Novel Fingerprint Development Techniques

By

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Abstract

There are numerous pre-existing fingerprint development techniques, however, often prints are difficult to develop, depending on their age or the surface upon which they have been deposited. Forensic scientists are relentlessly looking for new and better methods to enhance fingerprints. More recent technologies have higher sensitivity to very low levels of constituents present in residues and so are able to unearth significant details from a person's fingerprints at molecular level e.g. DNA, drug metabolites. Therefore, research continues in an attempt to generate novel, non-destructive processes that can enhance latent fingerprints.

Exposing fingerprints to the *p*-block compounds selenium dioxide (SeO_2), phosphorus sulfides (P_4S_x) and phosphonitrilic chloride trimer $(NPCl_2)_3$, in the vapour phase resulted in latent prints being visualized on a range of media. Selenium dioxide revealed prints on metal surfaces (e.g. brass) which were enhanced further upon formation of a dark brown coating of copper-selenide formed on the surface when exposed to moisture, giving a better contrast. P_4S_3 vapour revealed a higher percentage of prints and samples had greater stability in air while although $(NPCl_2)_3$ was able to develop fingerprints, the low quality was undesirable. Initially it was thought that $(NPCl_2)_3$ has the potential for further derivatisation but was proven very difficult to interact with compounds especially those with the potential to induce fluorescence. However, all three compounds are commercially available and sublimation techniques are straightforward.

This led onto the use of a modified DNA analysis method to obtain DNA from latent print residues both before and after chemical treatment via sublimation of SeO_2, P_4S_3 and $(SN)_x$ polymer. A combination of Chelex® extraction; polymerase chain reaction (PCR) amplification using *Alu* insertion polymorphisms with 40 PCR cycles followed by gel electrophoresis, allowed successful detection of *Alu* polymorphism insertions from latent fingerprints both before and after chemical treatment using vapour phase techniques.

The use of ESDA, a non-destructive technique for the detection of latent fingerprints and indentations was re-investigated. ESDA was used to develop prints placed on paper and cotton that had been exposed to various gases e.g. SO_2, O_2, CO_2, N_2 and changes in relative humidity prior to ESDA. There were no improvements on developed prints using ESDA, it was likely that the change, if any, was so small that the ESDA was not sensitive enough to enhance latent prints.

Finally, a series of miscellaneous fingerprint development techniques were re-visited. A hydrazine crystal violet (HCV) formulation was used to develop latent fingerprints on paper and glass. The HCV formulation was able to reveal latent fingerprints on glass with ridge detail only after samples were re-exposed to atmospheric CO_2 over a number of days after the HCV dip. However, the quality of fingerprints developed was undesirable compared to those developed using the original crystal violet technique.

An optimised method of 10% phosphomolybdic acid (PMA) applied using an EcoSpray and developing prints at 100°C proved to have a higher success rate than the results originally reported by S. E Vincent in 1973. This technique has shown real promise to develop sebaceous fingerprints from problematic surfaces such as wood, metals and cotton with good quality ridge detail.

The use of cupric acetate and rubeanic acid to detect fatty acids and in turn develop latent fingerprints on paper and fabrics was re-investigated. Optimisation of the copper-rubeanic acid technique successfully enhanced latent fingerprints on a wide range of surfaces including cotton where in the original report attempts to do so were deemed unsuccessful. The optimised technique showed that dipping fingerprint samples in the copper solution for 1 minute prior to development in rubeanic acid was sufficient to reveal ridge detail to a grade 3 or 4 standard.

Finally, 0.10% ethanolic rubeanic acid has successfully developed whole palm prints with an impressive quality of ridge detail after gripping copper or lead for only a few minutes before transferring onto the gelatine lifters. This has created a platform for additional research using rubeanic acid and gelatine lifters and develop the technique further and potentially be used to aid criminal investigations involving metal theft.

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Abbreviations

2 – NH_2 – AN	2 – aminoanthracene
A	Ardrox
AN	Anthracene
AN – 9 – COOH	Anthracene – 9 – carboxylic acid
AWRE	Atomic Weapons Research Establishment
BATF	Bureau of Alcohol, Tobacco and Firearms
BR – 28	Basic Red 28
BY – 40	Basic Yellow 40
CA	Cyanoacrylate
CAST	Centre for Applied Science and Technology
CCD	Charge-coupled device
$CDCl_3$	Deuterated chloroform
CFC – 113	Chlorofluorocarbon – 113
CuSe	Copper selenide
CV	Crystal violet
DFO	1, 8, diazafluoren-9-one
DMAC	4 – dimethylaminocinnamaldehyde
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EPSRC	Engineering and Physical Science Research Council
ESDA	Electrostatic detection apparatus
FAM	6 – carboxy – fluoroscine (Fluoroscein amidite)
HCV	Hydrazine crystal violet
HEX	hexachloro – 6 – carboxy – fluoroscine
HFE – 7100	Hydrofluoroether – 7100 carrier solvent
H_2O_2	Hydrogen peroxide
HOSDB	Home Office Scientific Development Branch
hTERT	Human Telomerase reverse transcriptase

IPC	Internal PCR control
IR	Infra – red
KBr	Potassium bromide
MBD	4-(4-methoxybenzylamino-7-nitrobenzofurazan)
MGB	Minor groove binder
MMD	Multi – metal deposition
MPD	Modified physical developer
Nd:YAG	Neodymium doped yttrium aluminium garnet – $Nd:Y_3Al_5O_{12}$
NFQ	Non – fluorescent quencher
NMR	Nuclear magnetic resonance
$(NPCl_2)_3$	Phosphonitrilic chloride trimer
NWSD	Non water soluble deposit
PCR	Polymerase chain reaction
PD	Physical developer
PMA	Phosphomolybdic acid
P_4S_x	Phosphorus sulfides
P_4S_{10}	Tetraphosphorus decasulfide
P_4S_9	Tetraphosphorus nonasulfide
P_4S_3	Phosphorus sesquisulfide
PSDB	Police Scientific Development Branch
PTFE	Polytetrafluoroethylene
R6G	Rhodamine – 6G
RA	Rubeanic acid
RCMP	Royal Canadian Mounted Police
RH	Relative humidity
RNA	Ribonucleic acid
RP	Ruhemann's purple
RUVIS	Reflective ultra violet imaging system
SeO_2	Selenium dioxide
SKP	Scanning Kelvin probe
$(SN)_x$	Polysulfur nitride

S_2N_2	Disulfur dinitride
$^{35}SO_2$	Radioactive sulfur dioxide
SPR	Small particle reagent
TAPP	Tetra – *o* – aminophenylporphyrin
TBE	Tris boric EDTA
TET	Tetrachloro – 6 – carboxy – fluoroscine
TMS	Trimethylsilane
USACIL	US Army Criminal Investigation Laboratory
UV	Ultra violet
VCA	Vacuum cyanoacrylate
VMD	Vacuum metal deposition
WSD	Water soluble deposit

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Chapter 1 – Introduction

1.1. Structure of Friction Ridge Skin

A latent fingerprint is defined as an impression left by friction ridges of a human finger, which are invisible to the naked eye. A latent fingerprint deposited contains a complex mixture of natural secretions from the sweat glands and contaminants from the environment.

A friction ridge is the raised portion of the epidermis on the palmar surfaces of the hands and the plantar (sole) surfaces of the feet.

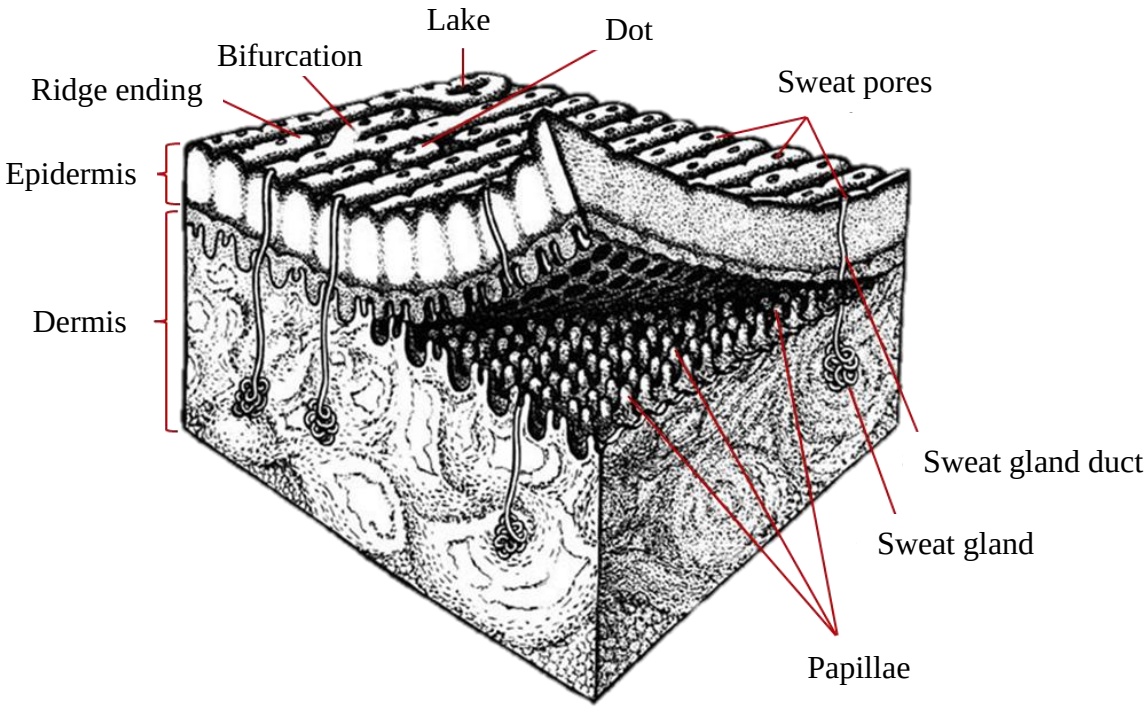


Figure 1: Cross section of friction ridge skin structure[1]

The epidermis is the outer layer of tough, ridged skin bearing only sweat pores. Its main functions are as a gripping aid and protection of the dermis. The dermis consists of the sweat glands, nerve endings and the papillary layer, which determines the unique fingerprint code. Each double row of papillae on the dermis is represented by one ridge on the epidermis.

1.2. Composition of Latent Fingerprints

There are three types of fingerprint evidence, visible, indented and latent fingerprints. Visible and indented fingerprints are usually able to be photographed using coloured or oblique lighting, although some treatment may be needed to enhance the marks e.g. fingerprints in blood may need chemical treatment. Latent fingerprints are the more common form of evidence but are the most problematic to develop due to the complex mixture of constituents in the trace residues left behind.

There are three types of sweat glands responsible for these natural secretions: eccrine, sebaceous and apocrine glands.

Table 1: Main constituents of the secretions of the sweat glands[2]

Source	Inorganic Constituents	Organic Constituents
Eccrine glands	Chlorides Metal Ions (Na^+, K^+, Ca^{2+}) Ammonia Sulphate Phosphate Water (>98%)	Amino acids Urea Uric acid Lactic acids Sugars Creatinine Choline
Sebaceous glands	-	Fatty acids Glycerides Hydrocarbons Alcohols
Apocrine glands	Iron Water (>98%)	Proteins Carbohydrates Cholesterol

When the friction skin is in contact with an object, some of these constituents present on the skin are transferred, placing an “invisible” image of the detail on that surface, i.e. as a latent fingerprint. The amount and composition of the residue varies extensively due to metabolism, climate, diet and mood.

1.3. Fingerprint Patterns

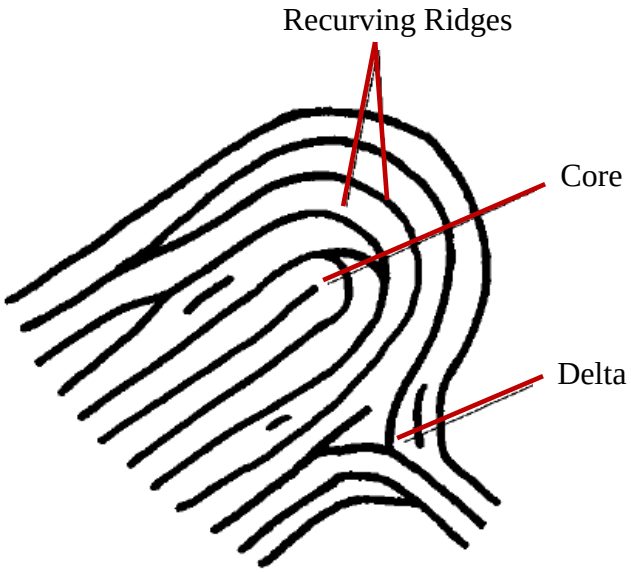


Figure 2: Elements of a fingerprint[3]

Fingerprint patterns consist of a combination of these three elements:

1. A re-curve is any ridge retracing its course and includes the bend in a loop or whorl. Arches and tented arches do not contain these free re-curves.
2. A delta is a ridge that converges on a point from three different directions. Arches and sometimes tented arches lack these.
3. The central area of a pattern is the core and contains the point of a core, which is determined differently for each fingerprint pattern.

These elements above form the eight fingerprint patterns in (Figure 3) and are classed as level 1 detail. A fingerprint examiner assessing fingerprint detail cannot use this alone to give a complete identification, but it can narrow down the search. Another detail is if a pattern contains a permanent scar, which forms if the dermis is damaged. Scars disrupt the flow of the ridge detail, which is irreversible, thus increases the chance of a positive identification due to this distinctive pattern.

Level 2 detail involves looking at the overall ridge flow and any major deviations such as those in (Figure 4). Lastly, level 3 detail involves looking at the shape of the ridge flow and location of the sweat pores. Usually a combination of all three categories is used to compare and correctly identify any fingerprint[4].

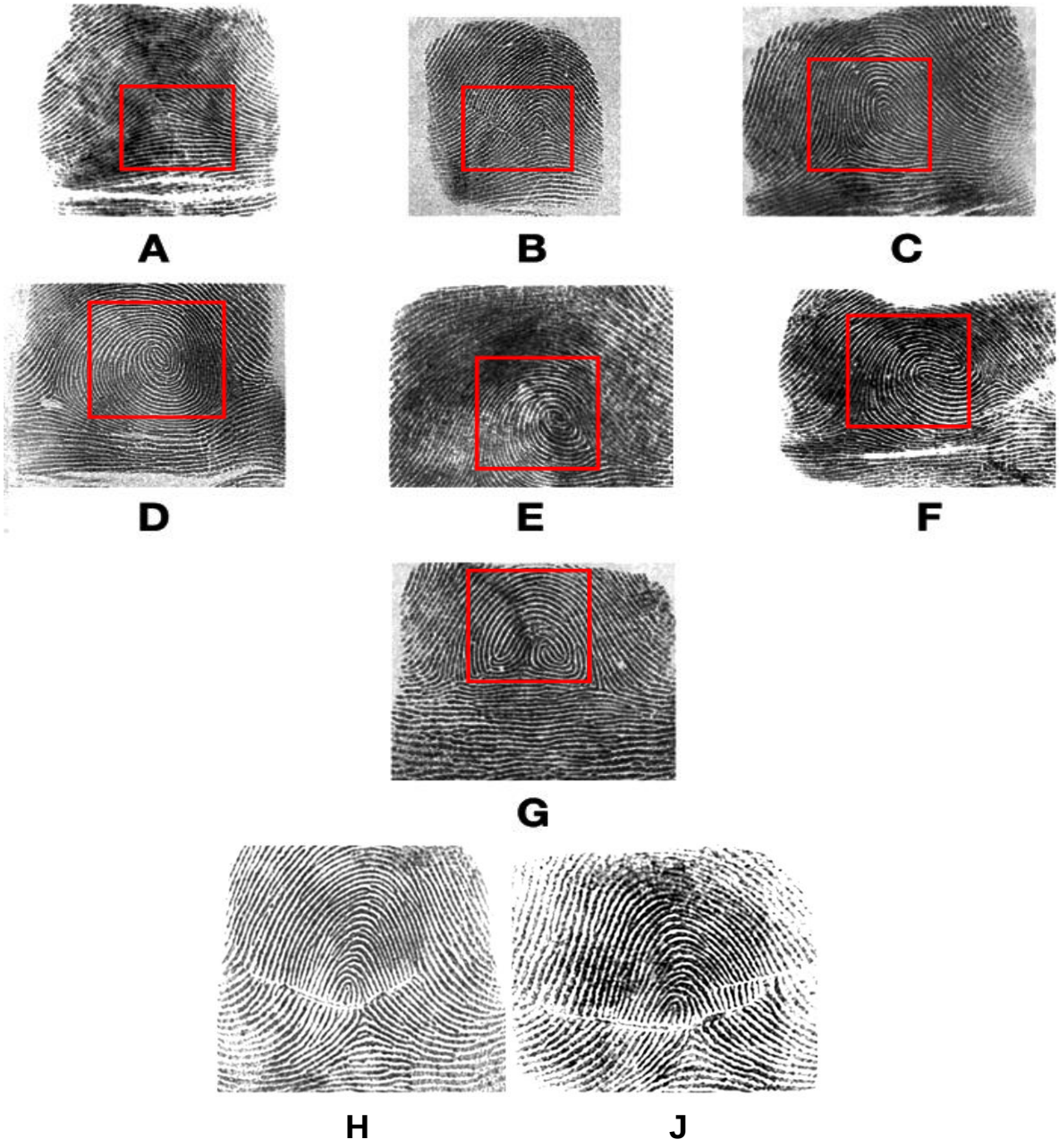


Figure 3: Level 1 detail: (A) arch, (B) tented arch, (C) ulnar/radial loop, (D) whorl, (E) central pocket loop, (F) double loop, (G) composite, (H) scarred fingerprint, (J) same fingerprint recorded 11 years later[3]

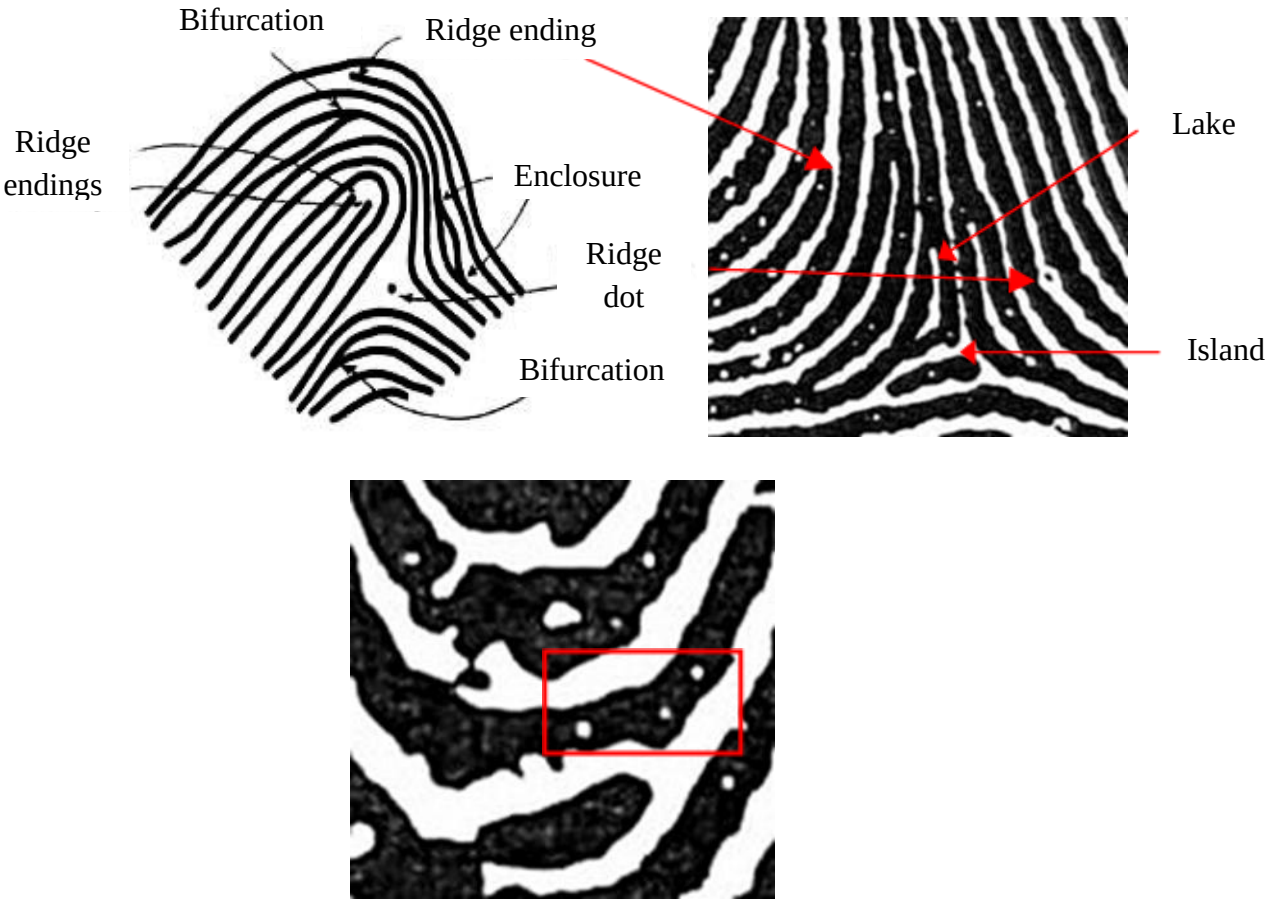


Figure 4: Level 2 fingerprint ridge detail (top) and Level 3 detail (bottom)[4]

Fingerprints are very important in a forensic context because our hands are sensory organs, the part of the body in almost constant physical contact with our surroundings and the part of the body that most frequently leaves our personal signature behind.

However, there are several factors, which influence the choices of techniques used to recover and process latent fingerprints to the highest quality possible. These include:

- The porosity of the surface e.g. porous, non-porous or semi-porous.
- The type of fingerprint e.g. eccrine (sweaty), sebaceous (oily) or usually a mixture of both.
- The age of a fingerprint (This may be unknown in most cases).
- Any exposure to extreme environments e.g. fire.
- Any exposure to the elements e.g. rain, snow.
- Any deliberate attempts to remove the fingerprints, by washing (with water or chemicals) or rubbing off a surface.

1.4. Surface Characteristics

In general, surfaces can be divided into three groups: porous, non-porous and semi-porous. Recently, latent fingerprint formation has been considered a physiochemical process between the ridge skin and deposition surface. When fingers touch a solid surface, secretions that are transferred may depend on certain factors[5]:

- Temperature of the surface: Sebaceous material adheres better to surfaces that are cooler than the human body.
- Surface Structure: The rougher the surface the greater the adhesive forces.
- Electrostatic forces on the print-receptive surface.
- Surface wettability: The ability for a liquid to maintain contact with solid surface depending on the balance of cohesive and adhesive forces.

1.4.1. Porous Surfaces[4]

A porous surface is defined as one that absorbs the latent fingerprint very quickly e.g. paper, cardboard and cotton. Water-soluble deposits (WSD) e.g. amino acids, urea, chlorine (NaCl) are taken into the first few surface layers within seconds of deposition. Over time, water evaporates leaving residues of WSD behind. The depth of penetration of the latent fingerprint depends on the environmental conditions e.g. relative humidity (RH), porosity of the surface. Once absorbed, the WSD are relatively preserved under "normal" conditions (RH < 80%). As the fingerprint ages, amino acids remain stable but other components migrate. The non-water soluble deposits (NWSD) e.g. fats, waxes, alcohols remain longer on the surface.

1.4.2. Non Porous Surfaces[4]

A non-porous surface is defined as one that does not absorb any component of the latent fingerprint deposits e.g. polyethylene plastic bags, glass, glazed ceramics, and glossy paints. An emulsion of WSD and NWSD remain on the surface for a significant period unless destroyed through environmental or aging effects, deliberately rubbed off or washed off using organic solvents and water.

1.4.3. Semi Porous Surfaces[4]

A semi-porous surface is defined as any surface with intermediate characteristics e.g. painted surfaces, polymer banknotes and varnished wood. The surface absorbs WSD slower than porous surfaces and NWSD do not remain on the surface as long as on non-porous surfaces.

1.5. Fingerprint Development Techniques

A fingerprint detection sequence should always be initiated with an optical assessment because this is non-destructive. The simple observation of an object under white light may reveal a visible finger-mark that can be photographed without any further treatment. Marks contaminated with coloured materials e.g. blood can be considerably enhanced using selective absorption techniques. Latent fingerprints can be revealed with more complex optical detection methods.

1.5.1. Optical Detection Techniques[4]

The absorption mode is useful for the enhancement of visible fingerprints. For example, fingerprints in blood can be optically enhanced as dry blood has a strong absorption peak at 415 nm. Fingerprints that have been chemically developed can also be optically enhanced using the absorption technique.

Lasers have been proposed for detecting untreated fingerprints on non-fluorescent surfaces.[6] Four types of lasers have been used in the past: argon ion, copper vapour, Nd:YAG and the tuneable dye laser. A good starting point is to illuminate an evidential object with a high-intensity blue light source (e.g. 450 nm) and observing the fluorescent prints through yellow/orange goggles. This type of visual examination should be carried out in complete darkness to get the best results.

Oblique lighting on shiny surfaces can reveal latent fingerprints, fingerprints contaminated with dust and fingerprints developed using powders, cyanoacrylate (CA) fuming and vacuum metal deposition.[7] Prints will be visible as light images against a dark background as the light is diffusely reflected from the fingerprint ridges.

Japanese workers originally proposed the optical detection of latent fingerprint by shortwave UV reflection. This technique requires the use of a UV-sensitive charge-coupled device (CCD) camera (with a quartz lens) and a UV light source. The Hamamatsu Company (Hamamatsu City, Japan) has marketed a complete Reflective UV Imaging System (RUVIS) for the detection of trace evidence e.g. fingerprints, footwear impressions and bite marks on skin. Other systems include, KrimeSite™ Scope marketed by Sirchie® and SceneScope™ marketed by SPEX Forensic Group in 1997. The technique is based on the contrast between the surface, which may absorb or reflect UV light and the fingerprint deposit that absorbs some UV radiation and diffusely reflects the remainder. Thus, it gives either light ridges on a dark background or dark ridges on a light background. In order to get satisfactory results, the type of UV lamp and angle of incidence of the UV illumination play critical roles. It was found that RUVIS was very good when used with CA-fuming and dye-stained surfaces that are fluorescent when excited with a light source at wavelengths between 400 and 580 nm.[8]

It has been reported that illumination of latent prints on paper using the 266 nm radiation from a frequency quadrupled Nd:YAG laser, with photographic detection of their fluorescence (emission in long wave UV region 300 – 400 nm) can produce images with good ridge detail.[9] It has also been reported that the aromatic amino acids tyrosine and tryptophan account for approximately 80% of the UV fluorescence from finger sweat extracts.[10]

Some surfaces such as white glossy paper contain optical brighteners, which absorb UV light and produce a strong fluorescence in the visible spectrum. This can reveal latent marks especially if they are contaminated with UV-absorbing material. Marks of this type will be seen as dark images against a bright fluorescent background and only a UV lamp is required to view this.[11]

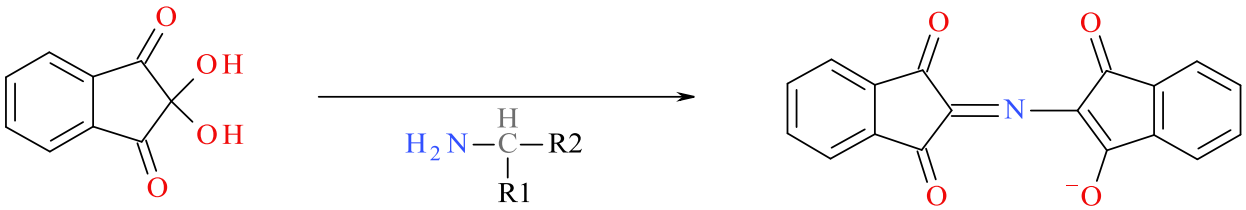
1.5.2. Fingerprint Detection Techniques on Porous Surfaces[4]

Two of the most common compounds used to develop latent fingerprints are ninhydrin and 1, 8-Diazafluorene-9-one (DFO). Both of these compounds react with amino acids present in latent fingerprint residues, however, unlike ninhydrin, DFO produces a fluorescent product thus increasing its sensitivity. Other techniques also exist which react with sebaceous constituents present in latent fingerprint residues. Physical developer (PD) and multi-metal deposition (MMD) are more commonly used. Both of these methods have a great advantage over ninhydrin and DFO in that they can recover fingerprints from wetted surfaces.

Ninhydrin

Ninhydrin was first synthesized in 1910 by Siegfried Ruhemann, a professor of chemistry at the University Chemical Laboratories at Cambridge University. Ruhemann discovered that this compound reacted with ammonia and amines to yield coloured products but it was not until 1954 that two Swedish scientists, Odén and von Hofsten recognized that it was a useful reagent for developing fingerprints. Since then, ninhydrin has become the most popular technique for finger-mark detection on porous surfaces.

Ninhydrin reacts with primary and secondary amines e.g. amino acids, peptides and proteins to give a dark purple product called Ruhemann's purple (RP) (Figure 5). Ninhydrin is a nonspecific amino acid reagent.



Ninhydrin (colourless) **amine** **Ruhemann's Purple (dark purple)**

Figure 5: Reaction of ninhydrin with a secondary amine[12]

Although the structure of RP was determined by Ruhemann in 1911, X-Ray studies carried out by Grigg et al. (1986) further confirmed the structure of the final

product.[12] Even though the mechanism for its formation has been explored by many groups for over 90 years, the accepted mechanism for the formation of Ruhemann's purple was first proposed by Friedman and Williams as illustrated in Figure 6. Although this mechanism seems simple, it may be more complex due to different pH conditions of the solution.

Early formulations of ninhydrin posed several problems such as low sensitivity, high background staining, solvent mixtures were highly flammable and organic solvent mixtures caused ink and dye in the paper to run thus obscuring fingerprints. Several of these problems were overcome by using a CFC113-based formulation. However, CFC113 was banned in 1992 due to its destructive nature to the ozone layer. More recently, 3M™ Novec™ HFE-7100, a hydrofluoroether was introduced as a non-ozone depleting replacement.[13]

Ninhydrin can be applied and developed in several ways. The most popular method of application is dipping. Others applications include, spraying a mist, or swabbing the formulation onto the paper. For maximum sensitivity and less background staining, samples need to be left to develop in the dark at room temperature to prevent prints from fading in the light. However, full development can take weeks, which is not ideal for a forensic laboratory. Current methods use 65% relative humidity to accelerate print development using ninhydrin.

Many new formulations were made over a 40-year period by trying to improve the optical properties of ninhydrin, but these were unsuccessful. In 1982, the first three ninhydrin analogues synthesized specifically for fingerprint development were reported by Almog et al. They showed that modifying the aromatic ring was more favourable and still preserved the reactivity of the cyclic triketone.[14]

Also in 1982, Herod and Menzel discovered that ninhydrin developed fingerprints when treated with zinc chloride solutions were highly fluorescent under an argon laser as well as able to change colour.[15] This improved contrast on various backgrounds and increased the number of prints able to be visualised due to increased sensitivity of fluorescence methods over absorption methods.

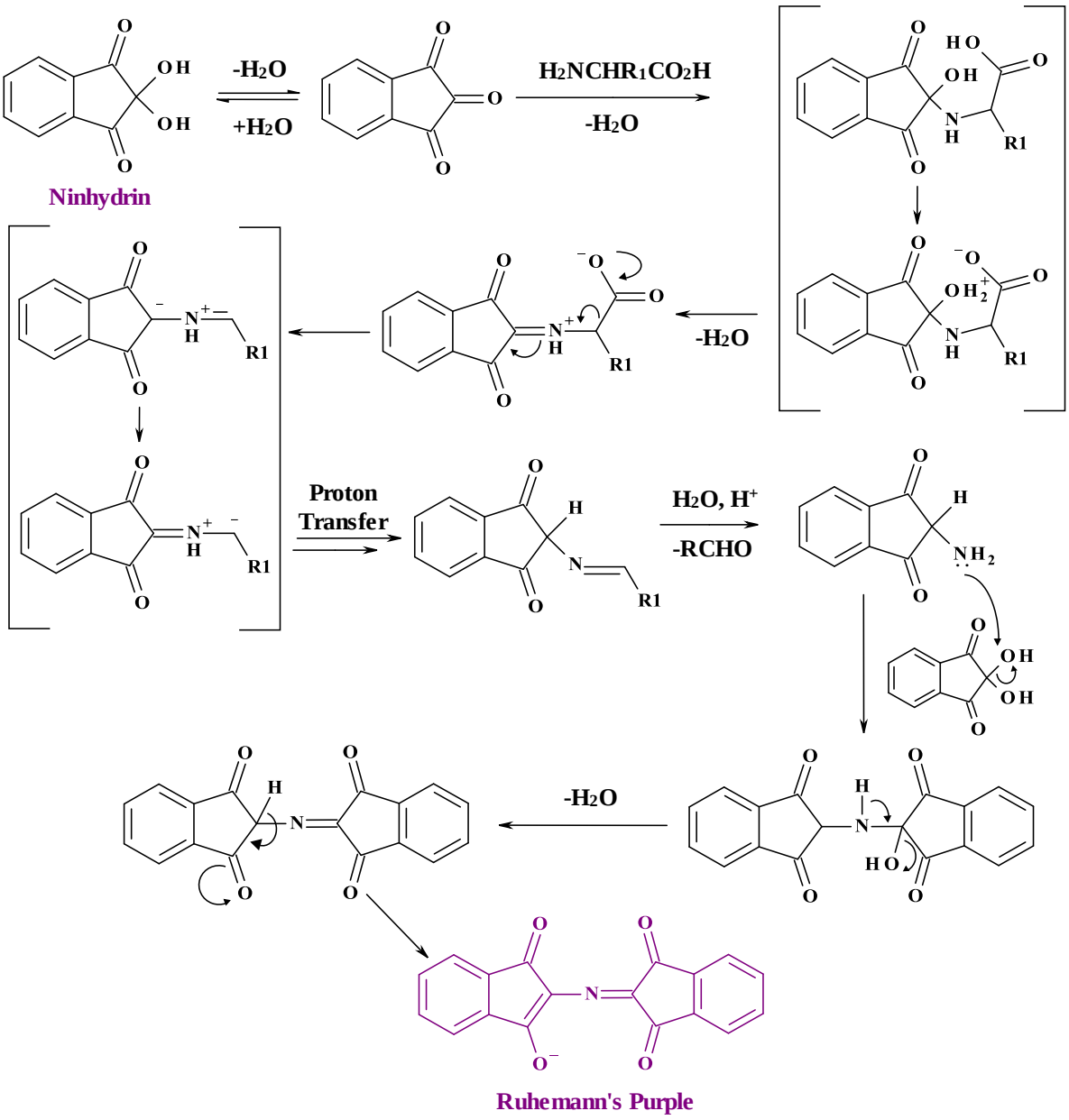


Figure 6: Mechanism for the reaction of ninhydrin with an amino acid[16]

Nearly 100 ninhydrin analogues have been synthesized since 1910. Benzo[*f*]ninhydrin developed dark green fingerprints and when combined with zinc chloride the complex formed exhibited fluorescence. 5-Methoxyninhydrin had lower intensity of colour thus no advantage over ninhydrin.[17] Thieno[*f*]ninhydrin displayed good chromogenic and fluorogenic properties after zinc chloride treatment.[18] Bis-ninhydrin analogues showed that each half behaved as an isolated ninhydrin

molecule.[19] 2-thienyl and 3-thienyl analogues showed promise due to their fluorogenic properties with no other treatment.[16]

The reaction of metal salts e.g. zinc (II) and cadmium (II) salts with RP produced fluorescent products. The intensity was improved by cooling prints in liquid nitrogen before observation.[16]

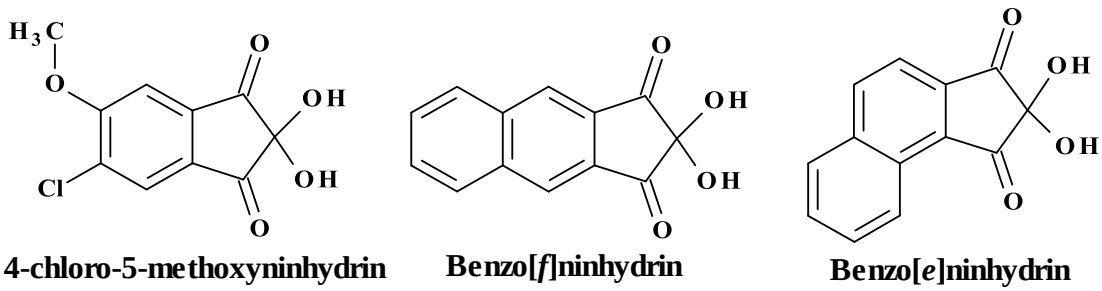


Figure 7: First ninhydrin analogues[20]

1, 8-Diazafluorene-9-one (DFO)

Although this is not a direct analogue of ninhydrin, the production of DFO was a major achievement because it showed increased sensitivity for amino acids and unlike ninhydrin, DFO developed fingerprints that were highly fluorescent without secondary treatment with metal salts. This increase in sensitivity has been shown to reveal more fingerprints than ninhydrin. In 1990, Grigg and Pounds prepared DFO using the Druey and Schmidt (1950) method as shown in Figure 8.

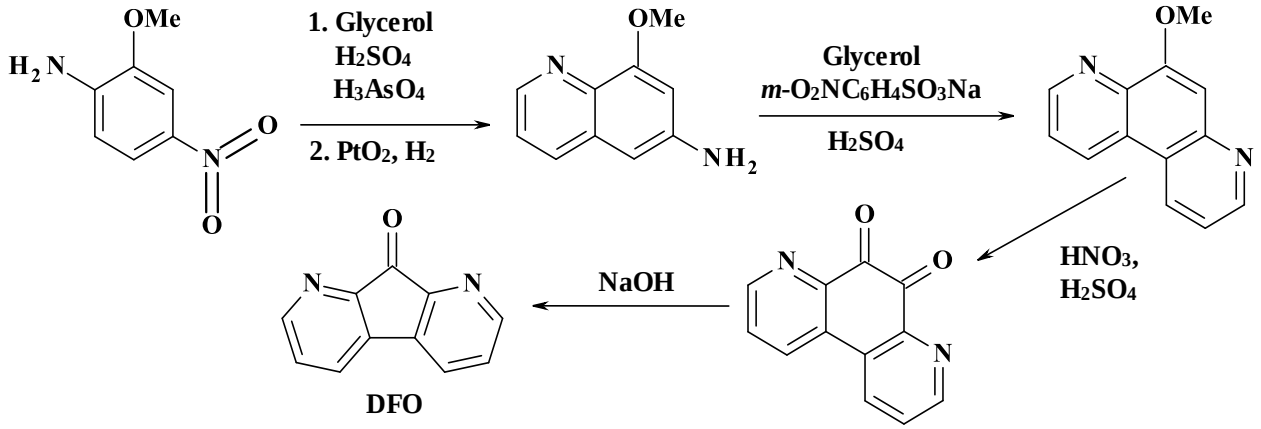


Figure 8: Mechanism for the formation of DFO[21]

DFO was found to react with α-amino acids to give a red product, an analogue to RP. However, DFO, whilst commercially available, it is significantly more expensive than ninhydrin and requires specialized light sources for accurate observations. In addition, its development is more demanding as high temperature and dry heat is needed to drive the development reaction. Therefore, it has been said that secondary treatment of DFO-developed prints with ninhydrin has been the most effective processing sequence to maximize the number and quality of fingerprints.

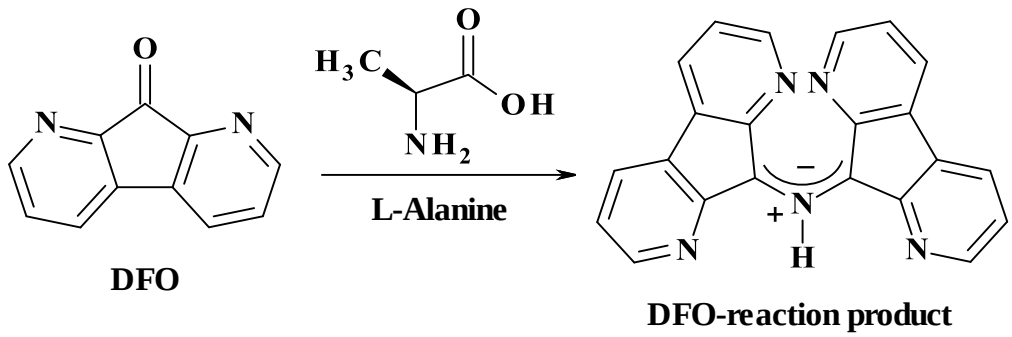


Figure 9: Reaction of DFO with an amino acid[20]

More recently, Bratton and Juhala (1995), reported applications of DFO in the dry state to develop latent marks on papers. The DFO is applied from DFO-soaked filter papers and processed with a steam iron filled with 5% acetic acid solution, then heated in a mounting press at 100˚C for 10 minutes. This "DFO-dry" procedure provides the same fluorescence intensity but prevents ink running, document damage or background-induced fluorescence.[22]

Physical Developer (PD)

This finger-mark processing technique was developed by the Atomic Weapons Research Establishment (AWRE) under contract to the Police Scientific Development Branch (PSDB – UK) in the 1970's. This technique is sensitive to the NWSD, so is still effective even if the surface is wet. It is thought to react with a combination of sebaceous and eccrine constituents in a latent finger-mark residue.[23] The finger-marks developed are of a dark grey/black colouration due to the deposition of silver metal along the fingerprint ridges. The PD solution is a delicate balance of ferrous, ferric and silver ions stabilized by the presence of citric acid and a cationic surfactant. The

citric acid is used to mop up the ferric ions and maintain a low pH. The surfactant (*n*-dodecylamine acetate), suppresses the growth of spontaneously formed silver colloids in solution by surrounding a negatively charged silver colloidal particle in a staggered manner, resulting in a positively charged micelle.

When a document is placed in the PD reagent, silver slowly deposits from the solution. A conclusive explanation as to why the stabilized PD reagent can selectively develop latent marks on paper is yet to be found.

$$Fe^{2+}_{(aq)} + Ag^{+}_{(aq)} \rightleftharpoons Fe^{3+}_{(aq)} + Ag^{0}_{(s)}$$

Figure 10: Formation of silver metal using PD[4]

As PD reacts with the NWSD, it is complementary to amino acid reagents such as ninhydrin so can be combined to increase the revelation of ridge detail. PD-developed marks can be enhanced with bleach as it can lighten the background and darken the print due to the formation of silver oxide. This can be valuable for improving the contrast on dark surfaces e.g. brown paper. PD is a sensitive technique and research is currently being undertaken by the U.S. Secret Service to develop a copper-based physical developer to lower costs and give fluorescent PD-developed marks.

Figure 11: Physical developer on an account book[24]

Multi-Metal Deposition (MMD)

MMD was developed by George Saunders in 1989. The technique itself involves a two-step process. In the first step, the item is immersed in a colloidal gold solution (pH ~ 2.7). Colloidal gold is prepared by treating gold chloride with sodium citrate and a detergent. The negatively charged colloidal gold particles are attracted to organic groups containing positive groups at low pH. Gold deposits on any fingerprint residue on the immersed item. Weak marks will be barely visible but stronger marks may be pale salmon pink in colour. The second step involves the developed finger-marks to being with a modified physical developer (MPD) solution. The bound colloidal gold provides nucleation sites around which silver precipitates from the MPD solution. This amplifies the visibility of the marks and the colour varies from light grey to black.[25]

This technique has improved in its sensitivity and specificity by using silanized glassware and 14 nm-colloidal gold particles instead of 30 nm-colloidal gold particles as in the original formulation. Furthermore, a PD solution based on silver acetate and hydroquinone is favoured over the conventional PD solution of silver nitrate and ferrous/ferric redox system.[26] These modifications have led to the optimized process recognized as, MMDII.

Figure 12: Example of finger-mark developed on a porous surface (white paper) using the old method (left) against the proposed optimized method (right)[26]

1.5.3. Fingerprint Detection Techniques on Non-Porous Surfaces

The most commonly used technique to detect latent fingerprints on non-porous surfaces is fingerprint powders. Fingerprint dusting formulations can be placed in four categories: regular, fluorescent, metallic and magnetic powders. Other techniques are small particle reagent (SPR), a non-vapour phase technique, vapour phase techniques such as vacuum metal deposition (VMD) and the most common development technique, cyanoacrylate (CA) fuming more commonly known as Superglue fuming. Usually these techniques are adapted or used in sequence to enhance latent fingerprints further.

Fingerprint Powders

One of the first powders used was a finely ground mixture of mercury and chalk, by Sir Edward Henry in 1905, which was applied using a camelhair brush. However, the toxicity of mercury has led to the abandonment of this powder. Most of these powders and methods of application were used without an understanding of the mechanism of adhesion of the powder to the fingerprint deposit.

Attempts to obtain this understanding were made by Blott and Scruton (1973) by measuring the force required to detach powder particles from a developed fingerprint. By comparing, the measured values with those calculated based on theoretical models of the possible mechanism; the major cause of adhesion can be deduced. Aluminium powder was used in this study. On the fingerprint ridges, most particles could be removed by a field of 3×10^8 V m^{-1}. Application of powder to fingerprints by brushing is a well-established technique but has one disadvantage; contact of the brush with the fingerprint has an inevitably destructive effect so a degree of care is required by the operator.[27]

Thomas reported a rather novel way of applying the powder without having to use a brush, which was originally devised by Roy (1975), known as the electrostatic deposition method.[27]

A new technique of developing fingerprints on paper and detecting the presence of fingerprints on a range of insulating substrates was developed by Morantz et a1. (1978).[28] If a charge is sprayed onto the examining surface, the material between the

surface and an earthed plate will become polarised. This reduces the effective charge density in varying degrees depending on whether or not a fingerprint is present and an electrostatic image of the fingerprint is produced. The resulting charge image on the membrane can easily be developed by applying an appropriate toner powder.[29] However, other methods of application now exist via the use of an atomizer, an aerosol spray, magna-brushes and magna-powders.

Fingerprint dusting formulations can be placed into four categories: regular powders, metallic powders, fluorescent powders and magnetic powders:[30]

Regular Powders

These powders consist of a resinous polymer e.g., starch, kaolin, rosin and silica gel for adhesion of the oils and moisture in sweat residues and a colorant (inorganic salts or organic derivatives used to adsorb onto the adhesive) for contrast. Formulations used previously are, Ferric oxide with rosin, manganese dioxide with rosin and titanium dioxide with kaolin. Lamp-black and Fuller's earth are two commercially available inorganic-based powders used for fingerprinting. Iron oxide black powder has been known to develop prints on the adhesive side of tape.[31] Over the years, commercial inorganic-based powders containing mercury, lead, titanium, cadmium and manganese salts were found to be toxic to the user so powders containing organic dyes became more popular. For improved results, powders with fluorescent and laser-active dyes such as Fluorescein and rhodamine B were used. Resins are very expensive so to lower costs, these were replaced with cheap insoluble salts and used with xanthen dyes.[32] This gave sharp, clear prints on paper, plastic, glass, Bakelite, enamelled metal and polished wood.

Fluorescent Powders

These consist of natural or synthetic organic derivatives that fluoresce or phosphoresce upon exposure to UV or laser light. Prints can be visualised on multi-coloured surfaces. Examples of these powders are crystal violet (gentian violet) (Figure 13), rhodamine B, acidine yellow, acridine orange and coumarin 6. Sodhi et al. (2001), found that fluorescent eosin blue dye develops weak prints under UV

light.[32] These powders allow the detection of fingerprints on surfaces, which would normally be difficult to obtain prints from e.g. wood, masking tape and polythene.

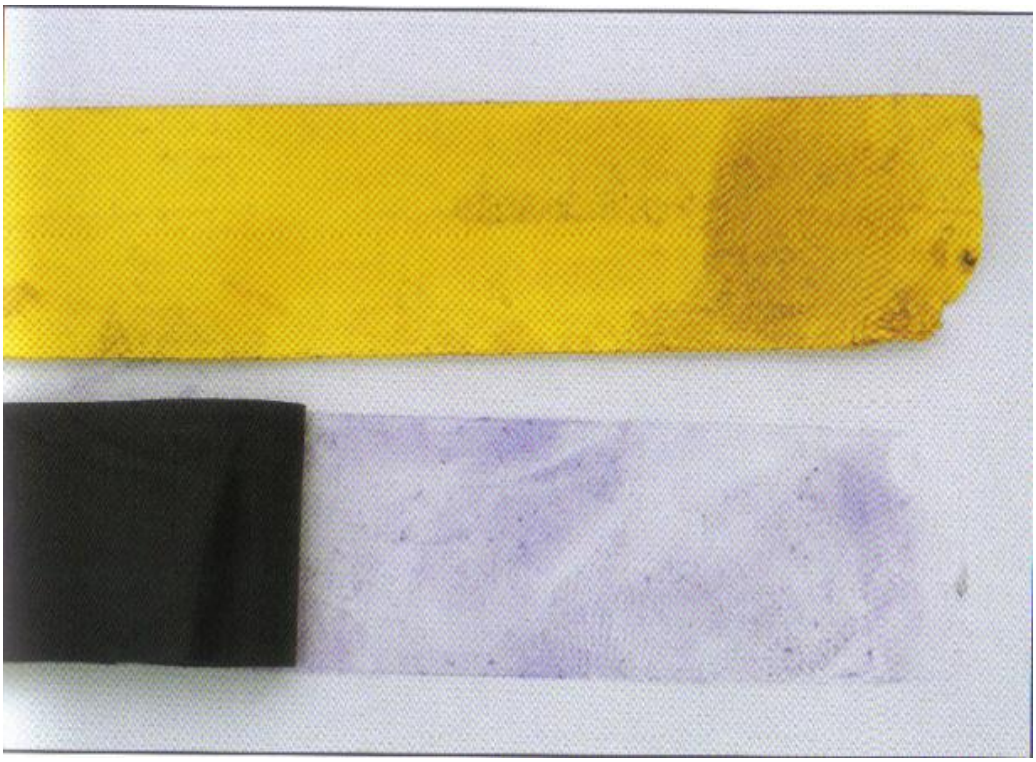


Figure 13: Gentian violet on yellow adhesive tape and transferred from black adhesive tape[24]

Metallic Powders

These formulations contain meshed metals, which are known to have a longer shelf life than organic-based powders. Silver powder is made up from aluminium flake and quartz powder; gold powder is made up of bronze flake and quartz powder and grey powder is made up of meshed aluminium and kaolin. Aluminium-based powders were made up with aluminium flakes (5-10 μm long, 0.5 μm thick) and stearic acid (3-5% w/w). By increasing the stearic acid content to 10% w/w, the powder was much more efficient and produced better contrast in developed prints due to lower background.[33] Toxicity of metallic powders depends on the metal, so the user has to take necessary precautions when using them.

Magnetic Powders[4]

These are generally made by mixing coarse iron grit with aluminium or copper flake powder and applied using a magnetic wand. The coarse magnetic particles form the "brush", while the fine powder develops the prints (Figure 14). The use of magnetic powders avoids brushing, thus is non-destructive. New magnetic applicators have been produced incorporating powerful rare-earth metals instead of using permanently magnetized steel rods. The new applicators with the magnetic flake powders provide a rapid and efficient means of developing prints over large surface areas. In addition, smooth surfaces can be removed of all traces of powder after fingerprint development by passing over the surface with a clean rare-earth magnetic applicator.

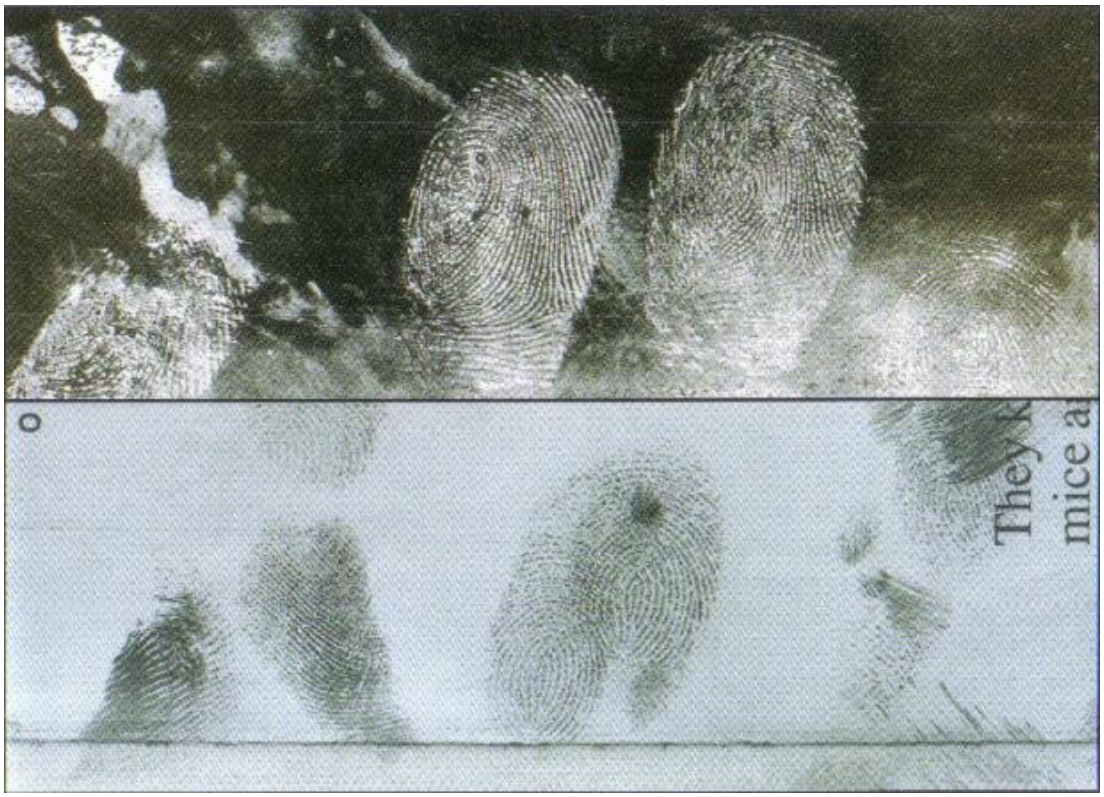


Figure 14: An aluminium powder lift (top), black magnetic powder on a magazine cover (bottom)[24]

Small Particle Reagent (SPR)

First reported by Morris and Wells in 1979, SPR consists of a suspension of fine molybdenum disulfide particles in detergent solution. The particles adhere to the fatty constituents of latent print residues and form a grey molybdenum disulfide (MoS_2) deposit. A detailed procedure and formulation were described later by Goode and Morris in 1983. Pounds and Jones (1981) recommended the use of MoS_2 dispersed in Manoxol OT ($C_{20}H_{37}NaO_7S$)[34] and the prints could be lifted with clean lifting tape once they were dry.

The SPR solution has been successfully used to develop latent prints on paper, cardboard, metal, rusty metal, rocks, concrete, plastic, vinyl, wood, and glass. Similarly, latent prints have been developed on sticky surfaces, such as soda cans and candy wrappers. Margot and Lennard (1994) found that the crystalline structure of the MoS_2 used in the recipe tend to have a significant effect on latent print development results. They recommended using ROCOL™ AS powder, made up as 10 g with 0.8 ml Tergitol 7 in a 100-mL stock solution. This stock is then diluted 1:10 for dipping and 1:7 for spraying.

Franck and Almog (1993) tested other particles rather than MoS_2 in suspension. The best results were seen with 0.66 g zinc carbonate, 20 ml water, 0.06 g Tergitol 7, and 55 g dimethyl ether. They further noted that particles of 2 μm average size adhered better than 6 μm.[35]

Springer and Bergman (1995) have described the preparation of SPR containing the fluorescent dyes Rhodamine 6G and Brilliant Yellow 40 (BY40). The preparations all worked initially, but the R6G reagent had poor shelf life. A BY40 suspension of 100 mL of 0.1% BY40 in ethanol mixed with 100 mL stock SPR showed consistently good results.[36]

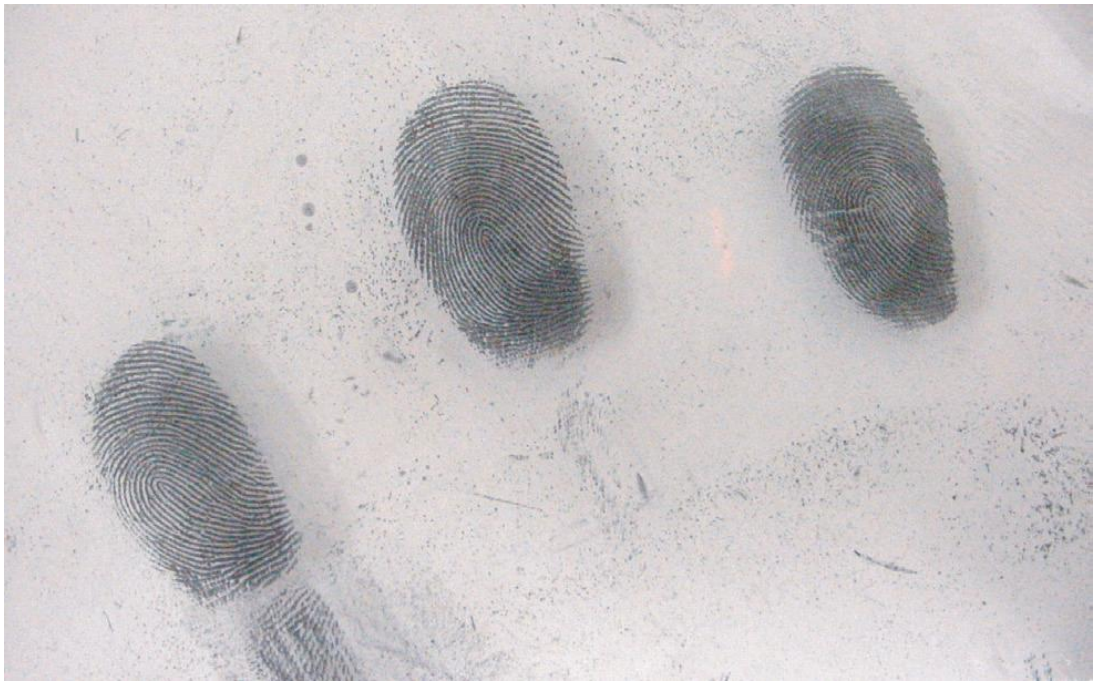


Figure 15: Latent finger-marks on wet glass developed using small particle reagent (SPR)[37]

Cyanoacrylate Fuming (Superglue Fuming)

The method was first devised by the Criminal Identification Division of the Japanese National Police Agency in 1978. In 1982, latent fingerprint examiners working at the U.S. Army Criminal Investigation Laboratory in Japan (USACIL-Pacific) and in the Bureau of Alcohol, Tobacco and Firearms (BATF) laboratory introduced a novel procedure to the U.S. that used alkyl-2-cyanoacrylate ester (superglue) as a means of developing latent prints. Since then, CA fuming has become the most widely used laboratory-based process for the development of fingerprints on non-porous surfaces.

Cyanoacrylate esters are colourless, monomeric liquids. CA liquid forms a vapour that reacts with specific eccrine and sebaceous (oils) components in a latent fingerprint deposit to form a hard, white polymer (poly-cyanoacrylate) (Figure 16).

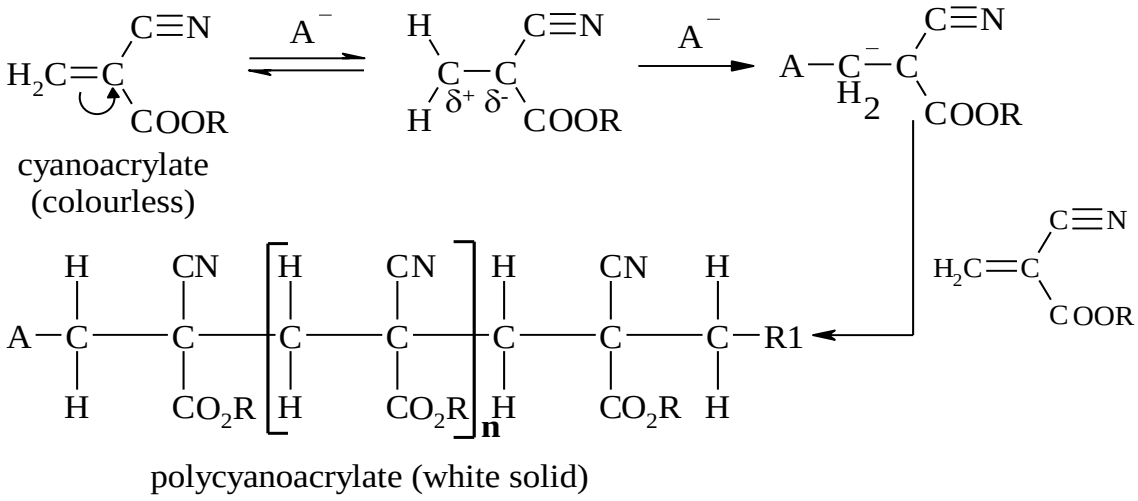


Figure 16: Cyanoacrylate polymerisation reaction[4, 20]

Numerous methods have been proposed and are commercially available. Simple homemade systems can be constructed at minimal cost by using a glass chamber that can be sealed to contain the CA vapour generated by a suitable heat source, e.g. a temperature-controlled hot plate. Items are placed in the chamber and a small amount of liquid CA glue placed in an aluminium boat is heated to 80-100°C to produce the vapour. The ideal relative humidity should be kept at 80% as it catalyses the polymerisation process.

However, although commercial chambers are more expensive, they are advantageous as they have better temperature and humidity control, an automatic vapour release mechanism and more efficient vapour circulation producing evenly developed prints.

Vacuum CA-fuming (VCA)

This technique was first developed by Watkin of the Royal Canadian Mounted Police (RCMP) Identification Division in 1990. Objects are placed in a large metal chamber with a small amount of liquid CA glue; the pressure is reduced to 0.2 torr via a rotary pump. The CA vaporises more easily and so evaporation is accelerated resulting in reduced development time. Under vacuum, the vapour spreads quickly to all parts of the chamber, thus fingerprints can be developed on surfaces not exposed directly to the fumes. Prints were more uniformly developed with sharper ridge and pore detail. Enhancements were carried out to improve the contrast of VCA-developed fingerprints as they were found to be smoother and translucent compared to

conventional CA-fumed prints, which were found to be more fibrous under a scanning electron microscope.

Enhancement of CA-Developed Prints

Enhancement using RUVIS has been reported, but in order to enhance the contrast of prints, coloured or fluorescent staining could be applied (Figure 17). Coloured stains for example, gentian violet could be used however, the use of fluorescent stains is preferred because it has greater sensitivity and can be applied to a larger range of surfaces. The most popular fluorescent stains used are Rhodamine 6G, Coumarin 540, Ardrox 970-P10 (A), basic yellow 40 (BY40), basic red 28 (BR28).

Low-level superglue fuming and staining with BY40 or superglue fuming followed by staining with rhodamine 6G or 4-(4-methoxybenzylamino-7-nitrobenzofurazan) (MBD) was also very effective in revealing prints from adhesive surfaces.[38]

Table 2: Approximate absorption and emission maxima of dyes and dye mixtures used to visualise cyanoacrylate developed latent prints[20]

Dye/Dye Mixture	Absorption Max (nm)	Emission Max (nm)
Rhodamine 6G (R6G)	525	555
Ardrox (A)	380	500
MBD (M)	465	515
R6G + A + M	460	555
BY40	440	490
BR28	495	585
Styryl 7 (S7)	555	680
BY40 + BR28	445	590
BR28 + S7	510	680
BY40 + BR28 + S7	445	680

Figure 17: Basic red 14 dyed superglue on a tiled wall[24]

Vacuum Metal Deposition (VMD)

Fingerprints on a surface can hinder the deposition of metallic films resulting from metal evaporation under vacuum. This phenomenon was known for some time, but it has only been applied to detect latent fingerprints since 1968 by Theys et al.[39]

Gold is evaporated under vacuum to form a very thin layer of metal on the surface and penetrates the fingerprint deposit. A second layer of zinc is deposited in the same manner. The zinc preferentially deposits on the exposed gold and does not penetrate the fingerprint producing a negative print. The phenomena of reverse development, whereby the zinc deposits onto the print ridges and not the background was reported by Kent et al. (1976)[40] but the cause of this has not yet been established. The quality of this technique depends highly on the polymer type e.g. high density, low density and the presence of additives because they each have an effect on the amount and structure of the gold film deposition on the surface, which in turn can effect zinc deposition.[41]

A preliminary study was carried out by Flynn et al. (1999) and reported that VMD could be used to detect aged latent marks on polymer banknotes.[42] This was confirmed and optimized by Jones et al. (2003) whereby, VMD treatment applied after cyanoacrylate (superglue) treatment and before application of a fluorescent stain was able to develop latent marks on polymer banknotes that were over 6 months old.[43]

Figure 18: 3 year-old latent finger-marks on glass after treatment by VMD (negative prints)[44]

1.5.4. Fingerprint Detection Techniques on Semi-Porous Surfaces

Latent finger-mark detection on intermediate semi-porous surfaces such as, waxed paper, glossy paper, matte-painted substrates, rubber/latex gloves etc. can be problematic. Jones (2002) found that MMDII was predominantly effective on developing prints on latex and nitrile gloves, expanded polystyrene and waxed paper.[45]

However, the best approach is to use a combination of techniques for porous and non-porous surfaces in a logical order in order to develop and visualise the best quality fingerprint.

1.5.5. Miscellaneous Fingerprint Detection Techniques

The techniques above are most commonly used to develop latent fingerprints. However, various other techniques are used alongside the common techniques on more problematic surfaces:

Gentian Violet (Crystal Violet)

Gentian violet is a fat-soluble stain and the conventional procedure involves treating the adhesive side of tape with a solution of gentian violet mixed in phenol as it aids the absorption of the stain by the sebaceous material in the latent print.[46] These surfaces are generally treated by immersion in the reagent, followed by rinsing with water to remove excess stain and an intense purple coloration results. Arima (1981) developed this technique further.[47] He suggested using a fluorescent dye, Mikephor for coloured and black electrical tape, whereby latent prints could be visualised under UV light. Ishiyama (1981) reported the first successful development of prints on the adhesive side of cellophane tape using Coomassie brilliant blue 250.[48] Section 5.1 discusses this technique in more detail.

Iodine Fuming

Iodine fuming has been used for latent print development for at least a century. The mechanism was initially thought to involve the reversible addition of iodine to the double bonds of the unsaturated fatty acids in fingerprint residue by halogenation. Recent research by Almog, Sasson, and Anah (1979) suggests that the mechanism of interaction involves physical absorption rather than a chemical reaction.[49] When warming iodine crystals, violet iodine vapours form by sublimation. The fumes are absorbed by the fingerprint secretion residues to give yellowish brown latent prints. The iodine vapour is toxic, corrosive, the technique has limited sensitivity, it will generally only detect marks up to several days old, and the colour is not stable and is short-lived in air unless the iodine is chemically fixed. Mashito and Makoto (1977) proposed that improvement of the contrast and stability of prints was possible by "fixing" the iodine-developed fingerprint with 7,8-benzoflavone (α-naphthoflavone) reagent.[50] Wilkinson et al. (1996), found that iodine fuming followed by the spray

application of a 7,8-benzoflavone solution gave the most consistent results on surfaces such as wallpaper, emulsion-painted walls and aged gloss-painted surfaces.[51]

Silver Nitrate

Silver nitrate reacts with chloride ions in latent fingerprints to form light sensitive silver chloride. The silver chloride decomposes to silver metal upon exposure to light, thus producing a black image of the fingerprint. This technique is most effective with prints < 1 week old; because in older prints the chlorides start to diffuse through the material.[52] However, it is a destructive method and silver nitrate is an expensive reagent, which is unpractical.

Lennard and Margot (1988) discovered that the silver nitrate technique is sometimes effective for improving the contrast in weak ninhydrin-developed finger-marks, particularly on raw wood.[53]

4-dimethylaminocinnamaldehyde (DMAC)

DMAC is urea sensitive and since urea is a major component of eccrine sweat, it was considered a possible technique for developing latent fingerprints by Sasson and Almog in 1978.[54] For example, biological evidence analysts have used DMAC since the 1950's as the basis for a presumptive test for urine. DMAC can also react with primary and secondary amines, and the bases for its reaction with latent residue have been questioned recently. Brennan et al. (1995) found that using DMAC as a fuming agent by heating DMAC crystals to 150 – 200ºC produced good ridge detail on a range of substrates. After treatment, items were to stand overnight and developed marks were visualised under white light initially and then in the fluorescence mode with an excitation range of 450 – 530 nm and observation using a 530 nm cut-off filter. This vapour phase technique has shown promise for detecting finger-marks on problematic surfaces such as thermal paper.[55]

Radioactive Sulfur Dioxide ($^{35}SO_2$)

Radioactive sulfur dioxide has been used to develop prints on surfaces such as paper and fabrics. Grant et al. reported that when developing autoradiographs of paper treated with $^{35}SO_2$, dark spots seen were identified as fingerprints. In 1963, a more

detailed method was published whereby 10 ppm of $^{35}SO_2$ was measured into a Schlenk tube and the pressure was increased to atmospheric by the addition of air at 66% humidity. The paper sample was exposed to this gas mixture for 12 hours and then placed against an x-ray film for one week.[56]

In 1971, Spedding suggested that SO_2 reacts with the lipids present in fingerprint deposits and noted that reactions occurred with oleic and linoleic acids. The method he proposed consisted of a treatment box within which samples could be hung on a rail with 60% relative humidity. The samples were exposed to $^{35}SO_2$ for 30 mins and then removed and placed in contact with x-ray film. Only then, it was discovered that $^{35}SO_2$ could be used to develop fingerprints on fabrics.[57]

An article published in New Scientist (1974) reported that radioactive SO_2 reacts with fingerprints in two ways:

1. The SO_2 dissolves in water in the fingerprint and is fixed as sulfate by a strongly pH dependent reaction.
2. The SO_2 reacts with the fats in the residue in the gas phase.

It was also found that the water reaction produced background noise and so other isotopes were looked at in an attempt to suppress the water reaction whereby it was found that ^{82}Br looked promising.

1.5.6. Fingerprint Detection Techniques on Cartridge Casings & Firearms

The detection of latent finger-marks on fired cartridge cases is problematic and has a low success rate. Wiesner et al. (1996) reviewed and presented various feasible reasons for this: friction between the surface of the cartridge case and the surfaces of the firearm at different stages of the firing process, high temperature and pressure generated at the moment of firing and exposure of the surface of the cartridge case to combustion gases and discharge residues. Their studies showed that the main cause of finger-mark deterioration is the friction between the cartridge case surface and the chamber at the point of ejection because the diameter of the cartridge case increases due to the high internal pressure generated.[58] It appears that the composition of the cartridge case also affects the ridge detail recovery.[59] One technique used to develop

latent prints on cartridge cases is the gun-blue method, but usually a combination of techniques is used to get the best results.

Gun Blue Method

Migron and Mandler (1997) first reported this technique. The active ingredients in gun blue include selenious acid (H_2SeO_3) and a cupric salt in acid solution. Both the selenious acid and cupric ions can oxidize zinc, aluminium and iron. When these two components are reduced in the presence of such metals, a black copper-selenide coating is formed. This coating only forms on a clean metal surface resulting in transparent latent marks against a dark background.[60]

Developed marks could be preserved by dipping the cartridge cases in clear varnish to prevent further oxidation and had to be photographed immediately as further oxidation can destroy ridge detail. It was also reported that aluminium and nickel coated brass cases did not respond to treatment with the selenious acid surface oxidation method. A sequential treatment of vacuum CA-selenious acid-BY40 was carried out on 21 post-fired cartridge cases. Post-firing fingerprint ridge detail was recovered from 17 cases and 5 of these were classified as identifiable. Saunders and Cantu (1996) found that superior results were obtained with CA-fuming followed by gun blue treatment.[61]

Finally, Cantu et al. (1998) discovered that acidified hydrogen peroxide could be used to remove excess gun blue deposit if a cartridge case had been overdeveloped by changing the insoluble Cu-Se back their soluble species. The overall reaction can be seen in Figure 19 and the acidified hydrogen peroxide solution could be used to visualise latent marks on post-fired cartridge cases via an etching ("cleaning") process which can subsequently be enhanced via the gun blue treatment.[62]

$$3H_2O_2 + 2H^+ + \text{Cu-Se} \rightleftharpoons H_2SeO_3 + Cu^{++} + 3H_2O$$

Figure 19: Overall reaction to remove Cu-Se using acidified hydrogen peroxide[63]

A much more recent study has reported that the chemical sequence of Cyanoacrylate-Black powder-Acidified hydrogen peroxide-Rhodamine 6G has been determined as the optimum sequence to develop fingerprints from fired brass cartridge casings with

fresh latent prints placed on them. The optimum sequence for obtaining prints from fired nickel casings is Cyanoacrylate-Rhodamine 6G-Acidified-hydrogen peroxide-Black powder. This sequence has also been successful in developing fingerprints from shotgun shells.[63]

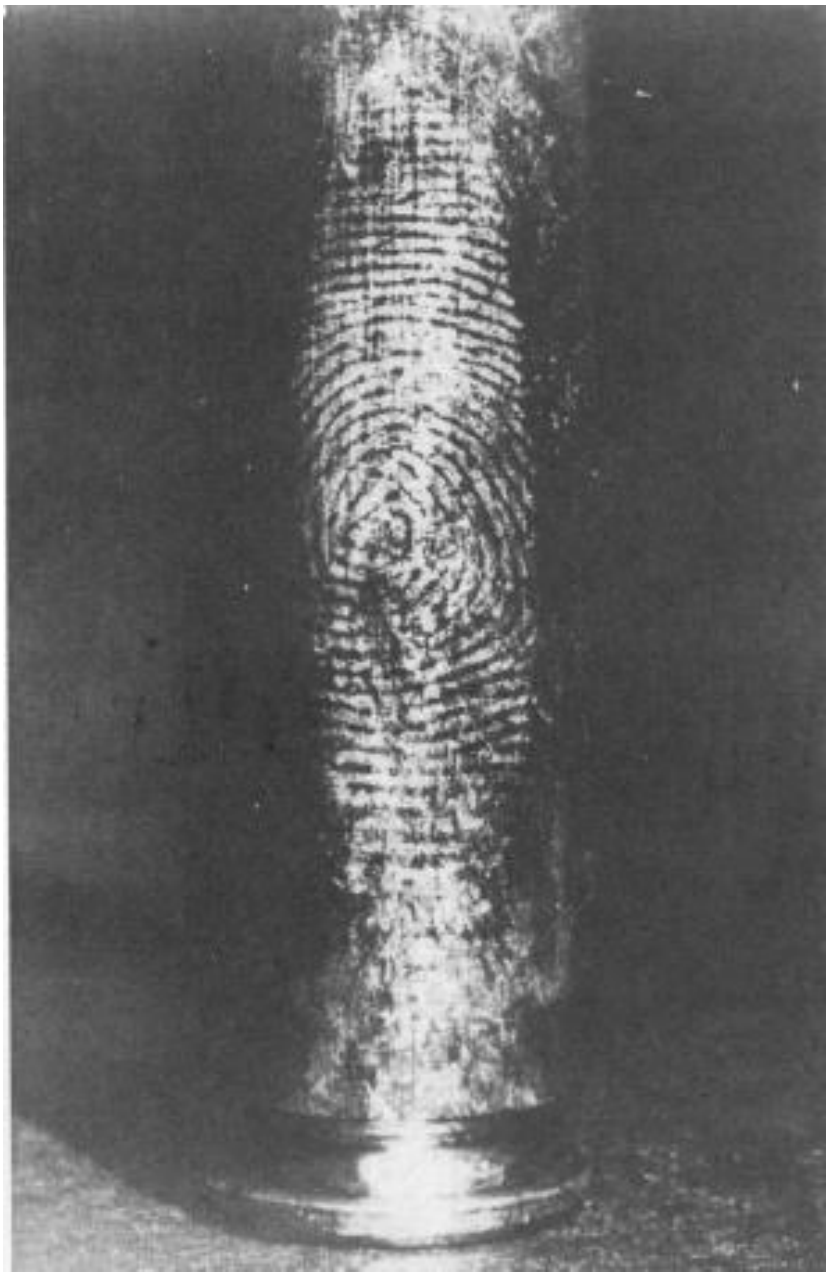


Figure 20: Latent finger-mark developed on a 7.62mm Nato cartridge case using selenious acid[61]

1.5.7. More Recent Fingerprint Detection Techniques

Overall, despite the existence of all these fingerprint development techniques, they all appear to have their limitations. Fingerprints deposited on cartridge cases that are more problematic are still difficult to develop using these techniques and as in many cases, sequential treatments are required, which can become very time-consuming. Techniques that are more recent involve the sebaceous components, as they are present for a much longer period of time, which means that older prints can now be developed on various surfaces.

Electro polymerisation process[64]

This method focuses on the fatty acids present in latent fingerprints acting as an insulator on the surface, where fingerprints are to be developed, which has led to the enhancement of aged prints on metal surfaces. The results are based on sebaceous prints deposited on metallic surfaces such as unfired weapons, gold, silver, iron and different alloys. The reaction consists in the electro polymerisation of pyrrole and substituted porphyrins e.g. tetra-*o*-aminophenylporphyrin (TAPP): radical cations are generated on superficial nucleation sites by oxidation of monomer, close to the electrode surface; subsequently the radical species react with the neutral monomer, which begins to diffuse to the electrode. PolyTAPP films are more suitable at enhancing fingerprints on silver and gold. Fingerprint legibility is affected by the morphological characteristics of the material of the polymer film. The outline sharpness, roughness and light reflection have to be determined by the polymerisation conditions: monomer concentration, potential scan rate and the supporting electrolyte. However, as of yet no further work has been published on using this method on fired weapons and ammunitions.

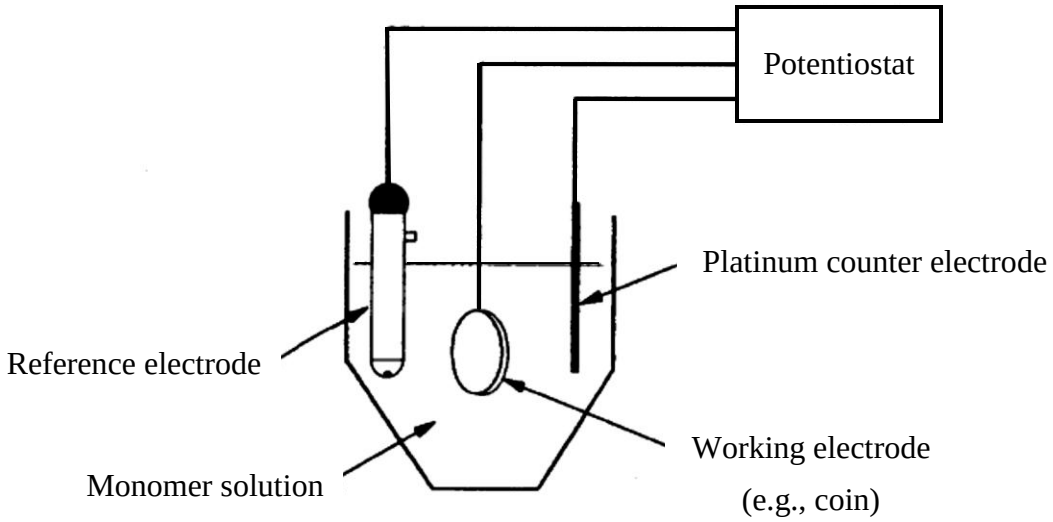


Figure 21: Schematic representation of the electrochemical cell[65]

Scanning Kelvin Probe[65, 66]

Electrochemical interactions between latent fingerprints and metal surfaces in air have been investigated by Volta potential mapping using the scanning Kelvin microprobe. The interaction between the metal surface and water soluble but non–volatile, inorganic salts (e.g. NaCl) present in latent fingerprint deposits resulted in a Volta potential decrease greater than 200 mV which may persist for months allowing the prints to be visualised by potential mapping. This was proven when fingerprints were heated to 600ºC as even though the organic components were volatilized the prints were still developed. It has been demonstrated that the SKP can effectively visualise latent eccrine finger-mark patterns on iron and brass for both smooth and roughened surfaces, discernible ridge detail can be retrieved even if the finger-marks had been physically removed by rubbing with a tissue and latent prints present beneath intact insulating films (e.g. paint, oil or grease) can still be visualised. In addition, although eccrine finger-mark detail becomes indistinct under smoke film contamination, sebaceous patterns remained clear even when covered with thick, optically opaque smoke layers and eccrine finger-mark deposits were visualised successfully on post-fired brass cartridge cases.

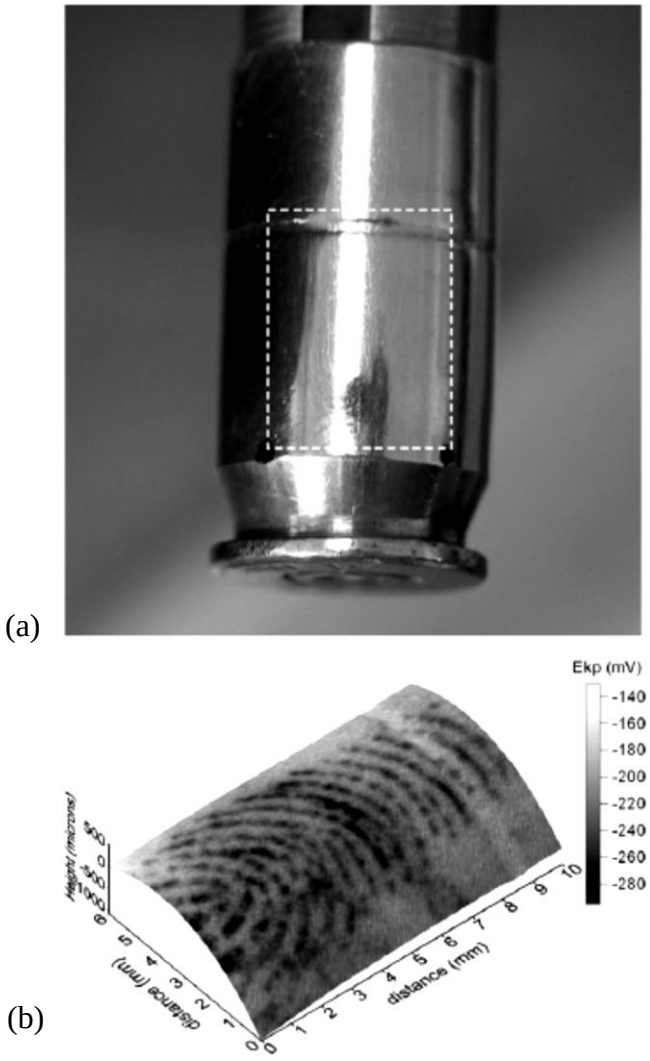


Figure 22: Image showing a region of a 0.45 in. calibre cartridge case with a latent eccrine fingerprint and its corresponding Volta potential pattern, superimposed onto a height profile, obtained by SKP[66]

Since 2001, extensive work has been carried out to visualise latent fingerprint corrosion of metallic surfaces by heating the metal up to temperatures of ~600ºC. The ionic salts present in fingerprint residue corrode the metal surface to produce an image of the fingerprint that is both resilient and resistant to cleaning of the metal. The degree of enhancement is dependent on the composition of the metal and the levels of salt secretion by the fingerprint donor. The corrosion itself can be exploited and fingerprint enhancement can be carried out based on the electrostatic charging of the metal and the preferential adherence of a metallic powder to the corroded part of the metal surface.[67]

Other electrostatic detection methods exist, for example, Electrostatic Detection Apparatus (ESDA). More details on ESDA can be found in Chapter 4.

Gelatine Lifting[68]

The concept of using gelatine lifting to recover fingerprints was first proposed in 1913 for lifting of marks powdered with lead acetate prior to treatment with hydrogen sulfide. The lifting medium used was paper coated with a gelatine/glycerol mix[69]. By the late 1970's several rubber and gelatine-based lifters were commercially available and PSDB conducted experiments utilising gelatine films to lift marks developed using vacuum metal deposition from patterned surfaces[70]. Physical developer was then used to enhance the lifted marks, which was successful provided a high contrast mark was produced by VMD in the first instance.

As a result of those preliminary studies, gelatine lifts are now marketed for lifting footwear marks, paint and micro traces, blood traces and recording patterns around bullet holes[71] and has shown to be effective at revealing indented writing and has often shown better performance than ESDA on thick, glossy paper types and to be capable of being used sequentially after ESDA on documents.

Gelatine lifters are available in black, white and clear forms, which can be an advantage if optimum contrast is needed depending on the powder or fuming technique used to reveal marks. In addition, the lifters are flexible and can be compressed against a surface, which prevents sample loss.

Surface with relief features, grease & loose particles (e.g. dust, fingerprint powder)

Apply black gel lift and smooth down

On removal of gel lift, loose particles/ grease adhere to the surface and relief features leave indentations

Figure 23: Schematic diagram to show how gelatine lifts can lift and reproduce surface features[68]

Disulfur Dinitride (S_2N_2)

S_2N_2, a four-membered square molecule is generated by thermal cracking of S_4N_4 through silver wool (N.B: Both S_4N_4 and S_2N_2 are friction sensitive). It has been revealed to interact with latent fingerprints on a wide range of surfaces resulting in the prints being visually imaged by polymeric $(SN)_x$, a dark blue/black polymer. Although the nature of the initial interaction between the S_2N_2 and the components in a latent fingerprint residue has not been fully established yet, the fact that prints can be obtained from paper soaked in water or ether and then dried suggests that more than one constituent can interact with the nitride. Prints have been successfully developed on paper, pottery, aluminium foil, cling film, glass, cotton, spent blank gun cartridges and many more. Clearly, there are limitations to the technique in that the S_4N_4 must be carefully handled and the apparatus required to generate the S_2N_2 is not portable. However, the advantages outweigh this because the technique itself is: non-destructive; non-solvent based, the exposure time is very short as prints start to develop within a few minutes; the developed prints can be seen with the naked eye, and they are air stable for many days but can also be preserved long term under a nitrogen atmosphere. However, there is still an ample amount of work being done using this $S_2N_2/(SN)_x$ system at present.[72]

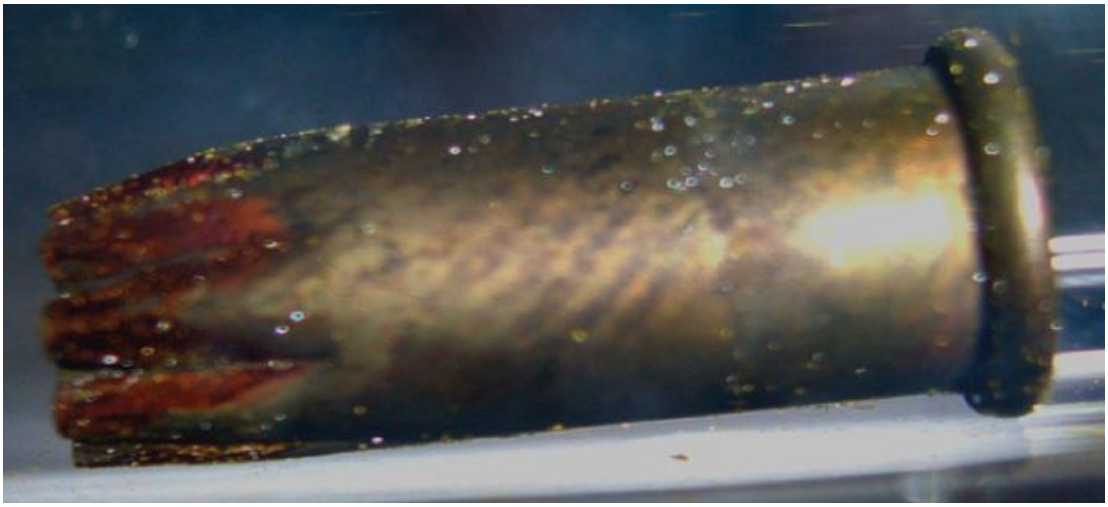


Figure 24: Fingerprints (deposited before firing) developing on a spent blank gun cartridge, photographed *in situ* during exposure to S_2N_2[72]

Antibody Bound Amino Acids Conjugated to Gold Nanoparticles[73]

Several techniques have been previously reported on the use of antibodies selectively binding to various components of friction ridge secretions to detect latent fingerprints. For example, blood typing techniques were adapted to produce a coloured deposit on fingerprint ridges that contained blood group antigens. However, the technique was unsuccessful for approximately 20% of the population, who do not secrete blood type carbohydrates in their perspiration[74]. In 2008, Reinholz published a method for the detection of latent fingerprints on porous substrates using anti-human serum antibodies[75]. This method produced visible purple ridge detail with some background staining on fresh samples; however, with aged and degraded samples it produced poor ridge detail. Recently, Leggett et al. successfully enhanced latent fingerprints on non-porous surfaces using anti-cotinine antibodies conjugated to gold nanoparticles[76], which has opened a pathway into illicit substance detection through latent fingerprint evidence. Lastly, Drapel et al. demonstrated that commercially available antibodies are able to bind fingerprint ridges containing keratin and/or cathepsin, which allowed the visualisation of fingerprints under long-wave UV radiation[77]. Each of these techniques target constituents that are not necessarily present within all latent fingerprints, therefore may not be applicable in all cases.

Hofstetter et al. have previously reported the production of antibodies that stereo selectively bind to L-amino acids[78]. Current research published by Spindler et al. in 2011 reports that enantioselective anti-L-amino acid antibodies raised in rabbits conjugated to 16 nm diameter gold-citrate nanoparticles are shown to facilitate the detection of latent fingerprints by interacting with L-amino acids which are omnipresent in friction ridge secretions. This antibody-based system is particularly effective for the enhancement of aged and dried fingerprints on non-porous surfaces. Once the antibodies have conjugated to nanoparticles, a reagent is generated that selectively targets amino acids in fingerprints. After incubation and washing steps, secondary anti-rabbit antibodies labelled with the fluorophore, Fluorescent Red 610 are introduced, which detect the bound conjugates. After a final incubation and washing step is carried out, samples are visualised by optical illumination using a

Xenon arc lamp at a wavelength of 590 nm and observed using a 650 nm band-pass barrier filter.

This technique has been most successful at revealing aged and degraded latent fingerprints on non-porous surfaces and could potentially be a new sensitive latent fingerprint enhancement reagent for casework samples of unknown age.

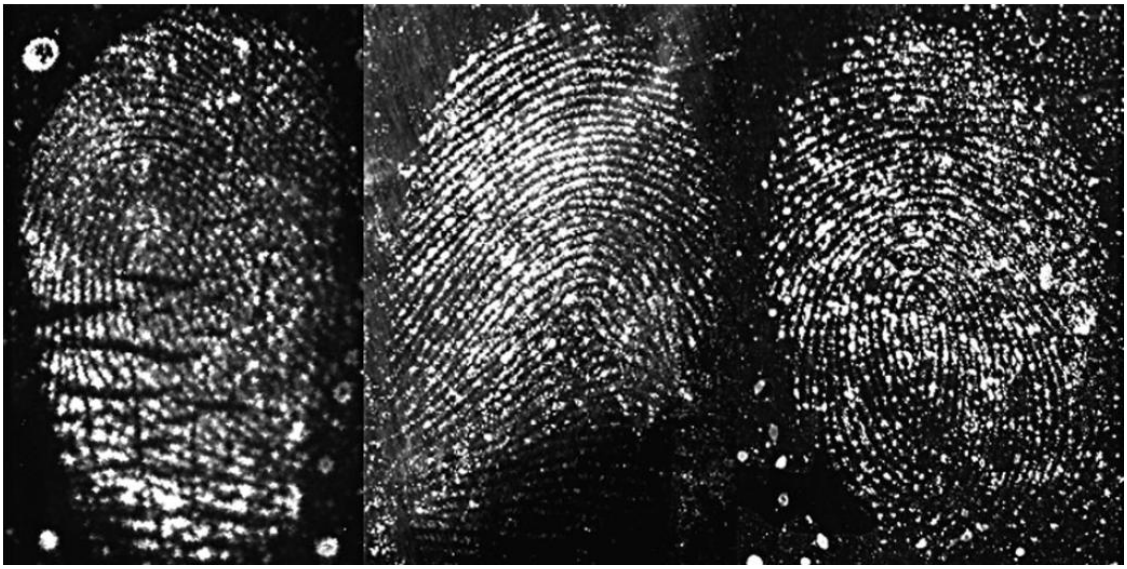


Figure 25: Finger-marks on aluminium foil developed using anti-L-amino acid antibodies conjugated to gold nanoparticles. A 4 month old fingerprint from a female donor (left), a 12 month old fingerprint from a male donor (middle) and a 2 week old fingerprint from a male donor (right)[73]

Despite the existence of a large selection of fingerprint development techniques, often prints are still difficult to develop, depending on their age or the surface upon which they have been deposited and degradation due to harsh environmental conditions e.g. high temperatures, moisture etc. Although, a series of sequential treatments are recommended, this becomes very time consuming. Forensic scientists are continuously looking for new and better methods to enhance fingerprints. Technologies that are more recent have higher sensitivity to very low levels of constituents present in residues and so are able to unearth significant information from a person's fingerprints at molecular level e.g. DNA, drug metabolites. Therefore, research continues in an attempt to generate novel, non-destructive processes that can enhance latent fingerprints.

1.6. Analytical Techniques

Throughout this research, several analytical techniques have been used: to compare compounds before and after sublimation of SeO_2, P_4S_3 and $(NPCl_2)_3$ onto latent fingerprints (Raman microscopy); to observe if derivatisation reactions have taken place with $(NPCl_2)_3$ (Nuclear magnetic resonance) and to identify copper precipitates (Elemental analysis; Infra-red spectroscopy). This section outlines the basic mechanics of these techniques and their application.

1.6.1. Infra-Red Spectroscopy

Infrared spectroscopy exploits the fact that molecules absorb specific frequencies that are characteristic of their structure. These absorptions are resonant frequencies, i.e. the frequency of the absorbed radiation matches the frequency of the bond or group that vibrates. The energies are determined by the shape of the molecular potential energy surfaces, the masses of the atoms, and the associated vibronic coupling.

The infrared spectrum of a sample is recorded by passing a beam of infrared radiation through the sample. When the frequency of the IR is the same as the vibrational frequency of a bond, absorption occurs. Examination of the transmitted radiation reveals how much energy was absorbed at each frequency (or wavelength). This can be achieved by scanning the wavelength range using a monochromator. Alternatively, the whole wavelength range is measured at once using a Fourier transform instrument and then a transmittance or absorbance spectrum is generated using a dedicated procedure. Analysis of the position, shape and intensity of peaks in this spectrum reveals details about the molecular structure of the sample.

This technique works almost exclusively on samples with covalent bonds. Simple spectra are obtained from samples with few IR active bonds and high levels of purity[79]. Complex molecular structures lead to more absorption bands and spectra that are more complex. The technique has been used for the characterization of very complex mixtures.

The instrumentation used to collect the data reported in this thesis was Perkin Elmer Spectrum 100; wavenumber range: 4000 – 300 cm^{-1}.

Samples were prepared by making KBr pellets using, KBr salt:sample ratio 10:1 and a Specac 15 Ton Manual Press.

1.6.2. Raman Spectroscopy[80]

When light passes through a material, some of it is absorbed and some of it is scattered. Nearly all of the scattered light has identically the same frequency as the incident light; this is called elastic scattering or Rayleigh scattering. However, a tiny fraction of the scattered light is shifted in frequency; this process is called Raman scattering and the associated frequency shift is called the Raman shift. Raman scattering is caused by the light interacting with some kind of oscillation inside the material, and the Raman shift measures the frequency of the oscillation involved. Many kinds of oscillations produce Raman shifts, e.g. molecular vibrations and rotations, lattice vibrations etc.; a complex structure like an organic compound can oscillate in many ways and can therefore produce a complicated Raman spectrum.

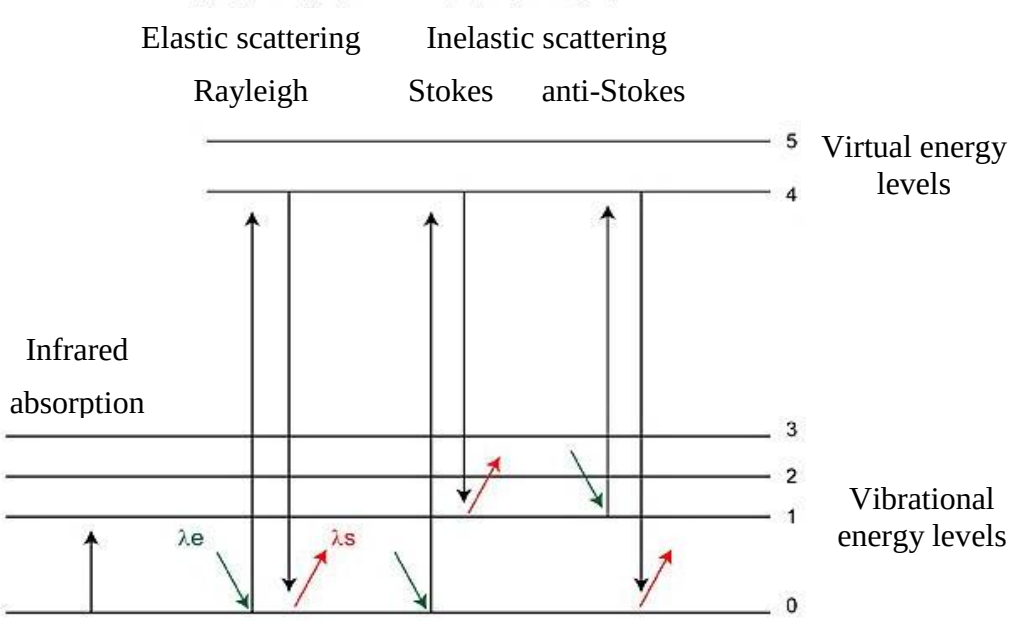


Figure 26: Molecular energy levels & Raman Effect[81]

A compound is only Raman active if changes in the polarisability of the molecule are observed. In Raman spectroscopy, an incoming photon causes a distortion of the electron distribution around a bond in a molecule causing temporary polarization of the bond, followed by re-emission of the radiation as the induced dipole disappears

upon relaxation. In a molecule with a centre of symmetry, a change in dipole is accomplished by loss of the centre of symmetry, while a change in polarisability is compatible with preservation of the centre of symmetry. Thus, in a centrosymmetric molecule, asymmetrical stretching and bending will be IR active and Raman inactive, while symmetrical stretching and bending will be Raman active and IR inactive. Hence, in a centrosymmetric molecule, IR and Raman spectroscopy are mutually exclusive. For molecules without a centre of symmetry, each vibrational mode may be IR active, Raman active, both, or neither. Symmetrical stretches and bends, however, tend to be Raman active.

The Raman spectroscopy reported in Chapter 2, was carried out on the following compounds: SeO_2, P_4S_3 and $(NPCl_2)_3$. All three of these compounds produce stretches which are Raman active and Raman spectra have previously been recorded and published by other researchers.[82,83,84]

The instrumentation and parameters used to obtain Raman spectra were: the Jobin Yvon Horiba LabRam HR800, HeNe internal laser, power output 20 mW, Charge-couple device (CCD) detector, polarised 500:1, λ: 632.817 nm, pinhole: 300 μm, Raman shifts: 0 – 1000 cm^{-1}, 10 cycles (unless stated otherwise).

1.6.3. Nuclear Magnetic Resonance (NMR)[85]

Nuclear magnetic resonance (NMR), is a phenomenon, which occurs when the nuclei of certain atoms are immersed in a static magnetic field and exposed to a second oscillating magnetic field. Some nuclei experience this phenomenon, and others do not, dependent upon whether they possess spin. Only those nuclei that possess spin will cause the nucleus to produce an NMR signal.

Spin is a fundamental property of nature like electrical charge or mass. Spin exists in multiples of ½ and can be positive or negative. Individual unpaired electrons, protons, and neutrons each possess a spin of ½. When placed in a magnetic field of strength B, a particle with a net spin can absorb a photon, of frequency ν. The frequency ν depends on the gyromagnetic ratio, γ of the particle.

$$\nu = \gamma B$$

Some of the nuclei routinely used in NMR are listed below however the spectra reported in this thesis focus on ^{31}P NMR and ^{1}H NMR:

Table 3: Nuclei routinely used in NMR Spectroscopy

Nuclei	Unpaired Protons	Unpaired Neutrons	Net Spin	γ (MHz/T)
^{1}H	1	0	½	42.58
^{2}H	1	1	1	6.24
^{31}P	1	0	½	17.25
^{23}Na	1	2	$^{3}/_{2}$	11.27
^{14}N	1	1	1	3.08
^{13}C	0	1	½	10.71
^{19}F	1	0	½	40.08

The signal in NMR spectroscopy results from the difference between the energy absorbed by the spins which make a transition from the lower energy state to the higher energy state, and the energy emitted by the spins which simultaneously make a transition from the higher energy state to the lower energy state. The signal is thus proportional to the population difference between the states. NMR is a rather sensitive spectroscopy since it is capable of detecting these very small population differences. It is the resonance, or exchange of energy at a specific frequency between the spins and the spectrometer, which gives NMR its sensitivity.

The chemical shift of a nucleus is the difference between the resonance frequency of the nucleus and a standard, relative to the standard. This quantity is reported in ppm and given the symbol delta, δ.

$$\delta = (\nu - \nu_{REF}) \times 10^{6} / \nu_{REF}$$

In NMR spectroscopy, this standard is often tetramethylsilane, $Si(CH_3)_4$, abbreviated TMS[86]. The instrumentation used to produce the spectra in this report is as follows: Bruker 400 Ultra-Shield, Avance- 400 spectrometer operating at 161.97 MHz for ^{31}P NMR and 400.13MHz for ^{1}H NMR and TOPSPIN software.

All samples were prepared in $CDCl_3$ and the chemical shifts recorded in ppm relative to phosphoric acid (H_3PO_4) 85% reference standard for ^{31}P NMR.

1.6.4. Elemental Microanalyses[87]

The samples to be analysed are weighed into disposable aluminium capsules. Each sample is injected into a high temperature furnace and combusted in pure oxygen under static conditions. At the end of the combustion period, a dynamic burst of oxygen is added to ensure total combustion of all inorganic and organic substances.

The resulting combustion products pass through specialised reagents to produce from the elemental carbon, hydrogen, and nitrogen; carbon dioxide (CO_2), water (H_2O) and nitrogen (N_2) and N oxides. These reagents also remove all other interferences including halogens, sulfur, and phosphorous. The gases are then passed over pure copper wire to remove excess oxygen and to reduce oxides of nitrogen to elemental nitrogen. After this stage, the gases enter a mixing chamber to ensure a homogeneous mixture at constant temperature and pressure.

The mixture then passes through a series of high-precision thermal conductivity detectors, each containing a pair of thermal conductivity cells. Between the first two cells is a water trap. The differential signal between the cells is proportional to the water concentration, which is a function of the amount of hydrogen in the original sample. Between the next two cells is a carbon dioxide trap for measuring carbon. Finally, nitrogen is measured against a helium reference.

Oxygen is also measured separately by pyrolysis in the presence of platinized carbon. The oxygen is finally measured as carbon dioxide.

The data collected in this report was obtained from an Exeter Analytical CE440 Elemental Analyser with a combustion temperature of 97.5°C and reduction temperature of 620°C.

Figure 27: Exeter Analytical CE400 Elemental Analyser[87]

1.6.5. CRi Nuance Multi-spectral Imaging System[88]

Microscopy combined with the CRi (Cambridge Research and Instrumentation Inc) Nuance multispectral imaging system, can spectrally characterize all the spectral components in a sample, and use this information to automatically separate and quantitate each signal into its own channel. This sensitive spectral analysis software is able to separate out and eliminate auto-fluorescence, which can obscure the detection of specific fluorescence signals, and allows apparently absent signals out of the noise to the fore, making them visible and quantifiable.

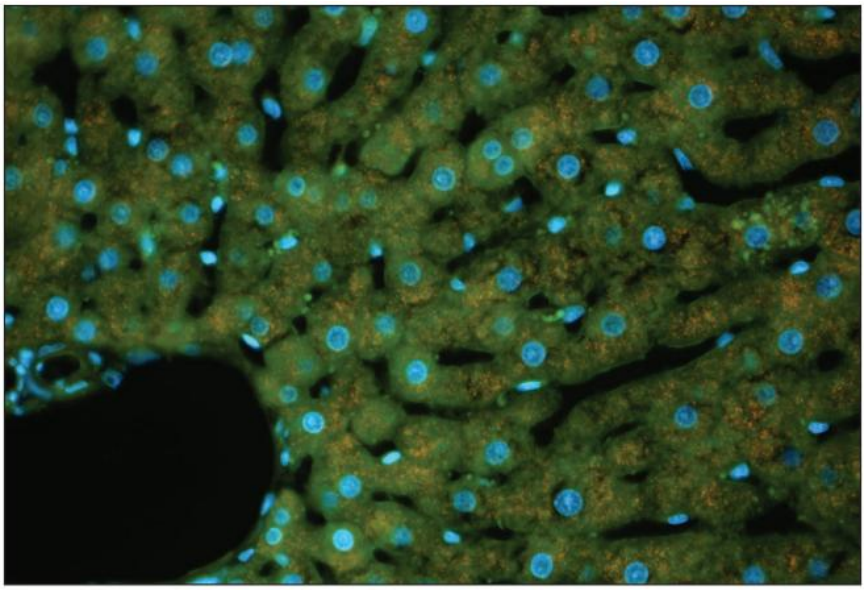


Figure 28: "Before" image of a liver sample captured with a conventional colour camera[88]

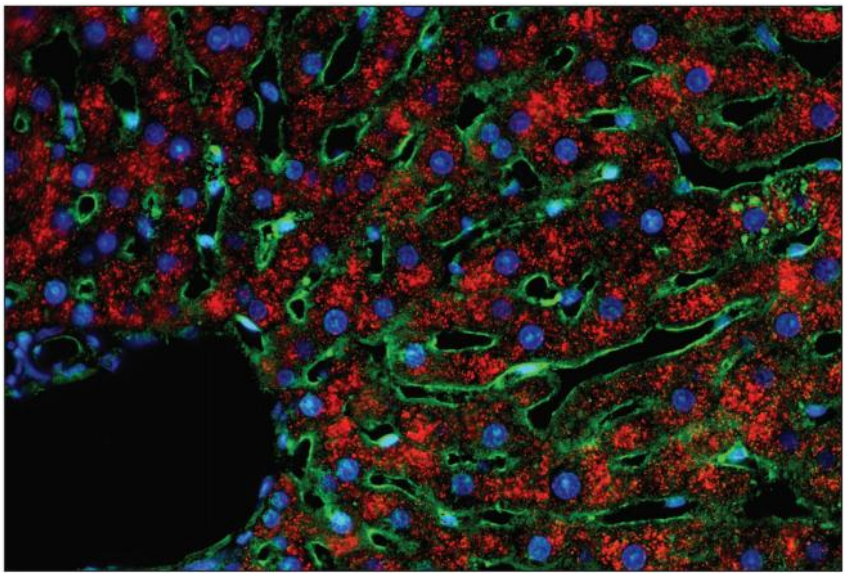


Figure 29: "After" image of a liver sample, where CRi Nuance software was used to remove auto-fluorescence and un-mix three fluorophores[88]

This software was used to reveal fingerprint ridge detail developed using rubeanic acid from cotton, nitrile glove and coloured carpet samples. The advantage of using this software was that any textured weave or pattern on a fabric sample was able to be removed in order to reveal fingerprint ridge detail that was otherwise lost when photographed with a conventional camera.

1.6.6. Fingerprint Grading System[89]

In order to make the techniques comparable, the fingerprints were subjected to a standard grading system devised by Home Office Scientific Development Branch (HOSDB).

Table 4 – Grading system used for determining the quality of ridge detail for developed fingerprints[89]

Grade	Comments
0	No development
1	Signs of contact but < 1/3rd of mark continuous ridges
2	1/3 – 2/3 of mark continuous ridges
3	> 2/3rd or mark continuous ridges, but not quite a perfect mark
4	Full development – whole mark clear with continuous ridges

Chapter 2 – *p*-Block Inorganic Systems

Superglue fuming, iodine fuming, S_2N_2 sublimation are all vapour phase (fuming) fingerprint development techniques, but each has its limitations. Cyanoacrylate (Superglue) chemically reacts with amino acids present in fingerprint residues, iodine fuming requires a fixing agent which may be destructive to fingerprints and S_2N_2 is friction sensitive and requires cautious handling. Vapour phase techniques are still preferred over chemical techniques because they have low impact on fingerprints. This section investigates other commercially available compounds in their vapour phase that could potentially be used to enhance latent fingerprints.

There are an immense number of *p*-block compounds available that have been exemplified in the recent extensive review by Chivers and Manners.[90] The review covers all structural binary compounds from group 13 through to group 17. However, despite the existence of a large range of compounds, many combinations are obscure, unstable and inapplicable to use in solution or in the gas phase therefore would not be classed as forensically viable. However, with the exception of iodine/iodine mono-chloride[50] and radioactive SO_2[57] that have been historically used to reveal fingerprints, it is still very rare for *p*-block compounds to react in the vapour phase. Nevertheless, in light of the success with S_2N_2 with its unique behaviour, there are other promising candidates. This has led to the research reported here on the potential use of commercially available group 15 and group 16 inorganic compounds in their vapour phase such as selenium dioxide (SeO_2), phosphorus sulphides (P_4S_{10}, P_4S_9, P_4S_3) and $(NPCl_2)_3$ to develop latent fingerprints. The P_4S_9 used was synthesised by a previous student however, the compound can be purchased from Ruiyuan Group Ltd, China. P_4S_{10}, SeO_2, P_4S_3 and $(NPCl_2)_3$ were purchased from Sigma Aldrich (Gillingham, UK).

An attempt to make $(NPCl_2)_3$ was carried out using the protocol below:

Synthesis of $(NPCl_2)_3$[91]

! **Caution:** This procedure was carried out in a fume-hood due to the hydrochloric acid (HCl) fumes liberated during the reaction.

$$n\mathrm{PCl_5} + n\mathrm{NH_4Cl} \rightarrow (\mathrm{NPCl_2})_n + 4n\mathrm{HCl}$$

Figure 30: Equation of the reaction to produce $(NPCl_2)_3$

The set up consisted of a 250 cm^3 round-bottom flask with a reflux condenser, a heating mantle and a scrubbing train consisting of one empty Drechsel bottle and one half-filled with 2M aqueous sodium hydroxide solution. 150 ml of 1,1,2,2-tetrachloroethane ($C_2H_2Cl_4$), *powdered* ammonium chloride (NH_4Cl; 75 mmol, 4 g), phosphorus pentachloride (PCl_5; 75 mmol, 15.6 g) and phosphorus oxychloride ($POCl_3$; 0.75 mmol, 1.15 g) were all added to the flask. The mixture was heated under reflux for 3.5 hours until no more HCl was evolved. The scrubbing assembly was disconnected, the mixture was cooled and any excess NH_4Cl was filtered off. The filtrate was evaporated using a rotary evaporator. The crude product was cooled in the refrigerator and the solid was extracted with 60 – 80 °C petroleum ether (3 x 25 cm^3). The extracts were combined and reduced to a ca. 10 cm^3 volume via a rotary evaporator. This solution was cooled in ice and an off-white solid was recovered. The $(NPCl_2)_n$ was filtered and vacuum dried (3.96 g, 46%). The melting point was recorded and compared against the literature melting point of $(NPCl_2)_3$ (lit. mp 98 – 100 °C[92], mp of crude product 83 – 87 °C). As the synthesised $(NPCl_2)_3$ was impure, the 99% pure $(NPCl_2)_3$ purchased from Sigma Aldrich (UK) was used.

2.1. Experimental

2.1.1. Sublimation Technique

Samples of 0.70 mm thick brass (63% Cu: 37% Zn), 0.40 mm thick copper and 0.50 mm thick 430 grade stainless steel (17% Cr) were obtained from Metal Off-cuts, Liverpool, UK and cut into strips of 20 mm x 60 mm in size and sheets of aluminium foil were wrapped around microscope slides. Fingerprints were deposited by pressing a thumb onto the metal surface, 1 cm from the bottom of the strip. The fingerprint donor washed their hands 10 – 15 minutes prior to deposition every time. Two types of fingerprints were placed:

1. Fresh "loaded" eccrine (sweaty) prints were generated by the donor placing their hand in a nitrile glove for 10 mins prior to deposition.
2. Fresh "loaded" sebaceous (oily) prints were generated by the donor rubbing their fingers on their forehead and around the nose prior to deposition.

The sublimation process for SeO_2, phosphorus sulfides and $(NPCl_2)_3$ was initially carried out on a microscope slide as follows:

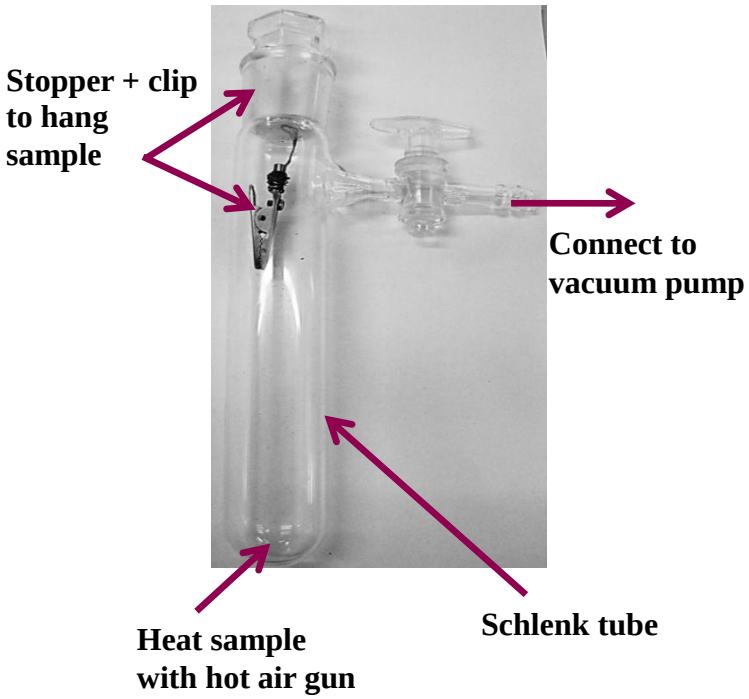


Figure 31: Sublimation Set-up

A microscope slide with a fingerprint deposited 1 cm from the bottom was hung from crocodile clip attached to a B34 stopper. Approximately 1/3rd of a spatula of the compound was placed in the bottom of a large Schlenk tube. The sample was hung from the clip and the whole apparatus was placed under a dynamic vacuum. The compound was heated with caution using a hot air gun until the fingerprint was revealed and digitally photographed. Any latent prints developed by $(NPCl_2)_3$ were scanned rather than digitally photographed.

2.1.2. Standard Protocol

A standard protocol containing six different tests was devised that fundamentally covered majority of conditions that fingerprints could be exposed to:

1. **Fresh prints:** Fresh eccrine and fresh sebaceous prints are placed on each surface sample as described in 2.1.1.
2. **Depletion method:** A fresh eccrine print is placed down on a series of 10 metal strips one after another without reloading in between. Prints 1, 5 and 10 were subjected to the sublimation technique. This was repeated with a fresh sebaceous print.
3. **Temperature effects:** Fresh eccrine and sebaceous prints were placed on two separate metal strips respectively with hand washing in between. The samples were placed in a furnace overnight at 100ºC and then once cooled they were exposed to the sublimation. This was repeated with samples being placed in a furnace overnight at 500ºC before the sublimation.
4. **Aging effects:** Loaded eccrine and sebaceous prints were placed on 4 metal strips each. Each sample was subjected to the sublimation after different periods and labelled accordingly: 1 hour (E1h/S1h), 1 day (E1d/S1d), 1 week (E1w/S1w) and 1 month (E1m/S1m).
5. **Other surfaces:** Loaded eccrine prints and sebaceous prints were deposited on a glass slide, black paper, black plastic, a nitrile glove, 100% black cotton, unfired 0.22 mm brass blank, post-fired 0.22 mm brass blank (print placed on brass blank prior to being fired) and a shotgun cartridge shell (print placed on a previously fired cartridge case). (0.22 mm blank brass casings purchased from Shooting &

Fishing Centre, Loughborough, UK). These particular surfaces are considered more problematic to recover latent fingerprints from, therefore needed to be investigated.

6. **Washed off prints:** Eccrine or sebaceous prints were deposited on brass and left in air over different time periods before being washed off with soapy water and dried with a tissue prior to development.

Raman Spectroscopy

Lastly, Raman spectroscopy was carried with the original powders of SeO_2, P_4S_3, $(NPCl_2)_3$ and fresh fingerprint samples on glass slides and brass after chemical treatment via sublimation of each compound. The spectra confirmed whether the original compound re-deposited onto the fingerprint ridges or if any chemical reactions took place with constituents in fingerprint residues. A useful spectrum from a single spot on the sample was produced in seconds. Raman shifts: 0 cm^{-1} – 1000 cm^{-1} (2 cycles with pure SeO_2 and P_4S_3, 10 cycles with all other samples).

2.2. Results and Discussion

2.2.1. Selenium Dioxide (SeO_2)

S_2N_2 vaporises as it is a small molecule and on cooling it polymerises. Although the mechanism for this is unsure, it does occur. This inspires the idea of SeO_2 due to the correlation between both structures. Selenium dioxide is a one-dimensional polymer that readily sublimes at 315ºC. It appears to break down to the dimer $(SeO_2)_2$ in the gas-phase[93] and then re-polymerise onto a cooler surface producing a white film.

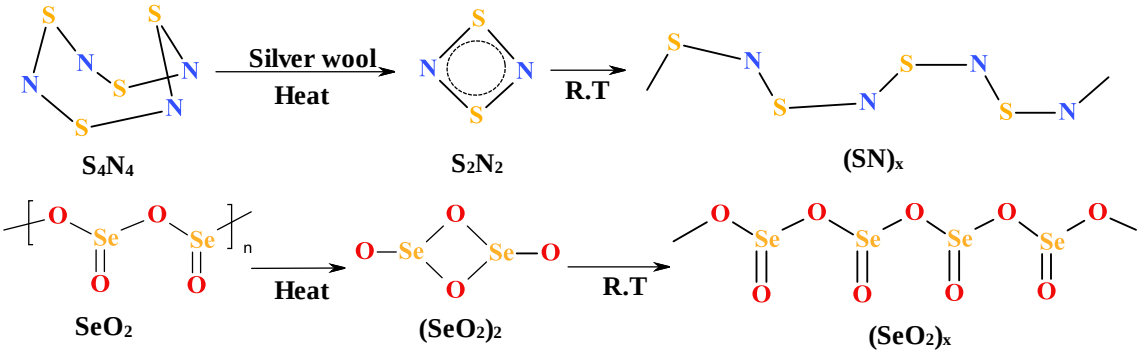


Figure 32: Polymerisation process for Sulfur nitrides[93] (top) and Selenium dioxide (bottom)

In both cases, the polymerisation process on cooling is affected by the presence of fingerprints. This is also the case with selenious acid, the active ingredient present in Gun Blue solution (Perma blue, Birchwood Casey). The gun blue method has its limitations, samples (metal surfaces with a latent fingerprint deposited) dipped in this solution, tend to become overdeveloped due to the rapid oxidation process.[60, 61, 62] SeO_2 has not been used in the vapour phase to reveal fingerprints which may have more promising results. However it must be noted that a major feature of S_2N_2 is the binary combination of group 15 and 16 elements in a molecular form however neither the compound S_4N_4 nor the precursor S_2N_2 are commercially available and both are instable and extremely friction sensitive so special precautions have to be taken during handling. This discovery not only serves to highlight the relative dearth of *p*-block compounds employed in fingerprint work, but also to inspire the search for similar reagents.

Preliminary Tests

In order to observe whether SeO_2 can develop fingerprints at all, the first sublimation attempts were carried out on glass microscope slides. These samples were left in air overnight in order to see whether the samples would remain intact. Then SeO_2 was sublimed onto fingerprints that had been placed on a vast array of media and exposed to alternative conditions to see whether the SeO_2 could reveal the latent fingerprints.

From these results in Table 5, it appears that SeO_2 is able to reveal fingerprints on a mediocre array of media, however has not been as successful with certain problematic surfaces e.g. fabrics and blank brass casings. There are several feasible reasons for the very poor success rate of revealing latent prints from blank brass casings:

- Friction between the surface of the casing and the surfaces of the firearm at different stages of the firing process may cause the print to rub off.
- One study has shown that the main cause of finger-mark deterioration is the friction between the casing's surface and the chamber at the point of ejection because the diameter of the casing increases due to the high internal pressure generated.[58]

- High temperature generated at the point of firing.
- Exposure of the surface of the casing to combustion gases and discharge residues at the point of firing.

Table 5: Fingerprint development using SeO_2 sublimation on various surfaces and under alternative conditions

Surface Type	Development Method	Fingerprint Grading
Glass microscope slide	Fingerprint placed 2 – 3cm from bottom of the slide.	4
Black laser paper	No changes	0
PTFE – used in ovens	No changes	0
Black bin liner	1. Wrap bin liner with print around cold finger and place in fridge. 2. Hang bin liner from clip.	1. 0 2. 0
Blue bin liner	Sample hung from clip.	4
1. Orange glass 2. Purple/red glass 3. Deep purple glass 4. Neutron density glass	1. No changes. 2. No changes. 3. No changes. 4. No changes.	1. 4 2. 2 3. 4 4. 4
Black paper	No changes	3
Black 100% cotton t-shirt	No changes	0
<u>Blank bullets:</u> 1. Unfired blank: 2. Post-fired blank:	1. No changes 2. Print placed then fired with a starting pistol.	1. 2 2. 0
Copper tubing	After SeO_2 sublimation, the print was enhanced by exposing the tube to air for few mins.	2
Black plastic stirrer stick	No changes	3
Black napkin/tissue	No changes	2
Shotgun cartridge/shell	Fresh fingerprint placed on both the plastic and brass sections.	2 (on brass)

Therefore, it was deemed quite crucial to trial the SeO_2 sublimation on the blank brass cartridges under various conditions and with different enhancing treatments.

Table 6: SeO_2 sublimation on pre and post-fired blank gun brass cartridges under various conditions

Surface	Development Method	Fingerprint Grading
Unfired blank casings	Simple SeO_2 sublimation, blank stored in vacuum	0
	SeO_2 sublimation + exposure to air	2
	SeO_2 sublimation + dipped in acidified H_2O_2	1
	SeO_2 sublimation + Schlenk backfilled with N_2	2
	SeO_2 sublimation + small volume of air overnight	0
	SeO_2 sublimation + air + Schlenk backfilled with N_2	2
	SeO_2 + air + rinse with distilled water	0
	SeO_2 sublimation + Anthracene sublimation	2
	SeO_2 sublimation + Anthracene-9-COOH sublimation	2
Post-fired blank brass casings	Simple SeO_2 sublimation	0
	SeO_2 sublimation + dipping in acidified H_2O_2	0
	SeO_2 sublimation + air + Schlenk backfilled with N_2	0
	SeO_2 sublimation + small volume of air overnight	0
	Fired blank left in air overnight then SeO_2 sublimation	0
	Fired blank left under a dynamic vacuum to dry overnight then SeO_2 sublimation carried out	1
	SeO_2 sublimation + Schlenk backfilled with N_2	0
	SeO_2 sublimation + AN sublimation	1
	SeO_2 sublimation + AN-9-COOH sublimation	1
	Print placed and left in air overnight, then fired and SeO_2 sublimation carried out	0
	Print placed and left in pistol for 4 hrs., fired and subjected to SeO_2 sublimation	0

From these results, it can be concluded that SeO_2 is able to reveal prints on various surfaces but is not very successful on fired blank brass casings. In order to compare results of different compounds, the standard protocol was used and some developed prints on brass were enhanced further upon releasing the vacuum and exposure to air.

Table 7: Results for SeO_2 sublimation using the standard protocol

Test	Results			
Test 1: Fresh prints	**Brass**	**Copper**	**Al foil**	**Stainless steel**
Fresh eccrine print	1	0	2	3
Fresh sebaceous print	3	3	3	3
Test 2: Depletion method Eccrine prints: E1, E5, E10 Sebaceous prints: S1, S5, S10	**Brass**	**Copper**	**Al foil**	**Stainless steel**
	E1: 2	E1: 0	E1: 2	E1: 0
	E5: 1	E5: 0	E5: 0	E5: 0
	E10: 1	E10: 0	E10: 0	E10: 0
	S1: 4	S1: 3	S1: 2	S1: 1
	S5: 3	S5: 1	S5: 3	S5: 1
	S10: 3	S10: 0	S10: 3	S10: 1

Test 3: Temperature effects	**Brass**		**Copper**		**Al foil**		**Stainless steel**	
100ºC	E: 0	S: 2	E: 0	S: 2	E: 1	S: 2	E: 0	S: 0
500ºC	E: 1	S: 3	E: 0	S: 0	E: 0	S: 0	E: 0	S: 0

Test 4: Aging effects	**Brass**		**Copper**		**Al foil**		**Stainless steel**	
1 hour	E: 2	S: 3	E: 0	S: 1	E: 2	S: 2	E: 1	S: 1
1 day	E: 1	S: 4	E: 2	S: 3	E: 1	S: 4	E: 0	S: 0
1 week	E: 3	S: 3	E: 0	S: 3	E: 2	S: 3	E: 0	S: 1
1 month	E: 1	S: 2	E: 0	S: 3	E: 1	S: 3	E: 0	S: 3

Test 5: Other surfaces	**Eccrine print**	**Sebaceous print**
Glass slide	2	2
Black paper	1	1
Black plastic	0	1
Nitrile glove	0	1
100% Black cotton	0	0
<u>Washed off prints:</u> Eccrine print on brass was left in air overnight. The print was washed with soap and water and dried with a tissue.		No print was visible before or after SeO_2 sublimation (Grade: 0)

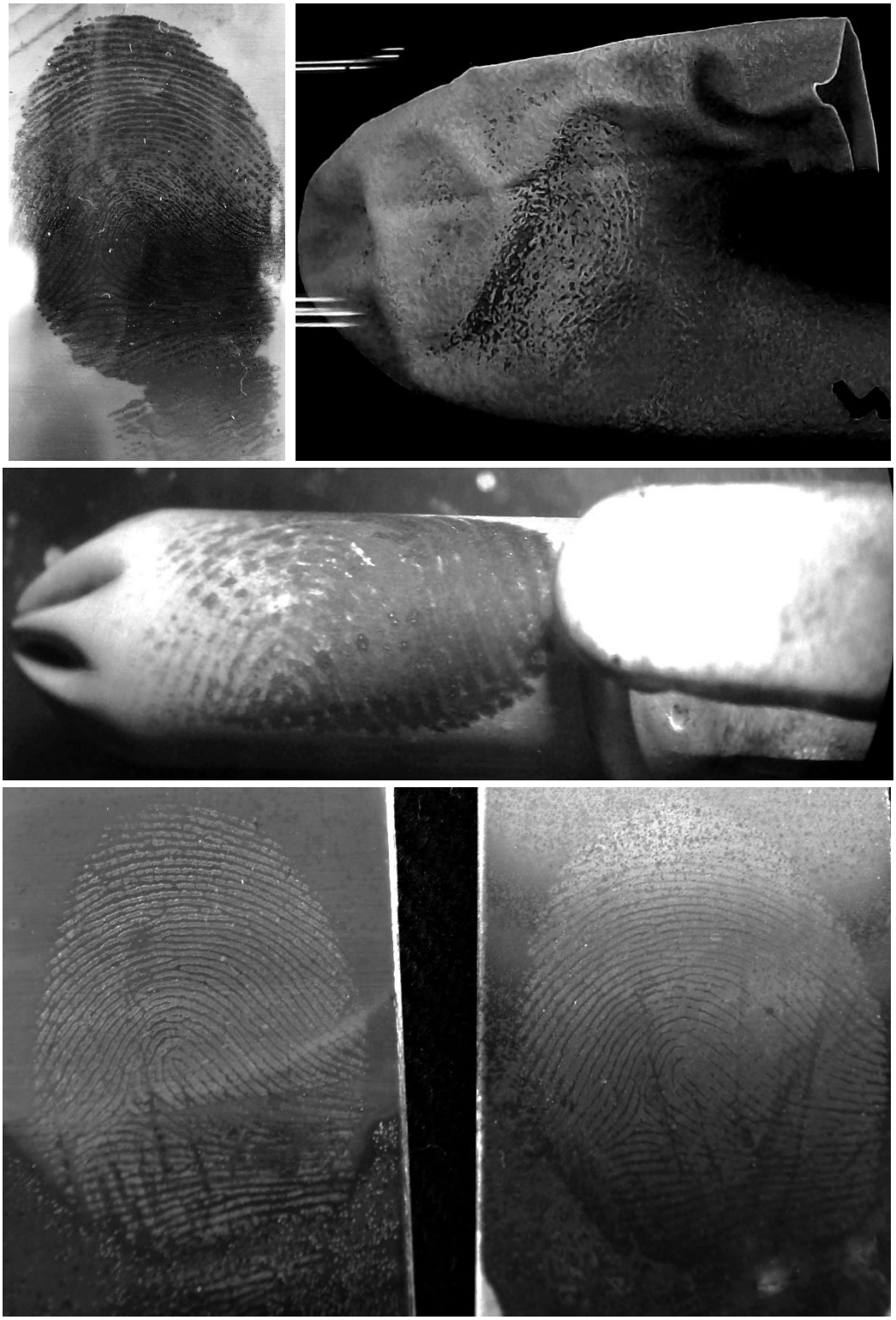


Figure 33: Photos after SeO_2 sublimation on: 1 month old oily print on Al foil (top left), sebaceous print on a nitrile glove (top right), an eccrine print on an unfired 0.22mm blank brass casing (middle), a comparison of a sebaceous print dipped in Gun-blue (bottom left) and a print exposed to SeO_2 sublimation on brass (bottom right)

From the results above it can be seen that at least 65% of the prints have been developed using this technique under various conditions. There were some issues of overdevelopment of eccrine prints where Cu-Se formed upon exposure to air, causing loss of ridge detail. Fingerprints were photographed whilst being stored under vacuum or immediately as the vacuum was released to avoid losing the prints. However, this was easily rectified through practice and most prints were preserved under nitrogen once they were developed to the right level. This compound worked better with sebaceous marks and brass as the contrast improved upon background formation of Cu-Se leaving a negative print.

SeO_2 predominantly reacts with water present in a fingerprint residue. After 24 hours, most if not all of the water is likely to have evaporated from eccrine prints which is supported by the results obtained from aging fingerprints in Table 7.

Raman Spectroscopy

Raman spectroscopy has been previously used to determine S-N peaks on samples exposed to S_2N_2[72]. SeO_2 is also Raman active due to its high polarisability. Raman spectroscopy was carried out on SeO_2 developed fingerprints. The advantage here was that the spectra could be directly compared to the original compound.

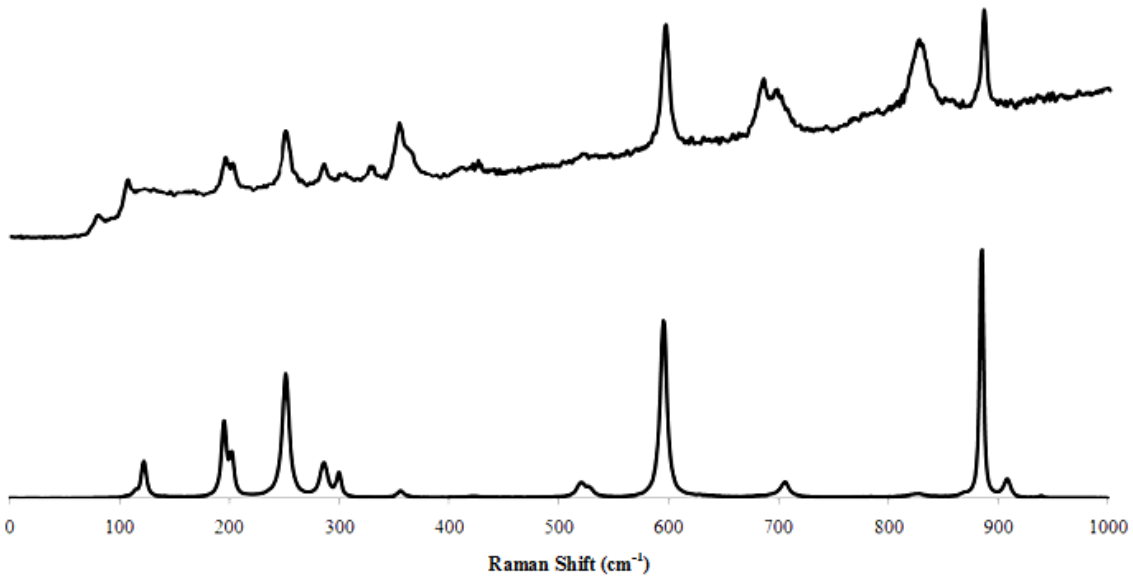


Figure 34: Comparison of the Raman spectrum of pure SeO_2 (lower) with that grown on an eccrine print on glass (upper), obtained through in situ Raman microscopy at room temperature

From Figure 34 it can be seen that SeO_2 grows on the eccrine print on glass in its original polymeric form as the peaks match the original SeO_2 spectrum although at much lower intensities, which is expected after sublimation. The peaks in the region 100 – 300 cm^{-1} correspond to various O-Se-O bending modes, with those at the higher wavelengths involving terminal oxygens. The peaks in the region 500 – 700 cm^{-1} correspond to various Se-O stretches involving bridging oxygens and the peaks in the region 870 – 920 cm^{-1} correspond to Se-O stretches involving terminal oxygens[82]. These peaks appear in both spectra however; there are some extra peaks in the spectrum of the latent fingerprints (top) compared to the SeO_2 powder spectrum (bottom). This coincides with the notion that the SeO_2 film on the fingerprints may be reacting with high levels of water present in eccrine prints, compared to the original powder, which is also showing some signs of this reaction but at much lower intensities.

Raman spectroscopy was carried out on a brass sample however, as soon as the vacuum was released the fingerprint was overloaded with copper-selenide and the Raman spectroscopy produced a spectrum of background noise only. The only way to avoid this, would be to carry out Raman spectroscopy under an inert atmosphere e.g. under nitrogen and at a lower temperature to obtain sharper peaks.

Ozin et al. (1971) have reported the findings of their investigation on SeO_2 vapour.[93] Mass spectroscopic data has shown that SeO_2 vapour is approximately 99% monomeric, but the remaining 1% is essentially all dimer $(SeO_2)_2$. Matrix Raman spectra of SeO_2 powder and vapour was compared. Ozin reported that they were able to obtain spectral data for the monomer, dimer and higher aggregates by carrying out diffusion-controlled polymerisation of SeO_2. The Raman spectra for SeO_2 powder at room temperature reported by Ozin et al. (1971) and Anderson et al. (2000)[82] both match the Raman spectra reported in this thesis. This confirms that there is only the polymeric SeO_2 present adhering to the fingerprint residues after sublimation.

2.2.2 Phosphorus Sulfides (P_4S_x)

Other examples of group 15 and 16 molecules with binary combination exist however; the most amenable are the phosphorus sulphides as they are easier to handle, unlike arsenic compounds, which are highly toxic. Phosphorus sulphides in the form P_4S_x (x = 3 – 10) are known.[90] Of these P_4S_{10} and P_4S_3 are the most stable in the series and commercially available. P_4S_3 is a readily available yellow solid and is made on a large scale for use in "strike anywhere" matches. P_4S_{10} is made on an even larger scale than P_4S_3 and is the primary source of a very wide range of organic P-S compounds.[94] In particular, P_4S_3 was used because it is a small molecule like S_2N_2 and volatile.

Tetraphosphorus decasulfide – P_4S_{10}

This yellow solid is the one of two phosphorus sulfides of commercial value. Samples often appear greenish-grey due to impurities. Due to hydrolysis by atmospheric moisture, P_4S_{10} evolves H_2S, thus P_4S_{10} is associated with a rotten egg odour. Aside from H_2S, hydrolysis of P_4S_{10} gives phosphoric acid:

$$P_4S_{10} + 16\ H_2O \rightarrow 4\ H_3PO_4 + 10\ H_2S$$

Figure 35: Hydrolysis products of P_4S_{10}[90]

This technique revealed over 80% of the prints (grade 1 – 4) and the samples were found to be more air stable than SeO_2. However, the disadvantages of using this compound are rapid hydrolysis and a much higher sublimation temperature is required than with SeO_2 thus requires more caution. The higher temperature required is most likely due to the possibility of larger, heavier molecules existing in the vapour phase compared to those present in SeO_2 vapour. It is unclear what is actually re-depositing on the fingerprints as Raman spectroscopy was not carried out on these samples, but it is feasible to assume that P_4S_{10} is re-deposits onto fingerprint ridges as the original powder. Smaller molecules such as, P_4S_9 and P_4S_3 may sublime at a lower temperature thus these compounds were also investigated.

Table 8: Results for P_4S_{10} sublimation using the standard protocol

Test	Results							
Test 1: Fresh prints	**Brass**		**Copper**		**Al foil**		**Stainless steel**	
Fresh eccrine print	2		1		1		2	
Fresh sebaceous print	3		2		3		4	
Test 2: Depletion method	**Brass**		**Copper**		**Al foil**		**Stainless steel**	
Eccrine prints:	E1: 2		E1: 1		E1: 3		E1: 2	
E1, E5, E10	E5: 3		E5: 0		E5: 1		E5: 3	
	E10: 4		E10: 0		E10: 1		E10: 4	
Sebaceous prints:	S1: 3		S1: 3		S1: 3		S1: 1	
S1, S5, S10	S5: 3		S5: 4		S5: 4		S5: 3	
	S10: 3		S10: 3		S10: 3		S10: 1	
Test 3: Temperature effects	**Brass**		**Copper**		**Al foil**		**Stainless steel**	
100ºC	E: 1	S: 1	E: 1	S: 3	E: 1	S: 2	E: 0	S: 1
500ºC	E: 1	S: 1	E: 0	S: 0	E: 0	S: 0	E: 0	S: 0
Test 4: Aging effects	**Brass**		**Copper**		**Al foil**		**Stainless steel**	
1 hour	E: 3	S: 3	E: 4	S: 3	E: 1	S: 2	E: 1	S: 2
1 day	E: 1	S: 4	E: 1	S: 2	E: 2	S: 3	E: 1	S: 4
1 week	E: 1	S: 4	E: 1	S: 3	E: 1	S: 3	E: 1	S: 3
1 month	E: 0	S: 4	E: 1	S: 3	E: 0	S: 4	E: 0	S: 4
Test 5: Other surfaces	**Eccrine print**				**Sebaceous print**			
Glass slide	3				3			
Black paper	0				1			
orange Perspex	2				3			
Nitrile glove	0				0			
100% Black cotton	0				0			
Washed off prints:					No print was visible before or after P_4S_{10} sublimation (Grade: 0)			
Eccrine print on brass was left in air overnight. The print was washed with soap and water and dried with a tissue.								

Tetraphosphorus nonasulfide – P_4S_9

P_4S_9 is a yellow crystalline solid, with mixed pyramidal (3 co-ordinate) and tetrahedral (4 co-ordinate) phosphorus geometries.

Table 9: Results of P_4S_9 sublimation using the standard protocol

Test	Results							
Test 1: Fresh prints	**Brass**		**Copper**		**Al foil**		**Stainless steel**	
Fresh eccrine print	0		4		2		1	
Fresh sebaceous print	3		0		4		3	
Test 2: Depletion method	**Brass**		**Copper**		**Al foil**		**Stainless steel**	
Eccrine prints: E1, E5, E10	E1: 2		E1: 0		E1: 2		E1: 2	
	E5: 0		E5: 0		E5: 2		E5: 2	
	E10: 0		E10: 0		E10: 1		E10: 2	
Sebaceous prints: S1, S5, S10	S1: 2		S1: 4		S1: 4		S1: 4	
	S5: 2		S5: 3		S5: 3		S5: 4	
	S10: 1		S10: 2		S10: 4		S10: 4	
Test 3: Temperature effects	**Brass**		**Copper**		**Al foil**		**Stainless steel**	
100ºC	E: 0	S: 3	E: 0	S: 1	E: 0	S: 3	E: 1	S: 4
500ºC	E: 2	S: 1	E: 0	S: 0	E: 0	S: 0	E: 0	S: 0
Test 4: Aging effects	**Brass**		**Copper**		**Al foil**		**Stainless steel**	
1 hour	E: 1	S: 4	E: 0	S: 4	E: 0	S: 3	E: 0	S: 4
1 day	E: 3	S: 4	E: 2	S: 4	E: 2	S: 3	E: 1	S: 4
1 week	E: 0	S: 4	E: 0	S: 4	E: 0	S: 3	E: 1	S: 4
1 month	E: 2	S: 4	E: 0	S: 2	E: 1	S: 3	E: 0	S: 3
Test 5: Other surfaces	**Eccrine print**				**Sebaceous print**			
Black paper	0				1			
Black Plastic	1				3			
Nitrile glove	0				0			
100% Black cotton	0				0			
Washed off prints:								
Eccrine print on brass was left in air overnight. The print was washed with soap and water and dried with a tissue.					No print was visible before or after P_4S_{10} sublimation (Grade: 0)			

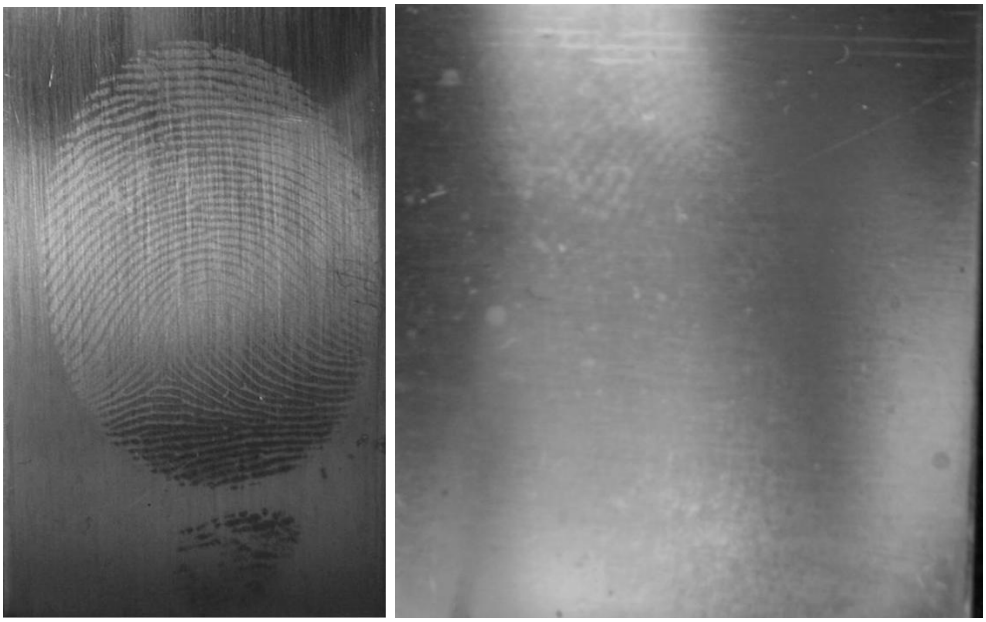


Figure 36: P_4S_{10} sublimation on: a 1 week old sebaceous print on brass (left), 1 month old eccrine print on copper (right)

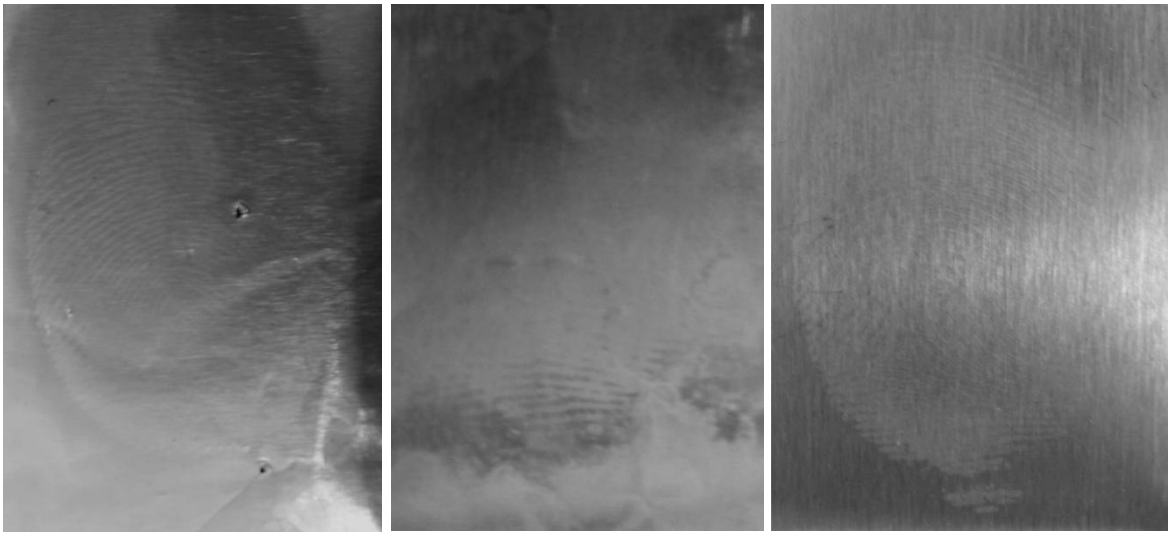


Figure 37: P_4S_9 sublimation on: a 1 week old sebaceous print on Al foil (left), a 10th eccrine print in a depletion series on Al foil (middle), 10th sebaceous print in a depletion series on copper (right)

Sublimation of P_4S_{10} resulted in a higher proportion of grade 3 – 4 prints than SeO_2 sublimation upon direct comparison of each of the conditions. The main disadvantage of SeO_2, P_4S_{10} and P_4S_9 is the rapid hydrolysis. Although P_4S_{10} and P_4S_9 both sublime at higher temperatures, P_4S_9 sublimation developed fingerprints to a poor quality. Therefore, the next step was to test out the smallest of the phosphorus sulfides, P_4S_3.

Phosphorus sesquisulfide – P_4S_3

P_4S_3 has a flash point of about 100ºC. It was stored at room temperature however, moisture in the air caused a liquid to form at the bottom of the bottle due to hydrolysis. To overcome this, the sample was stored in a desiccator in a refrigerator.

The results in Table 10 show that at least 80% of prints were revealed and photographed including a few ridges that were revealed on both pre and post-fired blank brass casings. In addition, the samples were much more air stable due to the slower hydrolysis, thus making the samples easier to handle, photograph and store under nitrogen. As P_4S_3 produced the best results, it seemed feasible to carry out Raman spectroscopy on some of the samples to see whether it was P_4S_3 re-forming on the fingerprint ridges and how they were forming.

Raman Spectroscopy

From Figure 39 it can be seen that the spectra of all samples subjected to P_4S_3 sublimation and the spectrum of P_4S_3 shows six broad peaks[95] at approximately 220, 285, 345, 420, 445 and 490 cm^{-1}. This shows that the P_4S_3 adheres to the latent print residue and does not react with the brass. It must be noted that it was much easier to carry out Raman microscopy on samples exposed to P_4S_3 compared to the samples subjected to SeO_2 sublimation because they hydrolyse much slower in air and therefore do not react with the brass when immediately exposed to air. There are ways to prevent overdevelopment of samples exposed to SeO_2 either by preserving them under vacuum, coating with a clear varnish or spraying with a clear lacquer to avoid any further reaction with moisture in the atmosphere however, no analysis can be carried out on the samples.

Table 10: Results for P_4S_3 sublimation using the standard protocol

Test	Results							
Test 1: Fresh prints	**Brass**		**Copper**		**Al foil**		**Stainless steel**	
Fresh eccrine print	3		1		1		1	
Fresh sebaceous print	3		3		4		3	
Test 2: Depletion method	**Brass**		**Copper**		**Al foil**		**Stainless steel**	
Eccrine prints:	E1: 2		E1: 2		E1: 1		E1: 1	
E1, E5, E10	E5: 1		E5: 1		E5: 1		E5: 1	
	E10: 1		E10: 1		E10: 2		E10: 3	
Sebaceous prints:	S1: 4		S1: 3		S1: 3		S1: 3	
S1, S5, S10	S5: 3		S5: 3		S5: 3		S5: 3	
	S10: 3		S10: 3		S10: 3		S10: 3	
Test 3: Temperature effects	**Brass**		**Copper**		**Al foil**		**Stainless steel**	
100ºC	E: 0	S: 1	E: 0	S: 0	E: 4	S: 3	E: 0	S: 1
500ºC	E: 1	S: 1	E: 0	S: 0	E: 2	S: 1	E: 0	S: 0
Test 4: Aging effects	**Brass**		**Copper**		**Al foil**		**Stainless steel**	
1 hour	E: 2	S: 3	E: 4	S: 3	E: 4	S: 3	E: 1	S: 1
1 day	E: 3	S: 3	E: 1	S: 1	E: 2	S: 3	E: 0	S: 3
1 week	E: 4	S: 3	E: 1	S: 3	E: 2	S: 3	E: 0	S: 2
1 month	E: 2	S: 3	E: 0	S: 2	E: 2	S: 2	E: 1	S: 2
Test 5: Other surfaces	**Eccrine print**				**Sebaceous print**			
Glass slide	3				3			
Black paper	1				1			
Orange Perspex	4				3			
Nitrile glove	0				0			
100% Black cotton	0				0			
Pre-fired brass blank	0				1			
Post-fired brass blank	0				1			
Shotgun Cartridge	-				3			

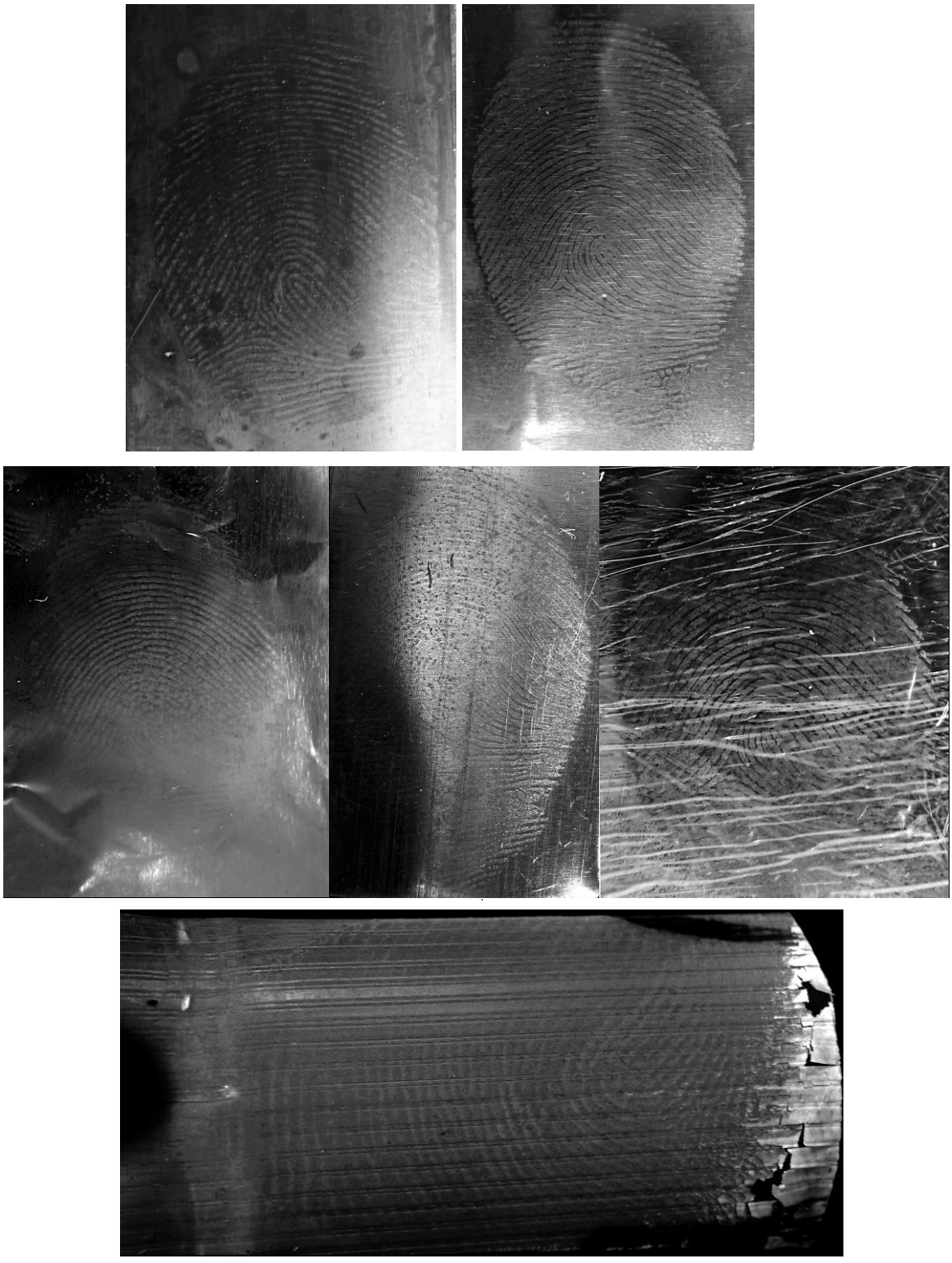


Figure 38: Photos after P_4S_3 sublimation on, Eccrine print on brass (top left), sebaceous print on Al foil (top right), 1 month old eccrine print on Al foil (middle left), 1 month old sebaceous print on copper (middle), sebaceous print on cling-film (middle right), sebaceous print on a shotgun cartridge (bottom)

Table 11: P_4S_3 sublimation on washed off prints on brass

Test	Results	
Washed off prints on brass: Eccrine print and sebaceous print placed on brass and washed with soap and water after x seconds and dried	**Eccrine print**	**Sebaceous print**
After 30 seconds	0	0
After 10 minutes	0	1
After 30 minutes	1	0
After 1 hour	1	2
After 24 hours	1	2

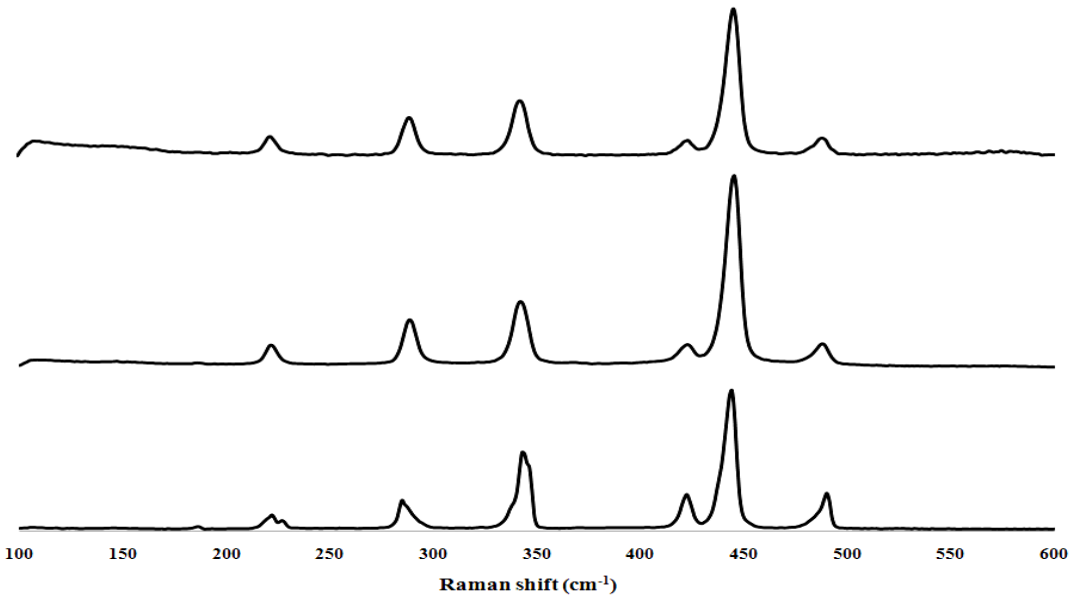


Figure 39: Comparison of the Raman spectrum of pure P_4S_3 (lower) with that grown on a fresh eccrine print (middle) and a fresh sebaceous print on brass (upper) obtained through in situ Raman microscopy at room temperature

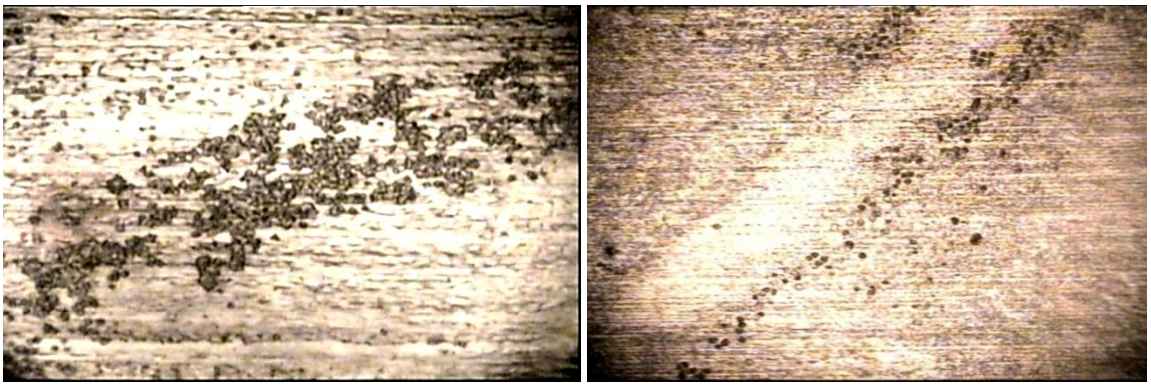


Figure 40: Magnified photos of P_4S_3 on a fresh eccrine ridge (left) & a fresh sebaceous ridge (right) on brass

From Figure 40 it is clear that the P_4S_3 crystals have adhered to the fingerprint ridges in two very distinctive ways due to the nature of the latent fingerprint being eccrine or sebaceous. The P_4S_3 appears to re-deposit on the eccrine fingerprint ridges producing a positive fingerprint whereas P_4S_3 appears to re-deposit either side of the sebaceous fingerprint ridges, producing a negative fingerprint. Phosphorus sulfides are known to hydrolyse in the presence of water; therefore, it is very likely that the preferential adherence of P_4S_3 to eccrine prints is because there is a higher concentration of water present. The preferential adherence of P_4S_3 to either side of the sebaceous ridges could mean that there are other constituents present in sebaceous prints that P_4S_3 adheres to. These constituents must only be present where the fingerprint ridges are situated, otherwise P_4S_3 would have adhered to the rest of the brass surface also.

2.2.3 Phosphonitrilic Chloride Trimer – $(NPCl_2)_3$

$(NPCl_2)_3$ is commercially available as a white crystalline powder. This trimer has been used as a ligand and/or ligand precursor for transition metals. Phosphazenes of this type have been extensively researched[96] and are the primary driving force for this work due to the fact that the polymerisation to $(NPCl_2)_x$ forms the basis for a range of polymers which are second in importance only to silicones. The trimer is derivatised via the reaction of the P-Cl bonds present therefore, polymerisation is important. Polymerisation may occur if a phosphazene derivative can be generated on a latent fingerprint. Such possibilities are utterly precluded in $(SN)_x$, SeO_2 and P_4S_3 because they do not have the necessary reactive sites at which to do chemistry.

Raman Spectroscopy

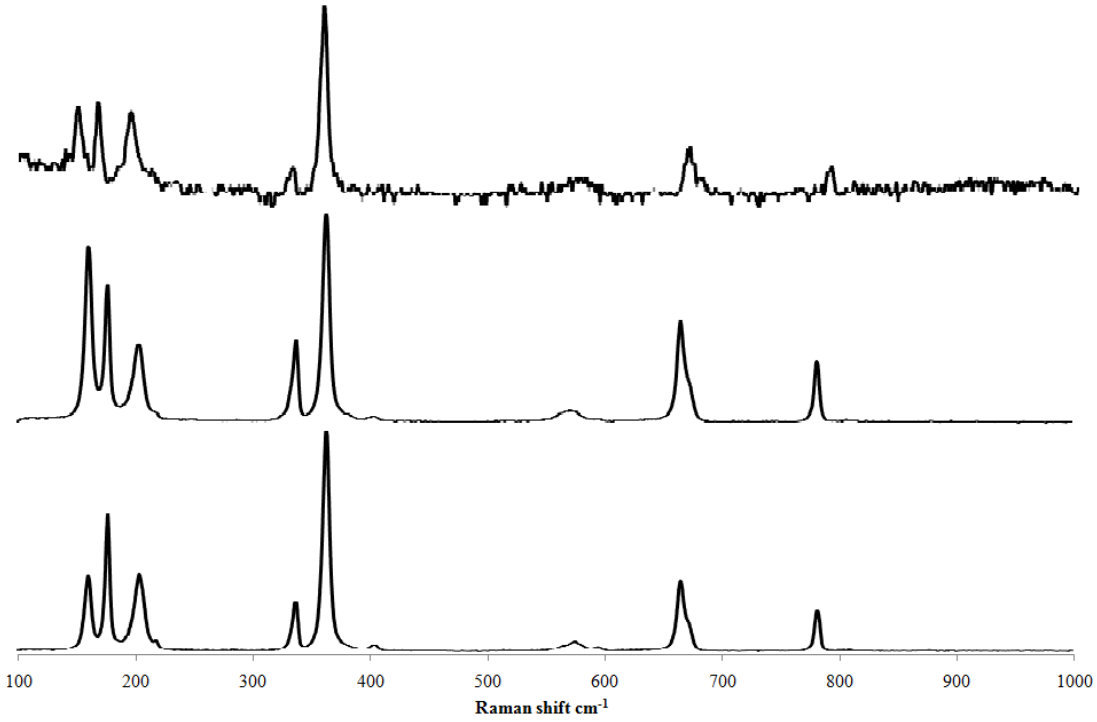


Figure 41: Comparison of the Raman spectrum of pure $(NPCl_2)_3$ (lower) with that grown on a fresh eccrine print (middle) and a fresh sebaceous print on brass (upper) obtained through in situ Raman microscopy at room temperature

From Figure 41 it can be seen that $(NPCl_2)_3$ appears to sublime and reform onto the fingerprints in its crystalline form. The peaks in the region 150 – 400 cm^{-1} correspond to PCl_2 stretching modes, the peaks in the region 590 – 700 cm^{-1} are likely to be ring

deformations and the peak at 790 cm^{-1} is likely to be due to the P-N-P ring vibrations[84]. Although this is the ideal situation, from the photos above it can be seen that the quality of the developed fingerprints is poorer than SeO_2 and P_4S_3.

Table 12: Results for $(NPCl_2)_3$ sublimation using the standard protocol

Test	Results							
Test 1: Fresh prints	**Brass**		**Copper**		**Al foil**		**Stainless steel**	
Fresh eccrine print	1		2		1		0	
Fresh sebaceous print	3		3		1		2	
Test 2: Depletion method	**Brass**		**Copper**		**Al foil**		**Stainless steel**	
Eccrine prints: E1, E5, E10	E1: 0		E1: 0		E1: 1		E1: 0	
	E5: 1		E5: 0		E5: 0		E5: 1	
	E10: 2		E10: 1		E10: 0		E10: 0	
Sebaceous prints: S1, S5, S10	S1: 2		S1: 1		S1: 1		S1: 2	
	S5: 2		S5: 1		S5: 1		S5:2	
	S10: 1		S10: 2		S10: 2		S10: 1	
Test 3: Temperature effects	**Brass**		**Copper**		**Al foil**		**Stainless steel**	
100ºC	E: 1	S: 1	E: 0	S: 0	E: 2	S: 0	E: 1	S: 0
500ºC	E: 2	S: 2	E: 1	S: 0	E: 3	S: 3	E: 3	S: 3
Test 4: Aging effects	**Brass**		**Copper**		**Al foil**		**Stainless steel**	
1 hour	E: 2	S: 2	E: 1	S: 2	E: 1	S: 0	E: 0	S: 0
1 day	E: 0	S: 0	E: 0	S: 1	E: 0	S: 0	E: 0	S: 0
1 week	E: 0	S: 2	E: 0	S: 1	E: 1	S: 2	E: 2	S: 0
1 month	E: 0	S: 0	E: 0	S: 0	E: 1	S: 1	E: 1	S: 0
Test 5: Other surfaces	**Eccrine print**				**Sebaceous print**			
Glass slide	2				1			
Black paper	0				0			
Orange Perspex	0				3			
Nitrile glove	0				0			
100% Black cotton	0				0			
Cling film	0				1			
Unfired brass blank	3				2			
Post-fired brass blank	0				2			

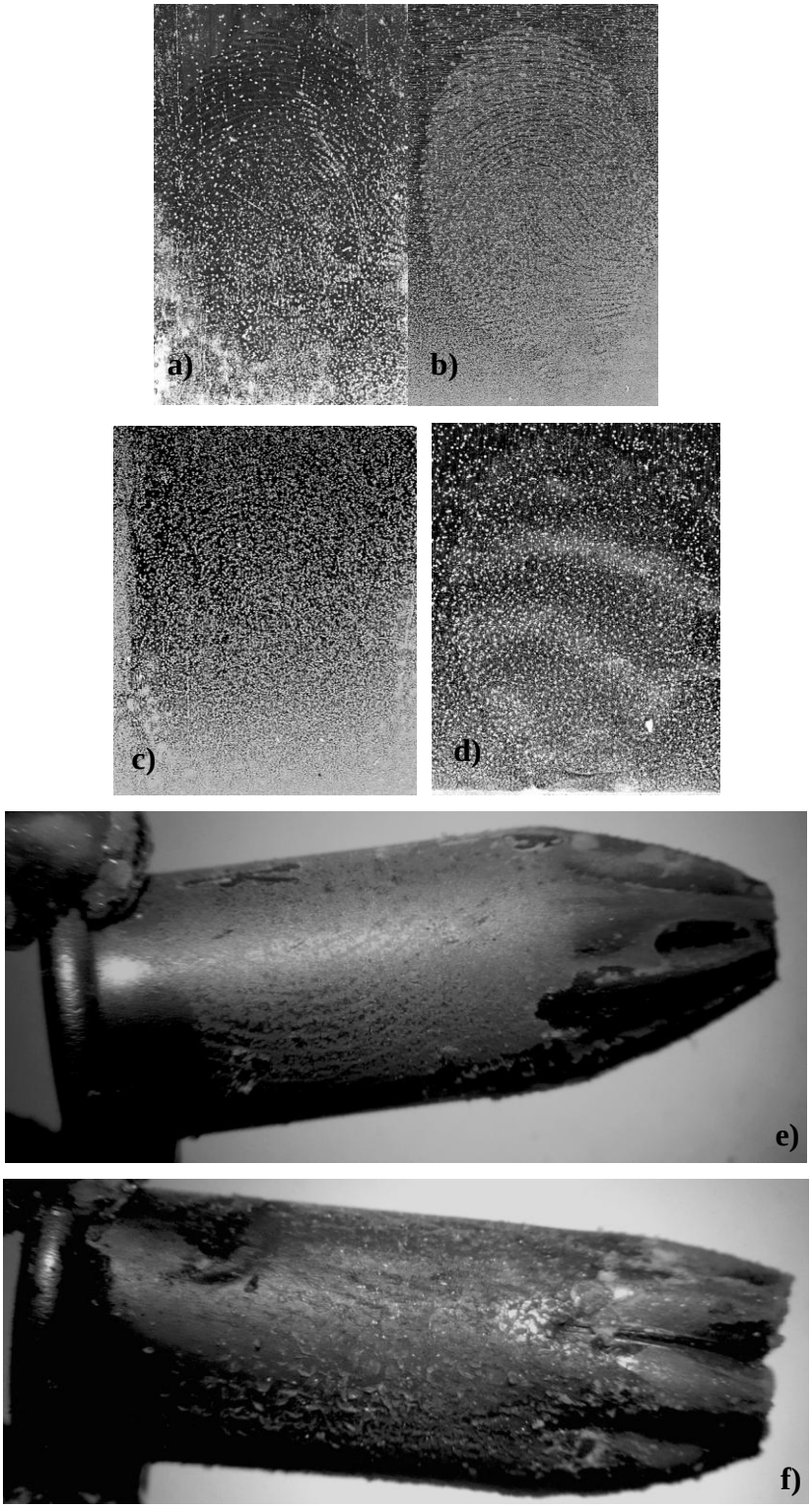


Figure 42: Scanned pictures after $(NPCl_2)_3$ sublimation of: (a) fresh eccrine print on brass, (b) fresh eccrine print on copper, (c) 10th eccrine depletion on brass, (d) 10th sebaceous depletion on Aluminium foil & digital photographs of (e) eccrine print on an unfired brass blank & (f) sebaceous print on post-fired brass blank

Looking at the constituents of $(NPCl_2)_3$, the reactive P-Cl bond allows scope for potential derivatisation in order to enhance the quality of fingerprints developed via this compound or even introduce molecules that may have fluorescence properties.

Reaction of $(NPCl_2)_3$ with 2 – aminoanthracene

Two reactions were carried out in order to see if any new products formed. One in solution phase and one in the vapour phase.

1. $(NPCl_2)_3$ was sublimed onto a glass slide. A few milligrams of 2 – aminoanthracene were dissolved in dichloromethane. The slide was then dipped into this solution to see if the 2 – aminoanthracene reacted with the trimer resulting in fluorescence under UV light. There was no fluorescence seen under UV light, the trimer appeared to wash off the microscope slide and mix into the solution.
2. An alternative approach was taken whereby a small amount of solid trimer and 2 – aminoanthracene were mixed together and then sublimed onto a glass slide. The trimer appeared to sublime first and then the 2-aminoanthracene sublimed on top of the trimer. The slide did fluoresce under UV light, but no reaction had occurred between the two compounds. The fluorescence was purely due to the 2-aminoanthracene re-forming on top of the trimer.

In order to see if any reaction occurred between the two compounds in solution, ^{31}P NMR spectroscopy was carried out.

NMR Spectroscopy

^{31}P NMR spectroscopy was carried out on two samples:

1. $(NPCl_2)_3$ dissolved in deuterated chloroform ($CDCl_3$)
2. $(NPCl_2)_3$ dissolved in deuterated chloroform ($CDCl_3$) with a few milligrams of 2 – aminoanthracene.

^{1}H NMR spectroscopy was carried out with sample 2 above plus the 3rd sample below:

3. 2 – aminoanthracene dissolved in deuterated chloroform ($CDCl_3$).

The sharp peak in both ^{31}P NMR spectra at 20 ppm can be assigned to the phosphorus present in $(NPCl_2)_3$. As the phosphorus atoms are in the same environment as each other, one sharp peak is present. The smaller peak at -6.5ppm is likely to be impurities as the manufactured compound was not 100% pure. The fact that both spectra are the same indicates that no reaction occurred between the P-Cl bond in the trimer and 2-aminoanthracene.

The ^{1}H NMR spectra for 2-aminoanthrancene (Figure 44) shows the typical aromatic protons between 6.8 – 8.2 ppm and the protons from the amino group at 3.9 ppm. The other peaks are likely to be impurities as the compound has been used and stored over a long period. The ^{1}H NMR spectra of mixture of 2-aminoanthracene and $(NPCl_2)_3$ confirms that there has been no interaction between the two compounds as the spectra is the same as obtained for 2-aminoanthracene.

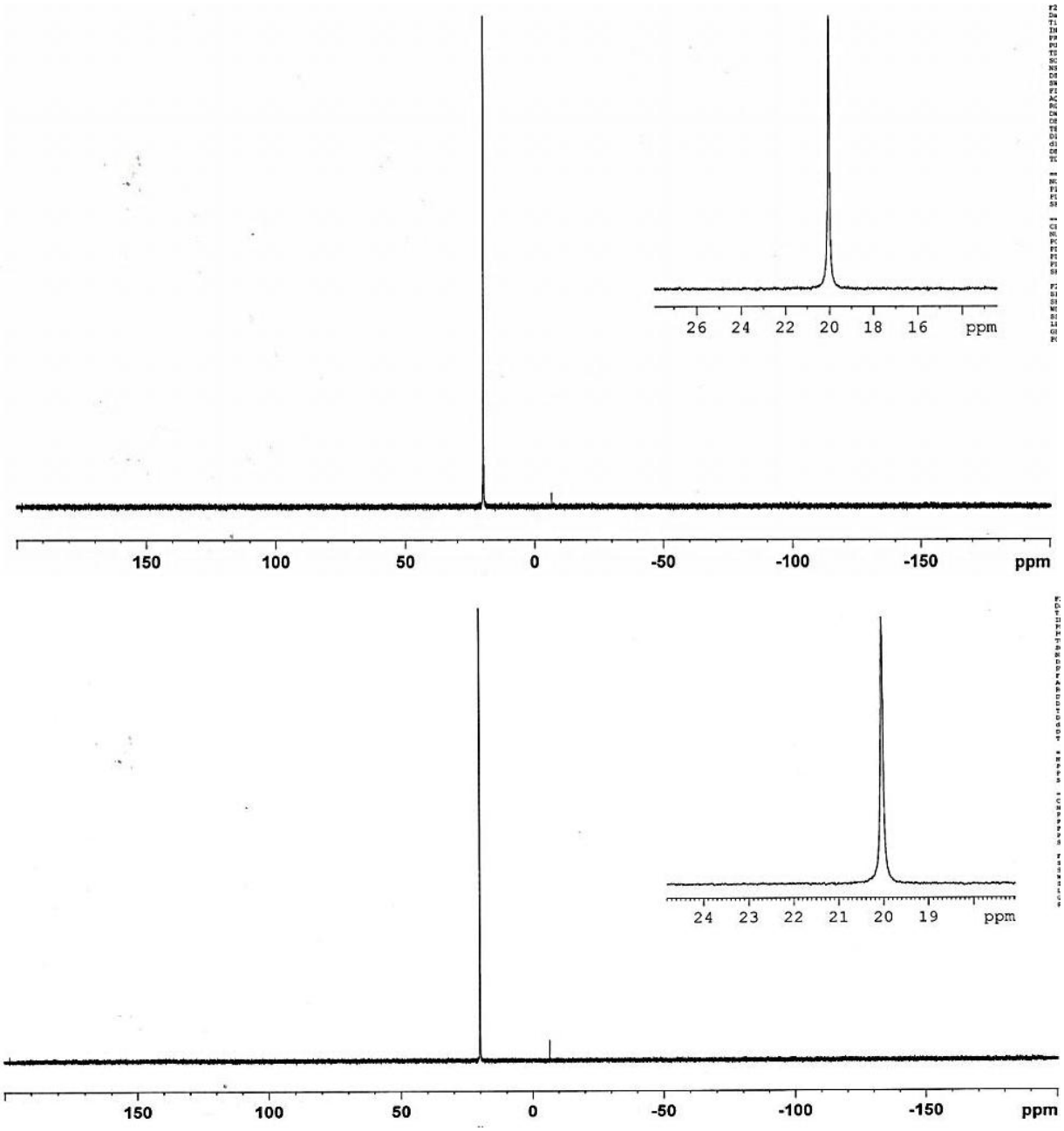


Figure 43: ^{31}P NMR Spectra for $(NPCl_2)_3$ dissolved in $CDCl_3$ (top) & $(NPCl_2)_3$ + 2 – aminoanthracene dissolved in $CDCl_3$

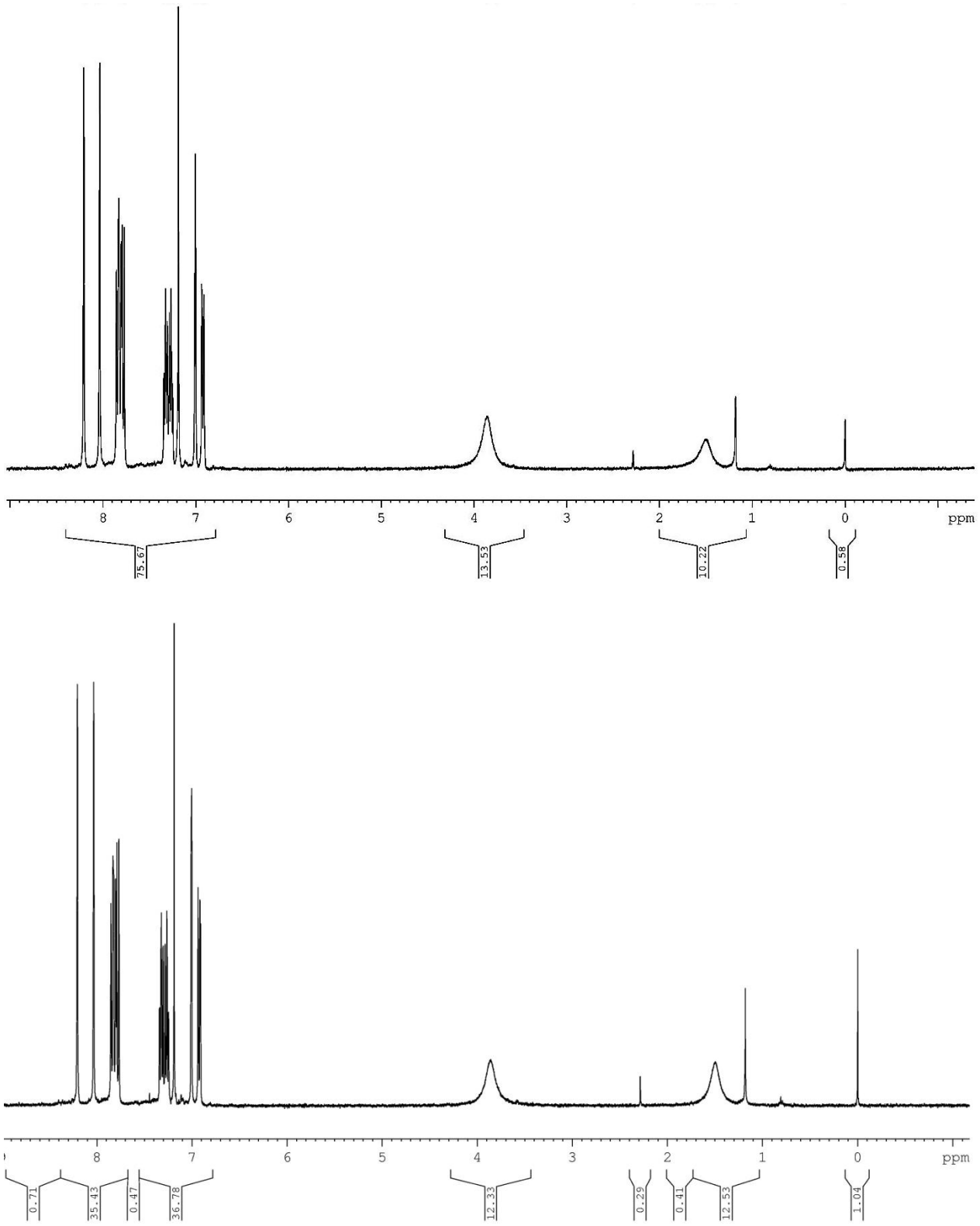


Figure 44: ^{1}H NMR spectra for 2-aminoanthracene in $CDCl_3$ (top) and 2-aminoanthracene + $(NPCl_2)_3$ in $CDCl_3$ (bottom)

2.3. Conclusions

Overall, developing fingerprints by SeO_2 sublimation works reasonably well on metals, however in order to preserve the fingerprint samples they must be stored under vacuum otherwise they tend to overdevelop within seconds of releasing the vacuum, which made the samples difficult to photograph. The Gun-Blue method produces better quality prints than SeO_2 sublimation despite the latter being more cost effective. Lastly, there has been little success with the 0.22 mm blank brass casings as these are too small to place a print on so larger spent cartridge cases would be the next thing to test. Raman spectroscopy revealed that SeO_2 re-polymerises onto fingerprint ridges.

From the results of all three phosphorus sulfides, it concludes that P_4S_3 is able to reveal the most prints of the best quality. This compound has advantages over the others because it sublimes at a lower temperature and hydrolyses at a much slower rate, producing less H_2S gas when left out in air during handling. This allowes a reasonable amount of time to capture better quality photographs of each sample. The only disadvantage is that care needs to be given when heating due to its low flash point, therefore any remaining P_4S_3 must be left to cool down before the vacuum is released to remove the sample otherwise the sample may ignite.

Raman spectroscopy confirmed that the P_4S_3 appears to sublime as a whole molecule and then re-deposit as crystals of P_4S_3 on the fingerprint. From the photographic images captured through the Raman microscope, it is evident that the P_4S_3 preferentially adheres to the ridges in eccrine prints whereas with sebaceous prints the P_4S_3 appears to adhere to the edges of the ridges to produce a negative print. Further work to decipher which constituents the P_4S_3 is preferentially adhering to is required.

Lastly with $(NPCl_2)_3$, although derivatisation at the reactive P-Cl site could be possible, it was considerably difficult to carry out and despite $(NPCl_2)_3$ being successful at revealing fingerprints from various surfaces, the low quality of fingerprints developed is undesirable and does not match the higher standard of developed latent fingerprints by both SeO_2 and P_4S_3. Raman spectroscopy revealed that $(NPCl_2)_3$ re-deposits onto fingerprints as the original compound.

Chapter 3 – Latent Fingerprints & DNA

There are several factors that affect whether DNA profiles can be obtained after an item has been processed with a range of latent fingerprint enhancement reagents:

- The type of latent fingerprint enhancement process used.
- The substrate or surface that the biological material has been deposited on.
- The type of biological material being dealt with.

It has become increasingly important to know what impact fingerprint enhancement processes will have on DNA present or the DNA profiling process. Several reagents such as magnetic powders, multi-metal deposition, shortwave UV irradiation and physical developer have all been found to interfere with DNA analysis methods. Other reagents such as DFO and 1,2-Indandione, originally thought to have a deleterious effect on DNA typing of saliva,[97] were proven not to have a detrimental effect if processed within 24 hours of fingerprint processing.[98] However, fingerprint processes do appear to affect the yield of DNA obtained post-treatment. Studies have shown that there is an average 60% decrease in DNA recovery following fingerprint treatment with DFO or ninhydrin.[99]

Other studies by Raymond et al. indicate that the recovery of DNA may be more dependent on the surface the fingerprint is deposited on rather than the processing technique. DNA profiles from fingerprints were able to be obtained from prints placed on plastic, glass, and adhesive tape regardless of the latent print processing treatment employed.[100] The least amount of DNA was recovered from paper and aluminium. The DNA extraction method used was found to be an important factor in the ability to obtain DNA profiles from fingerprints on paper.[99]

Some research has been carried out to attempt to remove those chemicals e.g. magnetic powders that inhibit the DNA amplification process successfully. This is due to research into multiplex PCR kits that have been reported as successfully providing DNA profiles for fingerprints and skin debris left on objects such as clothes and tools.[101] This shows that in the future, DNA fingerprinting in this way could replace routine fingerprint analysis methods. Lastly, with the sensitivity of modern

DNA testing methods, usable DNA profiles may still be obtainable from an item previously processed for latent fingerprints or even from latent fingerprints themselves.

DNA stands for Deoxyribonucleic acid. DNA consists of two long polymers of simple units called nucleotides, with backbones made of sugars and phosphate groups joined by ester bonds. These two strands run in opposite directions to each other (anti parallel). Attached to each sugar is one of four types of molecules called nucleobases (Adenine, Thymine, Guanine and Cytosine). It is the sequence of these four bases along the backbone that encodes information. This information is read using the genetic code, which specifies the sequence of the amino acids within proteins.

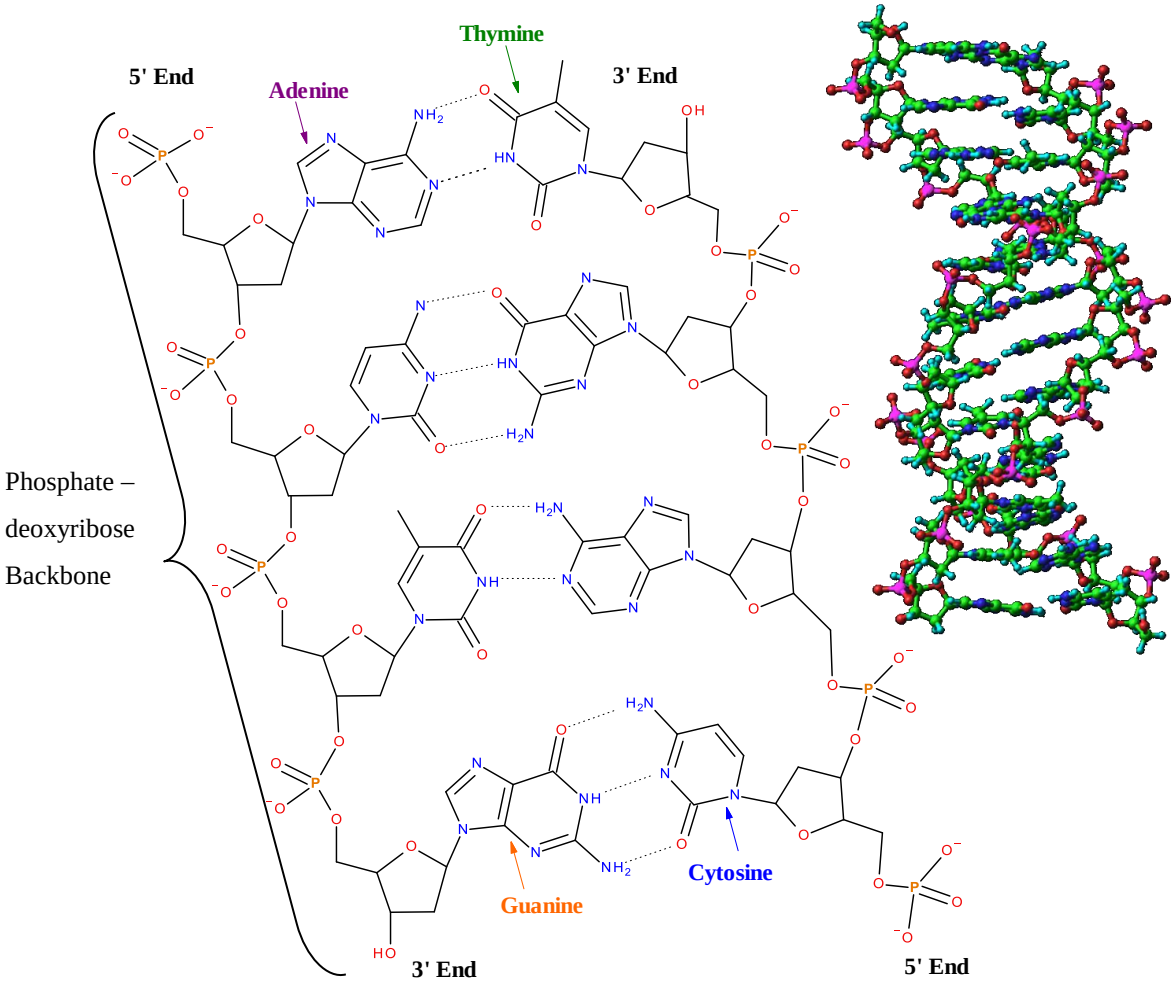


Figure 45: Molecular structure of deoxyribonucleic acid (DNA)[102]

3.1. DNA in Forensic Science

In forensic investigations, DNA profiles are obtained mostly from biological fluids left at a crime-scene e.g. blood stains, saliva stains, semen stains, urine and hair but also from objects that may have been touched e.g. cigarette butts thus leaving behind latent finger-mark residues or skin debris that can also be swabbed and processed to obtain a DNA profile.

There has been a significant research carried out on DNA extraction, quantification, amplification and sequencing from DNA swabs obtained from latent fingerprints. There tends to be only "trace" amounts of DNA present in latent fingerprint residues but new DNA profiling kits that are presently available are much more sensitive. Several published methods use various commercially available kits for each step in the process. DNA extraction kits such as InVisorb Forensic kit (Invitek)[103,104] and QIAmp DNA mini kit (QIAGEN, UK)[99] along with phenol:chloroform[105] and Chelex[106] extraction methods (see 3.2.2) have been successful at extracting "trace" amounts of DNA.

3.1.1. InVisorb Forensic Kit 1 (Invitek, Berlin)[103,104]

This kit involves DNA extraction based on a silica nanoparticle suspension matrix (InVisorb 50™ Carrier) which binds cellular DNA (genomic and mitochondrial). The kit comprises of Lysis Buffer D, which is used to disrupt the cellular membranes and inactivate the endogenous DNases. The washing buffer is made up of 96% Ethanol that is used to rinse the DNA-binding carrier suspension before Elution Buffer D is used to elute the DNA. This whole process produces contaminant-free DNA suitable for PCR. This kit has been successfully used to extract DNA from blood, fresh saliva, saliva swabs, sperm, chewing gum, cigarette butts, postage stamps, envelopes, human tissues, hair, bones, teeth, and stains on fabrics.

3.1.2. QIAmp DNA Mini Kit (QIAGEN, UK)[99]

This kit involves DNA extraction and purification that requires four steps and can be carried out using QIAmp Mini spin columns on a standard micro-centrifuge or on a

vacuum manifold. This kit can yield pure DNA ready for amplification in 20 minutes. The four steps comprise of:

- **Lysis** using QIAGEN Protease stock solution or Proteinase K enzyme along with lysis Buffer AL.
- **Binding** (adsorption) of the DNA to the QIAmp silica membrane using the spin or vacuum protocols.
- **Washing** of the DNA using buffers AW1 and AW2, which remove any residual contaminants.
- **Elution** of the DNA using Buffer AE (10mM Tris-Cl; 0.5mM EDTA; pH 9.0). The purified DNA can also be stored in the elution buffer at -20°C.

3.1.3. Phenol:Chloroform:Isoamyl Alcohol Extraction[105]

This organic extraction method allows DNA to separate from a solution that also contains proteins. The DNA dissolves in the aqueous layer and all other components go into the organic layer. The chloroform denatures the proteins and facilitates the separation of the aqueous (top layer) and organic phases. The isoamyl alcohol reduces foaming during extraction. To recover and concentrate the DNA from the aqueous phase, ethanol precipitation is carried out in the presence of sodium acetate (0.3 M). Once the ethanol has evaporated, the DNA pellet is then dissolved in TE buffer (mixture of Tris-Cl and EDTA at pH 8.0) which allows the DNA to be stored appropriately.

Quantification of DNA is also important as it could confirm that DNA is present. This is useful, as you need between 50 – 100 ng/µl of DNA to carry out the polymerase chain reaction (PCR). In addition, it can be used to see if the DNA has actually been successfully amplified after the PCR process. The Quantifiler Human Quantification kit[99] (Applied Biosystems, UK) and Quant-IT ssDNA assay kit[107] are designed for this purpose.

3.1.4. Chelex® Extraction

This is a very common DNA extraction method. The Chelex® resin is composed of styrene divinylbenzene copolymers and paired iminodiacetate ions that can bind with

metal ions such as magnesium. The removal of magnesium inactivates the nuclease enzymes that break down DNA e.g. Proteinase K, DNAse and polymerase. (See section 3.2.2 for the experimental of this extraction technique).

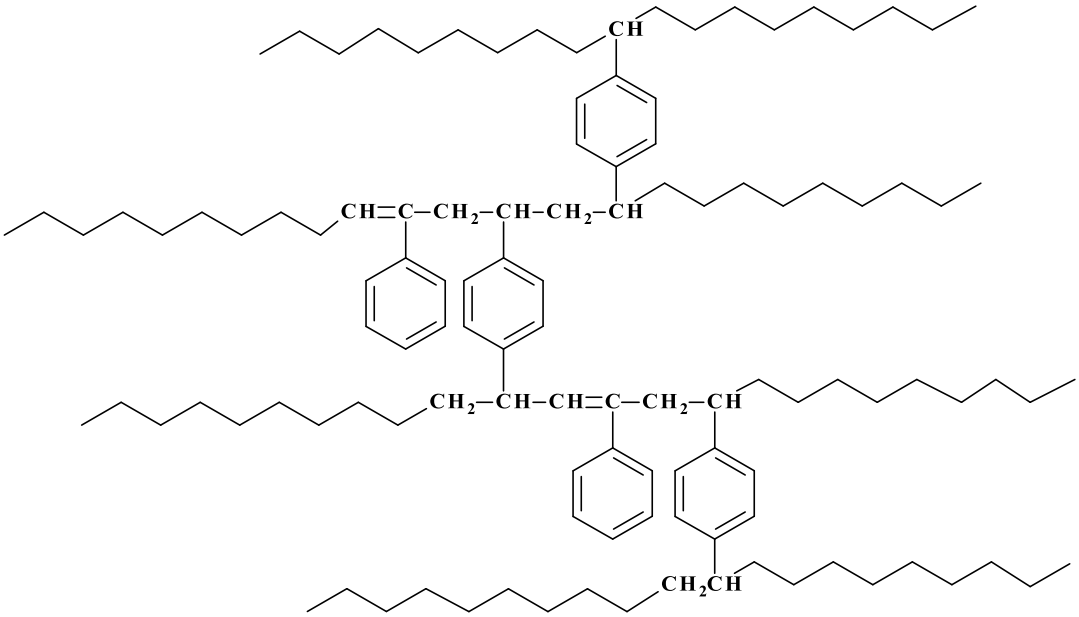


Figure 46: Styrene divinylbenzene copolymers[108]

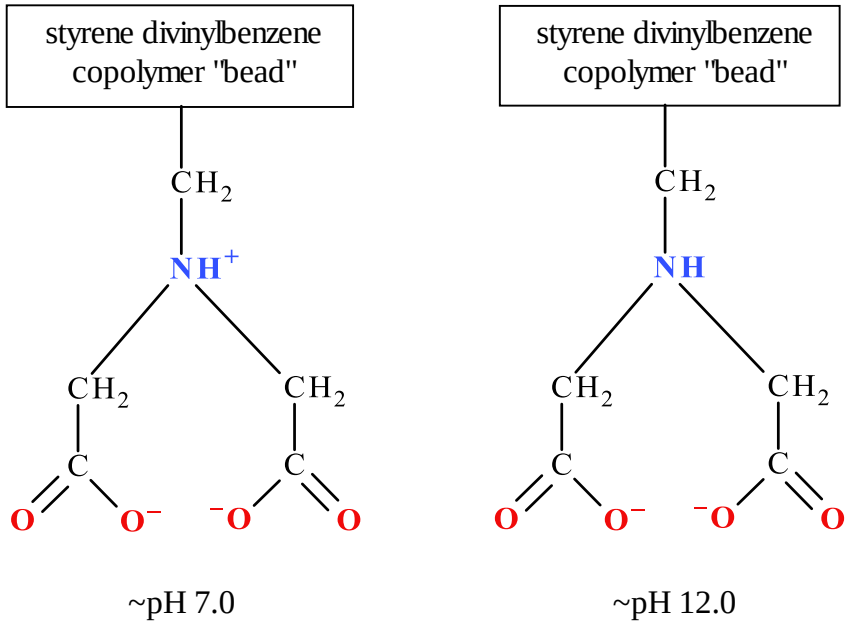


Figure 47: Chelex® resin structure[108]

3.1.5. Quantifiler™ Human Quantification Kit[99]

The DNA quantification assay combines two 5′ nuclease assays: a target-specific human DNA assay, and an internal PCR control assay. The target-specific assay for human DNA quantification consists of two 5′ primers, a forward and reverse primer, and one TaqMan® minor groove binder (MGB) probe. The 5′ end of the probe is linked with the reporter dye FAM™ for detecting the amplified sequence; VIC™ dye is substituted in the Internal PCR Control (IPC) assay as the reporter dye. The IPC sequence is synthetic. The 3′ end of the probe for both assays contains the non-fluorescent quencher (NFQ) along with the MGB. The target is Human Telomerase reverse transcriptase gene (hTERT), which consists of 62 bases in its length. The gene target is amplified throughout 40 cycles of PCR on the ABI Prism 7000[104], where real-time capture (CCD camera) of fluorescence between 500 – 660 nm (reporter dye) occurs during amplification (Due to excitement by the tungsten-halogen lamp). The SDS software applied data analysis algorithms to produce fluorescence spectra.

PCR cycles can be varied considerably in temperatures and the number of cycles depending on the equipment used and the type of kits used through the process as they may contain alternative enzymes that require temperatures to be slightly lower or higher to achieve the best outcome. Some examples of machines used to carry out PCR are the ABI Prism 7000[104] (used in real time PCR), DNA thermal cycler 480[109] and Eppendorf Mastercycler[104].

The advantage of using real time PCR is that the amplified DNA is detected as the reaction progresses in real time compared to standard PCR whereby the product of the reaction is detected at the end. In real time PCR, the DNA can be detected using non-specific fluorescent dyes or sequence specific DNA probes (oligonucleotides) that are labelled with a fluorescent reporter. Example applications of real time PCR are in the rapid detection of nucleic acids that are diagnostic of infectious diseases, cancer or genetic abnormalities.

In some cases, there tends to be random deletions that occur during the PCR step. An alternative method that is able to detect these anomalies is via multiplex PCR which

can be carried out by making the PCR master mixes or alternatively; Profiler®, PowerPlex® and AMPF/STR PowerPlex® Plus are some of the multiplex PCR kits[101] that are commercially available.

3.1.6. Multiplex PCR Kits[101]

Multiplex PCR amplifies genomic DNA samples using multiple primers. These primers are known as short tandem repeat (STR) polymorphisms in which two or more loci are amplified in one reaction from a single DNA sample. STR polymorphisms are short, repetitive sequence elements 3 – 7 base pairs in length. Usually the forward primers are labelled with a fluorescent dye marker e.g. FAM, HEX or TET and the reverse primers are not labelled. Each kit has specific amplification parameters for optimum results. PCR is usually carried out on a thermal cycler e.g. DNA Thermal Cycler 480. The separation is conducted using capillary electrophoresis and analysis and detection using a Genetic Analyser and laser-fluorescence technology respectively. These kits have significant importance in forensic investigations involving amplifying DNA from clothes, tools, grips of bags and especially degraded DNA samples.

The commercial kits have several advantages over manual multiplexing methods. Quality control measures are undertaken by the manufacturers of the kits and ensure that reactions are uniform across all kits. This avoids the preparation of PCR master mixes that can be very laborious and require pipetting which increases the risk of error and contamination. The DNA profiles obtained from commercially available kits are forensically viable to submit as evidence in court.

3.2. Aims

All of these kits above although commercially available they are very expensive. The aim of this research was to try to use a more cost-effective DNA analysis method to extract DNA from latent fingerprints both before and after chemical treatment. The next section describes the basic processes used to carry out the DNA analysis on cheek cell swabs and latent fingerprints.

3.3. Experimental: Forensic DNA Analysis[110]

3.2.1 DNA collection

The first step was to collect DNA from the donor by swabbing the inside of the cheek. To obtain DNA from a latent fingerprint residue on a glass slide, a cotton swab was wetted with RNAse/DNAse free water and swabbed over the fingerprint.

The next step involved cell lysis whereby the cells were broken down to release the DNA using centrifugation. This caused the insoluble cell debris to collect as a pellet at the bottom of the tube, and the supernatant consisted of DNA, RNA and protein. There were still large amounts of RNA and protein present that had to be removed to leave purified DNA. This is where Chelex® extraction took place.

3.2.2 Chelex® Extraction

A systematic protocol for the Chelex® extraction method used is given below:

1. 1.5 ml microfuge tubes (dependent on the number of samples) were labelled and 1 ml of RNAse/DNAse free water was pipetted into each tube using an automated pipette.
2. Each participant used a cotton swab and wiped the inside of their cheek for 2 minutes to remove loosely adhered cells. This was repeated with a second cotton swab.
3. The swabs were then placed into their specifically labelled microfuge tubes and rotated vigorously to dislodge the cells.
4. The samples were incubated at room temperature for 30 minutes with periodic stirring. The swabs were removed and discarded into a biological hazard waste bin.
5. The samples were then vortexed for 5 seconds each and then centrifuged for 2 minutes at 13000 revolutions per minute (rpm).
6. The supernatant of each sample was discarded leaving approximately 20 – 30 µl residual fluid. This small volume contained enough DNA for Chelex® extraction.

7. To each sample, 170 µl of 20% Chelex® (w/v) was added to give a final volume of 200 µl. The samples were then incubated for 30 minutes at 56ºC (heating block 1).
8. Each sample was vortexed for 10 seconds and incubated at 100ºC (heating block 2) for 8 minutes. The heating stages destroyed the cell proteins and denatured the DNA.
9. The samples were vortexed again and then centrifuged for 3 mins at 13000 rpm.
10. Lastly, a new set of clean 1.5 ml microfuge tubes were labelled to correspond with the original tubes and the supernatant of each sample was placed in each fresh tube respectively being careful to avoid any contamination. The resulting supernatant contained the single-stranded DNA ready to be amplified via PCR.

3.2.3 Multiplex PCR amplification of Alu Polymorphisms

PCR is used to amplify short regions of chromosomes to detect an insertion of a short DNA sequence called *Alu*. The *Alu* family of short interspersed DNA elements is distributed throughout the human genome. *Alu* elements are 300 base pairs in length and derive their name from a single recognition site for endonuclease *AluI* located in the middle of the *Alu* element. All *Alu* polymorphisms are dimorphic or bi-allelic, meaning each locus/marker contains only two alleles therefore only three possible genotypes can exist.

Three different PCR reactions were set up. Two of the reactions utilize oligonucleotide primers flanking *Alu* insertion sites that amplify the target DNA at four autosomal loci. The third reaction identifies the gender of the individual sample donors using an *Alu* polymorphism in the sex chromosomes.

Table 13: The oligonucleotide primers used in the PCR reactions[110]

Alu I.D.	Primer 1 (5' – 3')	Primer 2 (3' – 5')	+ Alu (no. of base pairs)	– Alu (no. of base pairs)	Chr. No.
ACE	ctggagaccactcccatccttct	gatgtggccatcacattcgtcagat	490	190	17
TPA25	gtaagagttccgtaacaggacagct	ccccaccctaggagaacttctcttt	400	100	8
Ya5NBC51Aa	tttccttacatctagtgcccc	cctccaagtaaagctacaccct	652	355	3
Ya5NBC182	gaaggactatgtagttgcagaagc	aacccagtggaaacagaagatg	563	287	7
*Alu*STYa	catgtatttgatggggatagagg	ccttttcatccaactaccactga	528	199	Y

The ACE/TPA25 and 51A/182 primers were chosen because they are amongst the most useful indicators of genetic variation between individuals and populations. In this report, the method of human gender identification is based on the presence or absence of the *Alu*STYa insertion into the non-recombining region X-Y homologous region. Fixed (non-polymorphic) insertions on the X or Y chromosome (Y in this case) provide a way of identifying that particular chromosome as this chromosome produces a larger fragment when the homologous region is amplified by PCR.

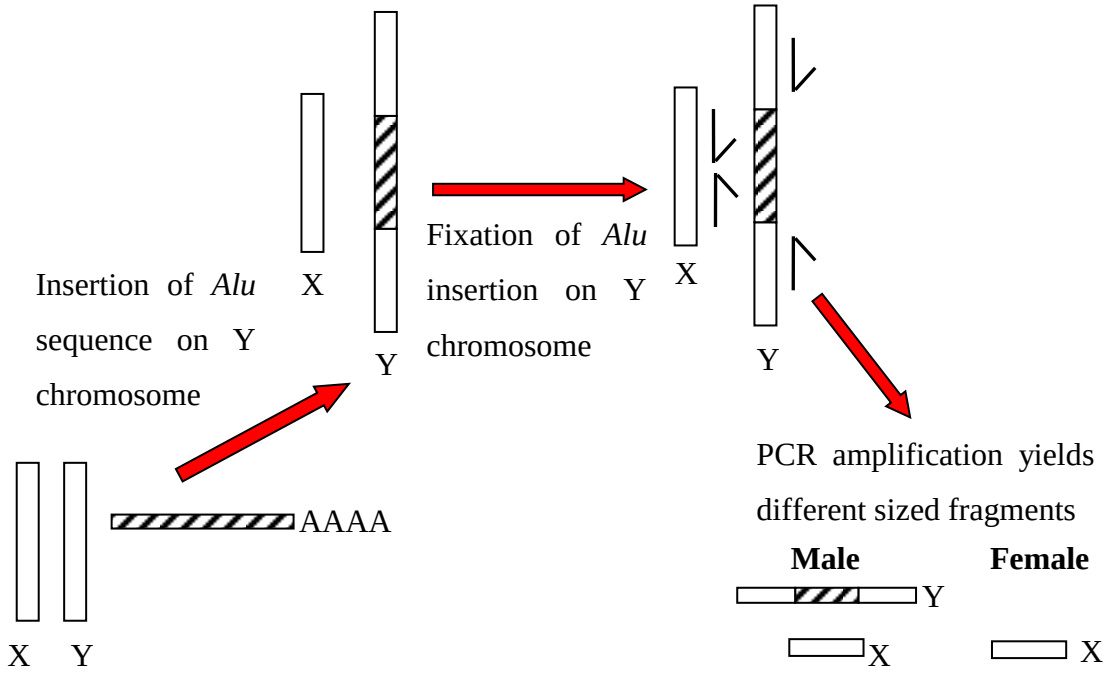


Figure 48: PCR amplification using *Alu*STYa to identify human gender[110]

Below is the protocol used to prepare the DNA extractions for PCR amplification:

1. 0.2 ml PCR tubes were labelled corresponding to the number of samples obtained from the Chelex® extraction.
2. The PCR master mixes for each set of reactions were made up. (Components and quantities can be found in Table 14).
3. 15 µl (unless stated otherwise) of the master mixes were dispensed into each labelled PCR tube. (e.g., 10 tubes contained ACE/TPA25 master mix, 10 tubes contained 51A/182 master mix and 10 tubes contained STYa master mix).
4. 5 µl (unless stated otherwise) of the extracted DNA was added and each tube was mixed gently.

Table 14: PCR table consisting of the master mixes[110]

Component	**ACE/TPA25 Master mix**			**51A/182 Master mix**			**Gender ID mix (*Alu*STYa)**		
Buffer	75 mM Tris-HCl buffer pH 8.8								
dNTPs	0.20 mM each of dATP. dCTP, dTTP, dGTP								
Taq	Taq DNA polymerase enzyme (1.25 units)								
$MgCl_2$	2.50 mM			2.50 mM			2.50 mM		
Primers	Per reaction (µl)	Final Conc^n (µM)	*Total volume (µl)	Per reaction (µl)	Final Conc^n (µM)	*Total volume (µl)	Per reaction (µl)	Final Conc^n (µM)	*Total volume (µl)
Forward – 1	ACE: 0.36	0.18	4.32	51A: 0.24	0.12	2.88	STYa: 0.40	0.20	4.80
Reverse – 1	ACE: 0.36	0.18	4.32	51A: 0.24	0.12	2.88	STYa: 0.40	0.20	4.80
Forward – 2	TPA25: 0.36	0.18	4.32	182: 0.20	0.10	2.40	-	-	-
Reverse – 2	TPA25 0.36	0.18	4.32	182: 0.20	0.10	2.40	-	-	-
2 x Buffer (2.5 mM)	10	1	120	10	1	120	10	1	120
RNAse/ DNAse free Water	3.56	-	42.72	4.12	-	49.44	4.20	-	50.40

∗Total volume = volume of primer per reaction (e.g. 0.36 µl) x number of samples (e.g. n = 12)

Table 15: PCR table consisting of the thermal cycling conditions[110]

	2720 96-well Thermal Cycler		Veriti® 96-well Thermal Cycler
Initial Denature	1 cycle of 3 mins @ 94°C	1 cycle of 3 mins @ 94°C	1 cycle of 3 mins @ 94°C
Cycles	32 cycles of	32 cycles of	32 cycles of
Denature	1 min @ 94°C	1 min @ 94°C	1 min @ 94°C
Anneal	1 min @ 55°C	1 min @ 55°C	1 min @ 58°C
Extension	1 min @ 72°C	1 min @ 72°C	1 min @ 72°C
Final Cycle	5 min @ 72°C	5 min @ 72°C	10 min @ 72°C
	Cooling to 4°C	Cooling to 4°C	Cooling to 4°C

3.2.4 Electrophoresis and Detection

Once the PCR samples were prepared, the next step involved using gel electrophoresis to produce a gel pattern (bands) of the markers, which were visible under UV light.

2% agarose gel preparation

Firstly, the gel-casting tray was prepared by placing an appropriate sized comb (e.g. 16 wells) in the slots and the two ends of the tray were sealed across with biohazard tape ensuring the tape was taut all the way across each side. Then, 2 grams of agarose was weighed out into a 250 ml conical flask. 100 ml of Tris Boric EDTA (TBE) buffer was added to this (pH 8.3). The flask was covered with cling-film and small holes were pierced in the film. The flask was heated in the microwave for 2 minutes, stirred, heated again for 1 min, stirred and heated again for 30 seconds. The solution went clear, however sometimes small bubbles were visible so the mixture was heated for a further 10 – 20 seconds paying careful attention to the flask to avoid the solution boiling over. The agarose was cooled to hand hot and 10 µl of SYBRSafe stain was added and the flask was swirled. The agarose was poured gently into the centre of the gel-casting tray to form even coverage and then allowed to set at room temperature for 30 mins.

Gel loading, electrophoresis & detection

The taped ends of the gel-casting tray were carefully removed and the gel was placed in a tank containing TBE buffer. TBE was added to the tank until it completely covered the top level of the gel. The combs were removed. 10 µl of each of the PCR samples were added respectively into each well, leaving the first well empty for the DNA ladder. 4 µl of ReddyRun Superladder low – 100 bp (Thermo Scientific, Surrey, UK) was loaded into the first well. The electrophoresis was run for 1 hour at 109 volts. The power was turned off and the gel was placed on a trans-illuminator to view the bands under UV light.

In some cases, no bands were seen. There could be several reasons for this such as, the Chelex extraction did not work; the PCR may not have worked; enzymes may have been inhibited where they should not have been; there may have been no DNA present because such small quantities are used and lastly, human pipetting which is a major source of error in these procedures. One method to determine the quantity of DNA present after DNA extraction if any is by using the Quant – IT ssDNA assay kit.

3.2.5 DNA quantification: Quant-iT ssDNA assay kit[107]

The Quant-iT™ ssDNA assay kit consists of:

1. Buffer
2. Dye
3. Standard 1
4. Standard 2
5. Qubit™ Fluorometer is used to measure the samples.

The assay range of this kit is between 1 – 200 ng and can measure samples concentrations as low as 50 pg/µl – 200 pg/µl. The actual protocol used to obtain the measurements to calculate the DNA concentration of the DNA extractions used above was:

1. In a clean microfuge tube, 199 µl x 6 (i.e. number of samples) = 1194 µl of buffer was measured out.
2. 1 µl x 6 = 6 µl of dye was also added to the same tube.
3. 190 µl of this buffer/dye mix was added to 6 Quant-iT tubes.
4. 10 µl of standard 1 was added to one of the Quant-iT tubes.
5. 10 µl of standard 2 was added to another Quant-iT tube.
6. 10 µl of 4 DNA extraction samples were added to Quant-iT tubes respectively.
7. Each sample was vortexed for 5 seconds and then the machine was calibrated using standards 1 and 2 following the onscreen instructions.
8. Then the DNA extraction samples were measured.

The DNA concentration was worked out as follows:

1. DNA concentration Quant-iT reading (ng/ml) x 200/X = DNA concentration (ng/ml)
   X = amount of DNA added to the buffer/dye mix i.e. 10 µl
2. DNA concentration value/1000 = DNA concentration (ng/µl)
3. Total DNA in PCR master mix (ng/µl) = DNA concentration (ng/µl) x 5µl (i.e. amount of DNA added to each PCR master mix.

The required amount for PCR is 50 – 100 ng/µl.

The next section incorporates all three of these methods above to see if any DNA could be extracted from cheek cell swabs and fingerprint samples both before and after chemical treatment with SeO_2, P_4S_3 and $(SN)_x$ before DNA extraction.

3.4. Results & Discussion

Set 1

The samples in this set consisted of:

2 x RNAse/DNAse free water: H_2O (1), H_2O (2) – control samples
(PCR tubes labelled 1A, 1B, 1C, 2A, 2B, 2C)
2 x cheek cell swabs from donor 1: JF_1, JF_2
(PCR tubes labelled 3A, 3B, 3C, 4A, 4B, 4C)
2 x cheek cell swabs from donor 2: BS_1, BS_2
(PCR tubes labelled 5A, 5B, 5C, 6A, 6B, 6C)
2 x eccrine fingerprints on glass from donor 2: BS_{E1}, BS_{E2}
(PCR tubes labelled 7A, 7B, 7C, 8A, 8B, 8C)
2 x sebaceous fingerprints on glass from donor 2: BS_{S1}, BS_{S2}
(PCR tubes labelled 9A, 9B, 9C, 10A, 10B, 10C)

Chelex® extraction was carried out on samples 3 - 10, and then the 3 different PCR master-mixes were made up. So the ACE/TPA25 master mix was added to set A, the 51A/182 master mix was added to set B, and the STYa master mix was added to set C. The PCR conditions were as listed in Table 14.

After electrophoresis, the gel was viewed under UV light and no bands were present at all. There are several reasons for this that have been mentioned previously, so two of the cheek cell swabs were investigated to see if DNA was conclusively present in the samples by quantifying the DNA using the method reported in 3.2.5. The readings proved that there was DNA present in the samples; it may be that some of the Chelex® may have also mixed into the samples, which would then inhibit the enzymes used for PCR amplification. This set of samples was discarded except for JF_2 and a fresh set of DNA extractions were carried out.

Set 2

This set of samples consisted of:

2 x cheek cell swabs from donor 1: JF_1, JF_3 (PCR tubes labelled 1Aa, 2Aa)

1 x cheek cell swab from donor 1 from set 1: JF_2 (PCR tube labelled 3Aa)

2 x cheek cell swabs from donor 2: BS_1, BS_2 (PCR tubes labelled 4Aa, 5Aa)

2 x eccrine fingerprints on glass from donor 2: BS_{E1}, BS_{E2}

(PCR tubes labelled 6Aa, 7Aa)

2 x sebaceous fingerprints on glass from donor 2: BS_{S1}, BS_{S2}

(PCR tubes labelled 8Aa, 9Aa)

2 x RNAse/DNAse free water: H_2O (1), H_2O (2) (PCR tubes labelled 10Aa, 11Aa)

The Chelex® extraction method was carried out with all the samples except JF_2 as the DNA had already been extracted previously. PCR amplification was carried out with only one duplex master mix this time. ACE/TPA25 PCR master mix was chosen as this is known to have a higher success rate with PCR.

The electrophoresis revealed no bands for the second time. The PCR amplification was carried out again however the master mix was made up with different volumes that can be seen in Table 16 below:

Table 16: PCR table for a new ACE/TPA25 master mix; n = 15

Component	**ACE/TPA25 Master mix**		
Buffer	75 mM Tris-HCl buffer pH 8.8		
dNTPs	0.20 mM each of dATP. dCTP, dTTP, dGTP		
Taq	Taq DNA polymerase enzyme (1.25 units)		
$MgCl_2$	2.50 mM		
Primers	Per reaction (µl)	Final Conc^n (µM)	Total volume (µl)
Forward – 1	ACE: 0.36	0.18	5.40
Reverse – 1	ACE: 0.36	0.18	5.40
Forward – 2	TPA25: 0.36	0.18	5.40
Reverse – 2	TPA25 0.36	0.18	5.40
2 x Buffer (2.5 mM)	10	1	150
RNAse/DNAse free Water	1.56	-	23.40

The final volume of master mix per reaction was reduced from 15 µl to 13 µl and the DNA content was increased from 5 µl to 7 µl. This new master mix was used to carry out PCR amplification using the DNA extractions previously obtained from the samples in set 2 and these were labelled 1At – 11At respectively.

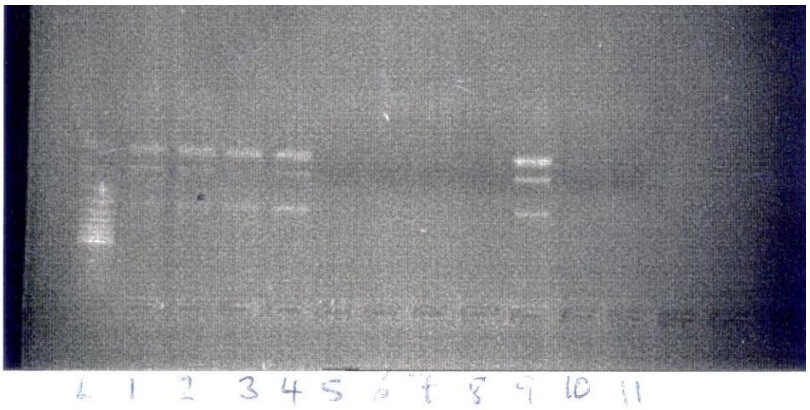


Figure 49: Gel pattern for samples 1At - 11At

After electrophoresis, there were bands visible from samples 1At – 4At and 9At. No bands were visible from samples 10At or 11At as these were the negative controls and 5At – 8At (fingerprint samples). This may be because the water in the master mix was diluting the DNA concentration thus there was an insufficient amount of DNA amplified to show any visible bands. Therefore, it seemed logical to try to improve the PCR method by increasing the DNA content further and removing water from the master mix.

Table 17: ACE/TPA25 master mix with no water; n = 15

Primers	Per reaction (µl)	Final Conc^{n} (µM)	Total volume (µl)
Forward – 1	ACE: 0.36	0.18	5.40
Reverse – 1	ACE: 0.36	0.18	5.40
Forward – 2	TPA25: 0.36	0.18	5.40
Reverse – 2	TPA25 0.36	0.18	5.40
2 x Buffer (2.5 mM)	10	1	150

The master mix volume per reaction was reduced to 10.72 µl and the DNA content was increased to 9.78 µl. Again the DNA extractions previously obtained from set 2

were added to each PCR master mix and labelled 1bT to 11bT respectively. The number of PCR cycles was also increased from 33 to 40 cycles in total.

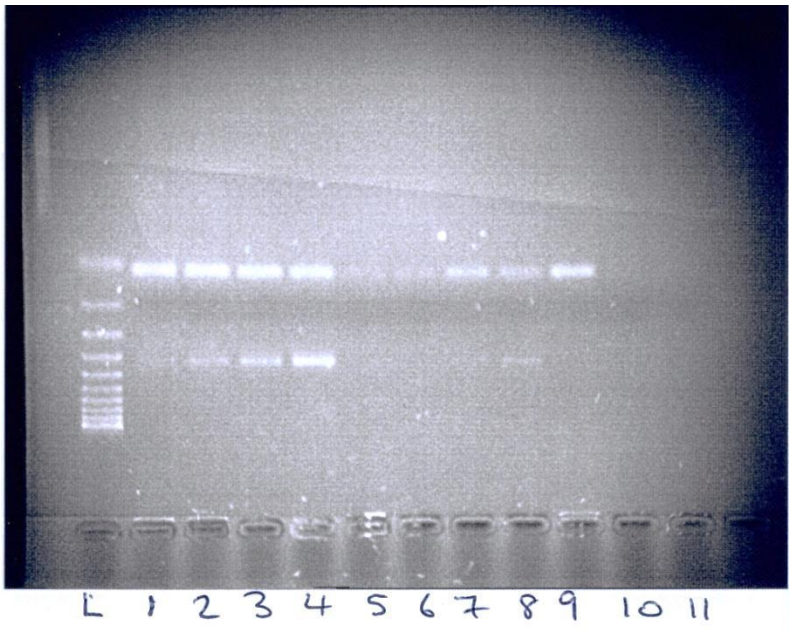


Figure 50: Gel pattern for samples 1bT - 11bT

The electrophoresis revealed bands with all of the samples bar the negative controls. However, the bands revealed from samples 5bT – 8bT were slightly ambiguous so one more alteration was made to the PCR method to see if the results could be improved.

This time dimethyl sulfoxide (DMSO) was added to each master mix. DMSO is used in PCR to inhibit secondary structures in the DNA template or the DNA primers. It is added to the PCR mix before reacting, where it interferes with the self-complementarity of the DNA, minimizing interfering reactions. Also due to the cost of these primers, a master mix of TPA25 was made only from this point onwards.

Table 18: TPA25 master mix with DMSO; n = 15

Primers	Per reaction (µl)	Final Conc^{n} (µM)	Total volume (µl)
Forward – 1	TPA25: 0.36	0.18	5.40
Reverse – 1	TPA25 0.36	0.18	5.40
2 x Buffer (2.5 mM)	10	1	150
DMSO (neat)	1	-	15

The final volume of the TPA25 master mix used in each reaction was 11.72 µl and the DNA extraction volume was 8.78 µl. This time the PCR tubes were labelled 1Tp – 11Tp respectively.

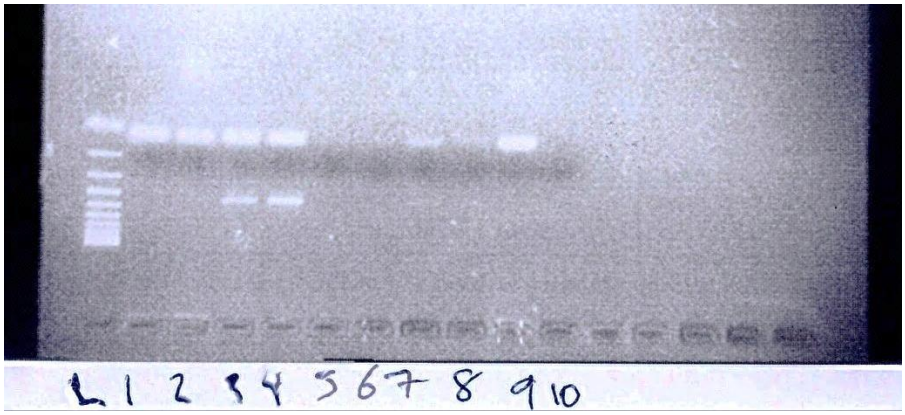


Figure 51: Gel pattern for samples 1Tp - 11Tp

The gel electrophoresis revealed all bands for 1Tp – 9Tp however they looked very similar to the previous gel pattern. The DMSO did not appear to improve the PCR in this case. The bands appeared very faint with samples 5Tp – 8Tp so it seemed logical to use the Quant-iT ssDNA assay kit to calculate the DNA concentration in those DNA extractions.

DNA quantification: 5Tp – 8Tp

Table 19: Total DNA content of samples 5Tp - 8Tp in the final PCR master mix

Sample I.D.	Reading (ng/ml)	DNA Concentration (ng/ml)	DNA concentration in 8.78 µl of DNA in PCR master mix (ng/µl)
5Tp	8.02	160.4	1.33
6Tp	14.20	284.0	2.35
7Tp	15.80	316.0	2.62
8Tp	6.22	124.4	1.03

From these results, it can be seen that the total DNA in each master mix from the fingerprint samples is much lower than what is needed for PCR. However, as it has been seen above the combination of making the TPA25 master mix with no water, using a total DNA content of 9.78 µl, and increasing to 40 PCR cycles appears to

reveal bands from both the cheek cell swabs and the fingerprint samples. Therefore these were the final conditions chosen to carry out DNA analysis from fingerprint samples that have been revealed by the sublimation of SeO_2, P_4S_3 and $(SN)_x$ polymer beforehand.

Set 3

This set of samples consists of:

2 x cheek cell swabs from donor 2: labelled 1F, 2F.

2 x cheek cell swabs from donor 3: labelled 3F, 4F.

1 x eccrine & 1 x sebaceous fingerprint on glass from donor 2: 5F, 6F.

1 x eccrine & 1 x sebaceous fingerprint on glass from donor 3: 7F, 8F.

1 x eccrine & 1 x sebaceous fingerprint on glass from donor 2, both revealed by sublimation of SeO_2 prior to DNA extraction: 9F, 10F.

1 x eccrine & 1 x sebaceous fingerprint on glass from donor 2, both revealed by sublimation of P_4S_3 prior to DNA extraction: 11F, 12F.

1 x eccrine & 1 x sebaceous fingerprint on glass from donor 3, both revealed by sublimation of $(SN)_x$ polymer prior to DNA extraction: 13F, 14F.

1 x RNAse/DNAse free water (for PCR only): 15F.

Chelex® extraction of samples 1F-14F was carried out, and the TPA25 master mix was made up as in Table 20 and 9.78 µl of each DNA extractions was added to 10.72 µl of the PCR master mix respectively.

Table 20: TPA25 master mix with no water; n = 18

Primers	Per reaction (µl)	Final Conc^n (µM)	Total volume (µl)
Forward – 1	TPA25: 0.36	0.18	6.48
Reverse – 1	TPA25 0.36	0.18	6.48
2 x Buffer (2.5 mM)	10	1	180

The gel electrophoresis revealed bands of samples 1F – 10F and 12F – 14F. However, no bands were visible from sample 11F and also some bands were very faint (samples 7F, 9F, 13F, 14F) and so slightly ambiguous as to whether these were visible bands or not. These particular samples are the eccrine fingerprint samples, there could be a

pattern emerging whereby eccrine fingerprints pre-treated to visualize them may be affecting the DNA present in the fingerprints however this is not conclusive.

The whole process was repeated with the DNA extractions from Set 3 to see if the same pattern emerged. These samples were labelled 1G – 15G for the PCR. The gel electrophoresis revealed bands from all of the samples 1G – 14G except for 11G. There could be several explanations for this. For example, the donor may have simply placed a "bad" eccrine fingerprint, the P_4S_3 could have interfered with the DNA present in the sample, there may not have been sufficient DNA in the sample to begin with, the Chelex® extraction may not have worked, human pipetting error, or the PCR process may have been inhibited by an enzyme or compound interfering with the amplification process. However, it would be an arduous and elongated process to distinguish which of these could be the cause for no bands being visible in the gel pattern for that particular sample.

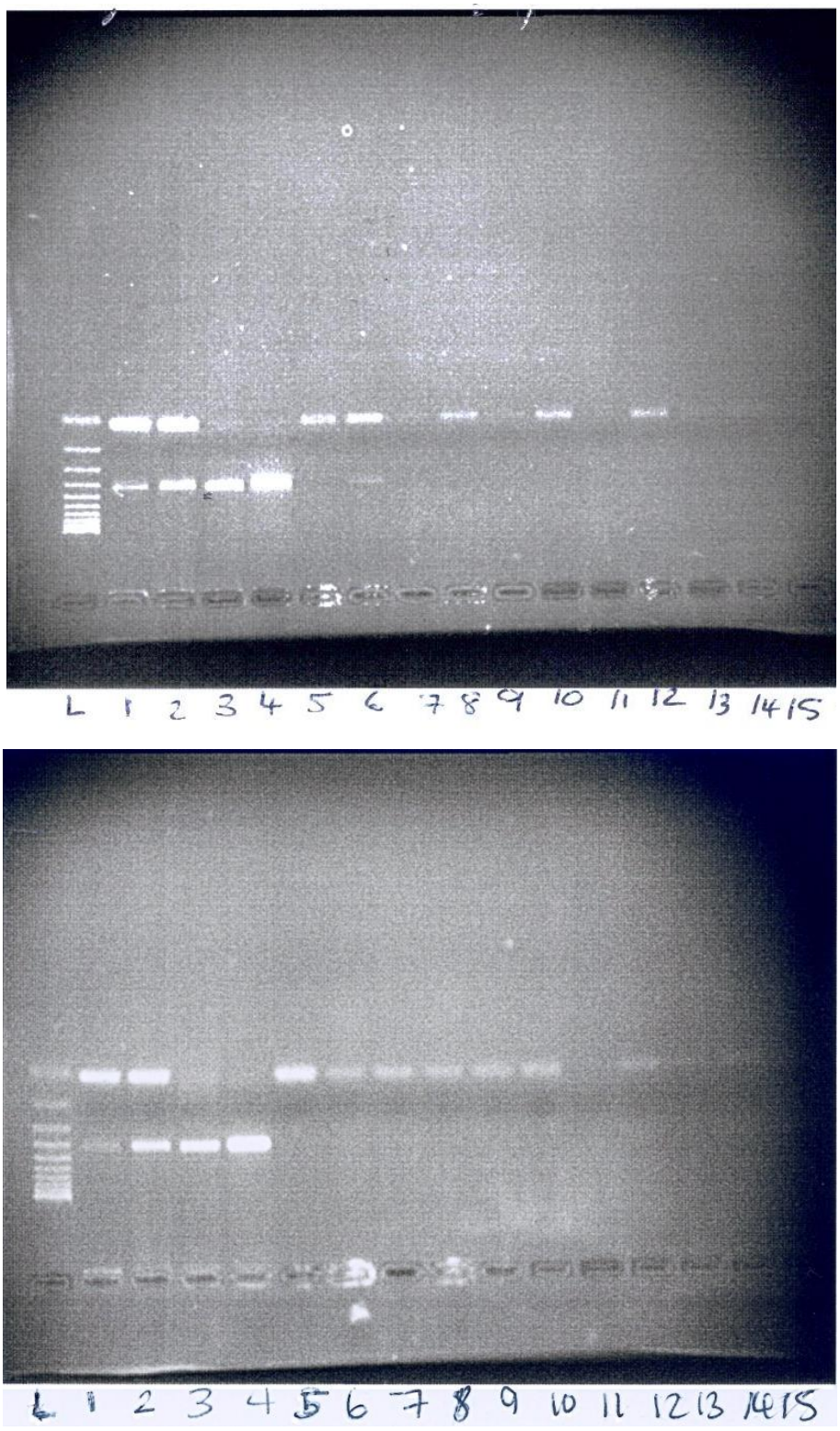


Figure 52: Gel patterns for samples 1F - 15F (left) & 1G - 15G (right)

3.5. Conclusions

Overall, the aim of this research was to investigate if it was possible to detect *Alu* insertion polymorphisms by PCR from latent fingerprints before and after chemical treatment prior to DNA extraction. It can be concluded that the *Alu* polymorphism insertions have been detected in the latent fingerprint samples before and after chemical treatment using the sequence of Chelex® extraction, followed by the addition of the TPA25 master mix as shown in Table 20, 40 cycles of PCR amplification and finally gel electrophoresis to reveal the bands.

This has created a platform to show that DNA can be extracted and amplified from latent fingerprints after they have been exposed to SeO_2, P_4S_3 and $(SN)_x$ polymer. It could be said that these compounds may not be interfering with DNA present in latent fingerprints. However, to conclusively state whether these fingerprint development techniques are non-destructive or not can only be confirmed after using specialist and more sensitive DNA profiling kits to see if any DNA profiles are obtainable.

Chapter 4 – Electrostatic Detection Apparatus (ESDA)

The concept of electrostatically charging fingerprints for their subsequent detection was first investigated in the 1970's with HOSDB working closely with the London College of Printing to develop the ideal equipment for this purpose[111]. HOSDB had discovered that the decay time for charged fingerprints on most materials was very short and so direct charging and toning was unfeasible. This was overcome by covering the surface being examined with a thin layer of Mylar (polyester) and producing the charge image on this thin polymer film. The thin film was then exposed to a corona-charging device and then treated with an electrostatic image developer, in this case, glass carrier beads mixed with carbon black as the cascade toner.

Initial research showed the process was able to develop fingerprints on papers and fabrics[112,113] but this was limited to fresh marks. However, HOSDB did observe that the technique was capable of revealing indented writing on paper and the ESDA results became superior to other techniques such as oblique lighting[114].

The indentations caused a local increase in capacitance due to a reduction in the distance between the charged surfaces and fingerprints, causing a local increase in capacitance because of the water in the fingerprint increasing the local dielectric constant[115]. However, very deep impressions sometimes do not develop with ESDA, which indicates capacitance variations are not the only mechanism.

It was later proposed that the indented writing effect could be explained by damage and abrasion of surface fibres caused by lateral movement between the sheets of paper during the writing process. Although the ESDA technique has successfully revealed indented marks, the technique has low sensitivity and is ineffective on finger-marks more than 24 hours old.[114] However, there is still a large interest in ESDA because it is non-destructive, low impact technique. This section investigates the use of vapours to enhance aging fingerprints, which in turn could potentially alter the ESDA response, whilst maintaining the low impact nature of the technique.

4.1. Experimental

The mechanism of ESDA has not conclusively been established, but Figure 54 and Figure 55 illustrate the stages in the process. The porous sample was held down on a sintered plate using vacuum and a thin (~3.5 μm) film of Mylar was laid over the top. This film was negatively charged by passing a charge-spraying device (Corotron) above the surface. The charging process set up electrostatic fringing fields around features in the exhibit. A mixture of carrier beads (fine glass spheres) and toner particles (carbon black) were cascaded across the surface. The toner selectively adhered to areas where the fringing fields were present.

Where samples were exposed to different gases (I_2, CO_2, N_2, O_2 and SO_2), a container with paper or cotton samples fixed to the lid was filled with a flow of gas from a gas cylinder to replace any air. The container was then sealed and left for different periods of time prior to using the ESDA.

Relative humidity was also investigated by placing samples (fixed onto the lid) in a container half-filled with hot water (RH > 80%) or placed in an oven (RH < 80%) prior to using the ESDA.

Initial test

The initial test was to place a depletion series of twenty fingerprints on plain paper. A depletion series is when a fingerprint is pressed on a surface 10 – 20 times successively, during which less residue is deposited with each touch. A depletion series of eccrine and sebaceous prints analysed using ESDA. Both sets were then exposed to sulfur dioxide (SO_2) gas for 1 hour and then run for a second time.

Direct comparison

Fingerprints were placed on paper as above but each fingerprint was split in half; one-half was exposed to a gas in a sealed container and the other half was exposed to air.

Figure 53: Illustration of how the direct comparison of fingerprints is undertaken for the ESDA process

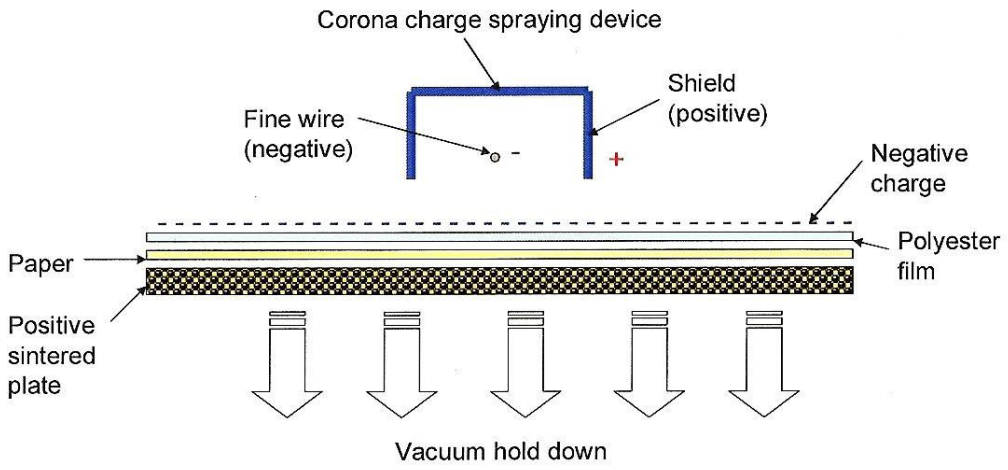


Figure 54: Schematic diagram of the general charging procedure for ESDA[114]

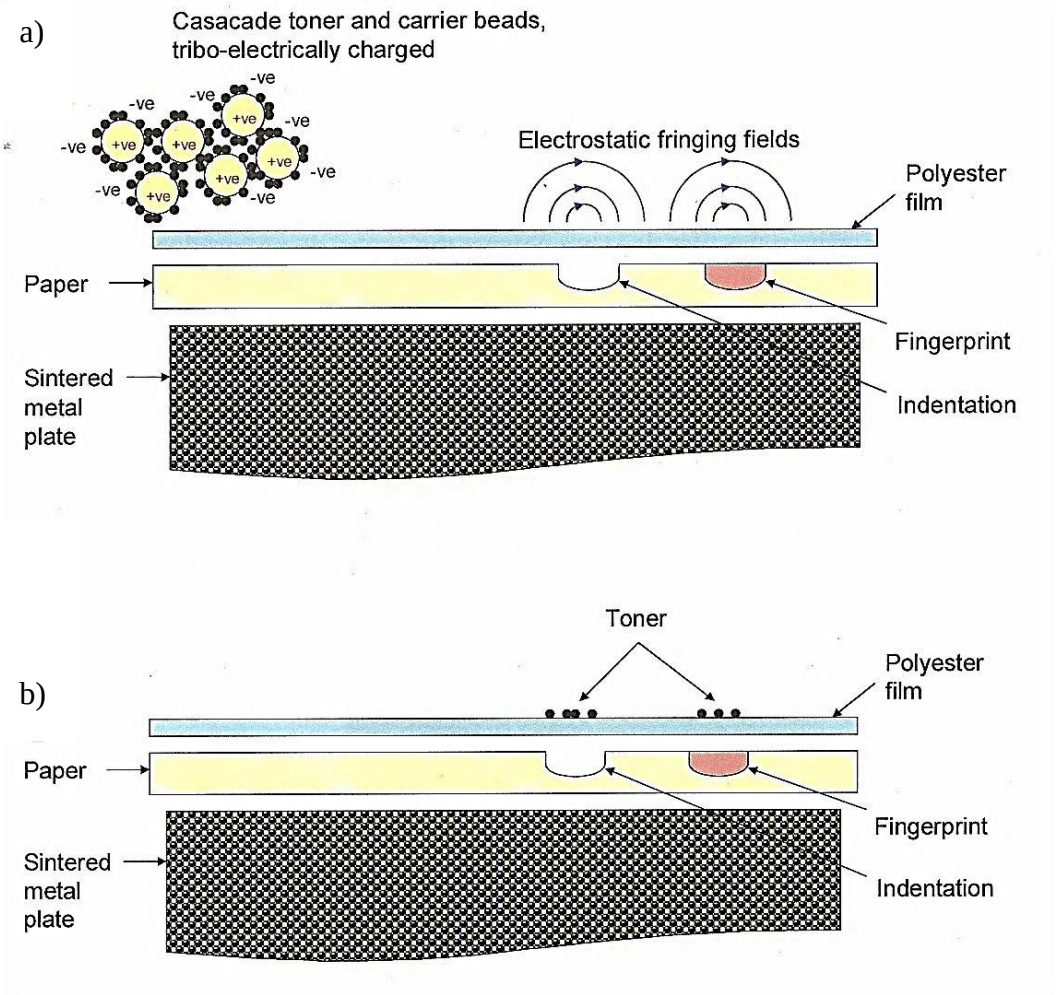


Figure 55: Schematic diagrams showing toner development of electrostatic images: (a) development of electrostatic fringing fields on the polymer film & (b) selective adherence of toner particles to regions where fields are present[114]

4.2. Results and Discussion

Initial test

The thought process behind this initial test was that if the intensities remained consistent each time the sample was run (see Figure 56) , then the same sample could be exposed to SO_2 and other compounds prior to using ESDA with confidence that the same response would be observed.

However, both sets appeared to produce lower intensities (i.e. lighter fingerprints with less ridge detail) after the second ESDA process. The main problem that was faced was making sure that the toner was cascaded across the sample with a constant flow allowing even coverage over the sample however, this technique was mostly perfected with practice.

Direct comparison

This was a much easier way of comparing the ESDA response rather than striving to produce a rating system for the change in intensity, as there were too many variables that were difficult to control.

4.2.1. The Effect on the ESDA Response on Exposure to Various Gases

Iodine Fuming – I_2

A depletion series of twenty eccrine and twenty sebaceous fingerprints were placed on plain white paper and one-half of each set was fixed facing up onto the lid of a container. The iodine crystals were placed in the bottom of the container and heated gently to accelerate the fuming process. The other half were placed in a separate container and simply sealed as mentioned in 4.1. Both sets were left for 30 minutes prior to using the ESDA.

Although iodine fuming reveals fingerprints, the ESDA response post-treatment was the measurement of interest. I_2 is non-polar and has a low dielectric constant and so would expect a poorer ESDA response on fingerprints exposed to I_2 due to a decrease in the local dielectric constant. The ESDA response decreased through the depletion

series in both cases, however, there was no difference in the response between each print. This showed that I_2 fuming had no effect on the ESDA response to fingerprints.

Carbon Dioxide (1) – Dry ice

The same method described above was used but this time some dry ice was placed in the bottom of the container and sealed. Both the eccrine and sebaceous prints were exposed to CO_2 for 30 minutes.

It was found that the eccrine prints exposed to CO_2 gave a reduced ESDA response (lower intensity) compared to their equivalent other half that had not been treated with any chemical. There was no change in the ESDA response with the sebaceous set of fingerprints. This method of introducing CO_2 may not have been the best way therefore to keep the method consistent; fingerprints were exposed to pure CO_2 gas via a CO_2 gas cylinder instead.

Carbon dioxide (2) – Pure gas

This time pure CO_2 gas was expelled into the container and immediately sealed. Various attempts were made whereby the fingerprints were exposed to CO_2 for different time periods of 24 hours, 6 ½ hours and 2 hours before running on the ESDA.

- **CO_2 exposure: 24 hours**

Both the eccrine and sebaceous fingerprints produced poor ESDA responses. It can be concluded that fingerprints left overnight produce a poor ESDA response regardless of the CO_2 exposure. The reason for exposing the fingerprints to CO_2 overnight was to allow ample time for the gas to interact with the fingerprints chemically to see if this changed the nature of the electrostatic behaviour of fingerprints.

- **CO_2 exposure: 6 ½ hours**

The ESDA response decreased in exactly the same manner with the fingerprints exposed to CO_2 and those that were not. It could be possible that the fingerprints are being exposed to CO_2 gas for too long so it seemed sensible to reduce the exposure

time to 2 hours. This meant that more samples could be run in a shorter period of time, it should have been more than enough time for the CO_2 to interact with the fingerprints and allowed a better ESDA response as the fingerprints will only be 2 hours old rather than ½ or 1 day old.

- **CO_2 exposure: 2 hours**

The ESDA response was very similar to those seen after 6 ½ hours. The similar ESDA response could be because as there is also CO_2 present in air so even though one side is exposed to pure CO_2 the amount present in the atmosphere could be enough to trigger the similar interactions if any and produce alike ESDA responses throughout. Therefore, it makes sense to try out the most abundant gas in the atmosphere, nitrogen (N_2) gas.

Nitrogen gas – N_2

Again, 2 hours was ample time to expose each gas to fingerprints before using the ESDA so this was also carried out with N_2 gas.

The same response was found in this case also, in that the intensities declined in the same manner as those exposed to air with both types of fingerprints. Another component that makes up the atmosphere is oxygen gas (O_2) so this was also investigated.

Oxygen gas – O_2

In this case, the eccrine fingerprints showed a consistent decrease in ESDA response through the depletion series, there was no significant difference when exposed to O_2. However, the sebaceous fingerprints that were exposed to O_2 showed a significant decrease in the ESDA response earlier on in the depletion series compared to those only exposed to air. As O_2 is non-polar it has a low dielectric constant, so there must be a decrease in the local dielectric constant upon interaction with latent fingerprints.

Lastly, sulfur dioxide gas as previously mentioned in 1.5.5 has been used in its radioactive form to develop fingerprints from surfaces such as fabrics. Although the mechanisms behind the processes are inconclusive, it was interesting to see whether

SO_2 being a slightly polar gas had any effect on the ESDA response as the local dielectric constant would be expected to increase upon interaction with fingerprints.

Sulfur dioxide gas – SO_2

- **Fabrics**

Since radioactive SO_2 has been used to develop fingerprints on fabrics, due to safety precautions, the initial test involved using non-radioactive SO_2. Two eccrine and two sebaceous fingerprints were placed on two pieces of black cotton (thicker weave) and the same on two pieces of white cotton (finer weave). One set of black and white cotton were exposed to SO_2 and left over two days in a sealed container. The other set were left open in air over two days.

Only the sebaceous fingerprints on cotton exposed to SO_2 were revealed and none of the fingerprints showed any ridge detail. However, from the set of fingerprints left open in air, fingerprints were only developed on the white cotton and had a small amount of ridge detail. The process was repeated and very similar results were observed the second time around.

This could be explained by the nature of the surface, as the black cotton was more porous than the white cotton and due to the size of the weave, they also had a significant difference in texture. Both of these factors affect how a fingerprint remains on the surface over time due to the vast number of organic and inorganic constituents present in a fingerprint residue.

- **Paper**

The same protocol was used to place fingerprints on paper. An alternative method was also attempted whereby both sets were left in air for approximately 6 hours, then one half of each fingerprint was exposed to SO_2 and the other half left open in air for a further 6 hours.

Using the original protocol of exposing the fingerprints to SO_2 for 2 hours, the ESDA response was poor in both cases. This was repeated three times, but there was very little success with the ESDA response being very poor revealing no fingerprints at all.

The second approach showed an increase in ESDA response down the depletion series of the eccrine fingerprints (see Figure 57). This was repeated twice more and a similar response was seen each time. The intensities appeared to increase slightly along the depletion series, which was not the case for all the other gases tested. The films were scanned and an attempt was made to enhance the quality of any fingerprints revealed by adjusting the brightness and contrast. However, on closer inspection, the results showed no significant difference between the two sets of fingerprints. This concluded that SO_2 did not appear to have any significant effect on the ESDA response to latent fingerprints.

4.2.2. The Effect of Humidity on the ESDA Response

The effect of increased humidity changes the absorption rate of the constituents in a latent finger-mark residue (see 1.4.1). In this case, as paper is a porous material, latent fingerprints absorb very quickly. The depth of penetration of the latent finger-mark into the surface depends on environmental conditions such as the relative humidity (RH) and degree of porosity of the surface. The higher the relative humidity, the faster the migration. If the temperature is raised, (i.e. relative humidity is lower than normal) then water evaporates quicker and migration slows down.

Effect of a higher relative humidity than "normal" on the ESDA response

The direct comparison method was used. One-half of each fingerprint was fixed onto the lid and the container was filled with 100 ml hot water and then sealed. The water vapour (steam) was allowed to interact with the fingerprints for 1 hour and the second half of each fingerprint was left open in air for 1 hour before running the samples on the ESDA.

No fingerprints were revealed after exposure to a higher relative humidity. The toner had a tendency to cover most of the paper surface instead. One theory that could be used to explain this is the "surface variation theory" whereby after humidification the paper behaves as a conductor rather than as a dielectric. The variation of electrostatic potential on the polymer film is a function of the degree of close contact between the polymer film and the paper. This could explain why deep indentations, where the film

does not contact the paper, do not produce results using ESDA. As the fingerprint residues are absorbed into the porous surface, their effect on the surface is reduced, this is increased some-what due to the increase in relative humidity inflicting faster migration[116]. It must be noted that none of these mechanisms have been conclusively proven.

Effect on the ESDA response under dry conditions

Here one half of each fingerprint were placed in an oven (temperature ~ 80°C) for 1 hour and the second half of fingerprints were left open in air for 1 hour prior to using the ESDA.

The set of fingerprints that were dried in the oven produced very faint ridge detail with some eccrine fingerprints compared to the set left out in air for 1 hour (see Figure 58), which is expected as the water evaporates quicker than normal and so the residues migrate much slower into the porous surface. However, the quality of the ridge detail was considerably low (grade 2 and below) to state that the ESDA response was affected significantly.

4.3. Conclusions

The ESDA response did not show any significant changes when eccrine fingerprints were exposed to various gases and changes in relative humidity. It was likely that any changes, if any, were so minute that the ESDA was not sensitive enough to detect any changes in local dielectric constants. The ESDA developed sebaceous fingerprints in most cases but again showed no significant change in response after the fingerprints were exposed to the different gases or humidity effects.

A major advantage of ESDA is that it is non-destructive. However, samples run using ESDA produced inconsistent results because consistency was a key issue i.e. how the toner was cascaded over the samples. It was impossible to expose the same volume of toner particles over each fingerprint on each sample, which meant there were always inconsistent results. ESDA is not recommended as a fingerprint development technique as the results are limited to the donor(s) used because the composition of fingerprints vary significantly from donor to donor.

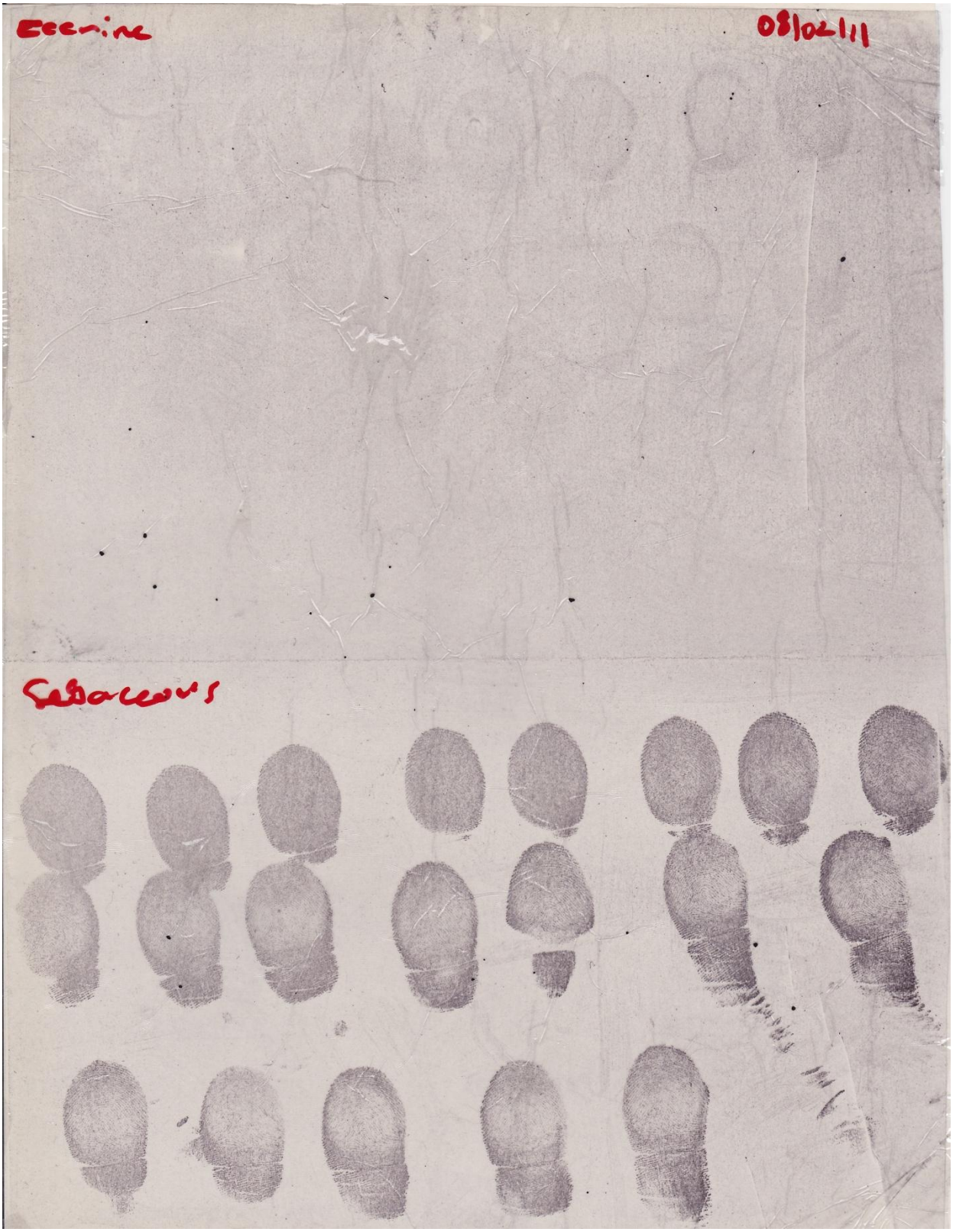


Figure 56: ESDA lift of depletion series (1-20) of eccrine prints (top) and sebaceous prints (bottom)

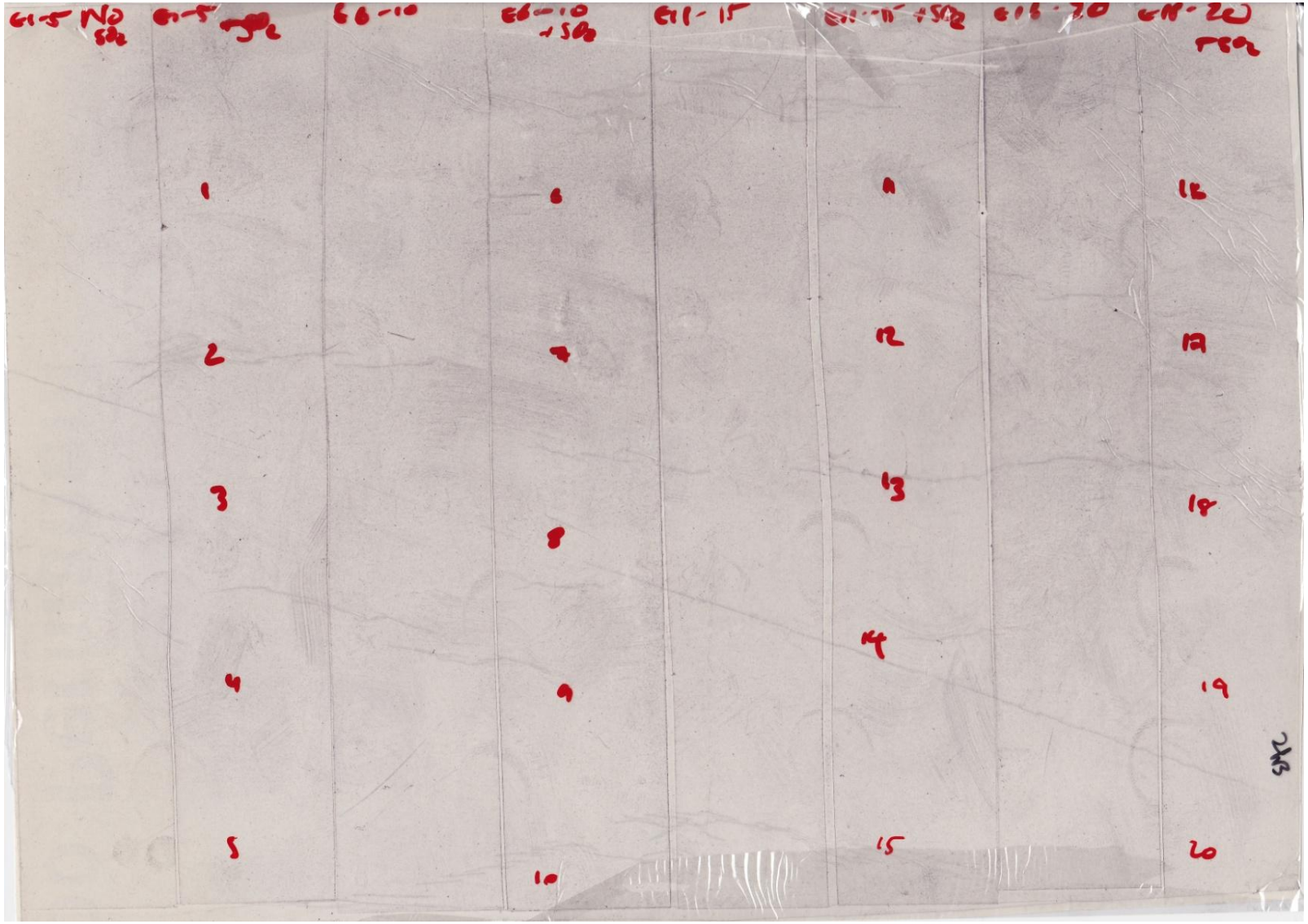


Figure 57: ESDA lift of eccrine prints exposed to SO_2 for 2 hours prior to using the ESDA

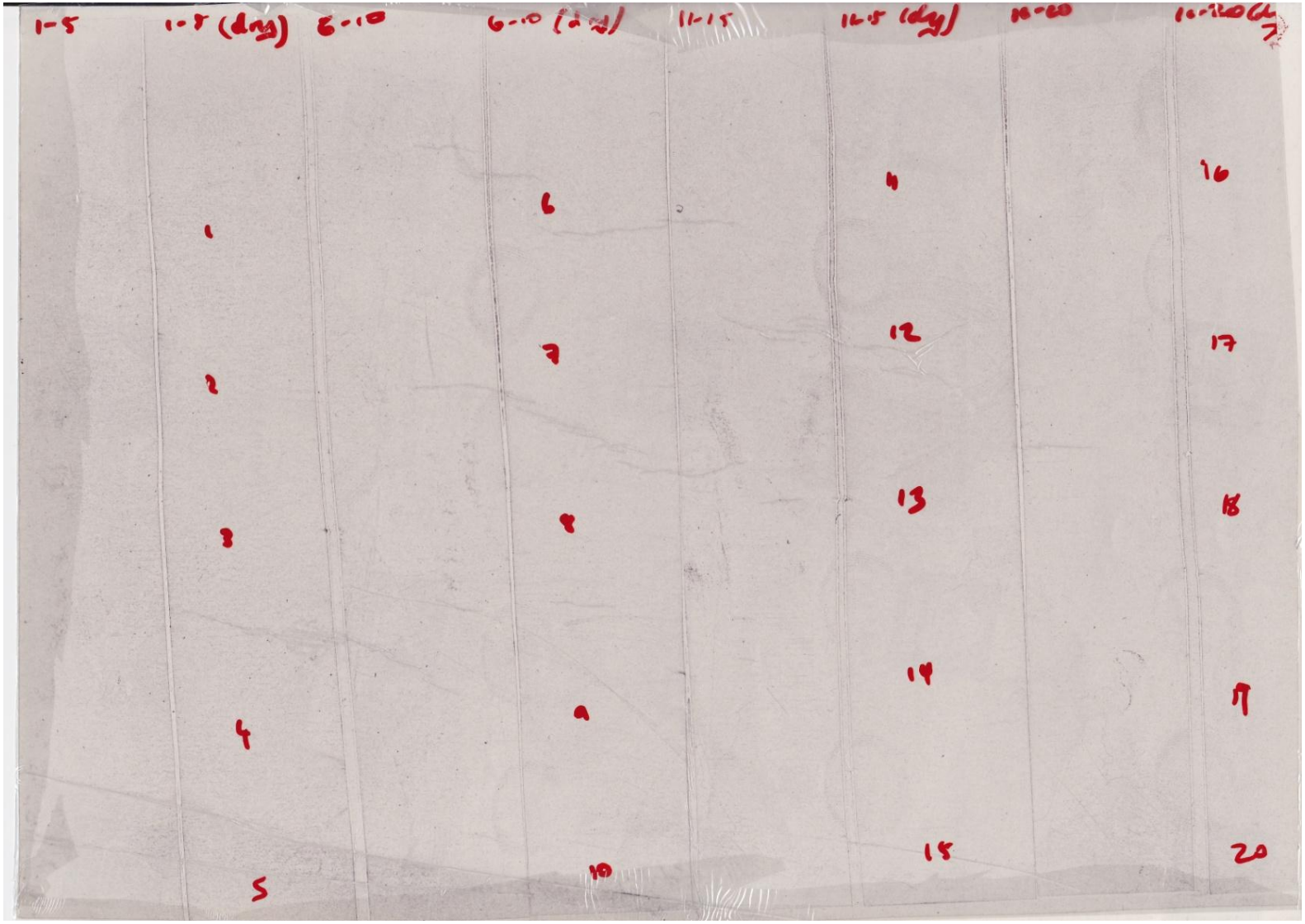


Figure 58: ESDA lift of eccrine prints after being oven-dried for 1 hour prior to using the ESDA

Chapter 5 – Miscellaneous Techniques

Several miscellaneous techniques have been used alongside commonly used techniques to visualise latent fingerprints (For details see section 1.5.5). These techniques have been found to enhance prints on problematic surfaces such as adhesive tapes (Crystal violet), painted surfaces (Iodine fuming), thermal paper (DMAC), fabrics (Radioactive SO_2), cartridge casings and metals (Gun-Blue, SKP, S_2N_2) and aging/degraded prints (Antibodies conjugated to gold nanoparticles). Some of these techniques require highly toxic compounds; require cautious handling; require compounds to be synthesised and some materials are too expensive to use in practice. This presents an opportunity for other novel techniques to be explored that could potentially avoid some of these issues mentioned.

This chapter investigates whether a technique used in the detection of carbon dioxide using a hydrazine-crystal-violet (HCV) mixture[118] could be adapted to enhance latent prints exposed to carbon dioxide. There was also extensive research carried out in 1973 by S. E. Vincent (University of Surrey)[119] reporting the use of commercially available compounds that can interact with specific constituents present in latent fingerprints and develop prints on fabrics. Although the compounds showed some promise, the findings were unpublished as further development was required. Therefore, these techniques were revisited and optimized.

5.1. Gentian (crystal) Violet[120]

Crystal violet reacts with lipids and has been used as a dye for staining epithelial cells as well as fatty acids present in latent fingerprint residues especially on adhesive surfaces. The exact mechanism of how the dye reacts with these constituents is unknown, however, one mechanism has been proposed for the interaction of the crystal violet dye molecule with lipids present in fingerprint residue.

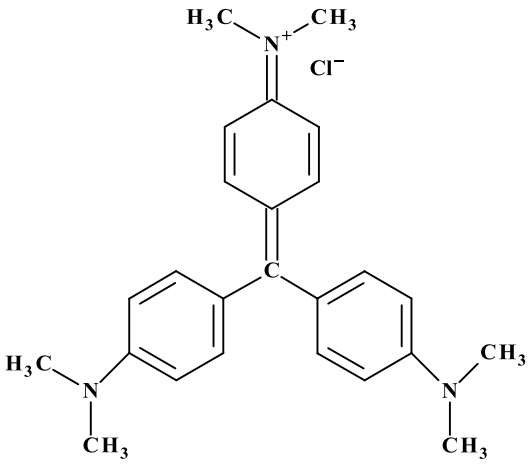


Figure 59: Structure of crystal violet[120]

The dye could link to fatty acids via hydrogen bonding between the nitrogen atom of the dye and the hydroxyl group in the fatty acid as shown below[121]:

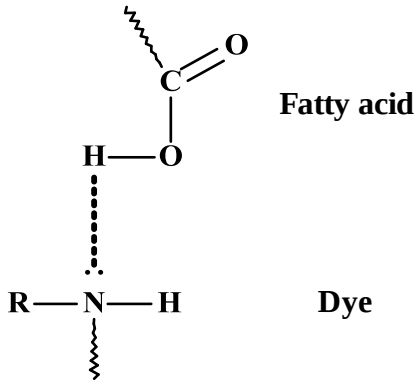


Figure 60: Alternative mechanism for the staining action of gentian violet[121]

This mechanism is applicable to the phenolic water solution used in the crystal violet formulation. This report uses the same formulation as recommended by CAST.

5.1.1. Experimental

Crystal Violet (CV) Formulation

The stock solution consisted of crystal violet (5 grams), phenol (10 grams) dissolved in 96% ethanol (50 ml). The working solution comprised of stock solution (1 ml) and distilled water (approximately 30 ml) or the water was added until the gold film that formed on the surface of the solution disappeared. The stock solution was stored in an amber bottle.

There were safety issues with this formulation in that crystal violet is carcinogenic and phenol is mutagenic. Extra precautions were made whereby all experiments were carried out in a fume-hood, any waste solutions and washings were kept in a separate bottle and disposed of separately.

In addition, if a gold film formed on the surface of the working solution, it was discarded as it may have resulted in high background staining on the sample surface.

This solution has already been reported to reveal fingerprints from the adhesive side of various tapes. However, a hydrazine crystal violet formulation recently reported, detects the presence of CO_2.[118] This initiated the notion of whether this reaction could be used for the enhancement of latent fingerprints exposed to CO_2.

Hydrazine Crystal Violet (HCV) Formulation[118]

Research has been reported whereby a formulation of crystal violet (1 mg) dissolved in methanol (15 cm^3) and hydrazine hydrate added drop wise to reach a colourless end point has been used to detect the presence of CO_2 as the solution goes from colourless to purple if CO_2 is present. Two reactions appear to take place, initially the hydrazine reacts with CO_2 to produce a carbamic acid. This intermediate product then reacts with crystal violet to give a purple colour change, as crystal violet is protonated.

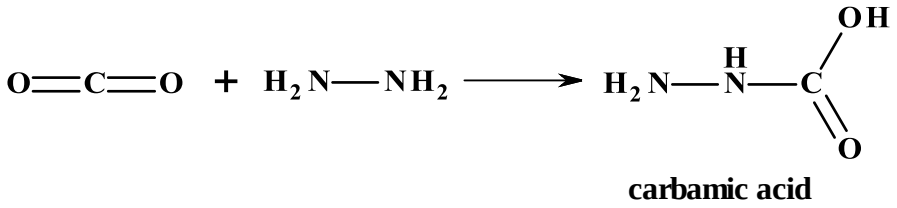


Figure 61: Reaction of hydrazine with CO_2 to produce a carbamic acid[118]

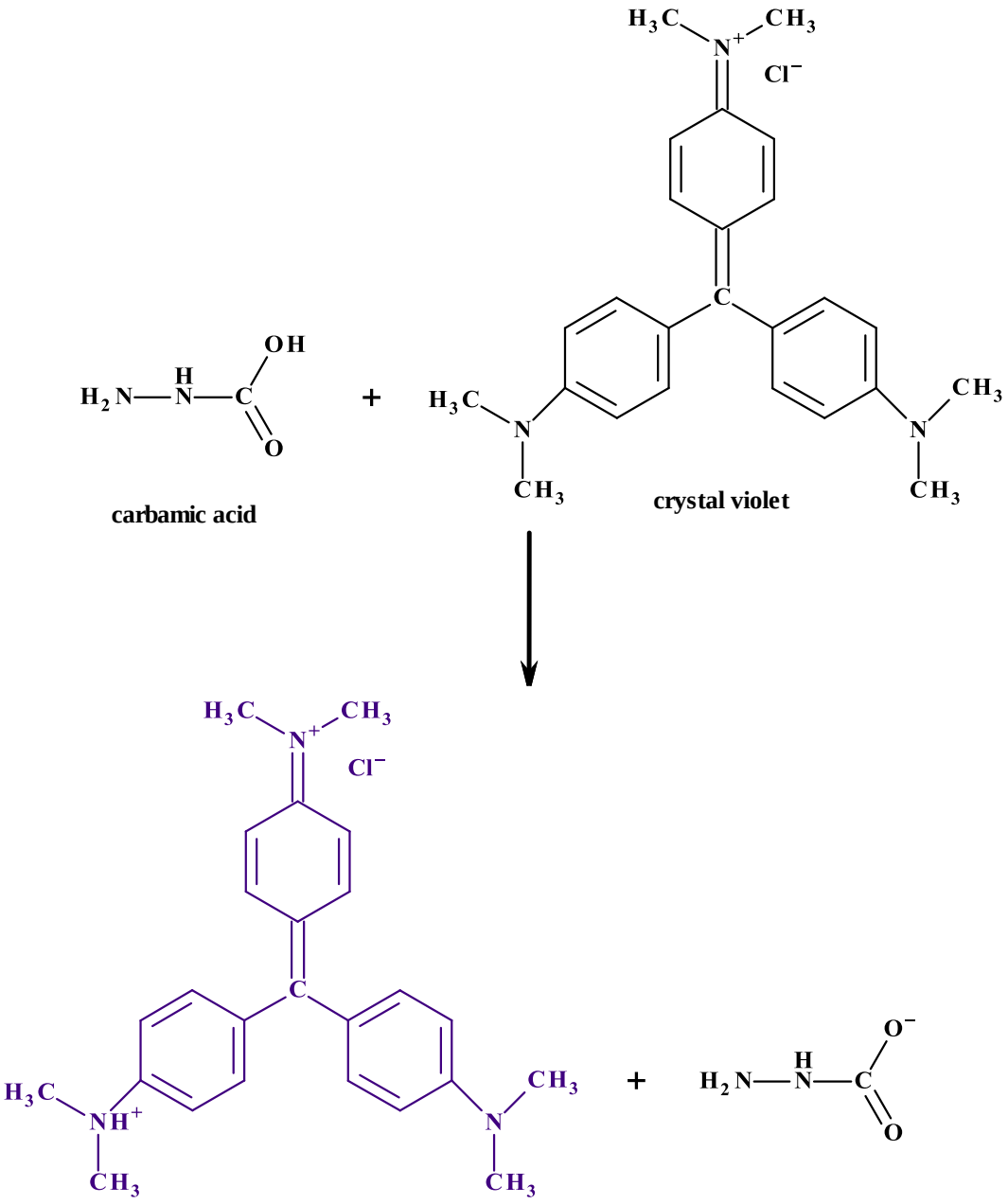


Figure 62: Reaction of carbamic acid with crystal violet to yield a purple colour change[118]

There have been no reported attempts in the use of the HCV mixture as a fingerprint enhancement technique. CO_2 is likely to react with any water present in latent fingerprint residue to produce a weak acid called carbonic acid. In theory, this means that CO_2 is "fixed" onto the fingerprint ridges. Therefore, the expectation was that the HCV solution would only react with the background surface not the fingerprint itself thus resulting in a "negative" mark on a purple background.

5.1.2. Results and Discussion

Fingerprint development on paper

A blank paper (no fingerprints) used as a control sample and a second piece of paper with fingerprints were placed in a sealed container with a CO_2 atmosphere overnight. The container was then opened to let the excess CO_2 escape and then both samples were dipped in the HCV mixture.

Overall, both paper samples turned purple very quickly and no white areas were seen where fingerprints could have been placed. It was evident that there was still a significant amount of CO_2 present on the surface of the paper in both cases.

Therefore, the next step was to repeat the experiment but leave both samples in air overnight with atmospheric CO_2 only rather than quenching the samples in pure CO_2 gas. This time only sebaceous prints were placed on the paper to see if any white areas could be yielded.

There were some white areas present however, as soon as the HCV mixture was applied to the paper, the hydrazine reacted with any CO_2 in the atmosphere, which only allowed the white areas to be present for a few seconds. The control sample turned purple slower in atmospheric CO_2 but it was still only a matter of seconds.

One last attempt was made whereby after fingerprints were exposed to atmospheric CO_2 overnight, the paper samples were dried in an oven for 5 and 30 minutes to attempt to remove any background CO_2 remaining on the paper and hopefully reveal fingerprints. These still produced background staining and no fingerprints could be seen. A new set of samples were left out in a fume hood for 5 and 15 minutes and then placed in an oven for 30 and 60 minutes. Only a white outline of sebaceous fingerprints were seen against a faint purple background after drying in the oven for 60 minutes, however the quality was insufficient to pursue further.

The next step was to test the HCV mixture on a non-porous surface for example, glass to see whether fingerprints could be revealed in one of two ways:

- Fingerprints were exposed to CO_2 overnight, then dipped in the HCV solution.
- Fingerprints were dipped in the HCV solution, then exposed to CO_2.

Fingerprint development on glass

Both eccrine and sebaceous prints were placed on glass slides and exposed to CO_2 for 2 and 24 hours and control samples of fingerprints not exposed to CO_2 were also made up. These were then dipped in the HCV solution and left to dry and photographed.

The samples that were not exposed to CO_2 did not produce a purple colouration, which is a feasible result however, the fingerprints became visible which may be due to the hydrazine interacting with constituents in the fingerprint residues.

Of the samples exposed to CO_2 after 2 hours, the sebaceous fingerprints were revealed with significant ridge detail however, the purple colouration developed further after the samples were exposed to CO_2 in air overnight. The eccrine prints were only partially visible after the longer exposure of atmospheric CO_2.

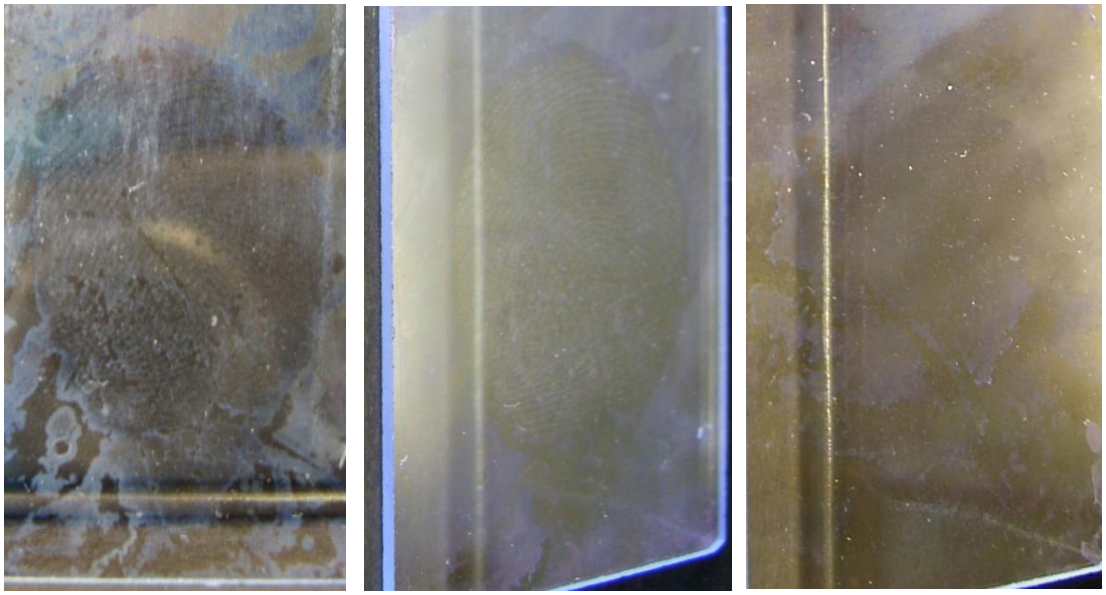


Figure 63: Sebaceous print revealed after 2 hrs. of CO_2 exposure (left), same sebaceous print re-photographed after exposure to CO_2 overnight (middle), eccrine print revealed after re-exposure to CO_2 overnight (right)

The second method involved fingerprints that were placed on glass being dipped in the HCV solution first and then exposed to atmospheric CO_2. The eccrine fingerprints were visible and developed a purple colouration over time. The sebaceous fingerprints were visible from the first dip, the purple colouration only darkened around the fingerprint. Photographs were taken after 1, 2, 4 and 7 days.

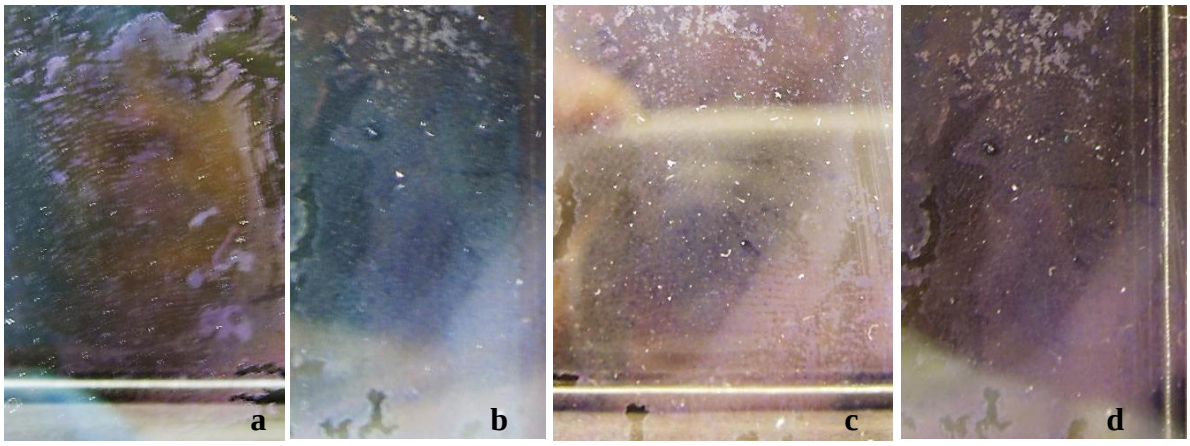


Figure 64: Eccrine print developed after HCV dip & exposure to CO_2 after: a) 1 day, b) 2 days, c) 4 days and d) 7 days

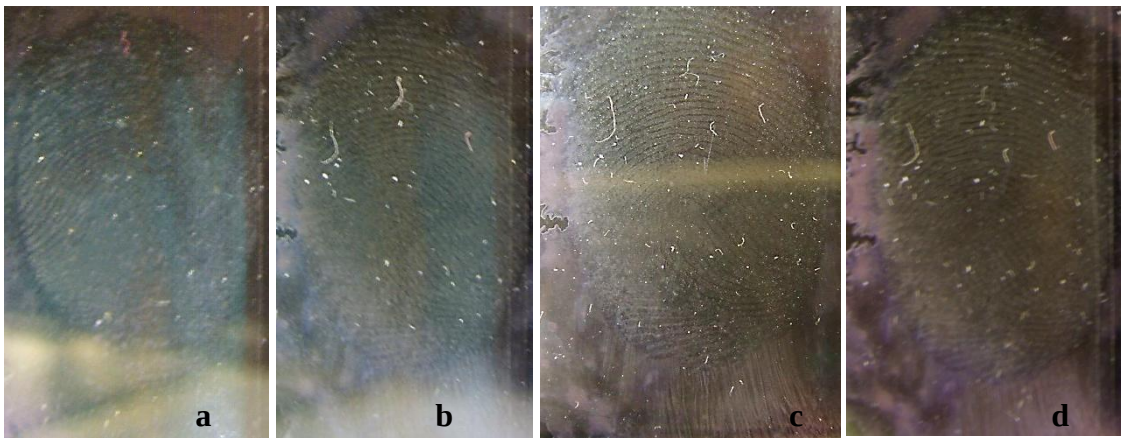


Figure 65: Sebaceous print developed after HCV dip & exposure to CO_2 after: a) 1 day, b) 2 days, c) 4 days and d) 7 days

5.1.3. Conclusions

The HCV formulation was able to reveal latent fingerprints on glass with ridge detail only after samples were exposed to atmospheric CO_2 over a number of days after the HCV dip. This supports the theory that the CO_2 must be reacting with the fingerprints initially and the sample only turns purple after the second exposure to CO_2 after the HCV dip. Overall, it can be seen above that the quality of the fingerprints developed is much lower than those developed using the original crystal violet technique as reported in 1.5.5 and so was not pursued further.

5.2. Phosphomolybdic Acid for Oxidizing Lipids, Steroids and Sterols[119]

The phosphomolybdic acid ($H_3PO_4.12MoO_3.24H_2O$) provides a site for oxidation forming a dark green precipitate on a light yellow/green background upon reduction of any lipids, steroids or sterols present.

It was reported that the overall performance of this technique was promising. This technique was able to reveal a print before and after the sample was washed with water. Only a small amount of solution was needed due to the spraying technique, but it was crucial that the solution was sprayed evenly. Although this technique was successful on paper samples, fabric samples showed mixed results.

Overall conclusions showed that other solvents were tested, but ethanol gave the best results, it was vital that a fresh solution was sprayed each day as old solutions tended to produce significant background noise and shielded any prints present. Lastly, further work was suggested whereby, the background could be lightened or removed by placing paper samples in a tank containing 25% ammonium hydroxide solution.

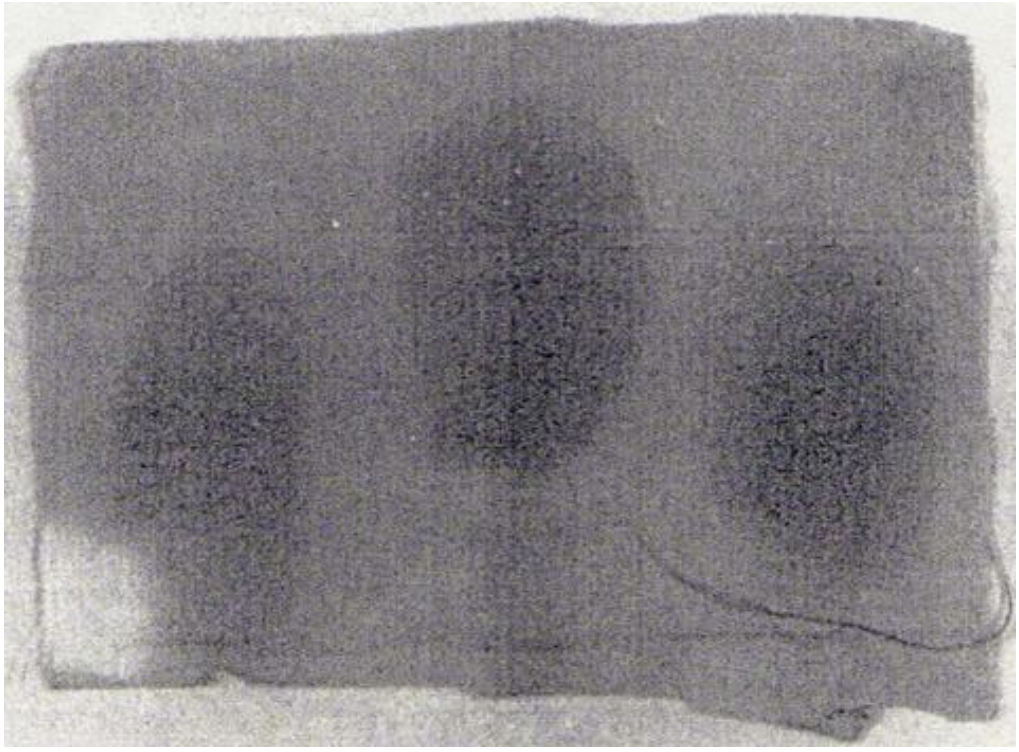


Figure 66: Developed fingerprints on a clean white cloth using the original method[119]

5.2.1. Experimental

The original study involved the use of phosphomolybdic acid (PMA), a yellow powder dissolved in ethanol to form a 10% ethanolic solution of the acid. Fingerprints were obtained by the participant stroking their face, which tends to secrete more oily constituents before placing them onto a given surface. The solution was then sprayed onto fingerprint samples placed on both paper and fabric and then placed in an oven at 120°C to develop[119].

As the type of solvent is the only variable that has been experimented with and this technique has not been revisited since, this has left several other variables which can be investigated such as, the concentration of the PMA solution, temperature effects and testing other surfaces. All safety precautions were met: any spraying was carried out under a fume-hood, with a container collecting any residual solution and any ethanol was left to evaporate, as it is highly flammable.

20 wt. % PMA solution in ethanol (Sigma Aldrich, Gillingham, UK), was diluted to 10% with ethanol prior to use. Samples were initially dipped in the PMA solution, but it was later found that using an EcoSpray MicroDiffuser (see Figure 71) was found to apply the PMA solution more evenly without overloading fingerprint samples.

5.2.2. Results and Discussion

Variable 1: Concentration of PMA solution

As 10% PMA, solution was used in the original method, it was deemed logical to make up 5%, 15% and 20% PMA solutions, all other variables were kept the same:

- Temperature - 120°C and left to develop for 5 minutes.
- Surface – Plain white paper
- A set of 5 fresh "loaded" sebaceous fingerprints were placed on one-half of the paper and a depletion series (1-5) of sebaceous fingerprints were placed on the opposite half.

Table 21: Fingerprint development results using different concentrations of PMA solution

Concentration of PMA solution	Quality of any developed fresh or depleted fingerprints
5%	Only 1 or 2 fresh fingerprints developed – grade 1 or 2 only, no depletion prints developed – grade 0.
10%	Revealed most of the fresh and depleted fingerprints to grade 3 or 4 standard and gave a good contrast against the background.
15%	Revealed all of the fresh fingerprints to a grade 3 or 4 standard, but only revealed some depleted fingerprints, but those revealed were grade 3.
20%	All the fresh fingerprints were revealed but overdeveloped and lost ridge detail, depleted fingerprints were developed to a grade 2 standard.

Therefore, it can be concluded that although 15% PMA solution produced similar results to 10% PMA solution, the 10% solution produced a better contrast against the background, therefore all other investigations were carried out using 10% PMA solution.

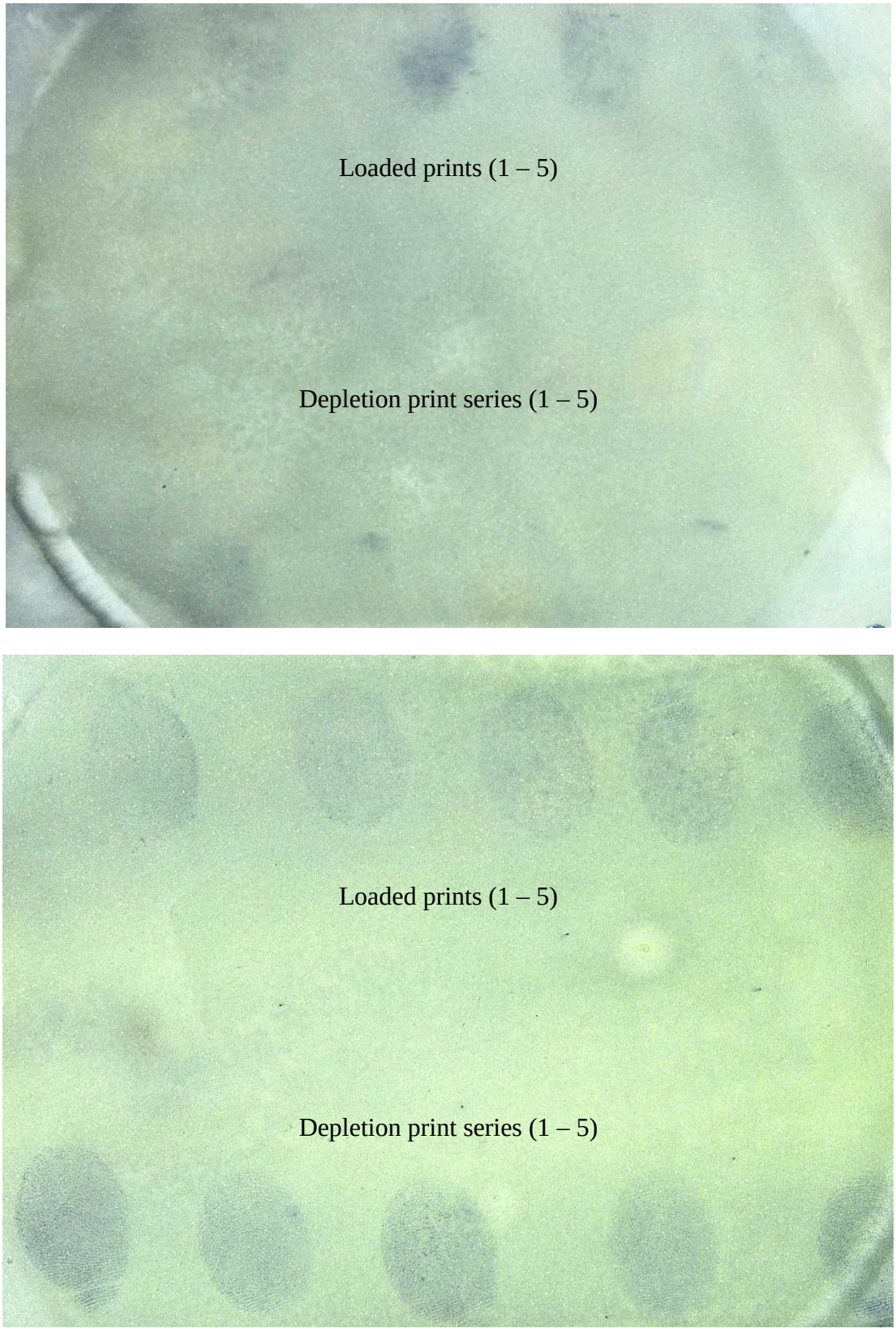


Figure 67: Developed fingerprints using 5% PMA solution (top) & 10% PMA solution (bottom)

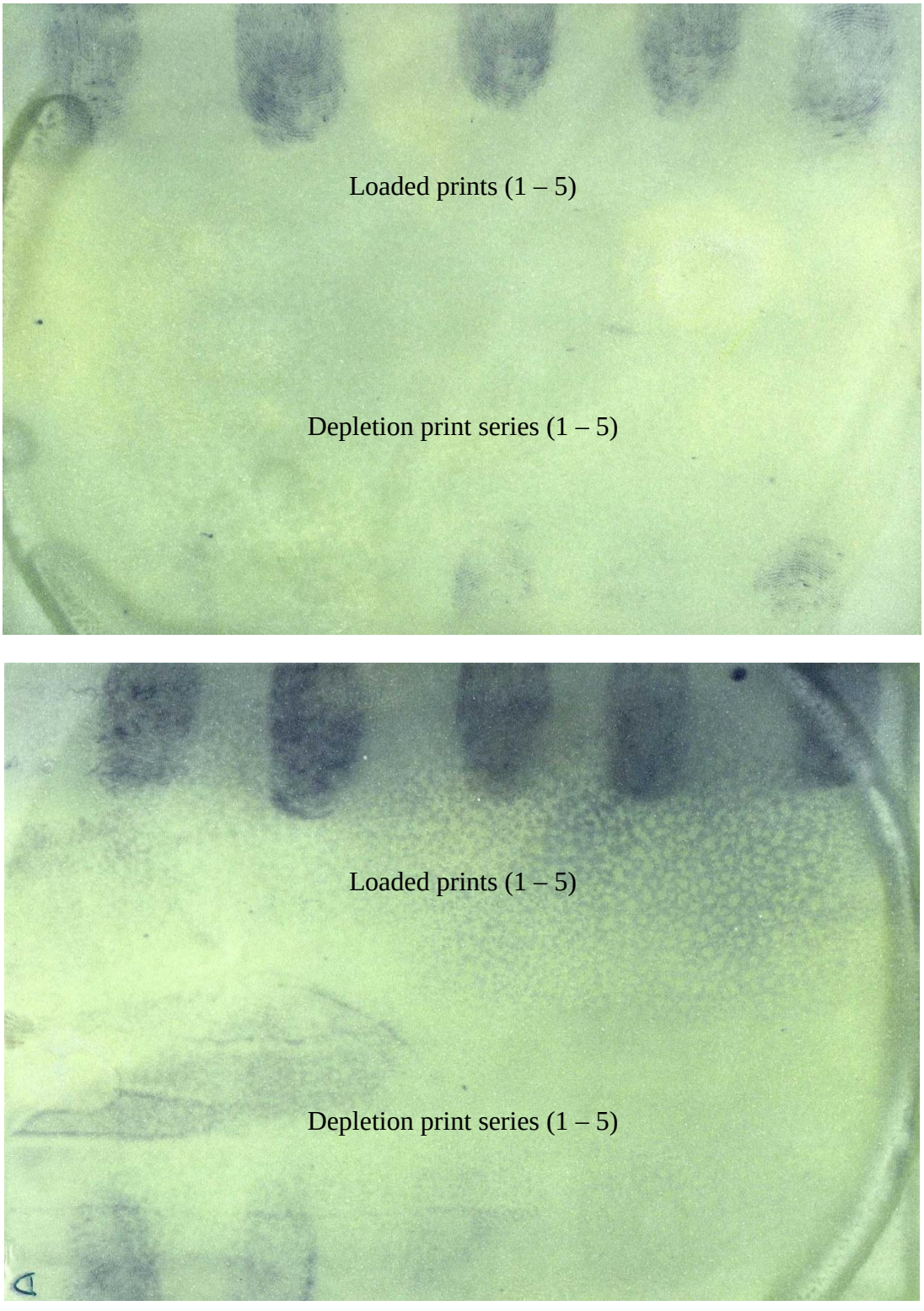


Figure 68: Developed fingerprints using 15% PMA solution (top) & 20% PMA solution (bottom)

Variable 2: Length of time given to develop prints

As stated previously, the samples were developed at 120°C for 5 minutes. It seemed feasible to test what effect the length of time in the oven had on the development stage. A 10% PMA solution was used and the oven temperature was kept the same. Samples were left to develop for 1, 2, 3, 4, 5 and 10 minutes.

As the samples were heated it was found that fingerprints fully developed from 5 minutes. However, the longer they were left to develop, the ridge detail that was present before started to disappear as the fingerprints began to overdevelop. Therefore, it was concluded that samples would be left to develop for a maximum time of 5 minutes.

Variable 3: Fingerprint development at different temperatures

It was decided that oven temperatures lower than 120°C should be analysed as higher temperatures may cause fingerprints to overdevelop. Therefore, fingerprint samples were developed for five minutes at: room temperature, 80°C, 90°C, 100°C, 110°C and 120°C.

Table 22: Results of fingerprints developed using 10% PMA solution at different temperatures

Temperature/°C	Quality of any fresh or depleted fingerprints developed
Room temperature ~ 21°C	No fingerprints developed.
80°C	Background was yellow, fingerprints were visible but no ridge detail was seen.
90°C	The fingerprints appeared slightly darker, ridge detail was only seen on fresh prints – grade 2, only prints 1 and 2 were seen in the depletion series – grade 1.
100°C	All fingerprints developed from grade 2 – 4. Background contrast was better than at 120°C.
110°C	The contrast began to decrease, making any fingerprints less visible – grade 2.
120°C	The contrast against the background was not as good as the fingerprints developed at 100°C.

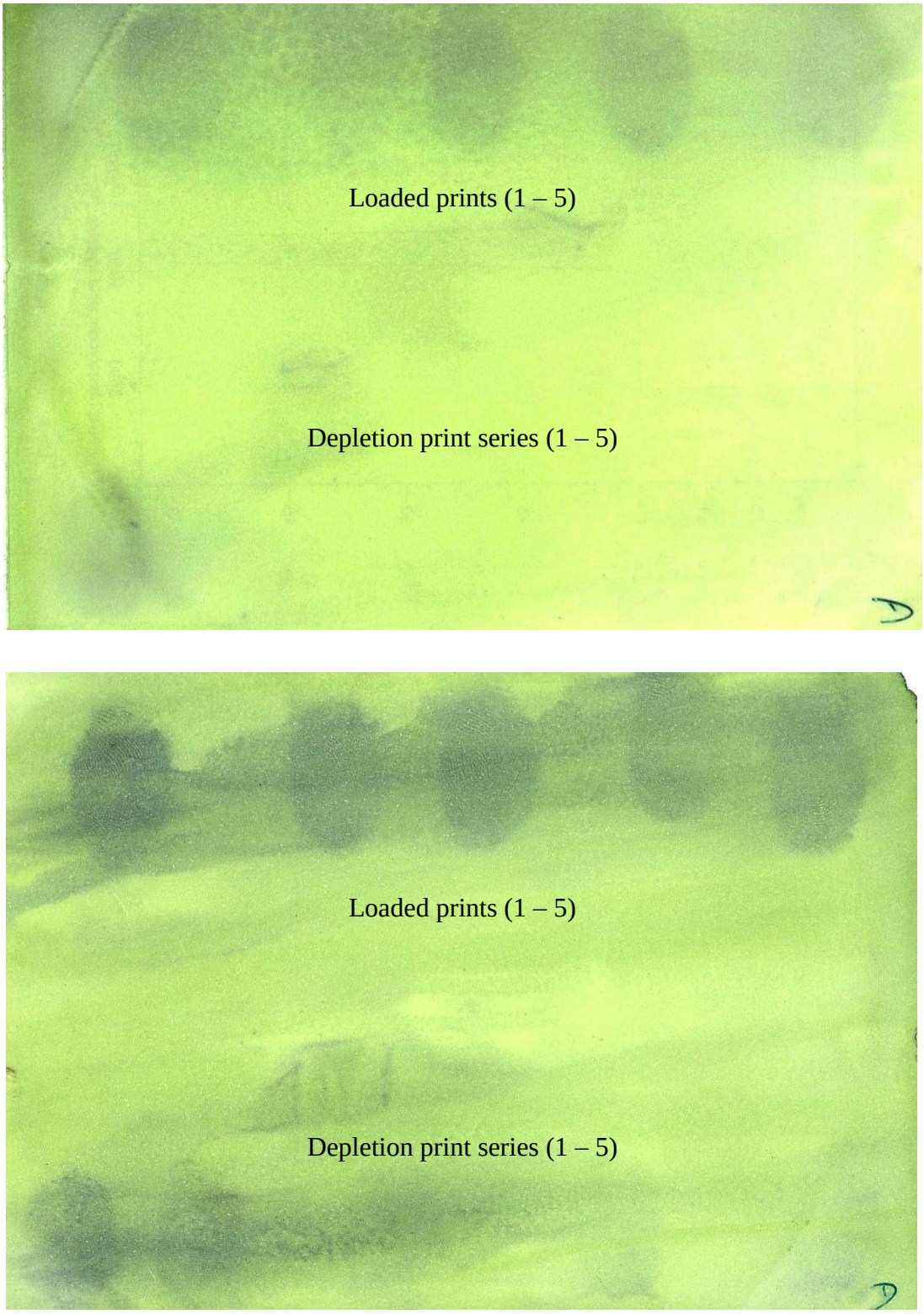


Figure 69: Fingerprints developed using 10% PMA solution at 80°C (top) & 90°C (bottom)

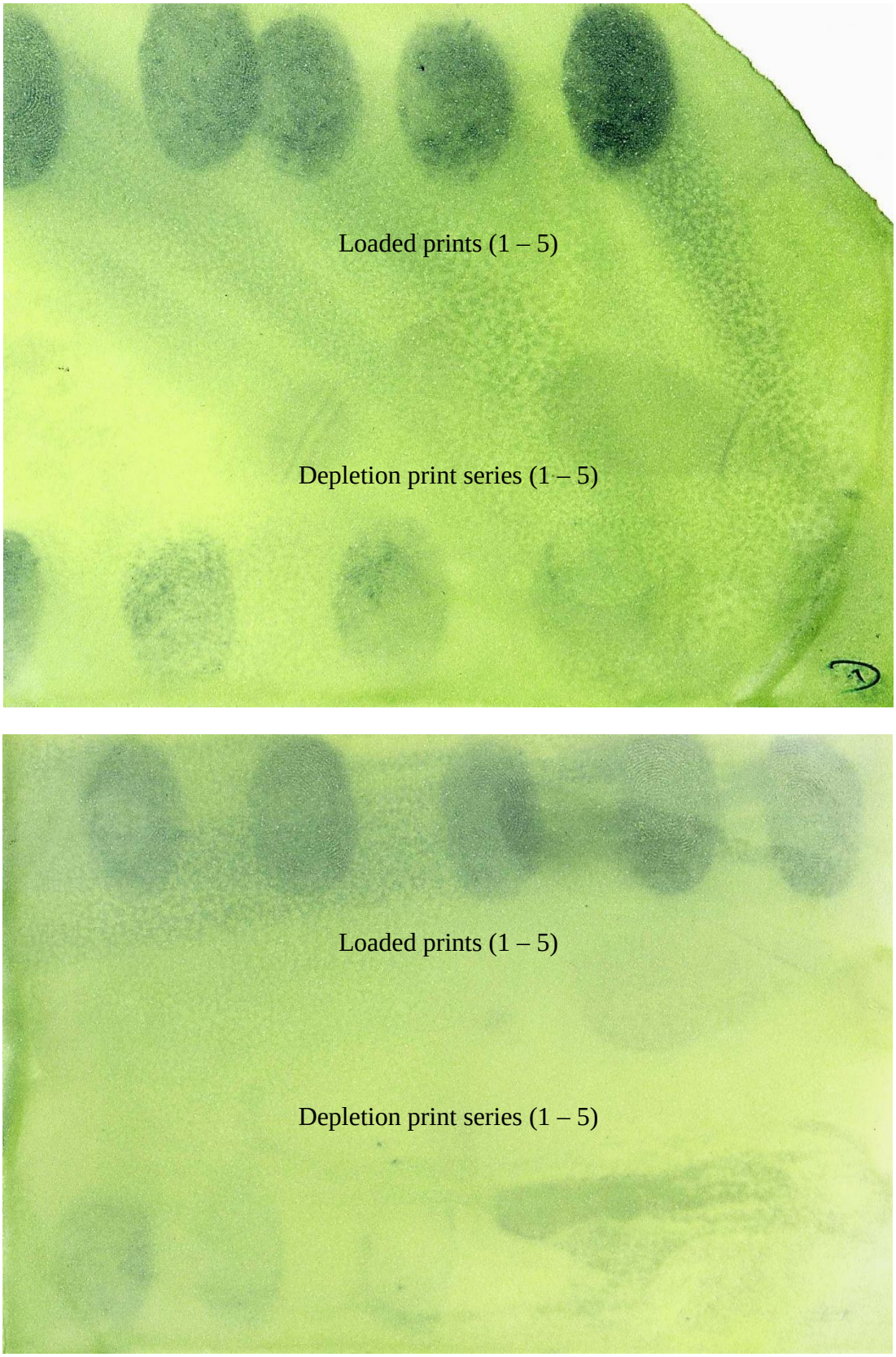


Figure 70: Fingerprints developed using 10% PMA solution at 100°C (top) & 110°C (bottom)

The optimised method to develop fingerprints was carried out as follows: 10% PMA solution sprayed and developed at 100°C for 5 minutes. This optimised method was used to develop fingerprints on a range of problematic surfaces such as wood, metals and fabrics. In order for the solution to be sprayed evenly, an EcoSpray MicroDiffuser (Tetra Scene of Crime Limited, UK) was purchased with an air canister allowing the solution to be sprayed as a fine mist, thus reducing background development and the amount of solution used became minimal.

Figure 71: Photograph of an EcoSpray MicroDiffuser (Tetra Scene of Crime Ltd, UK)

Variable 4: Developing fingerprints on problematic surfaces

- **Raw and laminated wood**

The optimised method showed promising results with both types of wood samples resulting in fresh fingerprints developed to grade 3 or 4 standard. However, a depletion series of fingerprints on raw wood showed significant promise whereby we were able to develop and visualise up to the 8th fingerprint depletion. It was clear to see that fingerprints further down the depletion series revealed better ridge detail, this is due to the fact that as fingerprints are placed sequentially so the amount of sebaceous residue transferred each time decreases, which is showcased as the prints at the start of the depletion series were overdeveloped and showed less ridge detail.

However, there are several optical techniques that may enhance these images to reveal more detail.

Figure 72: Fresh fingerprints developed at 100°C using 10% PMA solution on laminated wood (left) and raw wood (right)

Figure 73: One depletion series from left to right: print 1 – 4 (top), print 5 – 8 (bottom)

Figure 74: Depletion no. 7 and 8 re-photographed with enhanced lighting

- **Aluminium foil and steel**

With both of these metals, it was found that one short spray mist of the 10% PMA solution was enough to reveal fingerprints to a grade 2 – 4 standard otherwise it produced a highly speckled effect on the surface. As soon as more PMA solution was sprayed, the acid began to react with the metals and obscured some of the ridge detail.

Figure 75: A set of developed fingerprints on aluminium foil using 10% PMA solution with 1 short spray (left), 2 short sprays (middle), excessive spray (right)

Figure 76: Developed fingerprints on steel using 10% PMA solution with excessive spray (left) and 1 short spray (right)

- **Cotton**

Cotton is a very problematic surface in that as it is porous, it makes it very difficult to see any ridge detail. In this case, there were signs of fingerprints present but no detail could be seen. If these were enhanced, further it may be possible to reveal ridge detail. One positive outcome was that PMA was able to reveal up to the 5th depleted fingerprint on cotton, which is an improvement from the original study.

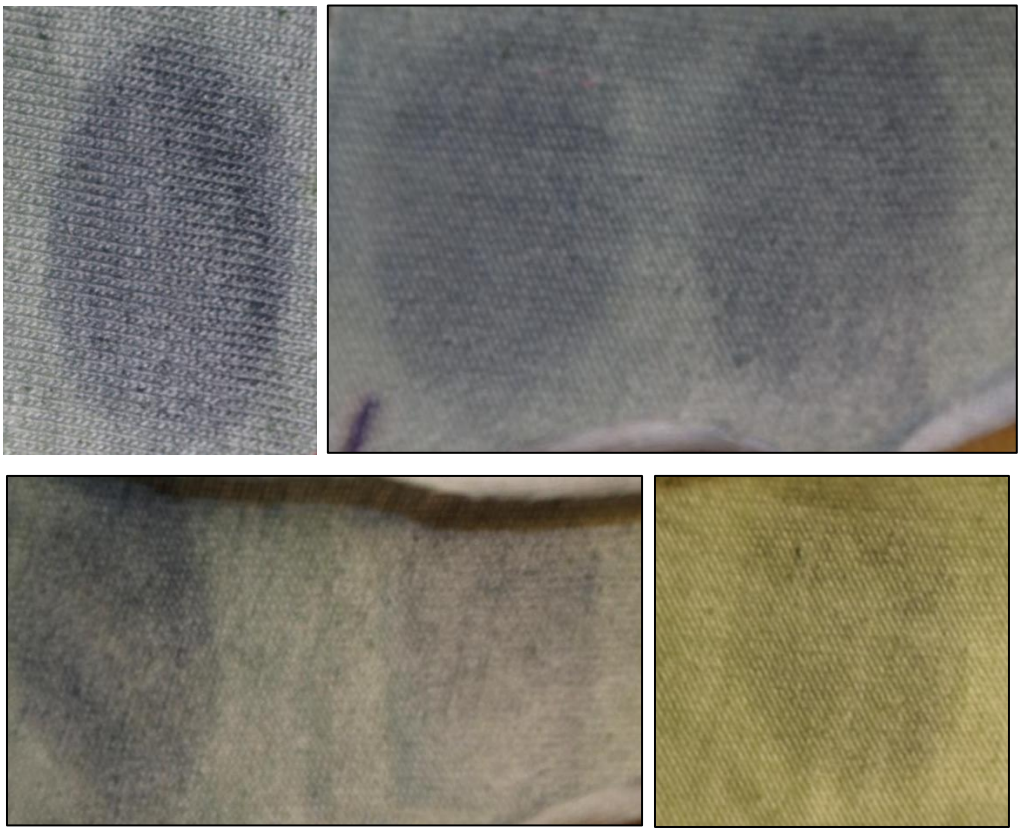


Figure 77: Developed fingerprints on cotton using 10% PMA solution: Fresh print (top left), Depletion prints 1 & 2 (top right), depletion prints 3, 4 & 5 (bottom)

5.2.3. Conclusions

Phosphomolybdic acid has shown promise to reveal sebaceous fingerprints from problematic surfaces such as wood, metals and cotton to a good standard of quality. The optimum conditions needed to achieve this were 10% ethanolic PMA solution sprayed using an EcoSpray MicroDiffuser and sample development at 100°C for up to 5 minutes. However, the formulation is flammable and only develops sebaceous fingerprints, which in retrospect are both significant limitations.

5.3. Cupric Acetate and Rubeanic Acid, for the Detection of Free Fatty Acids[119]

The original study described that two specific reactions take place:

- The adjacent SH groups in dithiooxamide (rubeanic acid) react with cupric ions in copper acetate to form a dark green coloured complex that has a cis configuration.
- Copper acetate is soluble in fatty acids that are present in latent fingerprint residues. This acidic environment is suitable for the reaction between rubeanic acid and cupric ions forming the dark green chelating complex, which is insoluble.

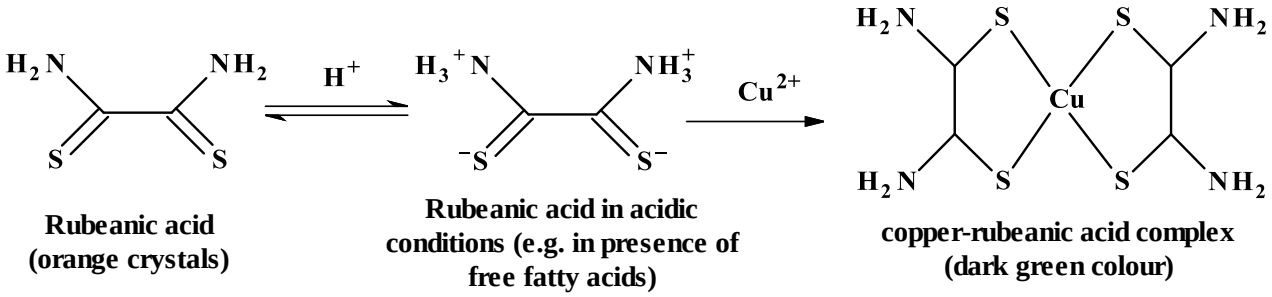


Figure 78: Reaction mechanism for copper (II) ions and rubeanic acid[119]

The report concluded that although the technique had some promise in developing fingerprints, fingerprints were poorly placed so appeared smudged when they were developed, the allotted washing time was insufficient to remove traces of copper from the background and fabrics being highly absorbent made it difficult to distinguish between the background and any fingerprints that may have developed.

However, the positive outcome of this technique was that low levels of fatty acids could be detected due to the preferential adsorbent nature of fatty acids to copper salts.

A recent news report in February 2012 revealed that in Britain, alone the number of metal thefts has gone up by 50% in the past year alone and prices of scrap metal have doubled since 2009. This is an ever-growing problem costing the economy an estimated £770 million a year[122]. This technique could potentially be used to detect

trace metals such as copper on suspected metal thieves' hands by testing palm prints. Alongside this, it can be used to detect traces of nickel and copper which tend to be used in bullets and jackets that in turn may be handled by a person. Thus, it seemed worthwhile to revisit this method and also attempt to resolve the original problems faced which have not been studied further for reasons unknown.

5.3.1. Experimental

The original report used 20 ml of a saturated solution of aqueous copper acetate (~1.44 grams copper acetate in 20 ml distilled water) that was diluted to 1 litre and a second solution of 0.1% w/v ethanolic rubeanic acid. Sebaceous fingerprints were placed on paper and dipped in the copper acetate solution for 10 minutes with frequent agitation. The paper sample was then transferred to a washing tank containing water for 30 minutes and then placed in a tank with the ethanolic rubeanic acid solution until the fingerprints were visible as a dark green colour against a light green background. It was found that the longer the paper was left in this solution, the darker it became. The paper was washed again to stop the reaction and air-dried. This was repeated on fabric samples also. As this technique has not been re-visited since it was reported, no one has attempted to optimise the technique by testing certain variables.

Optimisation of the copper-rubeanic acid technique

It was decided that the variables kept unchanged were the volumes of the saturated copper acetate solution, the volume of ethanolic rubeanic acid and the sample surface (plain white paper) which the latent fingerprints were placed on.

The variables investigated were the concentration of ethanolic rubeanic acid, the length of the dipping time in the copper solution, the length of washing time and lastly the washing method. There were two sets of fingerprints placed on paper samples in each case, fresh "loaded" sebaceous prints and a depletion series of sebaceous prints. These were then graded using the fingerprint grading system (see 1.6.5) for comparison.

Copper (II) acetate monohydrate and rubeanic acid (Dithiooxamide) were both obtained from Sigma Aldrich, UK.

Variable 1: Washing method and length of washing time

The original study showed that samples were dipped in the copper solution for 10 minutes and washed for 30 minutes with frequent agitation. However, it was noted that this was an insufficient amount of time to wash the excess copper from the samples. Therefore, the washing time was increased to 90 minutes and the water was changed every 10 – 15 minutes, may be crucial to removing the excess copper and not carried out in the original study.

Variables 2 and 3: Length of the dipping time in the copper solution and the concentration of ethanolic rubeanic acid

The original study had a dipping time of 10 minutes in the copper solution and fingerprints were developed in 0.10% ethanolic rubeanic acid. A combination of dipping times in the copper solution of 10 minutes, 5 minutes and 1 minute and concentrations of rubeanic acid of 0.10% and 0.05% were investigated. Each combination was repeated to test the reproducibility.

Investigation of the optimised copper-rubeanic acid method on different surfaces

The surface types that were investigated were placed into categories of:

- Paper and cardboard – magazine, gloves box, parcel box, gift bag and black card.
- Metals – aluminium foil, copper, brass and copper coins.
- Plastics – carrier bag, Clingfilm and transparent shampoo bottle.
- Fabrics – cotton, silk, carpets, linen and microfibre cloth.
- Other – nitrile glove, latex glove, grey duct tape, brown parcel tape, polystyrene, bank note and wood.

All experiments were carried out in the same way, the only difference was the length of time each sample was dipped in the 0.10% ethanolic rubeanic acid as this depended on how long it took for fingerprints to develop on each surface. Any changes made to the experimental are stated in section 5.3.2.

Variations of the optimised copper-rubeanic acid method on different surfaces

1. Using 0.15% ethanolic rubeanic acid.
2. Dipping in copper solution for 5 minutes and developing prints in 0.05% ethanolic rubeanic acid.
3. Doubling the copper solution concentration (~2.88g of copper acetate in 20 ml distilled water).
4. Using saturated cobalt (II) chloride solution. (~10.58g cobalt (II) chloride in 20 ml distilled water) instead of a saturated copper (II) acetate solution.
5. Using a combined solution of 20 ml, saturated cobalt and 20 ml saturated copper solution.

Using the optimised copper-rubeanic acid method on fingerprints that have been exposed to various conditions

1. Aging fingerprints for 1, 3, 5 days and 2 months.
2. "Normal" fingerprints – i.e. not loaded fingerprints.
3. Wet fingerprints – fingerprint samples washed before dipping in copper solution.
4. Temperature effects – fingerprint samples dried at 100°C for 2 hours before dipping in copper solution.

5.3.2. Results and Discussion

Variable 1: Washing method and length of time

Table 23: Results from increasing washing time to 90 minutes

Fingerprint ID	Fingerprint Grade
Fresh print 1	1
Fresh print 2	0
Fresh print 3	0
Fresh print 4	0
Depletion series print 1	0
Depletion series print 2	0
Depletion series print 3	0
Depletion series print 4	0

It can be seen that increasing the washing time did not show any improvements, therefore for all other tests, the washing time was kept constant at 30 minutes but the water was changed every 10 minutes to wash away any excess copper.

Variables 2 & 3: Length of the dipping time in copper acetate solution and the concentration of ethanolic rubeanic acid

Table 24: Results from combinations of variables 2 & 3

Combination of Variables 2 & 3	Fingerprint ID	Fingerprint Grading	Fingerprint ID	Fingerprint Grading
Cu dipping time: 10 minutes % ethanolic RA: 0.05%	F1	1	D1	1
	F2	2	D2	0
	F3	1	D3	1
	F4	1	D4	1
Cu dipping time: 5 minutes % ethanolic RA: 0.10%	F1	2	D1	1
	F2	3	D2	1
	F3	1	D3	1
	F4	2	D4	1
Cu dipping time: 5 minutes % ethanolic RA: 0.05%	F1	1	D1	2
	F2	1	D2	1
	F3	1	D3	0
	F4	2	D4	0
Cu dipping time: 1 minute % ethanolic RA: 0.10%	F1	2	D1	3
	F2	3	D2	2
	F3	3	D3	1
	F4	3	D4	0
Cu dipping time: 1 minute % ethanolic RA: 0.05%	F1	1	D1	1
	F2	0	D2	1
	F3	1	D3	0
	F4	1	D4	0

From the results, it can be seen that the only combinations that produced any grade 3 fingerprints were with two dipping time lengths of 5 minutes and 1 minute in the copper solution and a 0.10% w/v concentration of rubeanic acid. These two combinations were repeated twice more to confirm which one produced a higher proportion of grade 3 fingerprints each time.

Table 25: Results from copper solution dipping times of 1 & 5 minutes and 0.10% ethanolic RA

Combination of Variables 2 & 3	Fingerprint ID (set 1)	Fingerprint Grading	Fingerprint ID (set 2)	Fingerprint Grading
Cu dipping time: 5 minutes % ethanolic RA: 0.10%	F1	2	F1	2
	F2	2	F2	2
	F3	2	F3	3
	F4	3	F4	2
	D1	2	D1	2
	D2	2	D2	2
	D3	2	D3	1
	D4	2	D4	1
Cu dipping time: 1 minute % ethanolic RA: 0.10%	F1	2	F1	2
	F2	2	F2	3
	F3	3	F3	3
	F4	3	F4	3
	D1	2	D1	2
	D2	2	D2	1
	D3	1	D3	1
	D4	1	D4	1

From the results above it is clear that the optimum conditions for this technique are:

- Samples dipped in the copper solution for 1 minute.
- Samples washed for 30 minutes and the water is changed every 10 minutes.
- Fingerprint samples are developed in 0.10% w/v ethanolic rubeanic acid solution.
- As soon as fingerprints are developed, they are removed, rinsed under running water and then dried before being photographed.

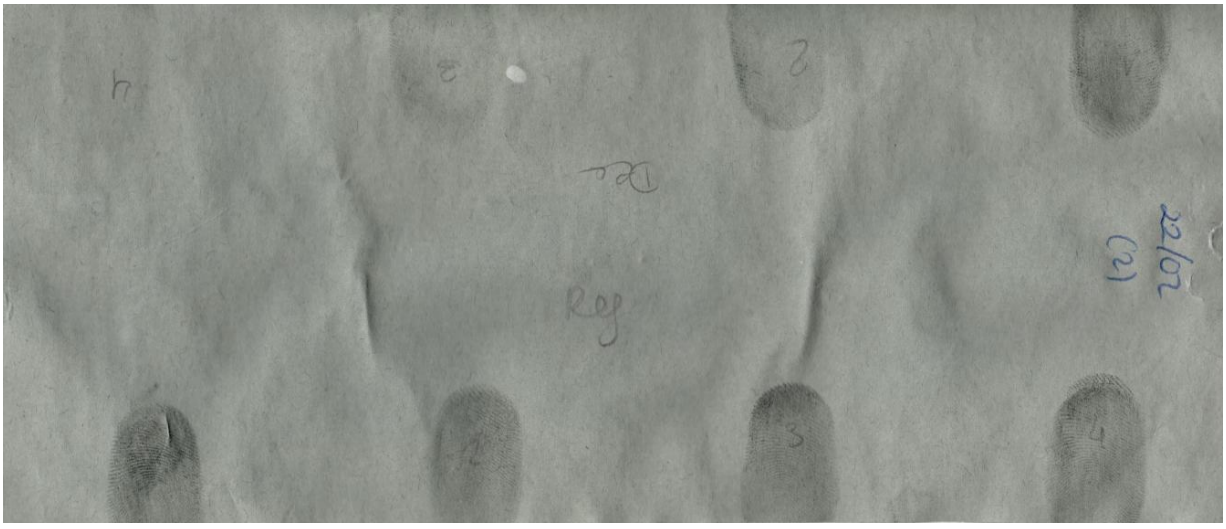


Figure 79: Fingerprint samples produced using the optimized Copper-Rubeanic acid method

Some of the fingerprint samples on paper were re-photographed at the Home Office CAST site (St Albans, UK) using a VSC6000 Video Spectral Comparator (Foster and Freeman, Evesham, UK). The "blue" images were produced using long wave ultra-violet illumination (365 nm), the "normal" images were produced under white light and the grey (reflected infrared) images were produced using white light with a long pass filter transmitting radiation above 830 nm.

Figure 80: Fresh sebaceous fingerprint photographed under white light

It can be seen that this method is able to develop sebaceous fingerprints on paper up to grade 3 standard and special imaging equipment was able to enhance some prints to a grade 4 standard. In order to conclude how much promise this technique has, the optimised experimental was used to develop fingerprints on a range of different surfaces especially problematic surfaces such as fabrics.

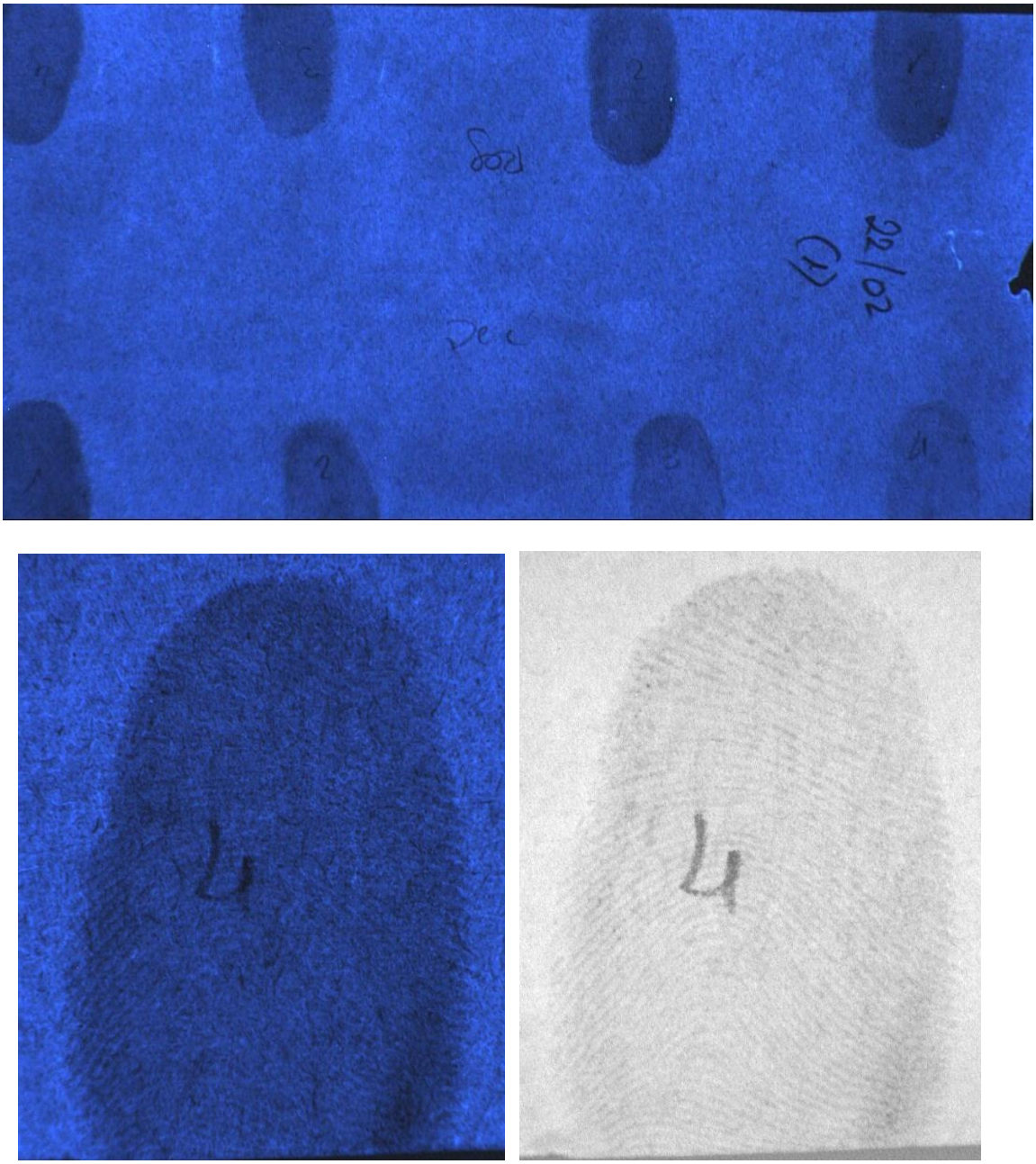


Figure 81: Fingerprints photographed using ultra violet (365 nm) light (top and bottom left) and using reflected infrared imaging (bottom right)

Investigation of the optimized copper-rubeanic acid method on different surfaces

Table 26: Optimised copper-rubeanic acid method on paper and cardboard

Surface and Conditions	Fingerprint Grading	
White paper – no changes to method	F1	1
	F2	1
	F3	1
	F4	1
White paper – fingerprints placed on paper after touching a copper pipe – only dipped in 0.10% ethanolic RA	0	
White magazine paper (coated paper)	F1	3
	F2	1
	F3	2
	F4	3
Gift bag – (white internal side)	F1	1
	F2	2
Black card	0	
Gloves box – glossy cardboard	F1	3
	F2	4
	F3	4
	F4	4
Parcel box	F1	1
	F2	1

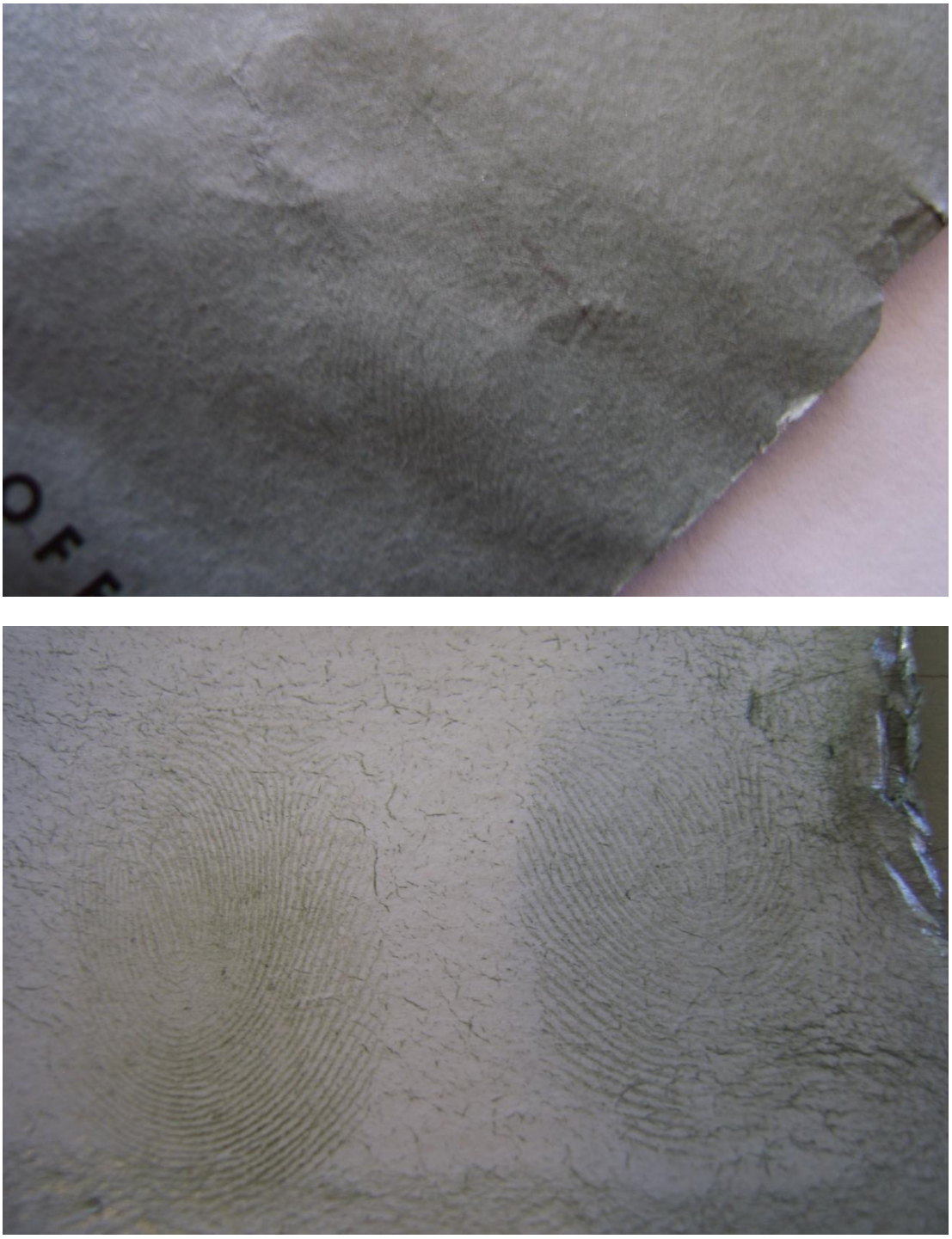


Figure 82: Prints developed on paper (top) and nitrile glove box using the optimized method

Less porous surfaces such as the glove box, which appeared to have a glossy coating allowed fingerprint ridge detail to be visible with a clear contrast against the background compared with normal paper, which is porous making it difficult to see clear ridge detail against the background.

Table 27: Optimised copper-rubeanic acid method on metals

Surface and Conditions	Fingerprint Grading
Aluminium foil (shiny side)	0
Aluminium foil (dull side)	3
Brass	2
Brass – washed prints off straight after placing them	0
Brass – washed prints off after 1 hour	0
Brass – Prints placed then dipped in 0.10% ethanolic RA only	1
Brass – washed prints off after 1 hour then dip in 0.10% ethanolic RA only	2
Copper – place fingerprint then dip in 0.10% ethanolic RA	2
Copper coin – place print then dip in 0.10% ethanolic RA	0
Copper coin	0

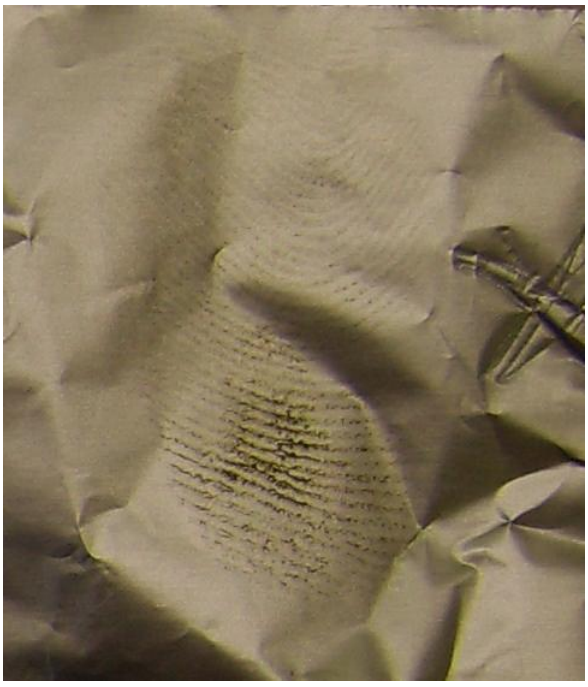


Figure 83: Prints developed on aluminium foil (dull side) using the optimized method

Fingerprints were only developed on the dull side of aluminium foil and on copper strips. There are other compounds such as, S_2N_2 that are better at revealing fingerprints from metals.

Table 28: Optimised copper-rubeanic acid method on plastics

Surface and Conditions	Fingerprint Grading
White carrier bag	3
Clingfilm	3
Clear hard plastic	2
Pink shampoo bottle (opaque)	2

Figure 84: Prints developed on a white carrier bag (left) and Clingfilm (right) using the optimized method

As plastics are non-porous, the contrast between the background and the fingerprint was very good allowing ridge detail to be seen clearly. The excess copper tends to wash off, as it cannot be absorbed by the non-porous surface. This shows that the copper ions must be either adsorbing onto or interacting with components in the latent fingerprints which stops the copper ions from being washed away before being dipped in the 0.10% ethanolic rubeanic acid.

Table 29: Optimised copper-rubeanic acid method on fabrics

Surface and Conditions	Fingerprint Grading
White cotton	1
White cotton – lightly placed fingerprints	1
White cotton – washed under running tap	1
White cotton – lightly placed fingerprints (depletion series 1 – 10)	D1 – D5 – 1 D6 – D10 – 0
White cotton – strongly placed fingerprints (depletion series 1 – 10)	1 (All prints)
White cotton – 2nd donor – lightly placed fingerprints (depletion series 1 – 10)	D1 – D5 – 1 D6 – D10 – 0
White silk	1
White silk – lightly placed fingerprints	1
Purple cotton	1
White linen	1
Yellow microfibre cloth	1
Purple carpet – 100% polyester	1
Carpet – 91% polyester:9% linen	0
Red striped carpet – 59% polyester:35% acrylic:6% viscose	0
Red carpet – 44% polyester:43% acrylic:14% cotton	0
Plum carpet – 42% polyester:43% acrylic:15% cotton	0
Brown carpet – 57% polyester:13% acrylic:27% cotton:3% linen	1
Light brown carpet – 100% polyester	0
Olive green carpet – 100% polyester	1
Lime green carpet – 10% polyester:37% cotton:53% viscose	1
Stone coloured carpet – 23% polyester:77% viscose	1

This technique was unable to reveal fingerprints from fabrics of any quality but was able to indicate that fingerprints were present by the dark green fingerprint shaped marks developed. However, with specialist imaging equipment (CRi Nuance multispectral imaging system with the Nuance operating software) available at Home

Office CAST, a white cotton sample and a purple carpet sample were enhanced to reveal some minute ridge detail, although these results are not significant enough compared to other techniques available, they show progression towards revealing fingerprints from problematic surfaces.

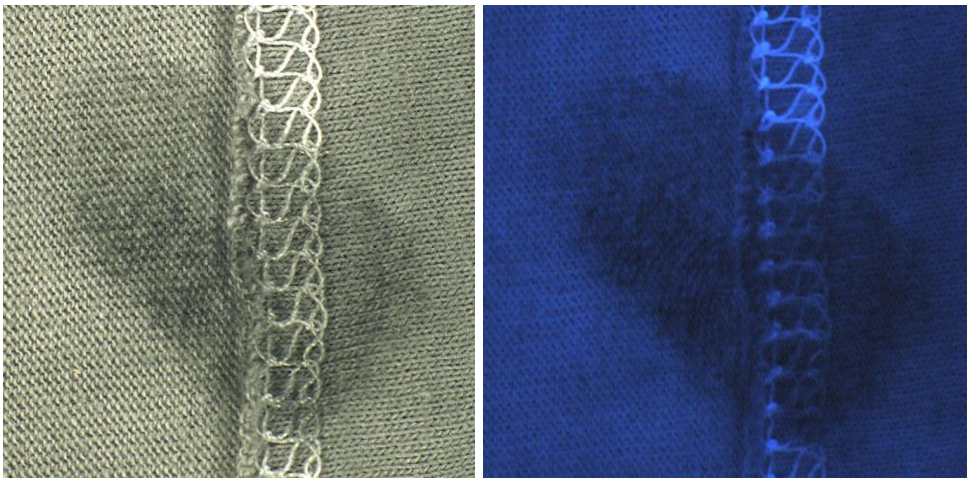


Figure 85: Fingerprint on white cotton under white light (left) & under ultra-violet light (right)

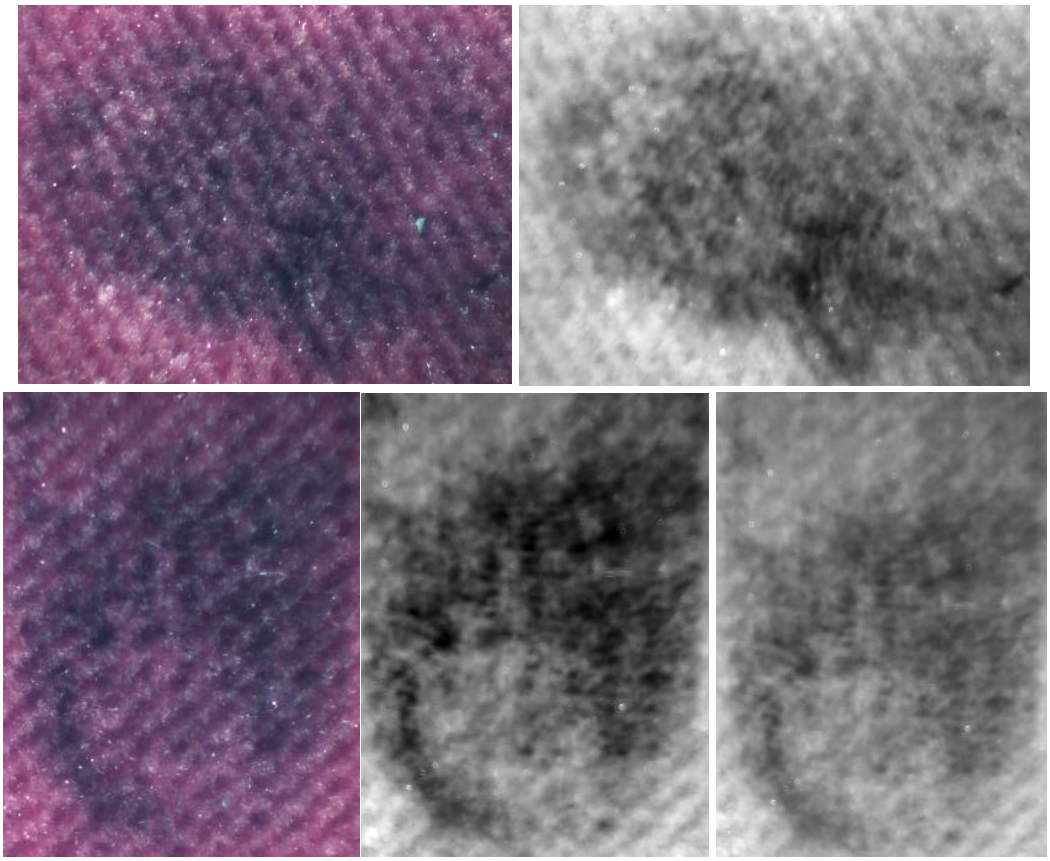


Figure 86: Purple carpet - 2 different developed fingerprints and enhanced images using the CRi Nuance multispectral imaging system with the Nuance operating software

The CRi Nuance multispectral imaging system and Nuance operating software was able to pinpoint two contrasting colours on the sample and convert the two colours into black and white images that uncovered hidden detail.

Table 30: Optimised copper-rubeanic acid method on other surfaces

Surface and Conditions	Fingerprint Grading
Grey duct tape	3
Grey duct tape (depletion series 1 – 3)	D1 – 3 D2 – 2 D3 – 1
Brown parcel tape	2
White latex glove	1
Blue nitrile glove	2
White polystyrene - packaging	2
Wood	0

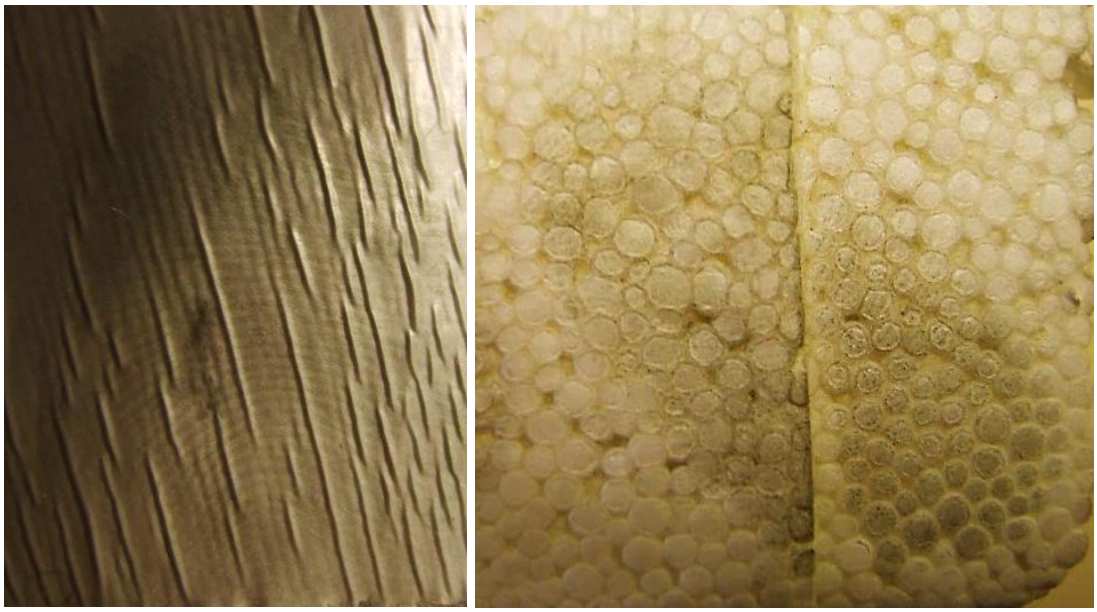


Figure 87: Fingerprints developed on grey duct tape (left) and polystyrene (right) using the optimized Copper-Rubeanic acid method

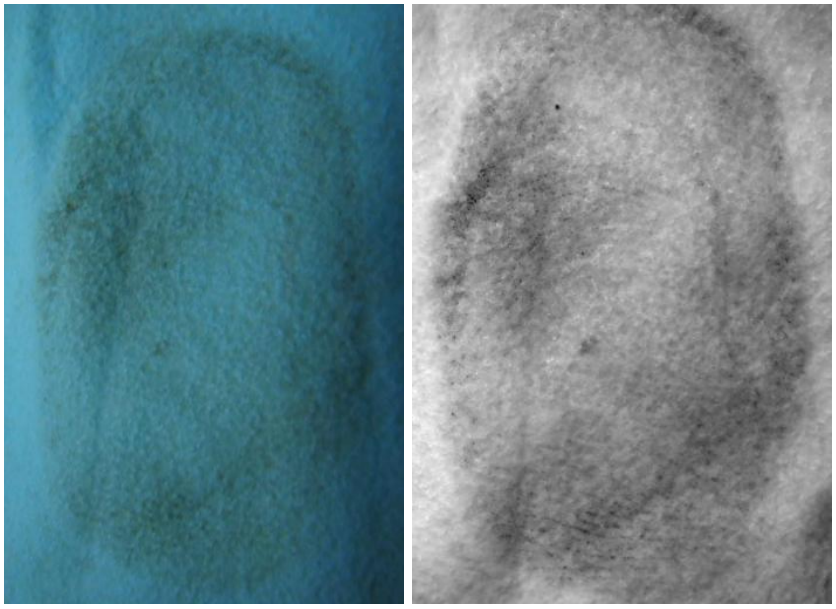


Figure 88: Nitrile glove before (left) and after enhancement images using the CRi Nuance multispectral imaging system and Nuance operating software (right)

Variations of the optimized copper-rubeanic acid method on different surfaces

The optimized method above did not reveal a high proportion of fingerprints on different surfaces, therefore other combinations of the concentration of copper acetate solution and ethanolic rubeanic acid solutions were also investigated to attempt to improve some of the results above.

1. Using 0.15% ethanolic rubeanic acid

Table 31: Graded fingerprints on different surfaces developed using 0.15% ethanolic rubeanic acid

Surface and Conditions	Fingerprint Grading
White paper	3
White paper (depletion series 1-4)	2
White magazine paper	2
White cardboard	1
Gift bag	2
Filter paper	3
Grey duct tape	1
Grey duct tape (depletion series 1-4)	0

Figure 89: Developed fingerprints on filter paper using 0.15% ethanolic rubeanic acid

This variation worked best on filter paper and normal white paper samples. However, duct tape revealed fingerprints of a lower quality than with the original optimized method.

2. 5 minutes in copper solution and 0.05% ethanolic rubeanic acid

Table 32: Graded fingerprints on different surfaces developed using 0.05% ethanolic rubeanic acid

Surface and Conditions	Fingerprint Grading
Glove box	1
Gift bag	2
White cardboard	1
Grey duct tape	3
Green plastic recycling bag	2
Red plastic recycling bag	1
Nitrile glove	1

These conditions developed better quality fingerprints on duct tape. The fingerprints developed on paper and cardboard samples were of a lower quality than with the previous conditions used.

3. Double concentration of copper acetate solution

Table 33: Graded fingerprints on different surfaces developed using a doubled concentration of copper solution

Surface and Conditions	Fingerprint Grading
White paper	3
White cardboard	3
Filter paper	3
Brown parcel tape	1
White cotton	1

This variation developed a higher proportion of fingerprints on paper with significant ridge detail compared with the previous method. The higher concentration of copper ions are able to react with the fingerprints thus improving the development of them once dipped in 0.10% ethanolic rubeanic acid. The fabric and tape samples showed no change.

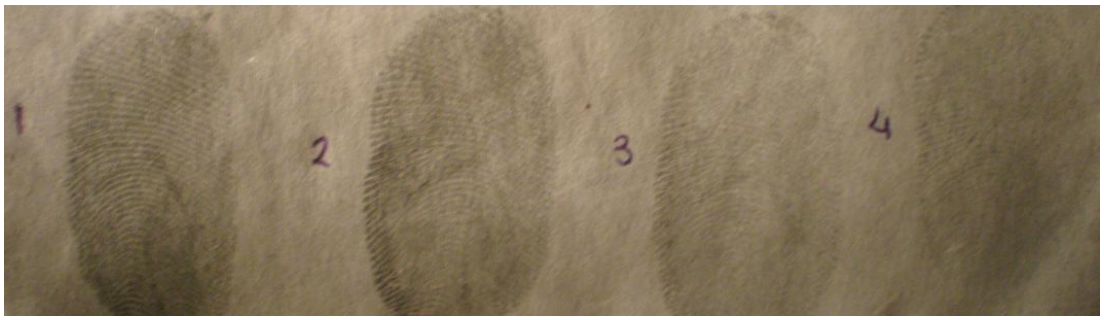


Figure 90: Fingerprints developed on filter paper using a doubled concentration of copper solution

4. Using 20 ml saturated cobalt (II) chloride solution

Rubeanic acid can also be used detect cobalt ions. A saturated solution of cobalt (II) chloride was made up to one litre and fingerprint samples were dipped in a 20 ml cobalt solution in the same fashion as the copper-rubeanic acid method.

The cobalt solution revealed better quality fingerprints on majority of the surfaces except the red plastic bag. The lack of fingerprint visibility is most likely due to the background colour of the sample. The cobalt solution revealed more ridge detail than the copper solution, as there was a better contrast against the background.

Table 34: Graded fingerprints on other surfaces developed with saturated cobalt solution

Surface and Conditions	Fingerprint Grading
White paper	3
Orange paper	4
Glove box	2
Thermal paper (receipt)	3
Glass microscope slide	3
Clear plastic	3
Red plastic recycling bag	0
Copper	3
Aluminium foil (shiny)	3
Aluminium foil (dull)	2
Stainless steel	4
Brass	3

Figure 91: Comparison of fingerprints developed with copper (II) (left) vs. cobalt (II) solution

Figure 92: Fingerprints developed using Cobalt-Rubeanic acid method on glass (top left), copper (top right), thermal paper (middle) and stainless steel (bottom)

It was interesting to see whether the two saturated solutions combined had the ability to reveal better quality fingerprints overall.

5. 20 ml saturated Cobalt (II) chloride solution + 20 ml saturated Copper (II) acetate solution

Table 35: Graded fingerprints on different surfaces developed using combined cobalt and copper solutions

Surface and Conditions	Fingerprint Grading
White paper	1
White paper – lightly placed fingerprints	0
Filter paper	1
Gift bag	2
Aluminium foil (shiny)	1
Aluminium foil (dull)	1
Grey duct tape	0
Latex glove	0
White cotton	0
White silk	1
Clear plastic	1
Green plastic recycling bag	1
Red plastic recycling bag	0

It can be seen that these results are much poorer than if the copper or cobalt solution is used alone. Most of the samples developed a mixture of mustard and green coloured fingerprints, which made it harder to see any ridge detail as they were masked by the two colours.

Other metal ions were also investigated such as iron chloride and zinc acetate however, the ferric ions did not react with rubeanic acid. A spot test was carried out with zinc acetate to see if zinc ions reacted with rubeanic acid. Dark spots were only revealed under ultra-violet light however, this was not pursued further. These particular salts were tested because they are water-soluble and are present in alloys such as steel, brass and bronze, which are found in church buildings, public statues,

railings, manhole covers. These items are amidst a rising list of targets in metal theft cases.

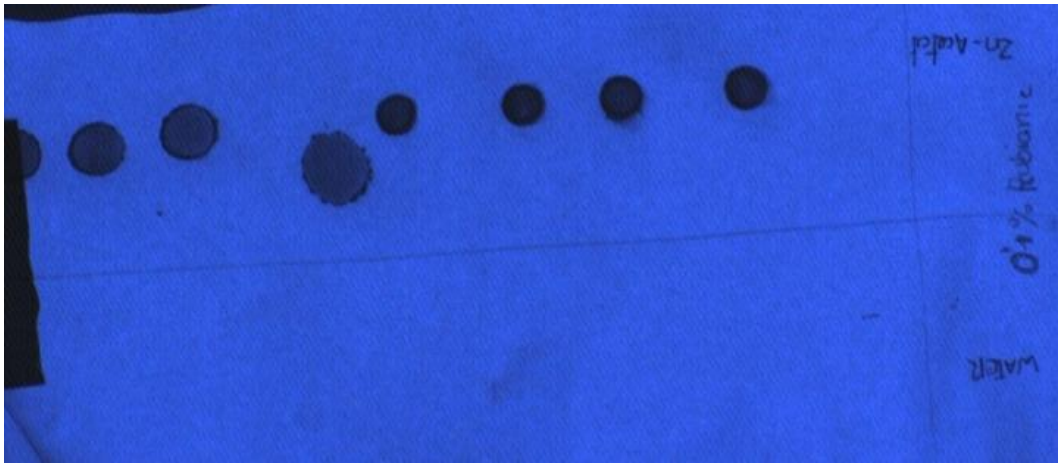


Figure 93: Spot test using Zinc acetate and 0.10% ethanolic rubeanic acid

Using the optimised copper-rubeanic acid method on fingerprints that have been exposed to various conditions

1. Investigation with Aging Fingerprints

"Loaded" fingerprint samples were left open in air for three days, five days and two months before development using the optimized copper-rubeanic acid method. Depleted fingerprints were also aged for 5 days and two months respectively.

Table 36: Results for aging fingerprints developed using optimized copper-rubeanic acid method

Surface and Conditions		Fingerprint Grading
3 days	White cotton	1
	Filter paper	1
5 days	White cotton	1
	White paper	1
	Filter paper	2
	Brown parcel tape	2
2 months	White paper (depletion series 1-2)	1
	White cardboard (depletion series 1-3)	D1 – 3, D2-D3 – 2
2 months	Filter paper(depletion series 1-4)	1
	Brown parcel tape (depletion series 1-3)	D1 – 3, D2-D3 – 2

Figure 94: 2 month old fingerprints on filter paper developed using the optimized copper-rubeanic acid method

This technique has shown promise in revealing fingerprints that are several weeks old. It appears that the mode of development may differ from fresh marks, as aging fingerprint ridges appear light against a darker background. This is perhaps because constituents in the print residue migrate through the porous paper over time so the copper ions are unable to adhere to the fingerprint ridges. However, more surfaces should be investigated to provide a better conclusion about this technique for aging fingerprints. "Normal" i.e. natural, unloaded fingerprints and wetted fingerprints were also investigated on different surfaces.

2. Investigation of "Normal" fingerprints

Table 37: Results for "normal" fingerprints using the optimized copper-rubeanic acid method

Surface and Conditions	Fingerprint Grading
White paper	0
Filter paper	0
White cotton	0
Brown parcel tape	0

It is clear from the results that "normal" fingerprints that are less likely to have a high sebaceous composition are not successfully developed using the copper-rubeanic acid technique.

3. Investigation of Wet Fingerprints

The samples were dipped in water prior to the copper-rubeanic acid technique. In this case, the concentration of the copper solution was doubled. As sebaceous components are insoluble in water, you would expect this technique to reveal the fingerprints in the same way as the others. From the results below, it can be seen that this was not the case. Although fatty acids have a hydrophobic tail (hydrocarbon chain), they also contain a hydrophilic head (carboxyl group). It is known that fatty acids with shorter hydrocarbon chains can be soluble in water and so may have been washed off in the process. This could have resulted in the copper ions not being able to adsorb onto the fingerprint residue surface.

Table 38: Results for wet fingerprints developed using doubled concentration of copper solution

Surface and Conditions	Fingerprint Grading
White paper	1
Filter paper	1
Filter paper (depletion series 1-5)	D1-D2 – 1, D3-D5 – 0
Copper	1
Brass	0
Stainless steel	1

4. Investigation of Temperature Effects

All of the fingerprints samples were placed in an oven for two hours at 100°C prior to development. In this case, the concentration of the copper solution was doubled, all other parameters were kept constant.

Table 39: Results for oven dried fingerprints using doubled concentration of copper solution

Surface and Conditions	Fingerprint Grading
White paper (depletion series 1-5)	D1-D3 – 1, D4-D5 - 0
Filter paper (depletion series 1-4)	0
Glass	1
Brown parcel tape (depletion series 1-3)	0

High temperatures affect fingerprints drastically as the majority of saturated fatty acids present in sebaceous fingerprints have melting points below 100°C so most of the latent fingerprint residue may have evaporated over the two hours, thus the copper ions were unable to adsorb onto the surface of the latent fingerprint residue.

5.3.3. Interaction between Cupric Ions and "Free" Fatty Acids

A series of "free" fatty acids commonly present in latent fingerprints were supplied by Home Office CAST. The first four were dissolved in acetone, the next two in ethanol and the last two in dichloromethane. The solutions of the fatty acids below were made up in separate sample vials by dissolving a few milligrams of each fatty acid in a solvent and then adding 1 ml of saturated copper acetate solution:

1. Decanoic acid – $CH_3(CH_2)_8COOH$
2. Dodecanoic (Lauric) acid – $CH_3(CH_2)_{10}COOH$
3. Myristic acid – $CH_3(CH_2)_{12}COOH$
4. Palmitic acid – $CH_3(CH_2)_{14}COOH$
5. Octadecanoic (Stearic) acid – $CH_3(CH_2)_{16}COOH$
6. Eicosanoic (Arachidic) acid – $CH_3(CH_2)_{18}COOH$
7. Docosanoic (Behenic) acid – $CH_3(CH_2)_{20}COOH$
8. Tetracosanoic (Lignoceric) acid – $CH_3(CH_2)_{22}COOH$

These were left for a few days to see if any precipitates were formed. A blank of water with saturated copper acetate solution was also made up for comparison. After a few days, blue precipitates started to form which was not present in the blank sample. The precipitate formed in the decanoic acid solution was filtered and air-dried. To identify this precipitate, infrared spectroscopy, and elemental microanalysis were carried out and compared with the starting materials of solid copper (II) acetate monohydrate and decanoic acid.

Figure 95: "Free" fatty acids mixed with saturated copper solution (top) and the same samples re-photographed after a few days showing blue precipitates formed (bottom)

A hypothesis was made in that the blue precipitate formed is likely to be copper (II) decanoate, $(C_{10}H_{19}O_2)_2Cu$. The carboxyl ion in the fatty acid is likely to share its electrons with the copper (II) ions present in the solution.

Micro Elemental Analysis

Three 1g samples (Copper (II) acetate monohydrate, decanoic acid and the blue precipitate) were submitted for elemental (CHN) analysis. An example calculation is shown below to show what percentage of each element %C, %H, %R (remainder: %Cu + %O) should be expected for each compound.

E.g. Copper (II) acetate monohydrate: $C_4H_6CuO_4.H_2O$, M_r = 199.65 $gmol^{-1}$

Expected % C = ((12.01 x 4) / 199.65) x 100 = ~24%

Expected % H = ((1.008 x 8) / 199.65) x 100 = ~4%

Expected % Cu = (63.55/199.65) x 100 = ~32%

Expected % O = ((16 x 5) / 199.65) x 100 = ~40%

Expected % R = % Cu + % O = ~ 72%

Expected results for decanoic acid: % C = ~70%, % H = ~11%, % R (= % O) = ~18%

Expected results if the blue precipitate is copper (II) decanoate:

% C = ~60%, % H = ~9.5%, % Cu = ~ 15%, % O = ~ 15%, % R = ~30%

The elemental analysis results highlight whether the results obtained for the precipitate are a good match to expected results that would be obtained for copper (II) decanoate.

Copper (II) acetate monohydrate: % C = 24.04%, & % H = 4.03%, % R = 71.82%

Decanoic acid: % C = 69.97%, % H = 11.87%, % R = 17.95%

Precipitate: % C = 60.80%, % H = 9.69%, % R = 29.27%

Although the molecular mass of the precipitate was not measured using mass spectrometry, the elemental analysis results for the blue precipitate above are a near perfect match for copper (II) decanoate if you compare them to the expected results.

Infrared Analysis

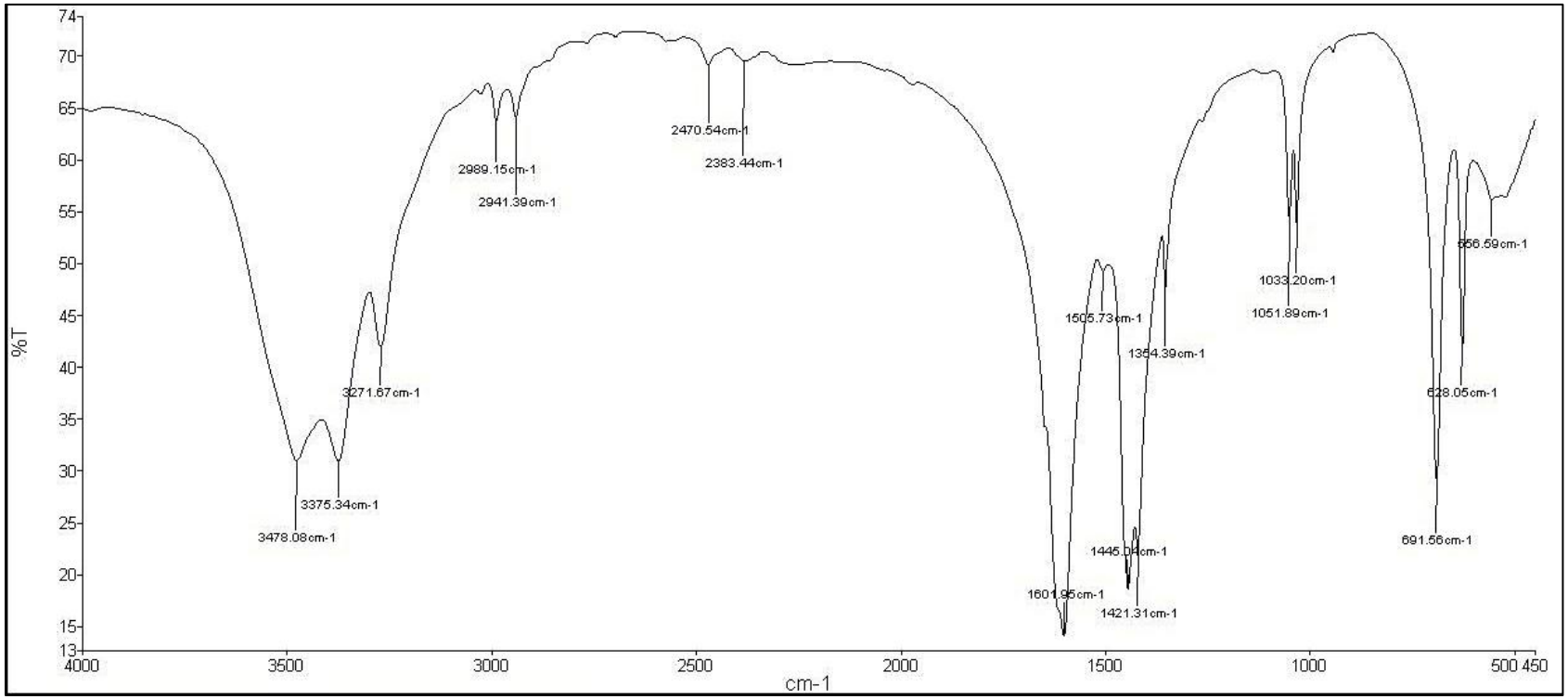


Figure 96: Infrared spectrum for copper (II) acetate monohydrate

There are several distinct peaks in this spectrum. Two bands at ~ 1100 cm^{-1} represent the acetate group, a band at ~1600 cm^{-1} represents the carbonyl group from the copper carboxylate salt, bands at 3375 cm^{-1} and 3478 cm^{-1} represent O-H stretches possibly due to hydrogen bonding with the acetate group and a water molecule, bands present at ~1350 cm^{-1} represent the methyl group in the acetate ligands, two sharp bands present at 1421 cm^{-1} and 1445 cm^{-1} represent symmetric stretching bands between the two acetate ligands CH_3CO=C-O-Cu-O-C=$OCCH_3$ and lastly, bands present between 650 – 695 cm^{-1} represent Cu-O-H vibrations.

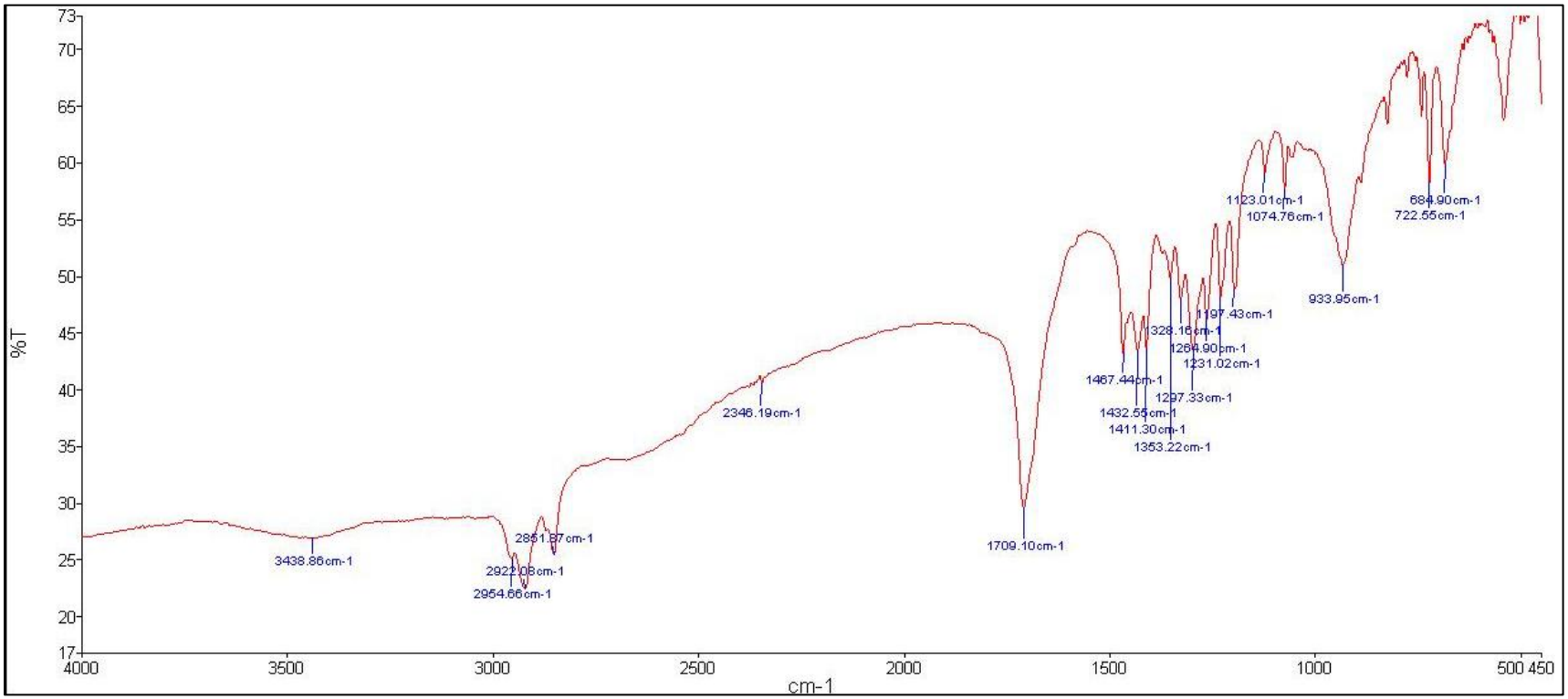


Figure 97: Infrared spectrum of decanoic acid

The characteristic bands of decanoic acid are: the broad peak at ~ 2950 cm^{-1} which represents the O-H stretch, the strong peak at 1709 cm^{-1} is a distinctive peak for the C=O stretch in carboxylic acids and the large number of peaks around 1200 – 1400 cm^{-1} represent the C-H stretches in the hydrocarbon chain.

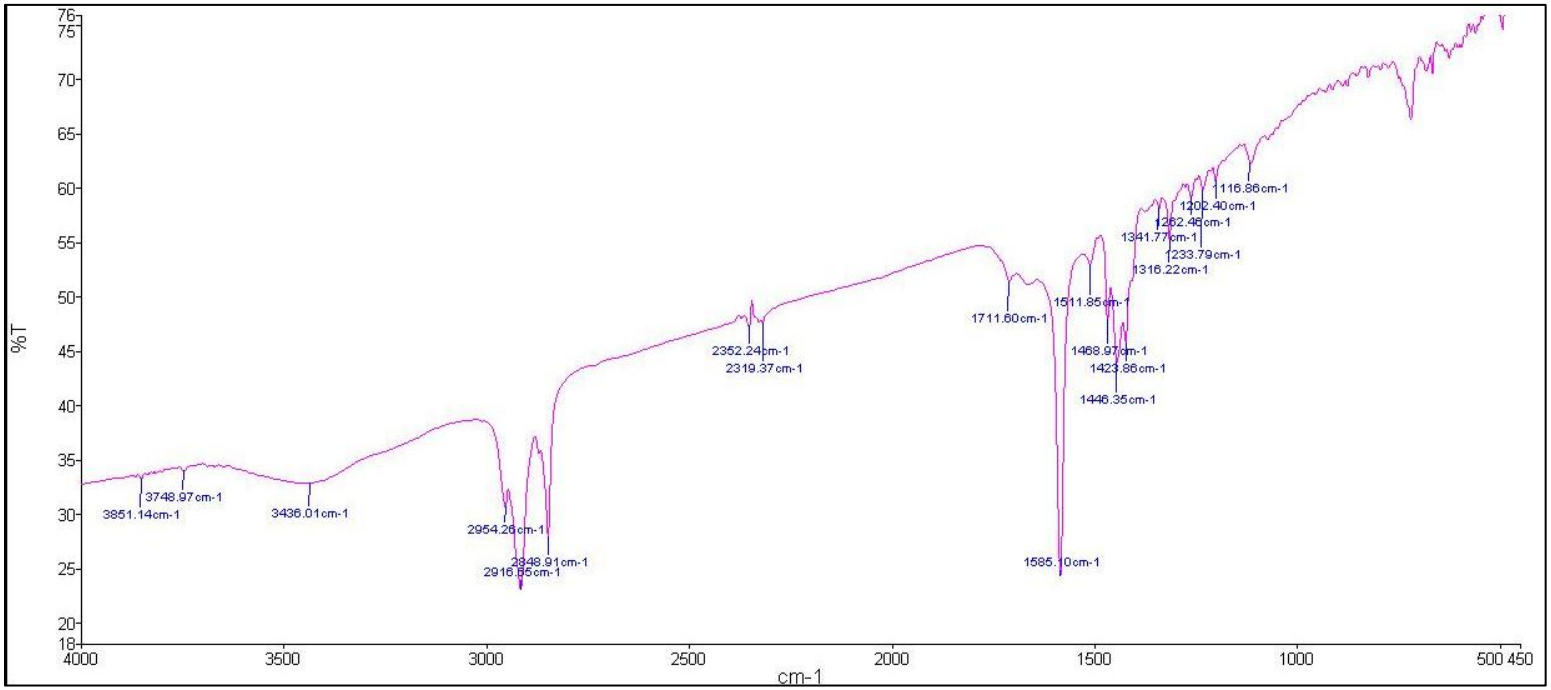


Figure 98: Infrared spectrum of the blue precipitate

The characteristic bands of this blue precipitate are: the sharp peak at 1585 cm^{-1} represents the C=O stretch in a carboxylate salt, the peaks in the range 1200 – 1400 cm^{-1} and 2915 – 2954 cm^{-1} represent C-H stretches in a hydrocarbon chain and the broad peak at 3436 cm^{-1} represents the O-H stretch you would expect to see in a carboxylic acid due to hydrogen bonding.

This spectrum appears to show a mixture of copper (II) decanoate and possibly some traces of the starting materials in the sample. In 2008, M. Ramos Reisco et al. reported that there is an indication of a fusion of two polymorphs of the copper (II) decanoate present in the precipitate which arise because of a difference in the cell parameters and in the packing of the chains (bilayered or columnar).[123]

5.3.4. Interaction between the Copper-Carboxylate salt and Rubeanic Acid

Some spot tests were carried out using the solutions of the "free" fatty acids and copper (II) acetate solution (see Figure 95). Each solution was spotted onto filter paper. The filter paper was then dipped in rubeanic acid to compare each solution.

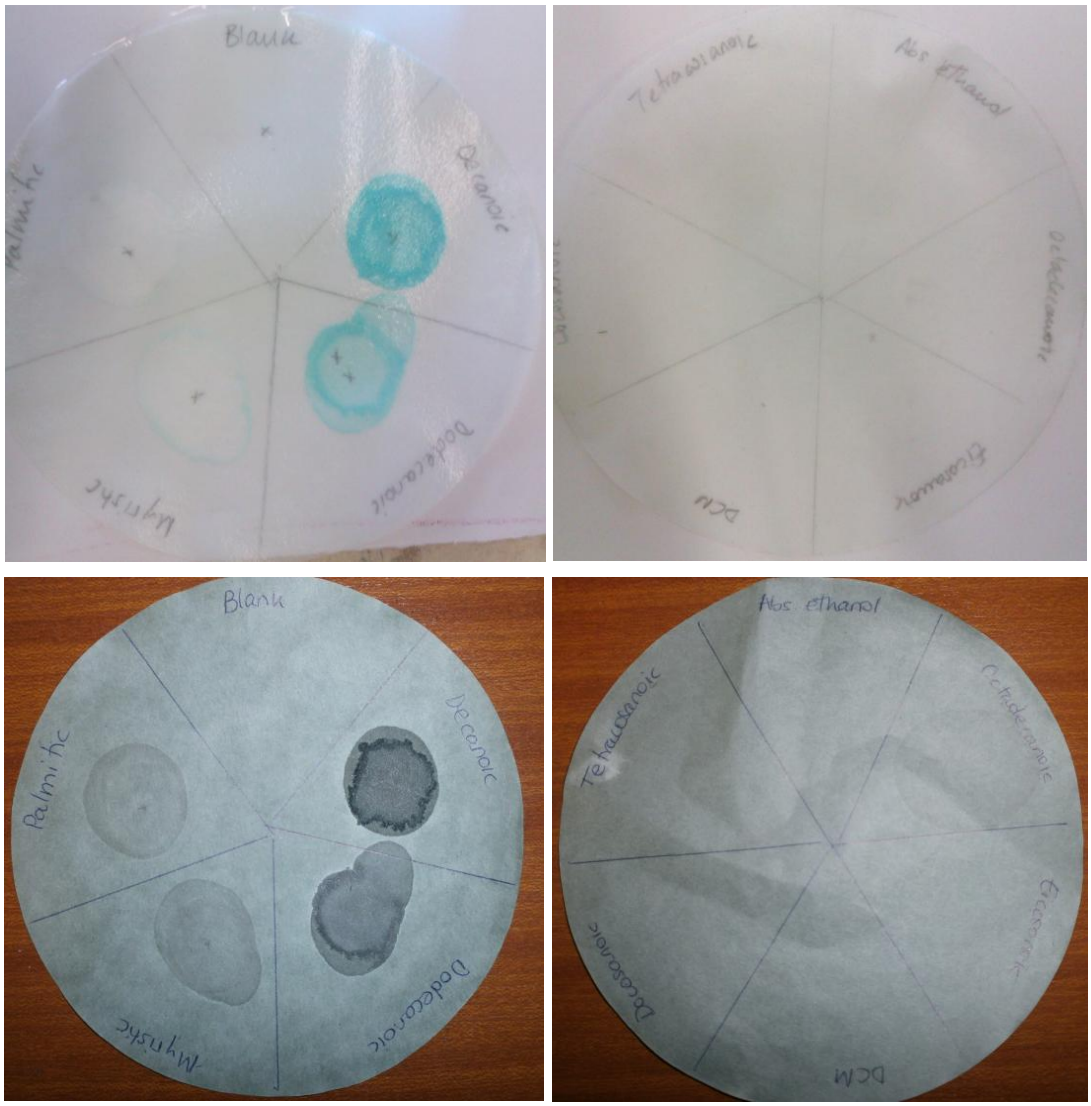


Figure 99: Spot tests of "free" fatty acids (see list of fatty acids in section 5.3.3) + copper solution (top), the same samples developed with 0.10% ethanolic rubeanic acid (bottom)

The solutions containing shorter chain length fatty acids such as decanoic acid and dodecanoic acid produce darker spots with rubeanic acid than the longer chain length fatty acids. This is explained by the fact that shorter chain length fatty acids are soluble in water and so are more likely to form a carboxylate salt with the copper

ions. This means that a higher concentration of copper (II) ions are likely to be adsorbed rather than being washed away, thus able to react with rubeanic acid and produce a more concentrated complex. Every individual's sebaceous fingerprints have a different composition of fatty acids, so the success of the copper-rubeanic acid technique used to develop latent fingerprints will vary considerably from donor to donor.

5.3.5. Application of gelatine lifters with rubeanic acid

As previously mentioned, the number of cases of metal theft is forever increasing in the UK and Europe and gelatine lifters are currently being used to recover latent fingerprints post-treatment[71]. The fact that the gelatine lifters are able to lift any particulates and sebum from latent fingerprint deposits means that coupled with rubeanic acid it could potentially be used to detect whether a person has been in contact with copper or lead.

Preliminary tests were carried out whereby, a donor had to thoroughly wash their hands and place a finger onto a gel lifter. This was dipped in rubeanic acid and dried. No fingerprints were present, this was used as the blank sample.

The same donor had to touch a copper plate for 30 seconds and then place a fingerprint onto the gelatine lifter. The gelatine lifter was then dipped in 0.10% ethanolic rubeanic acid until the fingerprint was visible. A grade 4 fingerprint was developed.

Figure 100: Grade 4 fingerprint developed on a gel lifter with rubeanic acid after touching a copper plate for 30 seconds

This was then repeated but this time, a very old copper pipe in poor condition was held for 30 seconds before the full handprint was placed on a gelatine lifter. This was then dipped in rubeanic acid. It was found that samples continued to develop and more ridge details started to appear after being left to air dry over a few days.

Figure 101: Palm print developed with rubeanic acid after holding an old copper pipe for 30 seconds

The same experiment was carried out with a donor touching a block of lead for 30 minutes, placing the whole hand onto a gelatine lifter and then dipping in rubeanic acid. This time, the handprint took over a week to develop and a brown coloured complex appeared to form. At this stage, it is inconclusive as to whether heat, light or exposure to air is aiding the development of fingerprints when left exposed. The resultant ridge detail was to a grade 3/4 quality.

Figure 102: Palm print developed with rubeanic acid after contact with lead for 30 minutes

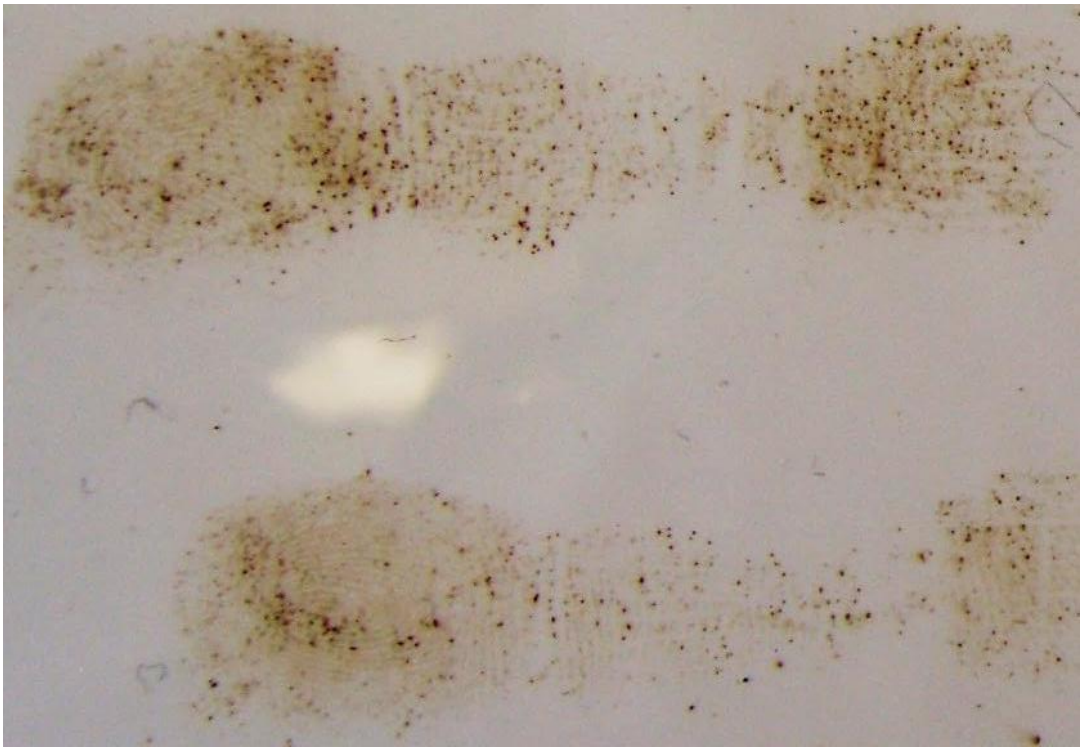


Figure 103: Palm print (close up) developed by detecting lead with rubeanic acid

5.3.6. Conclusions

Overall, the copper-rubeanic acid technique has shown great promise for developing latent fingerprints from a wide range of samples including cotton where in the original report attempts to do the same were deemed unsuccessful. The optimised technique does not require as much time as the original method suggests. It is sufficient to dip fingerprint samples in the copper solution for 1 minute prior to development in rubeanic acid.

This technique has successfully developed whole palm prints after being in contact with copper or lead for only a few minutes before transferring onto the gel lifts to be developed by 0.10% ethanolic rubeanic acid. The impressive quality of ridge detail not only could be used to identify a suspect but it also creates opportunity for research to be continued using gelatine lifters to attempt to positively identify if a person has been in contact with copper or lead. This in turn could lead onto creating a new system for identifying whether a person has been involved in metal theft especially as the number of cases are radically increasing and is undeniably affecting the economy world-wide.

Overall Conclusion & Further Work

Exposing fingerprints to the *p*-block compounds selenium dioxide, SeO_2, phosphorus sesquisulfide, P_4S_3 and phosphonitrilic chloride trimer, $(NPCl_2)_3$ in the vapour phase results in latent prints being visualized on a range of media. Developing fingerprints by SeO_2 sublimation works reasonably well on metals, however samples tend to overdevelop within seconds of releasing the vacuum so a method for storing and preserving samples needs to be devised. $(NPCl_2)_3$ developed the poorest quality fingerprints, derivatisation was attempted but was unsuccessful and so this compound should not be explored further since other compounds produced better results. Lastly, it can be concluded that P_4S_3 is able to reveal the most prints of the best quality. However, as P_4S_3 has a low flash point a safer method for heating the P_4S_3 needs to be devised which avoids using a heat gun so the temperature can be controlled better, bearing in mind that the temperature must also be high enough for P_4S_3 to sublime and the vacuum could be stronger to shorten the time taken for P_4S_3 to sublime.

While all three systems show some potential, they appear much less versatile than the S_2N_2 technique. However, even though the success is limited is does show potential with *p*-block systems in the vapour phase and there could be many more combinations that could be explored in this way.

The aim of the DNA analysis research in Chapter 3, was to test if it was possible to use a simpler and cost-effective DNA analysis method of Chelex® extraction, PCR amplification and gel electrophoresis to detect *Alu* insertion polymorphisms in DNA from latent fingerprints both before and after sublimation of SeO_2, P_4S_3 and $(SN)_x$ polymer. This was successful with all three compounds and leaves scope for further investigations with techniques reported in sections 5.2 and 5.3 to see if these produce similar results. Further work could also be done with individuals that are considered "bad fingerprint donors" to test if this DNA analysis method is sensitive enough. This could lead onto using DNA profiling kits that have greater sensitivity to produce real data and thus prove whether these techniques are non-destructive which could be a major advantage in a forensic investigation.

Chapter 4 explored the use of ESDA as a means to detect latent fingerprints after exposure to various gases and changes in relative humidity. The ESDA response did not show any significant change when latent fingerprints were exposed to the various conditions. Despite ESDA being a non-destructive technique, majority of samples ran using ESDA produced inconsistent results because it was problematic trying to keep parts of the method consistent i.e. how much of the toner was cascaded over the samples which was only achievable through continually practising. Therefore, ESDA is not recommended as a fingerprint development technique.

Finally, of the miscellaneous techniques revisited, the HCV formulation was deemed unsuccessful compared to the original crystal violet technique. The phosphomolybdic acid formulation has shown potential to develop fingerprints on problematic samples such as wood, metals and cotton samples. The EcoSpray MicroDiffuser was the best application method for the 10% ethanolic PMA solution. The main downfall with this technique is that it can only be used to develop sebaceous fingerprints as the PMA specifically interacts with lipids that are present in latent fingerprints. Further work could be carried out using the standard protocol reported in 2.1.2 to gain a better perception of whether this technique has any real promise. An optimized version of the copper acetate and rubeanic acid technique developed fingerprints from a wide range of samples including cotton and carpet. Specialist spectral imaging software was able to unveil ridge detail that was previously obscured due to the texture of fabric samples. Further work should be continued on aging fingerprints, as this still needs to be explored more thoroughly.

However, of all the techniques explored, by far the one with the most potential is the use of ethanolic rubeanic acid to develop whole palm prints on gelatine lifters of donors that have been in contact with copper or lead. The lead samples developed over a number of days, therefore whether heat, light or air could be causing this needs to be investigated further. In light of the extraordinary amount of ridge detail observed on the gel lifters, further work needs to be carried out to see if other metals produce the similar results.

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