of potential reactions with amino acids was possible. However, it is possible to speculate that the compound can exist as an activated electrophile as outlined in Figure 6.10, which could then react with nucleophilic species present in the latent fingermark.

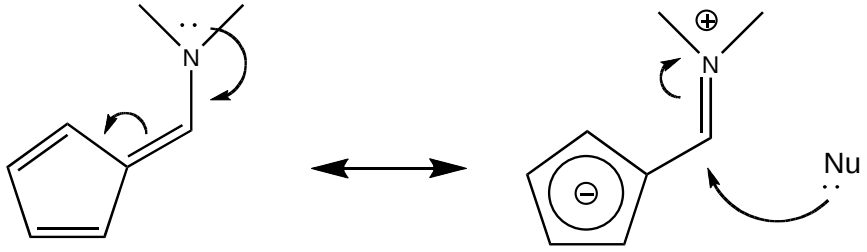


Figure 6.10: Speculative generalised initial step of reaction mechanism of 6-N,N-dimethylaminofulvene with a nucleophile.

6.4 CONCLUSION

This chapter has outlined three systematic approaches in screening and evaluating some natural dyes and other selected compounds that have the potential to lead to new fingermark detection reagents.

Alizarin was found to produce visible fingermarks in the luminescence mode, and this was achievable with limited solubility of the compound in the working solution. Juglone produced partial ridge development with weak luminescence and therefore did not perform as well as the other naphthoquinones tested in previous chapters.

An investigation of the use of retrosynthetic approaches was found to be unsuccessful at producing indigo from isatin. Despite this lack of success, this approach still has the potential to lead to the synthesis of novel reagents and thus expand the scope of research in this area. This provides an insight into natural dyes as an area of interest that could potentially lead to the identification of novel fingermark detection reagents.

Latent fingermarks treated with 6-N,N-dimethylaminofulvene produced a brown impression with luminescence characteristics. Ambiguity between some of the results has yet to be explained and requires further research. At this stage it has not been established that 6-N,N-dimethylaminofluvene reacts with amino acids. However, 6-N,N-dimethylaminofulvene as a compound has the advantage over other treatments in that it can be dissolved directly into non-polar solvents, and this is worthy of further investigation. In addition, it bears no relation in any way to current treatments for latent fingermarks on paper and this may form the basis for a new class of fingermark detection reagents.

CHAPTER 7: SUGGESTIONS FOR FUTURE WORK

Given the value of fingerprint evidence in criminal investigations, and the proven benefits that come from targeting the amino acids in the deposit, active research in this area will continue into the foreseeable future. In relation to this study, there are several areas that would benefit from further investigation, all of which are outlined below.

7.1 NAPHTHOQUINONES AS FINGERMARK DETECTION REAGENTS

Chapters 3 and 4 investigated naphthoquinones as potential fingermark detection reagents. The work presented in these chapters identified a number of selected naphthoquinones that had the ability to target the amino acid residues present in latent fingermarks, resulting in a coloured and/or luminescent product. This approach could have significant advantages if implemented in routine fingermark detection protocols. However, in order for this to be established, further research is required on an individual basis, investigating optimal working conditions and reagent stability for each compound. One avenue in assisting this process is determining the product(s) associated with each naphthoquinone when reacted with amino acids.

Attempts were made, as discussed in Chapter 5, which resulted in a better understanding of the chemistry involved. However, this was not sufficiently conclusive to deduce the exact product responsible for the observed colour and luminescence. It is important that future studies continue to utilise surface analysis techniques in order to examine the reaction intermediates and products formed *in-situ* rather than in solution in order to obtain results that are applicable to fingermark detection on porous substrates. A better understanding of reaction mechanisms will potentially allow for optimised developmental conditions and the design of novel amino acid reagents with enhanced properties. For example, addition of selected substituents to position 6, 7 and/or 8 of the aromatic ring of lawsone and its derivatives could provide a

route to additional fingermark reagents. Depending on the substituent, this should not significantly disrupt the reactive site but could modify the electronic properties, thus altering the solubility, resulting colour and/or luminescence properties.

Furthermore, in order to ascertain the potential of each new reagent in an operational context, comparative studies must be carried out, not only to establish their performance against current fingermark reagents, but also to evaluate their suitability as a complementary method. From this work, if a reagent were found suitable, investigations would be required into their position in fingerprint detection sequences. However, before this comparative evaluation, it is important to further optimise the working solution for lawsone. Difficulties highlighted with reagent stability and potentially shelf life must be addressed in order to accurately access lawsone against other reagents. Along with this, further investigation on post-treatments, in particular the use of citric acid, could be vital in improving the contrast in developed fingermarks.

7.2 NATURAL DYES AND MISCELLANEOUS COMPOUNDS

As discovered in Chapter 6, the alizarin reaction product may possess luminescence characteristics that are advantageous for fingermark detection. In order to assess alizarin as a potential fingermark reagent, a significant amount of work must be carried out into the reagent formulation, to ensure a stable working solution. Furthermore, it is important to establish whether alizarin is reacting with the amino acid deposits in the fingermark, which requires investigation with the use of amino acids, not just fingermarks. The discovery of lawsone and alizarin as fingermark reagents could effectively prompt further research into the use of other natural compounds for fingermark development and, in turn analogues related to these.

Research into compounds such as 6-N,N-dimethylaminofulvene may instigate a new trend in investigating novel fingermark reagents. While not particularly successful in this study, 6-N,N-dimethylaminofulvene has the advantage of being soluble in non-polar solvents, which is a desirable attribute for a fingermark detection reagent. While results with this compound were disappointing, the preliminary study did suggest that further investigation was warranted. Further work may provide some insight into the use of non-polar compounds as fingermark detection reagents. This would provide operational advantage in terms of simplified reagent formulations and non-polar solutions that are less destructive on treated documents.

7.3 NOVEL APPROACHES FOR FINGERMARK RESEARCH

Since the introduction of ninhydrin as a fingermark reagent, research into new reagents has primarily focused on analogues thereof, or other compounds that are known to react with the skin. This research methodology, although very effective, may reach a point in the future where it only provides incremental improvements in detection sensitivity. In order to develop significantly improved fingermark reagents, new research approaches must be considered. One approach, as discussed in Chapter 6, could be via retrosynthetic approaches. By selecting a compound of interest that possesses the ideal colour and luminescence characteristics that could complement the products formed from current reagents, researchers could investigate potential precursors that, on reaction with amino acids, produce this specific compound. Understandably, this notion may be far-fetched and difficult to implement; however, instigation of this approach could be undertaken with a proof of concept study, with the use of synthetic chemistry, before applying it to fingermark detection.

Synchrotron ATR-FTIR has been shown to be a valuable tool in detecting trace components linked to the reaction product of naphthoquinones with amino acids, as discussed in Chapter 5. This may provide an investigative platform to be used with current and prospective fingermark reagents where there may be some conjecture as to the reaction products that are formed, as is the case with 1,2-indanedione and genipin. Furthermore, Synchrotron ATR-FTIR could have a broader application in a number of scientific disciplines relating to *in-situ* analysis on cellulose-based substrates. Although this study was unable to identify the reaction products associated with the various naphthoquinones and amino acids, the information obtained, in conjunction with synthetic studies, has provided a significant insight into the potential products.

It is important to scale-up the synthetic studies reported here, in order to obtain a significant amount of the yellow and red products from the lawsone reaction with glycine, so that more in-depth analyses are possible. This would allow the application of various characterisation methods, along with fluorescence-based studies. Investigating the luminescence characteristics would ensure that the products being produced in solution correspond to the products observed on porous surfaces. Furthermore, the synthesised products could be placed onto TLC plates for subsequent analysis by Synchrotron ATR-FTIR. As a result, a direct comparison between fingermark treated samples and synthetic samples could be made. As seen in Chapter 5, a direct comparison between the ATR-FTIR spectra of synthetic samples and the Synchrotron samples was difficult due to the likelihood that matrix differences affect vibrational frequencies.

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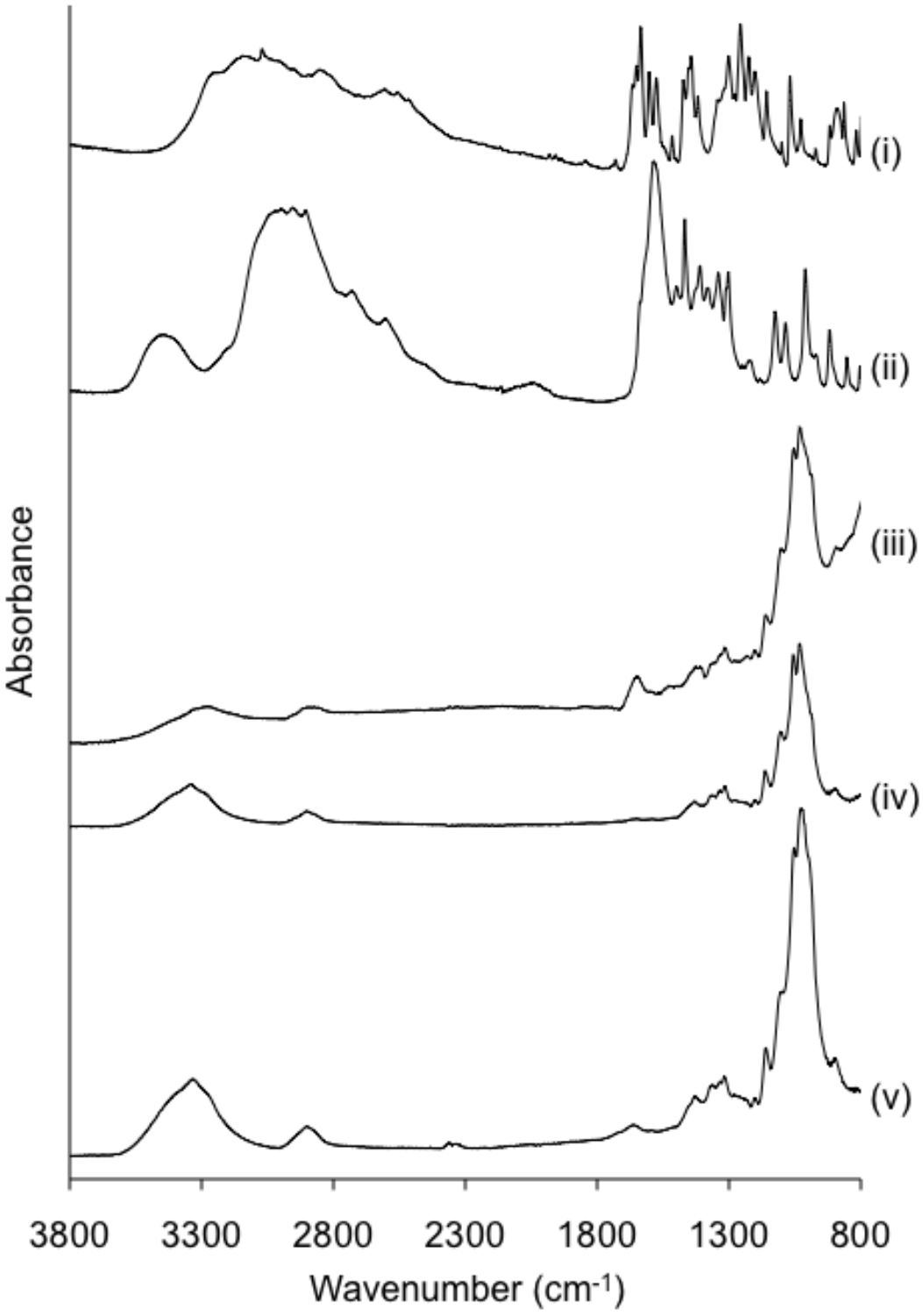
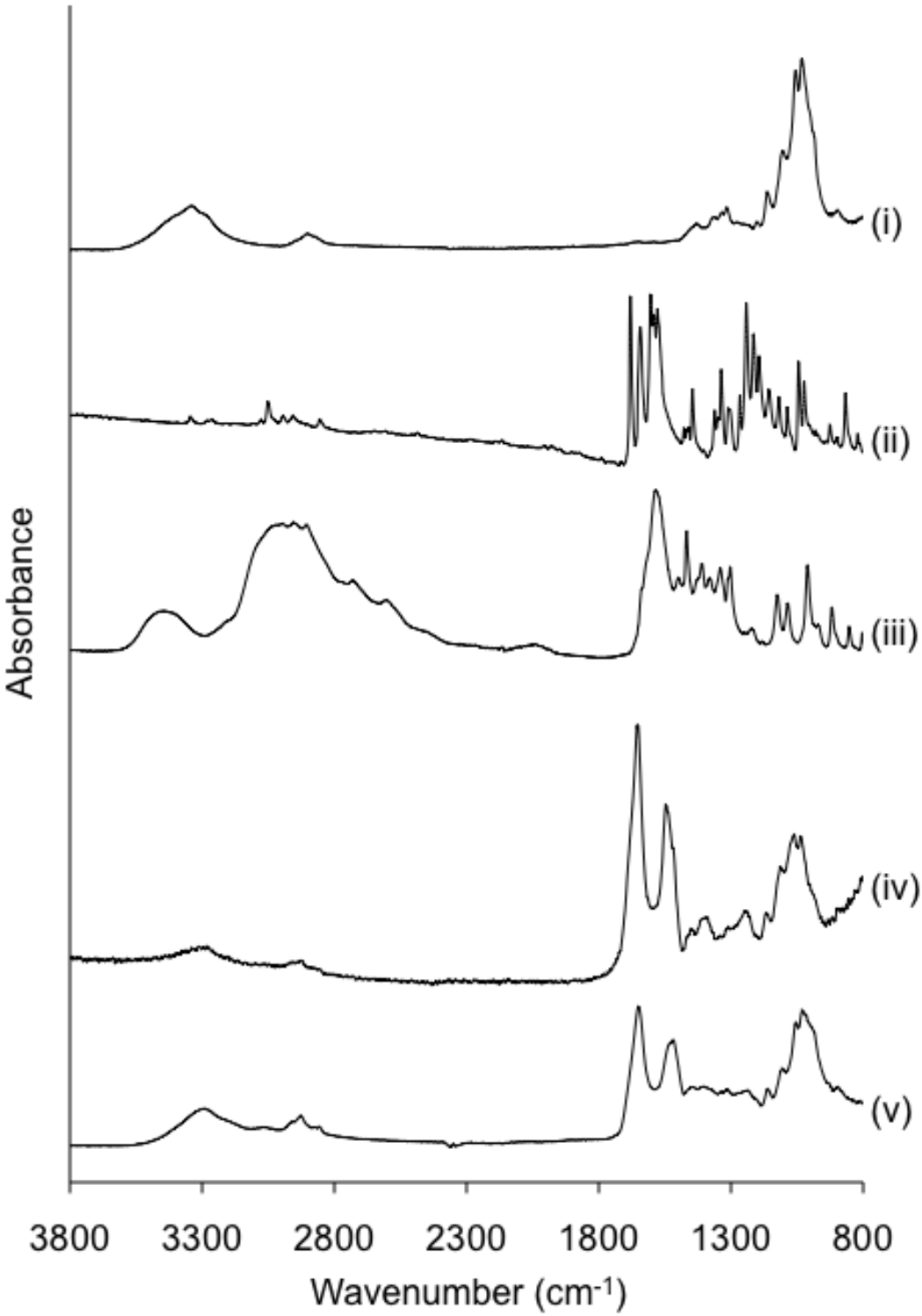
APPENDICES

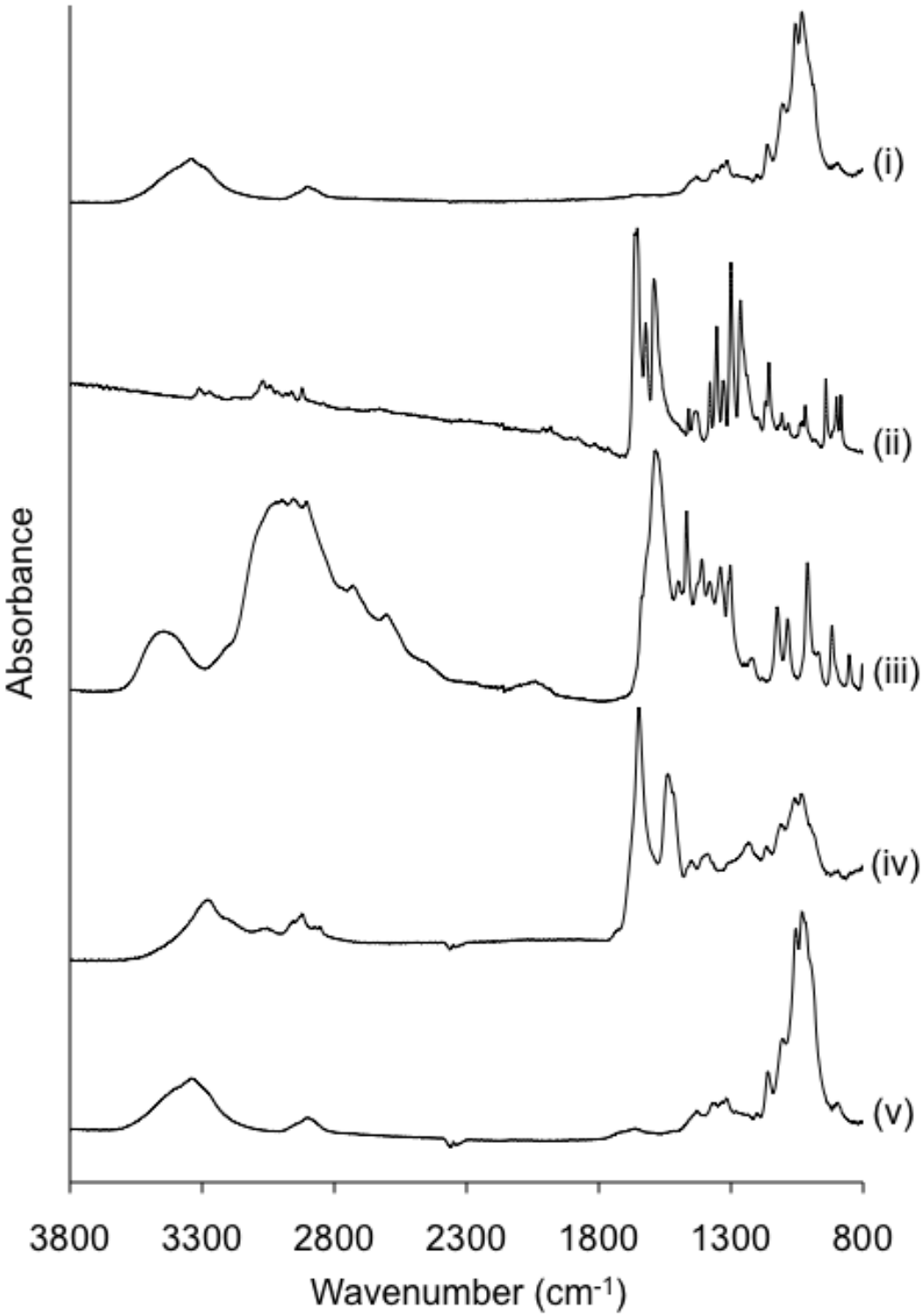
APPENDIX 1: FTIR SPECTRA



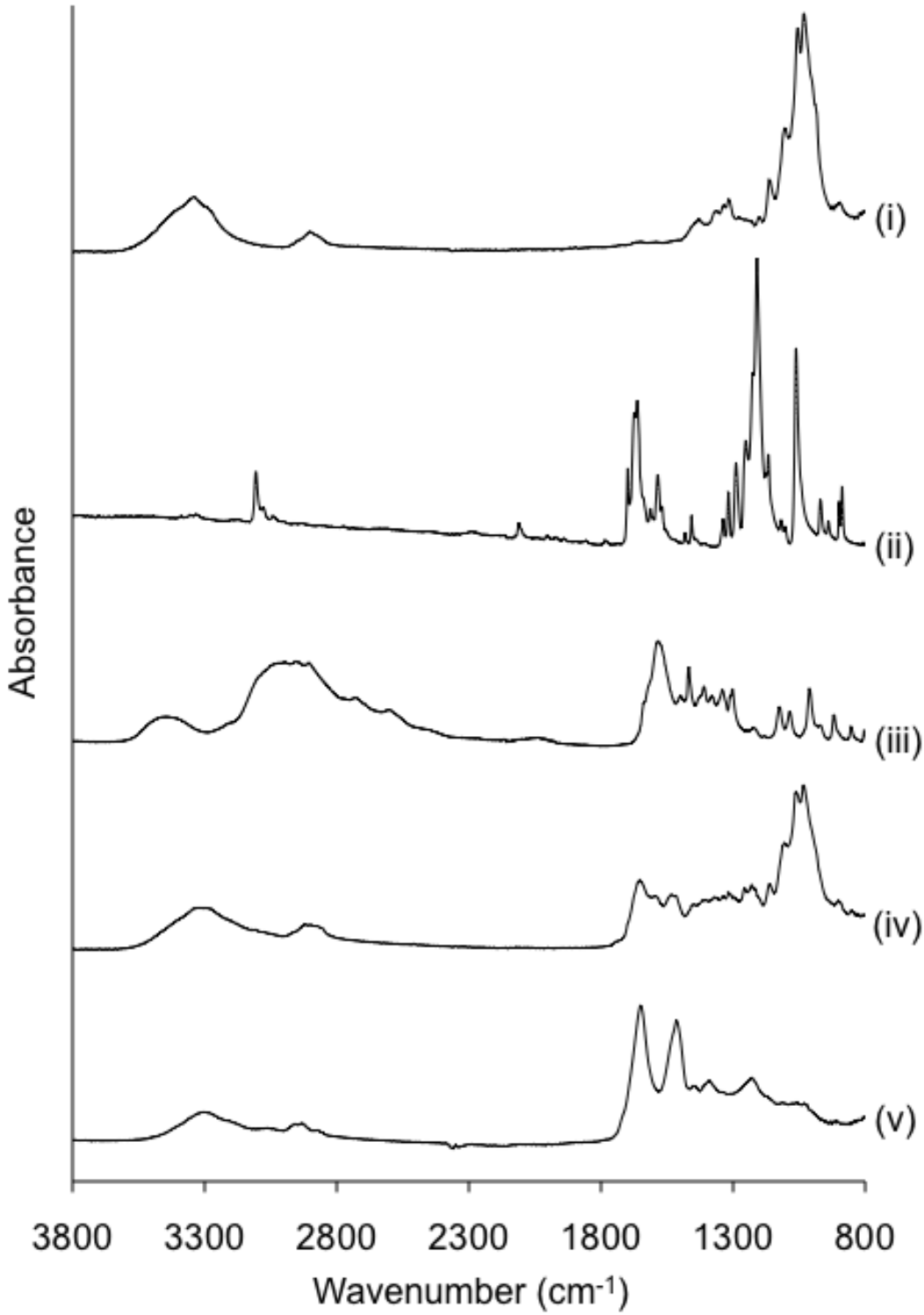
Appendix 1.1: Synchrotron ATR-FTIR spectra of: (iii) 1,4-dihydroxy-2-naphthoic acid treated serine spot on TLC; (iv) TLC cellulose; (v) 1,4-dihydroxy-2-naphthoic acid treated fingermark on TLC; and conventional ATR-FTIR spectra of bulk samples of: (i) 1,4-dihydroxy-2-naphthoic acid; and (ii) serine. Spectra have been normalised and offset to illustrate similarities and differences.



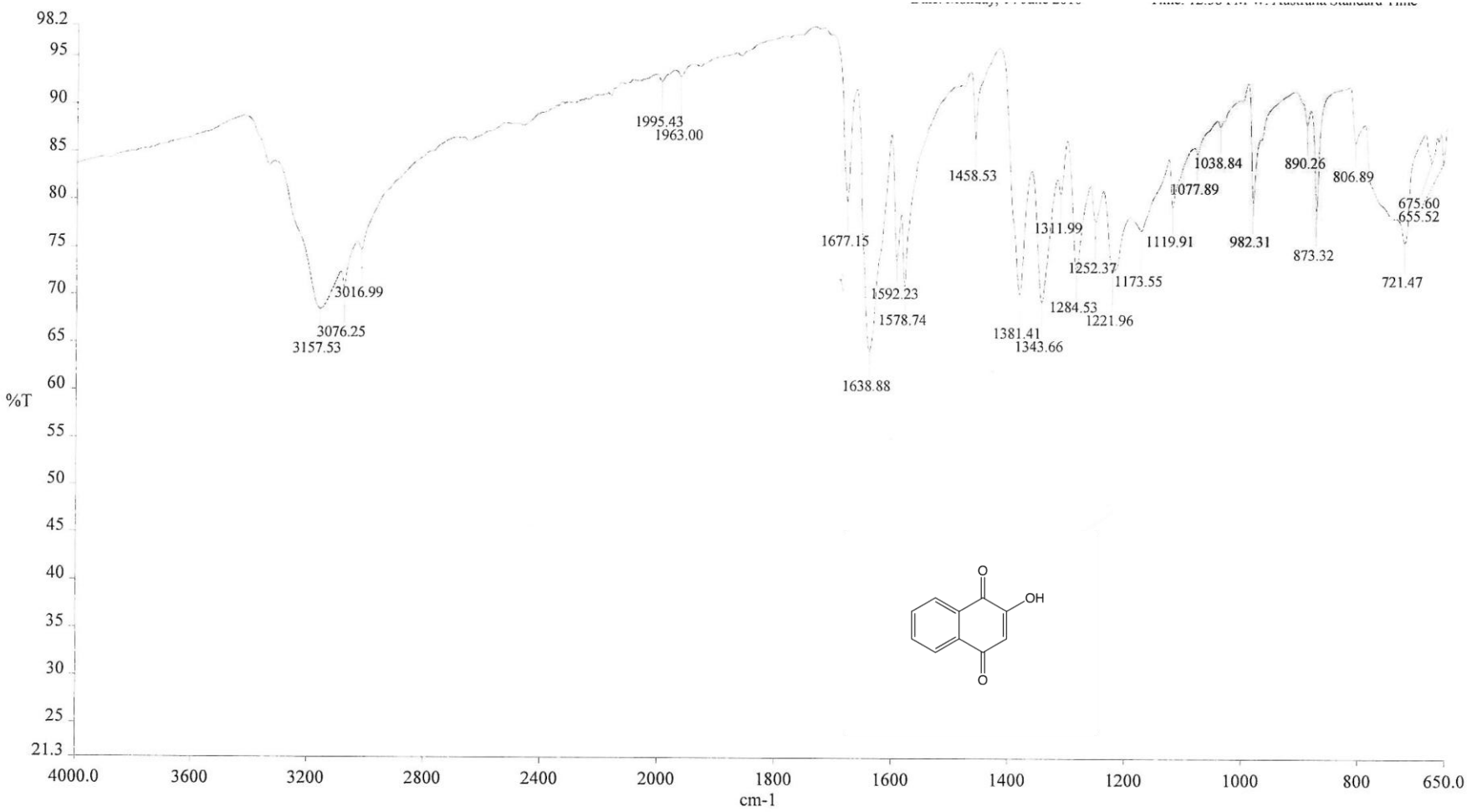
Appendix 1.2: Synchrotron ATR-FTIR spectra of: (i) TLC cellulose; (iv) 2-methoxy-1,4-naphthoquinone treated serine spot on TLC; (v) 2-methoxy-1,4-naphthoquinone treated fingermark on TLC; and conventional ATR-FTIR spectra of bulk samples of: (ii) 2-methoxy-1,4-naphthoquinone; and (iii) serine. Spectra have been normalised and offset to illustrate similarities and differences.



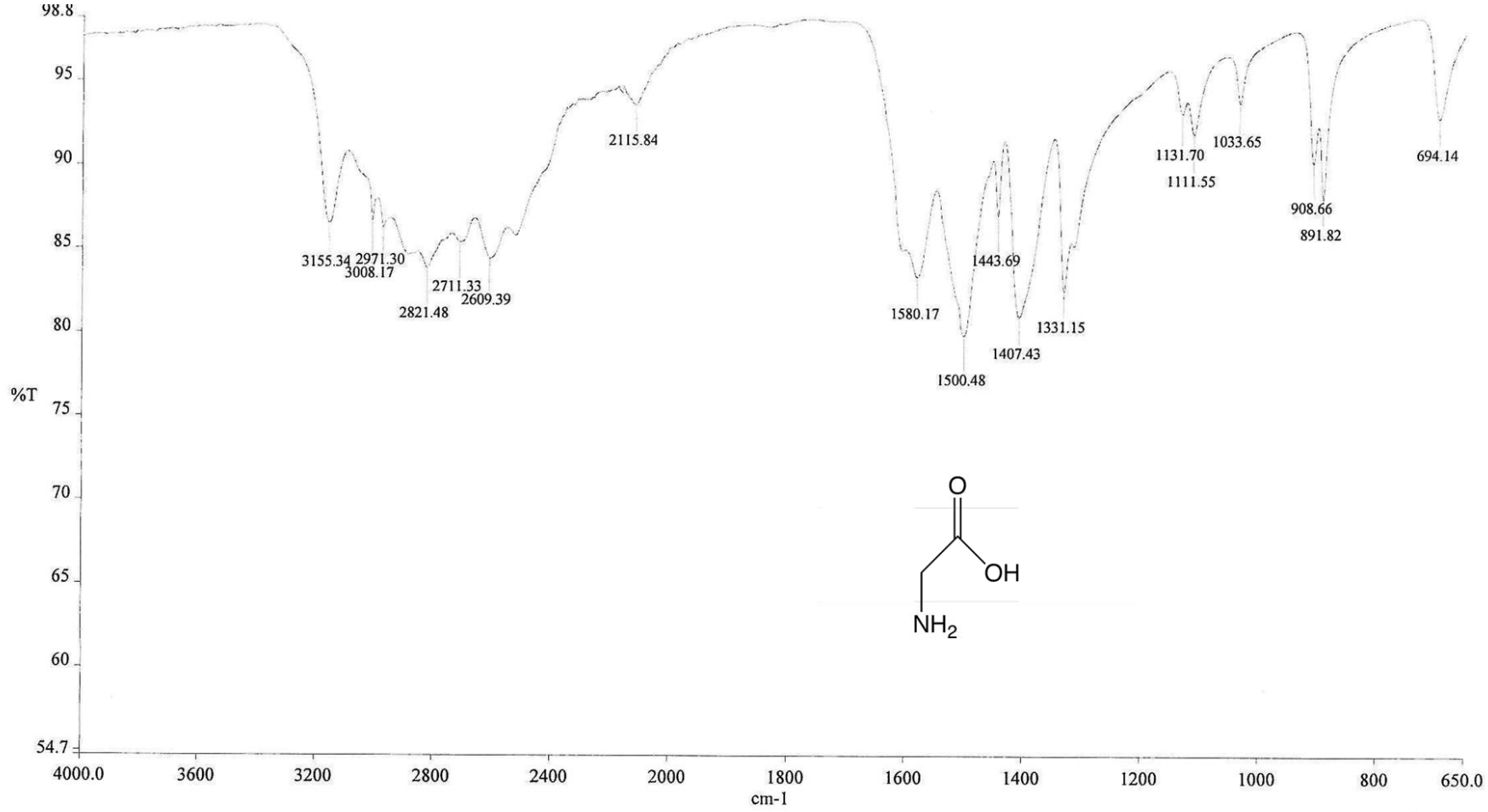
Appendix 1.3: Synchrotron ATR-FTIR spectra of: (i) TLC cellulose; (iv) 2-methyl-1,4-naphthoquinone treated serine spot on TLC; (v) 2-methyl-1,4-naphthoquinone treated fingermark on TLC; and conventional ATR-FTIR spectra of bulk samples of: (ii) 2-methyl-1,4-naphthoquinone; and (iii) serine. Spectra have been normalised and offset to illustrate similarities and differences.



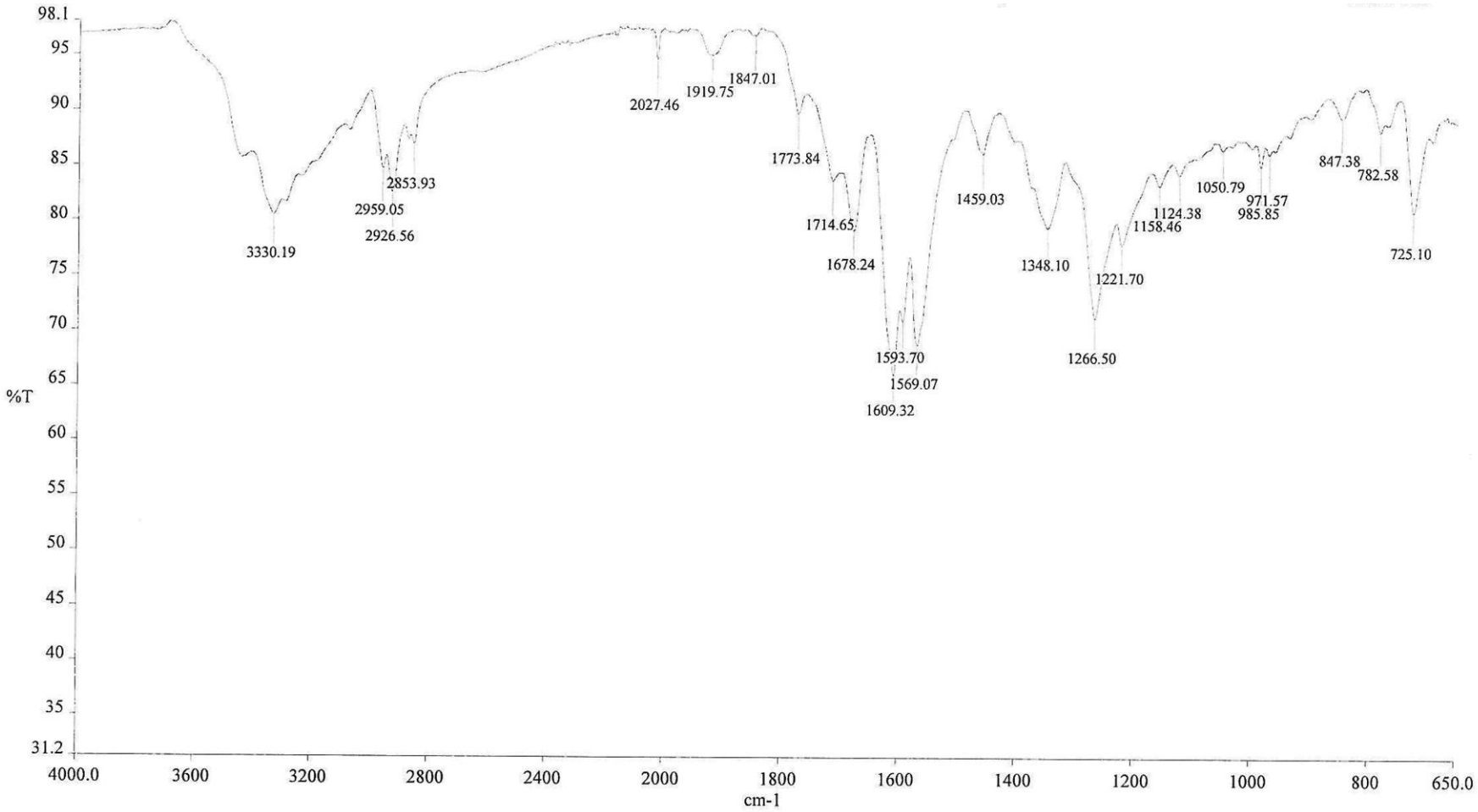
Appendix 1.4: Synchrotron ATR-FTIR spectra of: (i) TLC cellulose; (iv) 1,4-naphthoquinone-4-sulfonate treated serine spot on TLC; (v) 1,4-naphthoquinone-4-sulfonate treated fingermark on TLC; and conventional ATR-FTIR spectra of bulk samples of: (ii) 1,4-naphthoquinone-4-sulfonate; and (iii) serine. Spectra have been normalised and offset to illustrate similarities and differences.



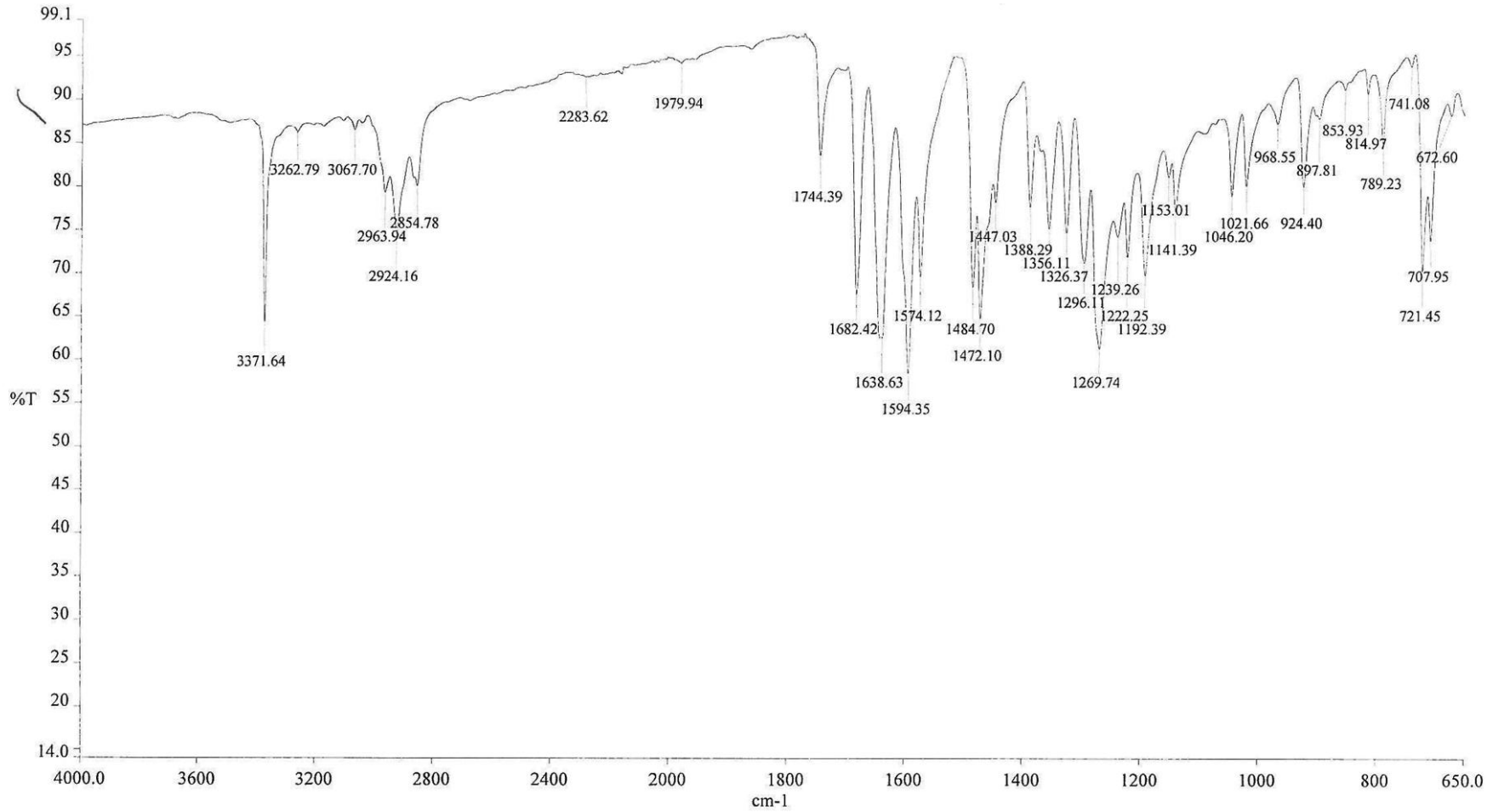
Appendix 1.5: ATR-FTIR of lawsone.



Appendix 1.6: ATR-FTIR of glycine.

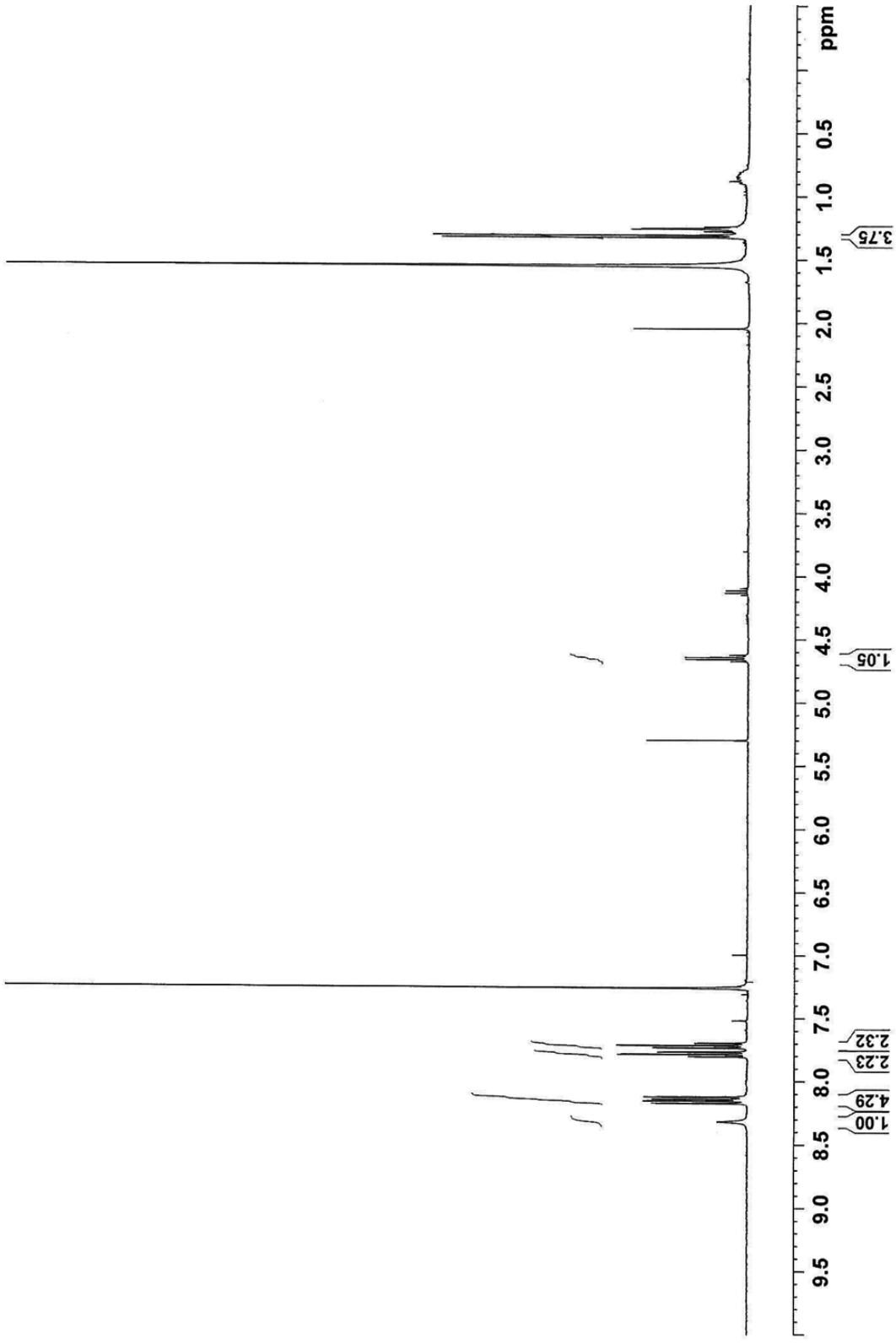


Appendix 1.7: ATR-FTIR of yellow product.

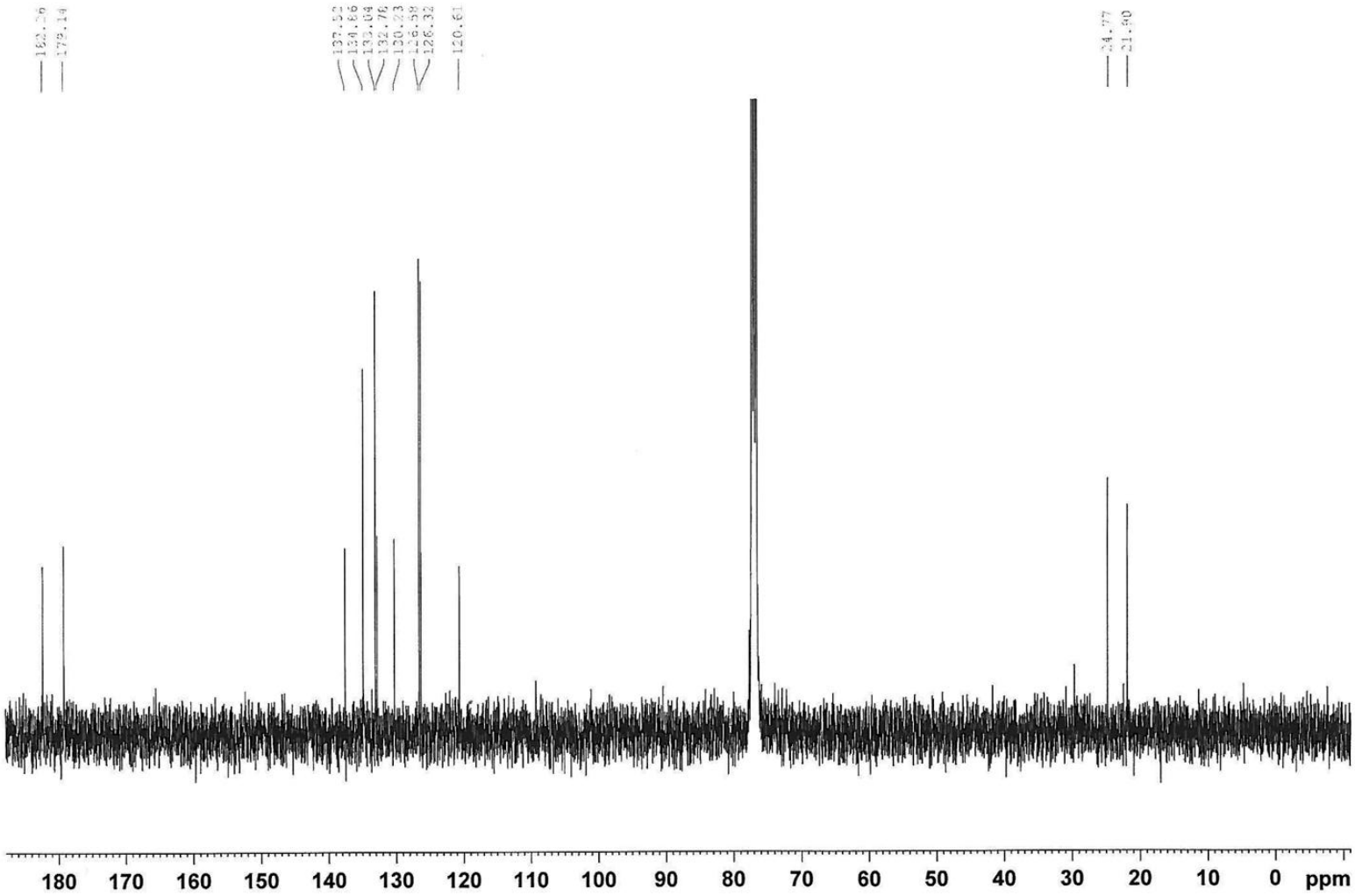


Appendix 1.8: ATR-FTIR of red product.

APPENDIX 2: NMR SPECTRA

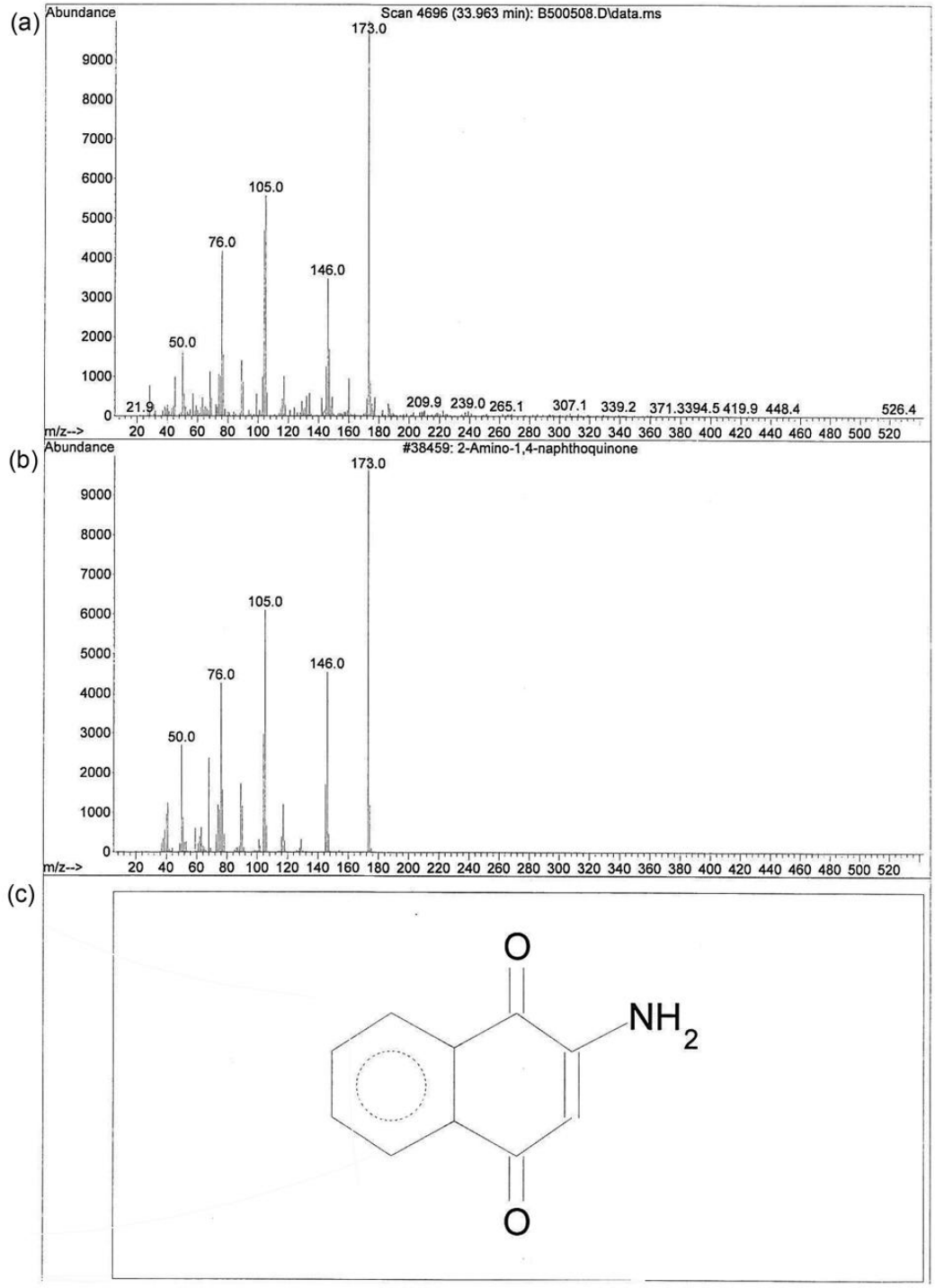


Appendix 2.1: ^{1}H NMR of red product.



Appendix 2.2: ^{13}C NMR of red product.

APPENDIX 3: MASS SPECTRA



Appendix 3.1: a) experimental mass spectrum of yellow product; b) library reference mass spectrum of 2-amino-1,4-naphthoquinone (89% match to yellow product); and c) structure of 2-amino-1,4-naphthoquinone.