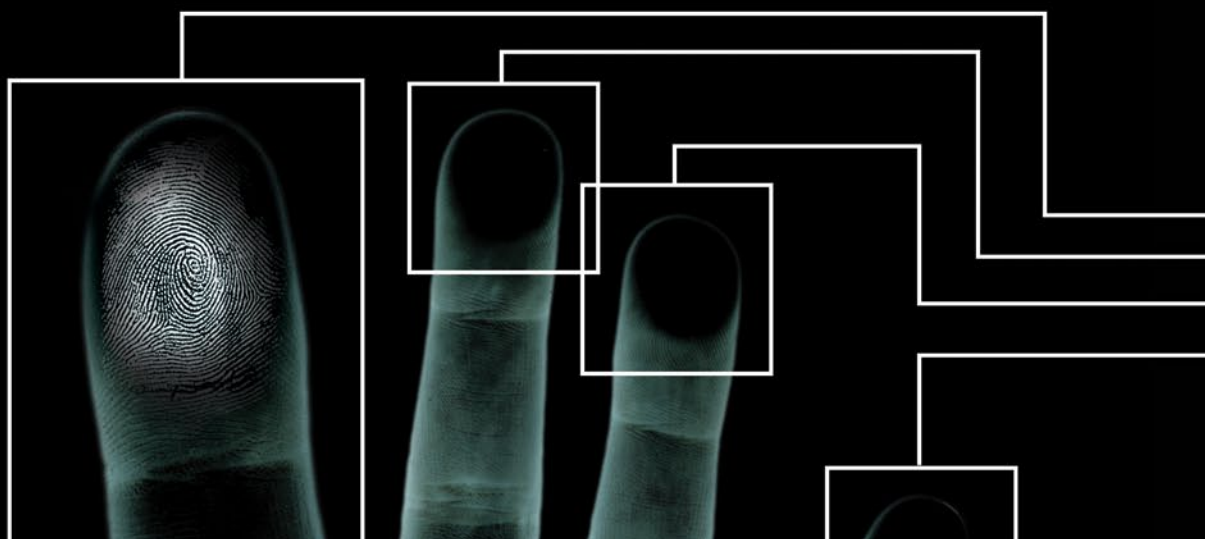
CHAPTER

LATENT PRINT DEVELOPMENT

BRIAN YAMASHITA AND MIKE FRENCH

CONTRIBUTING AUTHORS
STEPHEN BLEAY, ANTONIO CANTU, VICI INLOW, ROBERT RAMOTOWSKI, VAUGHN SEARS, AND MELISSA WAKEFIELD

CONTENTS



CHAPTER 7

LATENT PRINT DEVELOPMENT

BRIAN YAMASHITA AND MIKE FRENCH

CONTRIBUTING AUTHORS STEPHEN BLEAY, ANTONIO CANTU, VICI INLOW, ROBERT RAMOTOWSKI, VAUGHN SEARS, AND MELISSA WAKEFIELD

7.1 Introduction

Latent fingerprint development may be achieved with a wide array of optical, physical, and chemical processes, most having evolved during the past century. Because some techniques are often intricately related and continuously changing, it is imperative that those involved in laboratory and crime scene processing are well trained and well practiced (Trozzi et al., 2000, pp 4–9; Kent, 1998).

For those involved in crime scene and laboratory work, safety is paramount. It is important to follow safe work practices when using the processes described in this chapter. This can be accomplished by observing manufacturer warnings, reading material safety data sheets, and observing one's own institutional policies regarding evidence handling and fingerprint development. It is also important for those working with potentially hazardous materials or equipment to wear the appropriate personal protective equipment, such as gloves, lab coats, eye protection, and respirators; to use engineering controls such as fume hoods; and to practice proper laboratory procedures to reduce exposure to pathogens or harmful chemicals (Masters, 2002).

7.1.1 Types of Prints

Fingerprints found at crime scenes or developed in the laboratory are categorized by some examiners as patent, latent, or plastic impressions (Lee and Gaennslen, 2001, p 106), although all three types are routinely associated with the term *latent print.*

A patent print is simply a visible print. Many of these types of prints are wholly visible to the unaided eye, and only some form of imaging is needed for preservation. A good example of a patent print would be a greasy impression left on a windowpane. Patent prints can also be left in blood, paint, ink, mud, or dust. Lighting is a very important consideration in the search for this type of fingerprint; a good

flashlight or forensic light source is especially useful in the hunt for patent impressions.

The word *latent* means hidden or unseen. Latent prints are undetectable until brought out with a physical or chemical process designed to enhance latent print residue. Many of these processes and techniques are discussed in the remainder of this chapter.

A plastic print is created when the substrate is pliable enough at the time of contact to record the three-dimensional aspects of the friction skin. These impressions are formed when the raised friction ridges are physically pushed into the substrate, creating a mold of the friction skin ridge structure. Clay, putty, soft wax, melted plastic, heavy grease, and tacky paint are all substrates conducive to forming and retaining plastic impressions. Plastic impressions are usually photographed under oblique lighting that enhances the contrast of the ridges and furrows. These prints may also be preserved with silicone-type casting materials.

7.1.2 Deposition Factors

Deposition factors that influence the quality, or even the presence, of latent prints include the conditions surrounding the contact between friction skin and those objects that are touched. These conditions are described as follows:

Pre-transfer conditions include the condition or health of the donor's friction skin and the amount and type of residue on the skin (Olsen, 1978, pp 118–120). These conditions are affected by age, gender, stimuli, occupation, disease, and any substances the subject may have touched prior to deposition.

Transfer conditions also dictate whether a suitable impression will be left (Olsen, 1978, pp 117–122). These are the conditions of the surface (substrate) being touched, including texture, surface area, surface curvature or shape, surface temperature, condensation, contaminants, and surface residues. The pressure applied during contact (deposition pressure), including lateral force, also contributes to transfer conditions.

Post-transfer conditions, also called environmental factors, are forces that affect the quality of latent prints after deposition (Olsen, 1978, pp 121–122). Examples of these factors are physical contact from another surface, water, humidity, and temperature.

7.1.3 Surface Types

Correctly identifying the type of surface expected to bear a fingerprint is an important step toward successful development. Surfaces are generally separated into two classes: porous and nonporous. This separation is required to select the proper technique or reagent and the appropriate sequential order for processing.

Porous substrates are generally absorbent and include materials like paper, cardboard, wood, and other forms of cellulose. Fingerprints deposited onto these media absorb into the substrate and are somewhat durable. Amino acid techniques are particularly useful here because the amino acids tend to remain stationary when absorbed and do not migrate (Almog, 2001, p 178).

Nonporous surfaces do not absorb. These surfaces repel moisture and often appear polished. They include glass, metal, plastics, lacquered or painted wood, and rubber. Latent prints on these substrates are more susceptible to damage because the fingerprint residue resides on the outermost surface. Cyanoacrylate (CA), dye stains, powders, and vacuum metal deposition are usually the best choices to use on these surfaces.

A type of substrate that does not easily fit into the first two categories but should be mentioned is considered semiporous. Semiporous surfaces are characterized by their nature to both resist and absorb fingerprint residue. Fingerprint residue on these surfaces may or may not soak in because of the absorbent properties of the substrate and the variable viscous properties of the fingerprint residue. These surfaces include glossy cardboard, glossy magazine covers, some finished wood, and some cellophane. Semiporous surfaces should be treated with processes intended for both nonporous and porous surfaces.

Textured substrates can be porous or nonporous and present the problem of incomplete contact between the friction ridge skin and the surface being touched. (An example might be the pebbled plastic of some computer monitors.) This often results in fingerprints being discontinuous and lacking fine detail when developed. Additionally, these surfaces often do not respond well to a conventional brush and powder. The brushing action and tape lift typically develop the texture of the substrate, leaving fingerprints difficult or impossible to visualize.

Various techniques, such as the use of very fine powder or flexible lifting media, may be used to reduce the problems caused by textured surfaces (Guerrero, 1992; Kelly et al., 2001, pp 7–12; Knaap and Adach, 2002, pp 561–571).

7.1.4 Process Selection

Fingerprint reagents and development techniques are generally intended to be used in combination and sequential order. These methods are often specific to either porous or nonporous substrates; however, some techniques have universal applications. Deviation from the recommended order could render subsequent processes ineffective. Refer to Trozzi et al. (2000), Kent (1998), and Champod et al. (2004, pp 217–225) for examples of guidelines for sequential ordering, and to Champod et al. (2004, pp 105–179) for a recent review that includes many fingerprint development techniques. The following general procedures are appropriate during a systematic search for latent fingerprint evidence:

- Visual inspection with a bright light, forensic light source, or laser
- Sequential latent print processing
- Documentation of developed prints at each step

It is important to note that not all processes are used invariably. Some discretion will remain with individual agencies and practitioners both at the crime scene and in the laboratory. The following factors may influence the choice of development techniques as well as the level of resources used in any situation:

- Type of latent print residue suspected
- Type of substrate
- Texture of substrate
- Condition of substrate (clean, dirty, tacky, sticky, greasy, etc.)
- Environmental conditions during and following latent print deposition
- Length of time since evidence was touched
- Consequences of destructive processing methods
- Subsequent forensic examinations
- Sequential ordering of reagents
- Seriousness of the crime

7.1.5 Evidence Handling

Proper evidence handling begins with the use of latex, nitrile, PVC, or other suitable gloves. Some glove manufacturers or safety supply distributors will list gloves recommended for use with various chemicals. The use of gloves protects the evidence from contamination and the user from exposure to pathogens or hazardous chemicals. It does not, however, guarantee that latent prints will be preserved because even a gloved hand may destroy fragile latent prints on contact. This is especially true on nonporous surfaces where the latent print resides on the extreme surface of the evidence. To prevent damage to fingerprints on these surfaces, evidence should be handled in areas not normally touched or on surfaces incapable of yielding viable fingerprints. It should also be noted that the use of gloves does not preclude the transfer of friction ridge detail from the examiner to the exhibit (Willinski, 1980, pp 682–685; St-Amand, 1994, pp 11–13; Hall, 1991, pp 415–416).

7.1.6 Packaging

Packaging helps ensure the integrity of the evidence by keeping contaminants away, keeping trace evidence intact, and helping to guarantee chain of custody. Cardboard boxes, paper bags, and plastic bags are the most common forms of evidence packaging. Most experts recommend paper packaging because it is breathable and cost effective, although plastic bags are also widely used. Any items that have been wet should be allowed to air-dry prior to packaging because excess moisture trapped in any package will increase the probability of destructive fungal growth. Moisture can also be trapped in plastic bags when evidence is gathered in high-humidity environments.

Items of nonporous evidence should not be allowed to rub together. Nonporous evidence should be stored singly, secured inside an appropriately sized package in a manner that prevents shifting and rubbing. Under no circumstances should fillers such as shredded paper, wood shavings, or packing peanuts be used inside the package with the evidence because they may easily wipe off fragile fingerprints. (However, they can be used outside the evidence container, inside the mailing container.) Porous evidence can be secured in boxes, bags, and envelopes and can be stored together because latent prints are not likely to

rub off on contact. Once evidence is secured, the package should be sealed with evidence tape so that there are no entry points. The tape should be signed by the person securing the evidence, and the appropriate identifying information should be placed on the package as specified by the agency responsible for collection.

The remainder of this chapter is intended to describe, in some detail, the nature of latent print residue and the most commonly used fingerprint development techniques. Experimental and novel techniques have not been included, nor have processes considered by the authors to be redundant, impractical, or overly hazardous. However, the omission of reference to a particular technique does not indicate its unsuitability as a fingerprint development technique. Several formulations for various chemical solutions have been collected in Section 7.14.

7.2 The Composition of Latent Print Residue

7.2.1 Introduction

The composition of sweat that is deposited when friction ridge skin makes contact with a surface is a complex mixture (Ramotowski, 2001, pp 63–104; Bramble and Brennan, 2000, pp 862–869). Recent studies have identified hundreds of compounds present in human sweat (Bernier et al., 1999, pp 1–7; Bernier et al., 2000, pp 746–756). A considerable number of studies into the nature of sweat have been performed by both the dermatology and forensic science communities. In particular, a number of studies have investigated how the chemical composition of these residues changes with time, which is a critical problem for the fingerprint examiner. Although knowledge of the composition of sweat produced in the various glands throughout the body is of interest and provides a baseline for comparison purposes, this information does not accurately represent what is actually going on in the deposited print at a crime scene. Studies have shown that significant changes begin to occur in the latent print almost immediately after deposition. If the latent print is to be successfully visualized, a thorough understanding of these changes is needed.

This section will begin with a very brief overview of skin anatomy, which will be necessary to gain a better understanding of how the chemical compounds in a latent print are secreted onto the surface of friction ridge skin. Next, there will be a detailed look at the chemical composition of the secretions from each of the glands responsible for contributing to latent print residue. Another section will cover how the composition of some of these secretions changes as the donor ages. Finally, recent studies that have investigated how latent print residue changes with time will be summarized.

7.2.2 Anatomy of Skin

This topic is covered in more detail elsewhere in this sourcebook, so the treatment here will be very brief. Readers are directed to Ramotowski (2001, pp 63–104) for more detail.

Skin is the largest organ in the human body (Odland, 1991). The total area of skin on the body exceeds 2 m^2; yet, on most parts of the body, the thickness is no more than 2 mm. Skin serves several functions, including regulation of body temperature, moisture retention, protection from invasive organisms (e.g., viruses, bacteria), and sensation. It is composed of two primary and distinct layers, the epidermis and dermis.

The epidermis is composed of several distinct layers (Ramotowski, 2001, pp 63–104; Odland, 1991). The layer situated just above the dermis is the stratum germinativum (basal cell layer), and the top layer is the stratum corneum (cornified layer). In this stratum, eleiden is converted to keratin, which is continually sloughed off the surface of the epidermis, resulting in a constant need to replenish the keratin that is lost. A cell beginning in the stratum germinativum typically travels through to the stratum corneum in about 28 days.

The dermis is composed of a variety of different connective tissues, including collagen, elastin fibers, and an interfibrillar gel composed of glycosamin–proteoglycans, salts, and water (Odland, 1991). This layer also contains the two major sudoriferous and sebaceous glands.

7.2.3 The Production of Sweat

Three primary glands contribute to the production of sweat. These are the sudoriferous glands (eccrine and apocrine) and the sebaceous glands. Each gland contributes a unique mixture of chemical compounds. These compounds either exude from pores onto the friction ridges or are transferred to the friction ridges through touching an area (e.g., the forehead, underarm, etc.).

The eccrine gland is one of two types of sudoriferous (or "sweat") glands present in the body. Several million of these glands are distributed throughout the body, most commonly on the palms of the hands and soles of the feet and least numerous on the neck and back (Anderson et al., 1998, p 1561). These glands produce a secretion that is mostly water but contains many compounds in trace quantities (Brusilow and Gordes, 1968, pp 513–517; Mitchell and Hamilton, 1949, p 360; Sato, 1979, pp 52–131; Bayford, 1976, pp 42–43; Olsen, 1972, p 4). The average quantity of secretions produced during a typical 24-hour period varies between 700 and 900 grams. The pH of sweat has been reported to vary from 7.2 (extracted directly from the gland), to 5.0 (recovered from the skin surface at a low sweat rate), to between 6.5 and 7.0 (recovered from the skin surface at a high sweat rate) (Kaiser and Drack, 1974, pp 261–265).

The eccrine gland also secretes organic compounds. Of primary importance to the development of latent print ridge detail are the amino acids. Table 7–1 summarizes the average values of abundance for the amino acids listed (Hadorn et al., 1967, pp 416–417; Hamilton, 1965, pp 284–285; Oro and Skewes, 1965, pp 1042–1045). Serine is the most abundant amino acid, and thus all other values are normalized to a value of 100 for that compound. Proteins are also found in eccrine sweat (Nakayashiki, 1990, pp 25–31; Uyttendaele et al., 1977, pp 261–266). One study found more than 400 different polypeptide components present (Marshall, 1984, pp 506–509).

Lipids have also been detected in eccrine sweat. There is some difficulty in accurately determining the amounts of these compounds present in eccrine secretions because sweat often mixes with sebaceous compounds on the skin surface. However, one study reported detectable amounts of both fatty acids and sterol compounds (Boysen et al., 1984, pp 1302–1307).

Other miscellaneous compounds, including drugs, have been found in eccrine secretions (Sato, 1979, pp 52–131; Lobitz and Mason, 1948, p 908; Förström et al., 1975, pp 156–157). One study reported the presence of sulfonamides, antipyrine, and aminopyrine (Johnson and Maibach, 1971, pp 182–188). Another reported that L-dimethylamphetamine and its metabolite L-methamphetamine had been detected (Vree et al., 1972, pp 311–317). Ethanol has also been detected in eccrine sweat (Naitoh et al., 2000, pp 2797–2801), which has led to the suggestion of using sweat as a means of noninvasively determining a person's serum ethanol concentration (Hawthorne and Wojcik, 2006, pp 65–71). Acetaminophen has also been reported in a person's sweat a day after taking the medication (Mong et al., 1999).

Table 7–1

Relative abundance of amino acids in sweat.

Serine	100
Ornithine–Lysine	45
Alanine	30
Threonine	15
Valine	10
Glutamic acid	8
Phenylalanine	6
Tyrosine	5

The other sudoriferous gland present in skin is the apocrine gland. These sweat glands are associated with the coarse hair of the armpits and pubic area. They are larger than eccrine glands and secrete a thicker fluid (Anderson et al., 1998, p 1561). The gland's duct typically empties into a hair follicle (above where a sebaceous gland duct would be) before the secretions reach the skin's surface (Robertshaw, 1991). Because the contents of the apocrine gland often mix with sebaceous secretions prior to reaching the skin's surface, it is difficult to obtain uncontaminated "pure" apocrine secretions for analysis. One of the few published studies of apocrine secretions described them as milky in appearance and stated that they dried to a plasticlike solid, which fluoresced and had an odor (Shelley, 1951, p 255). Compounds reported to have been isolated from apocrine

secretions include proteins, carbohydrates, cholesterol, iron (Knowles, 1978, pp 713–721), C_{19}-steroid sulfates, and Δ16-steroids (Toth and Faredin, 1985, pp 21–28; Labows et al., 1979, pp 249–258).

Sebaceous glands are relatively small saclike organs and can be found in the dermis layer of skin. They are found throughout the body and are associated with body hair. They are particularly abundant on the scalp, face, anus, nose, mouth, and external portions of the ear (Anderson et al., 1998, p 1464). They are not found on the palms of the hands or soles of the feet. The secretions from the sebaceous gland typically empty into a hair follicle before reaching the skin's surface, although in some regions they do reach the skin's surface directly (e.g., lips). The purpose of sebaceous secretions appears to be to help prevent sweat evaporation (and thus retain body heat) and to lubricate hair and surrounding skin.

The primary compounds present in sebaceous secretions are lipids. Table 7–2 lists the approximate percentage values for the various lipid classes present in sebaceous secretions, as reported by Goode and Morris (1983). Knowles (1978, pp 713–721) reported similar concentration ranges.

Table 7–2

The approximate percentage of lipids in sebaceous secretions.

Lipid	Percentage
Glycerides	33
Fatty acids	30
Wax esters	22
Cholesterol esters	2
Cholesterol	2
Squalene	10

Free fatty acids in sebum are derived primarily from the hydrolysis of triglycerides and wax esters. About half of the fatty acids are saturated, with straight chain C16 and C14 being the most common (Green, 1984, pp 114–117). Monounsaturated fatty acids comprise about 48% of sebum, and polyunsaturated acids comprise the remaining 2–3% (Nicolaides and Ansari, 1968, pp 79–81). Branched chain fatty acids have also been reported (Green, 1984, pp 114–117).

Wax esters comprise about 20–25% of sebum. These compounds contain a fatty acid that has been esterified with a fatty alcohol. A significant percentage of these compounds (≈27%) have been reported to contain branched chain fatty acids (Nicolaides et al., 1972, pp 506–517). Sterol esters are thought to be produced secondarily by certain strains of bacteria (Puhvel, 1975, pp 397–400). Squalene, which comprises about 10% of sebum, is a major precursor for steroid production in the body (including the steroid alcohols, lanosterol and cholesterol).

7.2.4 Variation in Sebum Composition with the Age of the Donor

The free fatty acid composition in sebum changes dramatically with age of the donor (Ramasastry et al., 1970, pp 139–144). The approximate percentage of fatty acids in newborns (approximately 5 days old) has been reported to be only about 1.5% of the overall sebum composition. This value rises dramatically to about 20–23% in young children (age 1 month to 4 years). The value then stabilizes to 16–19% for adolescent and postadolescent subjects (up to approximately 45 years of age).

Triglycerides also vary significantly. Newborns were found to have triglycerides making up approximately 52% of their sebum. This value decreased to 38% in infants (1 month to 2 years of age). Subsequently, the value peaked at 50% in young children (ages 2–4 years) and then slowly decreased to 41% in postadolescent subjects.

In newborns, 26.7% of sebum was composed of wax esters. This value began to decrease in infants (17.6%) and continued until reaching a low of 6.9% in subjects between the ages of 4 and 8 years. The values then began to increase in preadolescents (17.8%) and continued to rise until reaching a maximum of 25% in postadolescents (up to 45 years of age).

The value of cholesterol in sebum tended to peak in preadolescents (7.2%). Newborns were reported to have 2.5% cholesterol in their sebum, whereas postadolescents had the lowest values, 1.4%. Cholesterol ester composition tended to vary in an unpredictable way. A value of 6.1% was reported for newborns, which increased to 10.3% for infants (1 month to 2 years of age). This value then decreased to 8.9% for young children (ages 2–4 years) and then increased to 14.6% in subjects of ages 4–8 years. This value then decreased dramatically to 5.7% in preadolescent children and continued to decline to 2.1% in postadolescent subjects (up to 45 years of age).

Although squalene composition changes with donor age, the range is not very significant. The concentration of squalene begins at 9.9% for newborns and reaches a low of 6.2% in children of ages 2–4 years. The concentration then slowly begins to rise in children 4–8 years in age (7.7%) and peaks at a value of 12% in postadolescents.

7.2.5 Latent Print Residue

A latent print is a mixture of some or all of the secretions from the three types of glands. The amount of material contained in a latent print deposit is rather small, typically less than 10 µg, and has an average thickness of about 0.1 µm (Scruton et al., 1975, pp 714–723). The latent print secretion is a complex emulsification of these numerous and varying compounds. When deposited on a surface, nearly 99% of the print is composed of water. As this water begins to evaporate rapidly from the deposit, the print begins to dry out. This process begins to alter certain reagents' ability to visualize the print. Fingerprint powder, for example, will not work as well on a dried-out latent print, but other processes, like ninhydrin and physical developer, have developed prints several years old (McDiarmid, 1992, pp 21–24).

Latent print residue is generally divided into two basic categories, water-soluble and water-insoluble. The water-soluble portion of the print deposit is typically composed of eccrine secretions like salts (e.g., NaCl) and amino acids (e.g., serine, glycine). Chemicals like ninhydrin (which reacts with amino acids) and silver nitrate (which reacts with sodium chloride) are effective reagents for visualizing this water-soluble portion of the residue. However, an eccrine-rich latent print that is exposed to water most likely will not be recovered with these methods. This is why, before the introduction of physical developer in the 1970s, there was no reliable method for recovering prints from water-soaked documents.

The water-insoluble portion can really be divided into two subcategories. One fraction of this residue is composed of large, water-insoluble molecules (e.g., proteins) and the other fraction is composed mainly of nonpolar lipids (e.g., fatty acids). Reagents like physical developer are thought to react with compounds similar to the first fraction, and reagents like Oil Red O or Nile Red react with lipids from the second fraction.

7.2.6 Aging of Latent Print Residue

A number of laboratories have looked into studying the changes in the composition of latent print residue and have determined that the composition of latent print residue can change dramatically over time. The foundation work in this area was conducted during the 1960s and 1970s by the U.K. Home Office Scientific Research and Development Branch and Central Research Establishment (Bowman et al., 2003, pp 2–3). Additional studies have been conducted by some of the Home Office Forensic Science Service laboratories and several Department of Energy National Laboratories.

7.2.7 Home Office Scientific Development Branch (U.K.)

The U.K. Home Office sponsored a number of research efforts, which were carried out by two groups: the Scientific Research and Development Branch (also known as the Police Scientific Development Branch [PSDB] and currently known as the Scientific Development Branch) and the Central Research Establishment. A number of studies conducted in the mid- to late 1960s looked at determining the amount of certain inorganic compounds (chlorides) (Cuthbertson, 1969) as well as lipids (Wilson and Darke, 1978) in latent print residue. These studies did not address the changes in composition with time. However, one study monitored the change in chlorides, amino acids (as leucine), and urea concentration in a deposit over the course of 236 days (Knowles, 1978, pp 713–721). After 236 days, the chloride concentration had changed from 0.223 µg/cm^2 to 0.217 µg/cm^2. The amino acid content had changed from 0.083 µg/cm^2 to 0.046 µg/cm^2, and the urea content from 0.083 µg/cm^2 to 0.028 µg/cm^2.

The PSDB recently sponsored some work in this area (Fitzgerald, 2003). A project was started in February 2002 between the University of Lincoln and PSDB to look into the gas chromatography–mass spectroscopy (GC–MS) analysis of the composition of latent print residue and how it changes over time.

7.2.8 Home Office Forensic Science Service (U.K.)

The U.K. Home Office Forensic Science Service (FSS) has also been active in the area of latent print chemistry. The FSS conducted an early preliminary study in conjunction

with the University of Lausanne in 1999 (Jacquat, 1999). This study compared the aging of prints exposed to light and darkness over the period of 1 month. The six most abundant peaks found in the residue were oleic acid, palmitic acid, cholesterol, squalene, and two wax esters. Data were collected from four donors at the time of deposition (t = 0), after 2 weeks, and finally after 4 weeks. Palmitic acid in a print kept in the dark and squalene in a print kept in the light showed a significant decrease over the first 2 weeks and then stabilized. Cholesterol and oleic acid showed a regular decrease in prints stored in the dark. No other observable trends were detected for the other compounds.

A recent study funded by the Technical Support Working Group (TSWG), an interagency working group that funds projects related to counter-terrorism, looked at the changes in lipid content of a print over time and under different environmental conditions (Jones et al., 2001a). The FSS study used methyl-N-trimethylsilyltrifluoroacetamide as a derivitizing agent. Samples were analyzed at selected intervals and stored in either light or dark conditions at 25 °C and 20% relative humidity. Several general trends were observed. Squalene was found to degrade rather quickly and was rarely detected in older prints. In some cases, certain fatty acid concentrations initially increased before tending to decrease over time. This may have been due to the breakdown of wax esters, which may have contributed fatty acids to the residue before the compounds began to break down. Similar trends were observed for samples stored in the dark; however, the decreases were less rapid than for samples stored in the light. The FSS is currently continuing to investigate this topic with a research grant issued by the U.K. Engineering and Physical Sciences Research Council.

Another effort conducted by the FSS involved the use of microfluidic systems for the chemical analysis of latent print residues (Valussi, 2003). The objective of this work was to develop a microfluidic device, based on capillary electrophoresis (CE), that would enable sampling, preconcentration, and analysis of latent print residues. The proposed micro-TAS (total analytical system) used microchip technology to allow for ultrafast and highly efficient separations. The analysis involved placing a print directly onto a gel-coated CE chip. An applied voltage caused polar components of the residue to migrate into the chip. After preconcentration, the residue was separated and then analyzed. The project demonstrated that the CE chip method is capable of separating certain components of latent print residue; however, additional refinements will be necessary to separate specific compounds (or groups of compounds) of interest.

7.2.9 Pacific Northwest National Laboratory

The Pacific Northwest National Laboratory (PNNL) performed a series of aging experiments for latent prints deposited on glass fiber filter paper (Mong et al., 1999). This TSWG-funded R&D effort was done during the late 1990s. The results obtained from the aging experiments were generally as expected. Most of the unsaturated lipids (e.g., squalene and fatty acids such as oleic and palmitoleic acids) diminished significantly during the 30-day study period. The saturated compounds (e.g., palmitic and stearic acids) remained essentially stable during the same 30-day period. Overall, as the sample print aged, there was a tendency to form more lower molecular weight breakdown products (e.g., octanoic and nonanoic acids) over time. It was hypothesized that these low molecular weight compounds would either break down further or evaporate.

7.2.10 Savannah River Technical Center

The Savannah River Technical Center (SRTC), in a project jointly funded by the Department of Energy and TSWG, also studied how latent print residue changes with time (Walter, 1999). This study focused on what changes occur as lipids in the print begin to age. The ultimate goal was to determine whether any of the breakdown products would be suitable for visualization by chemical reagents. A limited number of conditions (e.g., UV exposure, indoor and outdoor conditions, addition of a catalyst) were also evaluated as part of this study.

The primary breakdown products for the lipids studied by SRTC were found to be a class of compounds known as hydroperoxides. The standard mixture used in this experiment involved a combination of cholesterol, triglycerides, fatty acids, wax esters, cholesterol esters, and a catalyst, protoporphyrin IX dimethyl ester (approximately 0.01% of the overall mixture). This mixture was then exposed to the various environmental conditions. As with the PNNL study, the SRTC found that unsaturated compounds were rapidly depleted from the samples, even ones stored in relatively cool, dark conditions. One experiment that looked at the aging of squalene on a glass slide found that after 1 month of exposure to ambient laboratory conditions, 10% of the

sample had been converted to hydroperoxides. The SRTC was going to pursue chemiluminescent methods for visualizing these hydroperoxides. However, because hydroperoxides themselves are somewhat unstable, it is not known how long these compounds remain in aged print residues and whether additional compounds found in actual prints would speed up their breakdown.

7.2.11 Conclusion

The chemistry of latent print residue is very complex, yet its physical characteristics and properties are due to more than just the hundreds (or potentially thousands) of chemical compounds that comprise the residue. These compounds form a complex three-dimensional matrix, an emulsion of water and organic and inorganic compounds. The interaction of all of these different compounds as they are exposed to a variety of environmental conditions over a period of time can produce dramatic changes in the physical properties of the latent print. These changes can explain why some reagents, like powders, and iodine fuming, tend to work on relatively fresh prints, whereas a reagent like physical developer has been known to develop decades-old prints.

It is only by obtaining a better understanding of the chemical composition of latent print residue and how it changes with time that we can make improvements to existing reagents and design novel compounds for specialized conditions or surfaces. Such data will also assist in better understanding how latent print development reagents actually work (as well as what they actually react with in the residue). Only then can we develop a methodical approach for reagent design that will yield useful new techniques in the future for visualizing latent print residues.

7.3 Latent Print Powders

7.3.1 Background

Latent print visualization with powder, or "dusting", involves the application of finely divided particles that physically adhere to the aqueous and oily components in latent print residue on nonporous surfaces (Sodhi and Kaur, 2001, pp 172–176). This technique is one of the oldest and most common methods of latent print detection, with one of the earliest references dating back to 1891 (Forgeot, 1891, pp 387–404). Early practitioners used a variety of locally available ingredients to make their own dusting powders, including charcoal, lead powder, cigar ashes (Moenssens, 1971, pp 106–107), powdered "washing blue", powdered iron, soot (Lightning Powder Inc., 2002, pp 2–3), and talc (Olsen, 1978, pp 212–214).

7.3.2 Theory

Fingerprint dusting is relatively simple and relies on the adherence of powder to the latent print residue to provide good visibility and definition of fingerprint detail. Latent print powder has an affinity for moisture and preferentially clings to the residue deposited by friction ridge skin. It is well accepted that the mechanical attraction between these particles and the moisture and oily components in a print causes adhesion, with absorption being a factor (Olsen, 1978, pp 212–214; Lee and Gaensslen, 2001, pp 108–109). Particle size, shape, relative surface area (Olsen, 1978, pp 212–214), and charge (Menzel, 1999, p 143) appear to play roles as well.

Most commercial powders rely on at least two essential elements to provide adhesion to latent print residue without "painting" the substrate. These elements are referred to as pigment and binder. The pigment in fingerprint powder provides for effective visualization, offering contrast and definition against the background surface. The binder (also referred to as the carrier in some applications) provides for maximum and preferential adhesion to latent print residue (Menzel, 1999, p 143). Some pigment powders offer enough adhesion to be used individually. Background painting occurs when an undesirable amount of powder adheres to the substrate as well as the latent print, hindering detection.

Visualization will occur via reflected light (light powders), absorbed light (dark powders), and luminescence (fluorescent powders). Sometimes powders are combined for effectiveness on both light and dark substrates. This is the case with bichromatic powder, which uses highly reflective aluminum powder mixed with black powder to achieve visualization on both light and dark surfaces. A disadvantage of mixing different types of pigment particles is that extremely faint impressions, with few particles adhering to the print, may suffer from having only a fraction of the necessary pigment needed for visualization. This problem can be overcome by tagging a single type of pigment particle with a fluorescent dye stain, thus creating a particle with dual uses rather than combining different types of particles.

Commercial powder manufacturers tend to label powders by color, such as black, white, silver, gray, and so forth, rather than labeling the ingredients. Particles that serve as good fingerprint powders include carbon black (colloidal carbon), lamp black, talc, kaolin, aluminum, metal flake, and dolomite (Lee and Gaensslen, 2001, pp 108–109), among others. Good binders include iron powder (Lee and Gaennslen, 2001, pp 108–109), lycopodium, corn starch, rosin, and gum arabic (Menzel, 1999, p 143).

One of the most common latent print powders, known for its versatility and effectiveness, is carbon black. When mixed with a carrier, this powder works on a wide range of surfaces and causes little substrate painting (Cowger, 1983, pp 79–80). Carbon black mixtures produce a dark gray-black image that can be visualized on varying colored surfaces. This type of powder will also show up on glossy black surfaces, conversely appearing light in color (Cowger, 1983, pp 79–80). Interestingly, black fingerprint powder can also be prepared or "tagged" with a fluorescent dye stain (Thornton, 1978, pp 536–538), giving it the dual purpose as a photoluminescent technique as well.

Other effective and widely used latent print powders are flake metal powders made from aluminum, zinc, copper, brass, stainless steel, iron, cobalt, and nickel. Some data indicate that flake powders are more sensitive than nonflake powders (Kent, 1998). However, flake powders also sometimes tend to "paint" the substrate more than nonflake particles do.

Flake powders are manufactured by ball-milling spherical metallic particles into flakes ranging from 1 to 50 µm in diameter (James et al., 1991, pp 1368–1375). The increased surface area of the flake relative to the weight of the particle contributes to this powder's adhesion. It appears that commercially available flake powder with a mean diameter of 10 µm and an average thickness of 0.5 µm is optimum for latent print development. It is also important to note that the addition of stearic acid, intended to influence flake morphology during milling, increases the adhesion value of the flakes as well (James et al., 1990, pp 247–252). Aluminum flake powder that was washed of its stearic acid content resulted in poor fingerprint development, whereas aluminum flakes produced with approximately 10 weight-percent of stearic acid produced good results (James et al., 1991, pp 1368–1375). Another study indicated that a range of flake metals produced optimum results with 3–5 weight-percent of stearic acid levels (James et al., 1993, pp 391–401).

7.3.3 Application

All manufacturer warnings, including those in material safety data sheets, should be heeded when using fingerprint powder. Although commercial suppliers of latent print powder have discontinued using known hazardous ingredients such as lead, mercury, and cadmium, it is strongly recommended that the practitioner wear a dust mask or work on a downdraft table as minimum precautions while using any powder.

Powders are typically applied to nonporous surfaces with a soft brush. Powdering is not recommended for porous or highly absorbent surfaces such as uncoated paper or raw wood because other chemical treatments outperform powder on these surfaces. The softness of the bristles is particularly important to prevent damage to fragile latent print residue. Latent prints with a high moisture or oil content are easily damaged by a brush that is too stiff or is used with excessive force. Conventional brushes are typically made with animal hair, fiberglass filaments, or sometimes feathers. Although fingerprint brushes are largely taken for granted these days, a study of brushes has been carried out (Bandey, 2004).

Powders applied with a traditional filament brush consist of very fine particles and are usually low density or "fluffy" in nature. This enables particles to be easily picked up or "loaded" onto the brush filaments. The low density of this powder also allows it to easily become airborne during the dusting process, making a dust mask or respirator necessary at the crime scene.

It is important to keep brushes clean, dry, and relatively free of tangles. To apply fingerprint powder with a conventional brush, the filament tips are lightly dipped into a sterile, wide-mouth container holding a small amount of powder. This is called "loading" the brush. Excess powder is then shaken, spun, or tapped from the brush. The powder is then applied evenly to all areas of the substrate.

An area of the surface (or a substrate similar in nature) should be tested before fully processing the item. This is done to establish the optimum amount of powder to be used on that substrate and to avoid background painting. Brushing is accomplished with light and even strokes that resemble painting. It is important always to begin by lightly powdering and slowly building to heavier applications to minimize fingerprint damage.

When latent prints appear, they can be lightly brushed by adding powder and subsequently brushing excess powder away. This is done in the direction of the ridge flow to prevent damage to the impression.

Another type of powder, called magnetic or magna powder, allows for application with a magnetized rod that has no bristles. This type of powder can be light, dark, or fluorescent and utilizes the ferromagnetic properties of iron powder mixed with pigment powders. The magnetized applicator (magna brush) is dipped into the powder, picking up a ball of the iron and particle mixture, essentially forming its own brush (Figure 7–1). This ball serves as an effective carrier for pigment particles and is passed back and forth over the substrate to develop latent impressions.

It is important to note that the magnetic powder ball formed with a magna brush is much softer than conventional filament brushes and typically causes less damage to fragile latent prints (MacDonell, 1961, pp 7–15). Magnetic powders are usually less effective on ferromagnetic substrates such as steel or nickel and are therefore not recommended on those substrates. The magnetic attraction may cause contact between the applicator and substrate, damaging latent prints in the process. In addition, magnetized particles from the powder will cling to the substrate and resist removal.

There are two ways to record or preserve a powdered impression. The most common and simplest method is lifting. To lift a print, good-quality transparent tape is placed onto the surface bearing a powdered impression. Common tape size for fingerprint lifting is 1.5–2 in. wide. While it is being applied, the tape is rubbed to remove air bubbles and to ensure good adhesion to the latent prints. It is then removed and placed on a backing card that contrasts with the color of the powder. Probably the most common lift is of black fingerprint powder placed on a white backing card. Other adhesive lifting media are hinge lifters, where the adhesive square is attached to the backing card by a hinge; opaque adhesive gel lifters, typically black or white; and silicon-type materials that are spread onto the surface and allowed to harden to a flexible rubbery medium before lifting. Care must be taken during the comparison process to note which lifting techniques cause the print to appear reversed.

If the impression will be photographed in situ, the importance of powder color increases. Documenting powdered impressions this way requires combining proper selection of powder and photographic lighting that will produce ample contrast against the substrate.

Another type of powder that produces excellent results on a wide variety of surfaces is fluorescent powder. Fluorescent powder relies on the principle of luminescence to provide contrast between fingerprint and background. Fluorescent powders are typically created by adding a laser dye in solution to a binder and allowing the mixture to evaporate (Menzel, 1999, pp 62–65). The resulting dried mass is then ground into latent print powder.

Fluorescent powdering is highly sensitive when used with a good forensic light source and the appropriate barrier filters. In theory, luminescent fingerprint powder should be more sensitive than conventional methods (Menzel, 1999,

FIGURE 7–1

Magnetic applicator.

pp 4–7). It is important to test tape and lift cards used with fluorescent powders for any inherent fluorescence because fluorescence caused by lifting media will interfere with the quality of the impression.

Another use of fingerprint powder, or the components of fingerprint powder, is in a suspension, for use on wet surfaces or on adhesive tapes. Conventional small-particle reagent, for developing fingerprints on wet, nonporous surfaces, uses molybdenum disulphide in suspension, but other reagents have been developed (Frank and Almog, 1993, pp 240–244). A similar suspension, Sticky-side powder (Burns, 1994, pp 133–138), used to develop prints on the adhesive side of tape, has also been reformulated using fingerprint powder (Bratton et al., 1996, p 28; Wade, 2002, pp 551–559).

Finally, a word of caution may be in order. Although using fingerprint powder is quick and inexpensive, concerns have been raised recently concerning the possibility of contamination due to the transfer of DNA through the use of fingerprint brushes (van Oorschot et al., 2005, pp 1417–1422). Crime scene examiners are being warned to be aware of this possibility.

7.4 Ninhydrin and Analogues

7.4.1 Ninhydrin History

Ninhydrin was first described in 1910 when Siegfried Ruhemann mistakenly prepared the compound (Ruhemann, 1910a, pp 1438–1449). Ruhemann observed that the new compound reacted with skin and amino acids to produce a purple product (Ruhemann, 1910b, pp 2025–2031), and he published a series of papers detailing this and other reactions (Ruhemann, 1911a, pp 792–800; 1911b, pp 1306–1310; 1911c, pp 1486–1492). He proposed a structure for the deeply colored product (Ruhemann, 1911c, pp 1486–1492), today known as Ruhemann's purple, and commented on the possible application of the reaction to the detection of trace amounts of amino acids and protein products in biological samples (Ruhemann, 1911a, pp 792–800).

Following Ruhemann's discovery, ninhydrin found widespread use in analytical chemistry and biochemistry applications. As early as 1913, the reaction with amino acids was an important diagnostic test for the presence of protein and amine compounds in biological samples (Crown, 1969, pp 258–264; Friedman and Williams, 1974, pp 267–280). With the advent of chromatography, the reaction became even more useful for the location of amino acids on paper chromatograms or in fractions produced by liquid chromatography (Crown, 1969, pp 258–264; Smith and Agiza, 1951, pp 623–627).

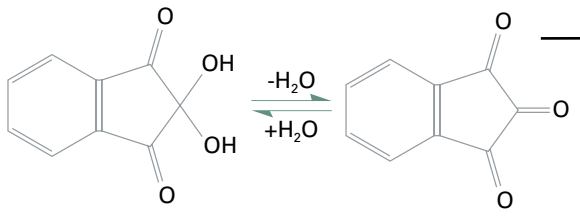
Ruhemann's purple and other by-products of the ninhydrin and amino-acid reaction were also used to quantitatively measure amino acid content of samples (Yemm et al., 1955, 209–214; Smith and Agiza, 1951, pp 623–627). The reagent was so powerful and versatile that some authors suggested it was the most widely used reaction in analytical laboratories (Friedman and Williams, 1974, pp 267–280).

This use of ninhydrin was frequently accompanied by warnings to avoid contact between bare skin and any surfaces to come into contact with the reagent (Crown, 1969, pp 258–264). This was due to the strong reaction between ninhydrin and sweat, which would cause the appearance of fingerprints on chromatograms (Crown, 1969, pp 258–264; Odén and von Hofsten, 1954, pp 449–450). Despite these warnings, which clearly indicated the ability of ninhydrin to develop fingerprints, the reagent was not applied in a forensic context until 1954 (Odén and von Hofsten, 1954, pp 449–450).

Following this initial report, ninhydrin rapidly became an indispensable tool in the detection of latent fingerprints, with widespread use among jurisdictions being documented as early as 1959 (Speaks, 1964, pp 11–13, 23). The technique is now amongst the most popular methods for fingerprint detection on paper and other porous substrates (Champod et al., 2004, pp 114–136). This method has limitations, however, and chemists have addressed these limitations by the synthesis of analogues—compounds structurally related to ninhydrin that exhibit similar reactions with amino acids—to improve the clarity of the developed fingerprint (Almog, 2001, pp 177–209). Several of these analogues were highly successful (e.g., 1,8-diazafluoren-9-one [DFO], 1,2-indanedione, and 5-methylthioninhydrin), although none have been able to completely replace ninhydrin as the most frequently used technique (Almog, 2001, pp 177–209).

7.4.2 Theory

7.4.2.1 Fingerprint Detection by Amino Acid Reagents. Some fingerprints are created by the deposition of sweat from the fingers when they come into contact with a surface. This sweat consists mainly of aqueous components,



Ninhydrin (2,2-dihydroxy-1,3-indanedione) **1,2,3-indanedione**

FIGURE 7–2

Equilibrium between hydrated and anhydrous ninhydrin structures.

which comprise 98% of the volume of a fingerprint (Pounds and Jones, 1983, pp 180–183). These aqueous deposits contain a small, but detectable, amount of amino acids, averaging about 250 ng per fingerprint (Hansen and Joullié, 2005, pp 408–417). After the water evaporates from the surface, the amino acids remain as solid material (Knowles, 1978, pp 713–720).

For porous surfaces such as paper, amino acids are desirable targets for fingerprint development reagents (Almog, 2001, pp 177–209). Although uncontrollable variables (such as the total amount of sweat deposited by the finger, the amino acid concentration of the individual's excretions, and the age of the fingerprint) influence the amount of amino acids transferred to the paper (Everse and Menzel, 1986, pp 446–454), amino acids are always present in perspiration in some amount (Speaks, 1970, pp 14–17). On contact with paper, these amino acids impregnate the surface of the paper, where they are retained by their high affinity for cellulose (Champod et al., 2004, p 114; Almog, 2001, pp 177–209; Hansen and Joullié, 2005, pp 408–417).

Because of this affinity, amino acids do not migrate significantly from their initial deposition sites; however, the amount of amino acids retained in the fingerprint decreases gradually over time (Knowles, 1978, pp 713–720). Furthermore, amino acids react with a wide variety of chemicals to produce colored compounds (Hansen and Joullié, 2005, pp 408–417). These qualities have been exploited to produce clear, sharp images of fingerprints that were up to 40 years old (Champod et al., 2004, p 114).

At least 14 amino acids may be present in fingerprint residues (Knowles, 1978, pp 713–720; Hier et al., 1946, pp 327–333). To produce the best-developed fingerprint, the ideal reagent must be nonspecific to a particular amino acid (i.e., reacts well with all).

Ninhydrin is one of many chemicals that acts as a nonspecific amino acid reagent and is, therefore, highly suitable for fingerprint development (Champod et al., 2004, p 114; Almog, 2001, pp 177–209).

7.4.2.2 Properties of Ninhydrin. Ninhydrin is a crystalline solid that is colorless to pale yellow in color and is highly soluble in polar solvents such as water and methanol (McCaldin, 1960, pp 39–51). When heated, the solid becomes pink to red in color at approximately 125 °C (Almog, 2001, pp 177–209), melts at 130–140 °C, and decomposes at 241 °C. The compound is found as the stable hydrate in the presence of any water but will assume a triketone structure in anhydrous conditions (Hansen and Joullié, 2005, pp 408–417). This equilibrium is illustrated in Figure 7–2.

7.4.2.3 Reaction of Ninhydrin with Amino Acids. The first observation of ninhydrin's reaction with skin to form a deep purple compound was reported in 1910 (Ruhemann, 1910a, pp 1438–1449). Subsequent studies indicated that the purple color resulted from the reaction between ninhydrin and amino acids and described the product of this reaction as diketohydrindylidene–diketohydrindamine (Ruhemann, 1910b, pp 2025–2031; 1911a, pp 792–800; 1911c, pp 1486–1492), which is now known as Ruhemann's purple. By-products of this reaction include an aldehyde derivative of the amino acid and carbon dioxide (Friedman and Williams, 1974, pp 267–280; Yemm et al., 1955, pp 209–214).

Multiple attempts have been made to determine the mechanism of this reaction (Friedman and Williams, 1974, pp 267–280; Hansen and Joullié, 2005, pp 408–417; McCaldin, 1960, pp 39–51; Retinger, 1917, pp 1059–1066; Bottom et al., 1978, pp 4–5; Grigg et al., 1986, pp 421–422; Grigg et al., 1989, pp 3849–3862; Joullié et al., 1991, pp 8791–8830; Schertz et al., 2001, pp 7596–7603). The mechanism that is most accepted today is the one proposed by Grigg et al. (1989, pp 3849–3862) and illustrated briefly in Figure 7–3. Acid and water are other reagents required for this reaction to occur.

Structural studies of the reaction product have confirmed that Ruhemann's original product structure was correct and that the reaction with amino acids produces the ammonium salt of Ruhemann's purple (Ruhemann, 1911c, pp 1486–1492; Grigg et al., 1986, pp 421–422; 1989, pp 3849–3862).

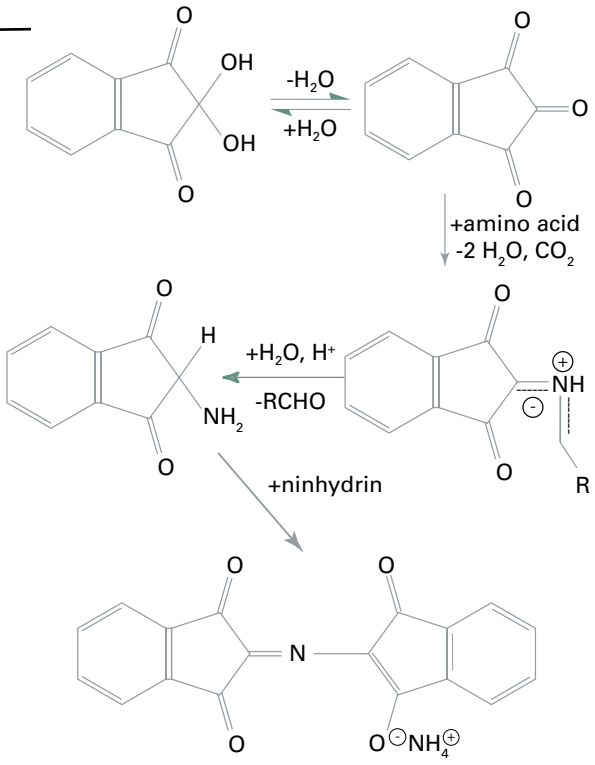
This reaction is complex and requires a finely tuned set of conditions in order to progress at a reasonable rate. The pH of the reaction must be above 4 (Friedman and Williams, 1974, pp 267–280; Bottom et al., 1978, pp 4–5) and ideally should be between 4.5 and 5.2 (Grigg et al., 1989, pp 3849–3862). Development in a high-humidity environment is of utmost importance (Champod et al., 2004, pp 116–117; Almog, 2001, pp 177–209) because water is a necessary reactant. Finally, because Ruhemann's purple is known to degrade in the presence of light and oxygen, the treated fingerprint should be stored in a dark, cool place (Friedman and Williams, 1974, pp 267–280; Joullié et al., 1991, pp 8791–8830). Ninhydrin-treated fingerprints are colored purple and exhibit excellent contrast and clarity of detail (Champod et al., 2004, p 117; Almog, 2001, pp 177–209).

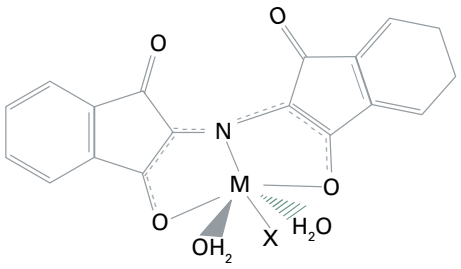
7.4.2.4 Optical Enhancement of Ninhydrin-Developed Fingerprints. Ninhydrin treatment provides excellent contrast under ideal conditions (e.g., fresh fingerprints on white paper). On colored paper or with aged fingerprints, however, the results can often be less than optimal (Crown, 1969, pp 258–264; Everse and Menzel, 1986, pp 446–454; Speaks, 1970, pp 14–17; Grigg et al., 1989, pp 3849–3862; German, 1981, pp 3–4; Herod and Menzel, 1982a, pp 200–204; Lennard et al., 1986, pp 323–328).

Several methods have been developed to increase the contrast between ninhydrin-developed fingerprints and a colored substrate or to enhance weakly developed fingerprints. The UV-to-visible light spectrum of Ruhemann's purple shows two *absorption maxima*—wavelengths of light that are strongly absorbed by the compound. These maxima, at λ = 407 nm and λ = 582 nm (Lennard et al., 1986, pp 323–328), can be used to increase the contrast between the developed fingerprint and a nonabsorbing background. When lasers became available to the forensic community in the late 1970s to early 1980s, a treatment with zinc chloride was described for enhancing weak ninhydrin prints by using the light of an argon ion laser (German, 1981, pp 3–4; Herod and Menzel, 1982a, pp 200–204). This method was capable

FIGURE 7–3

Accepted reaction mechanism of ninhydrin with amino acids.





M: Cadmium or zinc
X: Anion from the metal salt

FIGURE 7–4

Structure of Ruhemann's purple–metal salt complex.

of drastically increasing the number of identifiable latent fingerprints developed by the ninhydrin process. With the current ubiquity of forensic light sources, both absorption bands of Ruhemann's purple can be exploited to produce high-contrast fingerprints (Champod et al., 2004, p 117).

7.4.2.5 Post-Treatment with Metal Salts. The reaction between Ruhemann's purple and metal salts such as zinc, cadmium, cobalt, and copper was used in a biochemical context to preserve ninhydrin spots on chromatograms (Kawerau and Wieland, 1951, pp 77–78). Formation of a metal-salt complex alters the color of Ruhemann's purple from deep violet to red or orange, depending upon the salt used (Stoilovic et al., 1986, pp 432–445). The lighter hue may provide a greater contrast against a dark-colored background, especially when observed at 490–510 nm, where the metal–Ruhemann's purple complex has an absorption maximum (Stoilovic et al., 1986, pp 432–445).

It has been reported that viewing zinc-complexed ninhydrin-treated fingerprints under an argon ion laser could induce fluorescence of even weakly developed prints (Herod and Menzel, 1982b, pp 513–518). This discovery had a profound impact on fingerprint development because fluorescent reagents are more sensitive than chromogenic ones and can be viewed more clearly against colored backgrounds (Champod et al., 2004, p 120). Subsequent studies revealed that intense laser light was not necessary if the zinc-treated samples were cooled to the temperature of liquid nitrogen (-196 °C or 77 K); the fluorescence could be observed under a xenon arc lamp. This technique required submersion of the document in liquid nitrogen, a glass plate being placed between the sample and the light source and camera, and a heat source to prevent condensation on the glass (Kobus et al., 1983, pp 161–170). Later research showed that cadmium complexes provided an improved luminescence under these conditions (Stoilovic et al., 1986, pp 432–445).

Structural studies of the Ruhemann's purple–metal salt complexes have identified the structure in Figure 7–4 (Lennard et al., 1987, pp 597–605; Davies et al., 1995a, pp 565–569; 1995b, pp 1802–1805).

7.4.3 Application

7.4.3.1 Ninhydrin Formulations. Several ninhydrin formulations have been reported in the literature (Crown, 1969, pp 258–264; Odén and van Hofsten, 1954, pp 449–450; Speaks, 1964, pp 11–13, 23; Champod et al., 2004, pp 117–120; Almog, 2001, pp 177–209; Everse and Menzel, 1986, pp 446–454; Clay, 1981, pp 12–13). Ninhydrin solutions are typically prepared in two steps: first, a stock solution is prepared that has a high proportion of polar solvent to facilitate the stability of the mixture; second, a portion of the stock solution is diluted with a nonpolar carrier solvent to produce a reagent suitable for application to evidential items.

Application of ninhydrin working solutions can be performed by dipping, spraying, or brushing (Odén and van Hofsten, 1954, pp 449–450; Speaks, 1964, pp 11–13, 23), with the dipping method preferred in most instances. The item to be examined is briefly submerged in the working solution and allowed to air-dry to evaporate the solvent (Champod et al., 2004, pp 116–117).

Following treatment with ninhydrin solution, development should ideally proceed at room temperature, in a dark and humid environment (50–80% humidity), for a period of 1–2 days (Champod et al., 2004, pp 116–117). If ambient humidity is low, development in a specialized, humidity-controlled fingerprint development chamber may be necessary (Almog, 2001, pp 177–209). The development may be accelerated by the application of steam or heat, but this may result in a greater degree of background development, reducing the

clarity and contrast of the resulting fingerprints (Almog, 2001, pp 177–209). Steaming can be achieved by holding a steam iron above the exhibit; heat can be delivered in a press, oven, fingerprint development cabinet, or by a microwave oven and should not exceed 80 °C (Almog, 2001, pp 177–209).

Ninhydrin crystals may be ground in a mortar and pestle to form a fine powder and applied directly to the fingerprints with a fingerprint brush (Almog, 2001, pp 177–209). This method is slow and produces only faint prints but may be suitable for some types of heat- or solvent-sensitive paper (Wakefield and Armitage, 2005). Ninhydrin may also be applied by a fuming method; a forensic fuming cabinet is used to heat the ninhydrin until it sublimes, allowing gaseous ninhydrin to deposit on the fingerprint residues (Schwarz and Frerichs, 2002, pp 1274–1277). The reagent is most suited to paper, although any porous substrate may give visible results, and some nonporous substrates have been reported to produce visible fingerprints (Herod and Menzel, 1982a, pp 200–204; Speaks, 1966, pp 3–5).

7.4.3.2 Metal Salt Post-Treatment. The application of zinc or cadmium salts to ninhydrin-developed fingerprints will result in an immediate color change from purple to orange or red, respectively (Lennard et al., 1987, pp 597–605). Note that the use of zinc is preferred to cadmium because of cadmium's toxicity. Dipping the exhibit into the solution is preferred over spraying because of the toxicity of some of the reagents. If humidity is low, a short blast of steam may be required to produce development. However, the humidity must be carefully controlled if zinc salts are used because high moisture levels cause the formation of an unstable, nonfluorescent, red complex that will reduce the contrast of the resulting fingerprint (Stoilovic et al., 1986, pp 432–445; Davies et al., 1995a, pp 565–569).

Post-treated fingerprints may be further enhanced by viewing under 490 nm light (for zinc-treated residues) or 510 nm light (for cadmium-treated residues) (Champod et al., 2004, p 120; Stoilovic et al., 1986, pp 432–445). Fluorescence may be induced by submerging the article in liquid nitrogen and exciting the treated fingerprint with the above-mentioned wavelengths of light. The fluorescent emissions should be viewed using a 550–570 nm band-pass filter or a 550 nm long-pass filter (Champod et al., 2004, pp 121–124).

7.5 1,8-Diazafluoren-9-one (DFO)

7.5.1 History

1,8-Diazafluoren-9-one (DFO) was first prepared in 1950 (Druey and Schmidt, 1950, pp 1080–1087), but its reaction with amino acids was not explored until 1990, when it was first applied as a fingerprint development reagent. The preliminary results of this study were promising; DFO treatment resulted in faint red or pink fingerprints that were intensely fluorescent at room temperature (Pounds et al., 1990, pp 169–175; Grigg et al., 1990, pp 7215–7218). This presented clear advantages over the metal complexation-induced fluorescence of ninhydrin-developed fingerprints, and DFO was rapidly identified as the best fluorescent reagent for fingerprint development (Almog, 2001, pp 177–209). The reagent is now widely used in sequence with ninhydrin to develop fingerprints on porous surfaces (Wilkinson et al., 2005).

7.5.2 Theory

Although DFO is not a direct analogue of ninhydrin (Hansen and Joullié, 2005, pp 408–417), the structures of the two compounds, and the outcome of their reactions with amino acids, are similar (Grigg et al., 1990, pp 7215–7218; Wilkinson, 2000a, pp 87–103). Like ninhydrin, DFO contains a central ketone center activated by the nearby presence of electron-withdrawing groups. The structure of DFO is illustrated in Figure 7–5; the nitrogenous rings act similarly to the flanking ketone groups in ninhydrin (Hansen and Joullié, 2005, pp 408–417).

Mechanistic studies of DFO's reaction with amino acids have shown that the presence of methanol is essential. This allows the DFO to form a hemiketal (Figure 7–6), which is less stable than the parent structure and therefore more reactive, producing a more sensitive response to amino acid residues in fingerprints (Wilkinson, 2000a, pp 87–103). The red reaction product has been fully characterized and resembles Ruhemann's purple (Grigg et al., 1990, pp 7215–7218; Wilkinson, 2000a, pp 87–103).

The product of this reaction is pink to red in color with λ_{max} of approximately 560 nm and a weaker absorption at 520 nm (Pounds et al., 1990, pp 169–175; Wilkinson, 2000a, pp 87–103). Under excitation by either of these wavelengths, the product is strongly fluorescent at room temperature, emitting intense light of 576 nm (Stoilovic, 1993, pp 141–153). An illustration of a DFO-developed fingerprint in

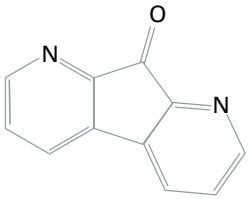


FIGURE 7–5

Structure of 1,8-diazafluoren-9-one (DFO).

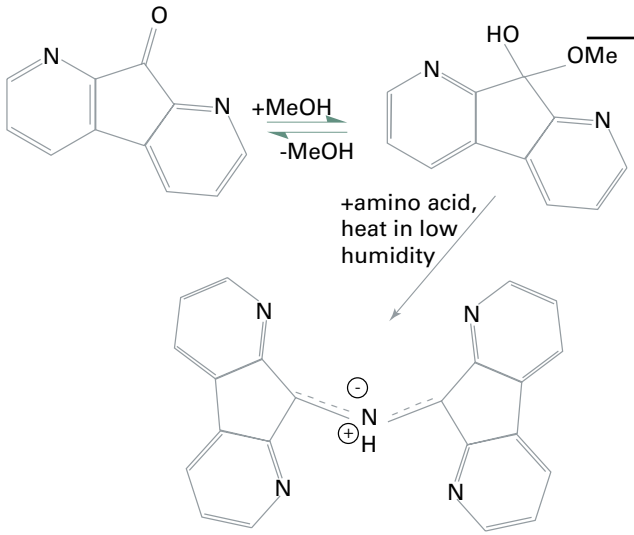


FIGURE 7–6

Hemiketal formation and reaction with amino acids.

both white light and under fluorescent conditions appears in Figure 7–7.

Unlike the ninhydrin reaction, the DFO reaction requires a high-temperature, low-humidity environment (Pounds et al., 1990, pp 169–175). Post-treatment with metal salts and subsequent cooling to liquid nitrogen temperatures does not significantly affect the intensity of the DFO product's fluorescence (Conn et al., 2001, pp 117–123).

DFO is reported to be a more sensitive fingerprint development reagent than ninhydrin, producing a greater number of identifiable latent fingerprints (Wilkinson et al., 2005; Stoilovic, 1993, pp 141–153; Cantu et al., 1993, pp 44–66). This sensitivity is due to the fact that a weakly fluorescing fingerprint is easier to see than a weakly colored fingerprint (Almog, 2001, pp 177–209). Despite this observation, if ninhydrin is applied after DFO treatment, additional development occurs, producing Ruhemann's purple. The conventional explanation for this phenomenon is that, although DFO-developed fingerprints are more visible when fluorescing, DFO does not react to completion with every amino acid in the fingerprint residue, thus leaving some amino acids available to react with ninhydrin (Wilkinson, 2000a, pp 87–103). The combination of DFO followed by ninhydrin develops more latent fingerprints than DFO or ninhydrin alone (Wilkinson et al., 2005), and this is the recommended sequence of examinations for porous surfaces such as paper (Champod et al., 2004, pp 128–131; Almog, 2001, 177–209).

7.5.3 Application

Several DFO formulations have been reported in the literature (Champod et al., 2004, pp 230–231; Almog, 2001, pp 177–209; Pounds et al., 1990, pp 169–175; Grigg et al., 1990, pp 7215–7218; Wilkinson et al., 2005; Wilkinson, 2000a, pp 87–103; Stoilovic, 1993, pp 141–153; Didierjean et al., 1998, pp 163–167). DFO solution can be applied to specimens by dipping, spraying, or brushing, although dipping is the preferred method (Champod et al., 2004, pp 128–131). The exhibit is allowed to dry and then heated to promote development. Several heating methods are suitable: heating in a 100 °C oven for 10–20 minutes (Champod

et al., 2004, p 128; Almog, 2001, pp 177–209; Pounds et al., 1990, pp 169–175; Didierjean et al., 1998, pp 163–167), applying a 160 °C iron for 20–30 seconds (Stoilovic, 1993, pp 141–153), or applying a 180 °C ironing press for 10 seconds (Almog, 2001, pp 177–209; Stoilovic, 1993, pp 141–153). The reaction must be carried out in a dry environment with low humidity because moisture interferes with the development reaction (Champod et al., 2004, p 129; Almog, 2001, pp 177–209; Wilkinson, 2000a, pp 87–103).

After DFO application and heating, developed fingerprints can be observed using 530 nm excitation light and a 590 nm barrier filter, or 555 nm excitation light and a 610 nm barrier filter (Almog, 2001, pp 177–209). The exhibit may then be treated with ninhydrin as previously described.

7.6 1,2-Indanedione

7.6.1 History

The fingerprint-developing capabilities of 1,2-indanedione were first considered after a related compound, 6-methylthio-1,2-indanedione, was found to produce fluorescent fingerprints (Hauze et al., 1998, pp 744–747). This prompted researchers to synthesize the parent compound and several other analogues and to evaluate their utility as fingerprint reagents (Ramotowski et al., 1997, pp 131–139). The results were similar to DFO in that a faint, pink-colored product was produced that fluoresced brightly at room temperature (Ramotowski et al., 1997, pp 131–139). Further research indicated that these reagents are more sensitive than other current methods and, because of the ease of synthesis, can be a cheaper alternative (Cava et al., 1958, pp 2257–2263; Dayan et al., 1998, pp 2752–2754; Joullié and Petrovskaia, 1998, pp 41–44). In the eight years following these discoveries, 1,2-indanedione has become a standard reagent in Israeli laboratories and has been investigated for use in many other countries (Almog, 2001, pp 177–209).

7.6.2 Theory

1,2-Indanedione is a close analogue of ninhydrin and is theorized to react with amino acids in a very similar fashion (Petrovskaia et al., 2001, pp 7666–7675). The structure of 1,2-indanedione has been characterized (Wilkinson, 2000b, pp 123–132) and is illustrated in Figure 7–8(A).

Mechanistic studies of 1,2-indanedione's reaction with amino acids have indicated that the presence of methanol desensitizes the reagent (Wilkinson, 2000b, pp 123–132). Like DFO, indanedione forms a hemiketal with methanol; however, unlike DFO, this hemiketal is more stable than the parent compound and thus its formation prevents the reaction with amino acids. Because 1,2-indanedione is completely converted to the less sensitive hemiketal (Wilkinson, 2000b, pp 123–132), some suggest that alcohols should be avoided in any indanedione formulations (Wilkinson et al., 2005; Wiesner et al., 2001, pp 1082–1084). Other studies have not corroborated this lack of sensitivity in methanolic solution (Roux et al., 2000, pp 761–769). Similar ambiguity exists on the addition of acetic acid (Lennard et al., 2005, p 43); some authors have found that a small amount of acetic acid improves the results (Hauze et al., 1998, pp 744–747; Joullié and Petrovskaia, 1998, pp 41–44), whereas others have experienced blurry, unclear fingerprints when using acidified solutions (Almog, 2001, pp 177–209; Wiesner et al., 2001, pp 1082–1084; Kasper et al., 2002). These discrepancies have been linked to the acid content of the paper produced in the authors' various countries (Wilkinson et al., 2005).

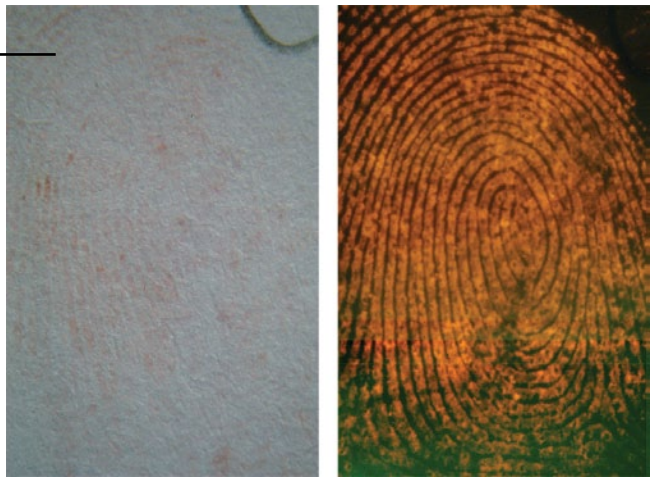


FIGURE 7–7

DFO-developed fingerprint. Left, under ambient light. Right, excited by a forensic light source and viewed through the proper viewing filter.

Production of the compound shown in Figure 7–8(B) during the reaction between amino acids and 1,2-indanedione has been confirmed. However, this compound does not fully explain the coloration of the developed print or its fluorescence. The possibility of a Ruhemann's purple analogue has not been ruled out (Petrovskaia et al., 2001, pp 7666–7675); such a compound is illustrated in Figure 7–8(C). Further studies are currently under way to elucidate the structure of the fluorescent species, which is expected to be polymeric (Wallace-Kunkel et al., 2005).

Whether or not metal salt post-treatment enhances the fluorescence of the developed fingerprint is another point of contention amongst authors. The varied results with each step of the indanedione development process indicate the influence that environmental conditions have upon the technique, and each research group should establish an optimal formula for use in its laboratory (Wilkinson et al., 2005; Lennard et al., 2005, p 43).

7.6.3 Application

Because of regional variations in humidity, acid content of paper, and other environmental factors, a single 1,2-indanedione formulation cannot be recommended. Application of the 1,2-indanedione reagent can be carried out by immersion of the exhibit or by spraying of the reagent. Development can occur at room temperature but may require 4–5 days (Roux et al., 2000, pp 761–769). In light of the established fact that heat treatment does not cause excessive background development, it is recommended that steam heat be applied to the treated fingerprints to expedite development (Almog, 2001, pp 177–209; Ramotowski et al., 1997, pp 131–139; Joullié and Petrovskaia, 1998, pp 41–44; Roux et al., 2000, pp 761–769). This heat can be applied in a humidity oven (100 °C at 60% relative humidity) (Wiesner et al., 2001, pp 1082–1084; Roux et al., 2000, pp 761–769; Almog et al., 1999, pp 114–118), by steam iron (Ramotowski et al., 1997, pp 131–139; Joullié and Petrovskaia, 1998, pp 41–44), or by a heat press (100 °C for 2–5 minutes [Kasper et al., 2002] or 165 °C for 10 seconds [Lennard et al., 2005, p 43]).

Fluorescence can be observed under 520 nm illumination and viewed through a 590 nm filter (Joullié and Petrovskaia, 1998, pp 41–44). Zinc salt post-treatment can be applied to enhance the color of the developed fingerprint (Roux et al., 2000, pp 761–769) and may increase the fluorescent intensity (Almog, 2001, pp 177–209; Hauze et al., 1998, pp 744–747; Ramotowski et al., 1997, pp 131–139; Lennard et al., 2005, p 43; Almog et al., 1999, pp 114–118).

1,2-Indanedione develops more fingerprints than DFO, ninhydrin, or the DFO–ninhydrin sequence combined (Wiesner et al., 2001, pp 1082–1084; Lennard et al., 2005, p 43). The indanedione-DFO sequence is capable of visualizing even more latent fingerprints than 1,2-indanedione alone (Roux et al., 2000, pp 761–769), and indanedione can also enhance ninhydrin-developed fingerprints (Kasper et al., 2002). However, ninhydrin treatment of indanedione-developed prints does not afford further enhancement (Wiesner et al., 2001, pp 1082–1084).

Finally, on a somewhat negative note, Wilkinson et al. had very poor results with indanedione for a study carried out across Canada (Wilkinson et al., 2003, pp 8–18).

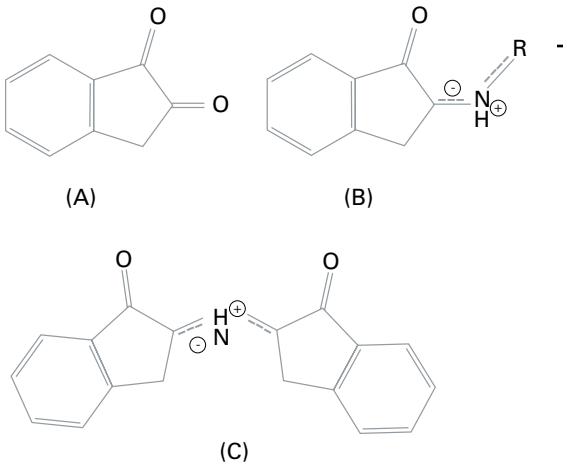


FIGURE 7–8

Structure of (A) 1,2-indanedione; (B) a known product of the reaction between 1,2-indanedione and amino acids; (C) a possible Ruhemann's purple analogue produced by the reaction.

7.7 5-Methylthioninhydrin (5-MTN)

5-Methylthioninhydrin (5-MTN) was first prepared and applied as a fingerprint reagent in 1990 as part of a U.S. Secret Service project (Cantu et al., 1993, pp 44–46). This analogue reacts with amino acids in a manner identical to ninhydrin because the reactive, chromogenic core of the molecule is not changed by the addition of the sulfur group (Figure 7–9) (Elber et al., 2000, pp 757–760). As a result, 5-MTN-developed fingerprints are a shade of purple similar to ninhydrin-developed fingerprints.

Development of 5-MTN-treated fingerprints requires heat and humidity, much the same as ninhydrin development. This can be delivered in the same manner described previously for ninhydrin or by microwaving the treated exhibit for 2–3 minutes alongside a container of water (Almog et al., 1992, pp 688–694). Care must be taken to avoid overheating the sample because significant background development may occur. The resulting fingerprint should appear deep purple in color, similar to a ninhydrin-developed fingerprint.

On treatment with a zinc salt, the 5-MTN-developed fingerprint changes color from purple to pink (Almog et al., 1992, pp 688–694). Accompanying this change is a strong fluorescence at room temperature when excited by light at 520 nm and viewed through a 590 nm filter (Cantu et al., 1993, pp 44–66; Almog et al., 1992, pp 688–694), with an intensity that is comparable to that of DFO. This is an obvious advantage over the continued use of ninhydrin (Cantu et al., 1993, pp 44–66). A recent study confirmed that 5-MTN could outperform ninhydrin but produced poorer fluorescent results than DFO or 1,2-indanedione (Wallace-Kunkel et al., 2006, pp 4–13). The fluorescence becomes even more intense if the exhibit is cooled to liquid nitrogen temperatures, but this step is not necessary (Almog et al., 1992, pp 688–694).

5-MTN can be synthesized in small-scale operations in the forensic laboratory following methods reported in the literature (Heffner and Joullié, 1991, pp 2231–2256; Della et al., 1999, pp 2119–2123). Alternatively, it can be sourced from commercial forensic suppliers. However, some suppliers provide the ethanolic hemiketal of 5-MTN, which dissolves more readily but may require some alteration of the given formulation (Section 7.14) to ensure the appropriate concentration of 5-MTN in the solution (BVDA, 2010).

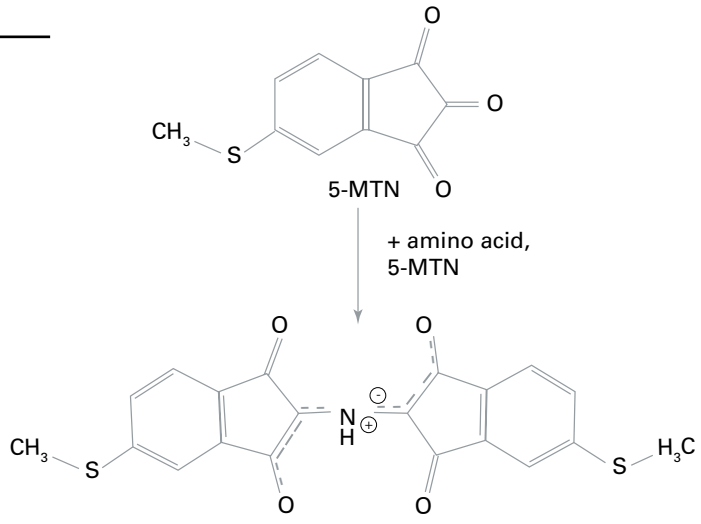
7.8 Modifications for Use on Chemically Treated Papers

7.8.1 Chemically Treated Papers

Chemically treated paper is a class that encompasses thermal paper and carbonless specialty papers (Stimac, 2003a, pp 185–197). These papers cannot be treated with the conventional amino acid reagent formulations described previously because the polar solvents react unfavorably with the chemical treatments applied to the paper during manufacture. This undesired interaction frequently causes the surface of the paper to blacken, obliterating the documentary evidence the paper contained (Stimac,

FIGURE 7–9

Structure of 5-methylthioninhydrin and its reaction product with amino acids.



2003a, pp 185–197). To address this limitation, several solvent-free or low-polarity formulations have been devised for the treatment of these difficult substrates.

7.8.2 Application of DFO to Chemically Treated Paper

DFO may be applied to chemically treated paper by a process known as "DFO-Dry" (Bratton and Juhala, 1995, pp 169–172). This technique does not require the application of a solvent to the exhibit under examination. Instead, filter paper is impregnated with a solution of 1 g DFO in 200 mL methanol, 200 mL ethyl acetate, and 40 mL acetic acid. The dried filter paper is applied to the exhibit, a towel is placed on top, and a steam iron filled with 5% acetic acid solution is applied for one minute. This transfers DFO onto the exhibit and provides the heat for development. This technique results in a less prominent color change but equal fluorescence to solvent-based methods (Bratton and Juhala, 1995, pp 169–172).

7.8.3 Ninhydrin Techniques

7.8.3.1 "Nin-Dry". This method was described in 1996 (McMahon, 1996, pp 4–5) and is similar to the previously described "DFO-Dry" process. Blotter or filter paper is soaked in a solution of 30–50 g ninhydrin dissolved in 1.5 L acetone and allowed to dry. An exhibit is placed between two sheets of the impregnated paper and then sealed into a plastic bag for 3 days to 1 week. This technique develops high-contrast fingerprints while preserving the integrity and appearance of the document and is applicable to any fragile paper types, including chemically treated papers.

7.8.3.2 Ninhydrin Fuming. The method proposed by Schwarz and Frerichs (2002, pp 1274–1277), and described above, can be applied to chemically treated papers with no loss of document detail.

7.8.3.3 Nonpolar Solution. A ninhydrin solution can be prepared in a mixture of the nonpolar solvents HFE 71IPA and HFE 7100. The exhibit is immersed in the working solution and allowed to develop in dark, humid conditions for 2–3 days, avoiding high temperatures (Stimac, 2003a, pp 185–197).

7.8.4 Indanedione Formulation

Indanedione is sufficiently soluble in nonpolar solvents that it can be effectively applied to thermal paper without causing any blackening (Stimac, 2003b, pp 265–271). The exhibit is immersed in the prepared solution and allowed to develop for at least 1 day in dark, cool conditions. Fluorescence is induced as described previously.

7.8.5 2-Isononylninhydrin (INON)

2-Isononylninhydrin, also known as INON, or commercially as ThermaNin, is a derivative of ninhydrin with greatly increased solubility in nonpolar solvents (Takatsu et al., 1991; Joullié, 2000). This compound, which is a product of the reaction between 3,5,5-trimethyl-1-hexanol and ninhydrin (Almog, 2001, pp 177–209; Hansen and Joullié, 2005, pp 408–417; Takatsu et al., 1992), has the chemical structure shown in Figure 7–10.

Solutions of this reagent do not have a long shelf life, so working solutions should be prepared as needed (BVDA, 2010).

The 2-isononylninhydrin solution is applied to the chemically treated paper by immersing the exhibit in the solution in an aluminum or plastic tray. The exhibit is allowed to dry and develop in dark, humid conditions for 24–48 hours. Under these conditions, the ninhydrin hemiketal reacts with water absorbed by the paper to form ninhydrin and 3,5,5-trimethyl-1-hexanol. The freed ninhydrin reacts slowly with the residues in the fingerprint to develop a fingerprint that is somewhat less intensely colored than a traditionally ninhydrin-developed print. This may be due to the relatively lower concentration of ninhydrin present after the hydrolysis reaction occurs (Al Mandhri and Khanmy-Vital, 2005).

7.9 Cyanoacrylate Fuming

7.9.1 Background

The liquid commercial adhesive, super glue, was inadvertently developed in the 1950s by researchers who were trying to develop an acrylic polymer for the aircraft industry. Besides its use as a glue, CA adhesive also found use as a field dressing in Vietnam in the 1960s, although it never received FDA approval for this use. In the late 1970s, researchers in Japan and the United Kingdom almost simultaneously discovered the latent fingerprint development capabilities of the fumes of the liquid adhesive. Shortly thereafter, latent print examiners from the U.S. Army Criminal Investigation Laboratory in Japan and the Bureau of Alcohol, Tobacco, and Firearms introduced this technique to North America. Once CA fuming proved practical, with the introduction of methods to make the technique faster

FIGURE 7–10

Structure of 2-isononylninhydrin (INON).

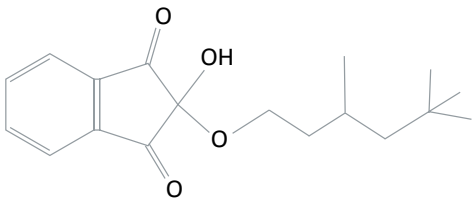
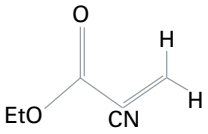


FIGURE 7–11

Ethyl cyanoacrylate monomer.



and more effective, it quickly gained acceptance worldwide (German, 2005; Jueneman, 1982, p 15).

Since those early discoveries, innumerable crimes have been solved through the routine use of CA ester (usually methyl or ethyl) fuming of evidence, and a substantial amount of research has been aimed at identifying the ideal environment for the technique.

Today, CA fuming continues to be a versatile and effective development technique on virtually all nonporous surfaces, including glass, metal, coated papers, and all forms of plastics. The method is particularly effective on rough surfaces where physical contact with a fingerprint brush tends to develop the texture of the material along with the latent fingerprints. CA vapors are extremely sensitive to fingerprint residue, adaptable to many different crime scene and laboratory situations, and are relatively inexpensive to employ.

Studies into the explicit polymerization initiators and the role of water in the development of latent prints are ongoing. These studies should eventually lead to a better understanding of latent print polymerization as it relates to latent print composition, pH, aging, and humidity.

7.9.2 Theory

Super glue or CA development of latent prints is best explained as a three-stage process to produce polymer growth, thus enabling latent print visualization.

The first stage occurs when fumes of CA ester monomers (see diagram of ethyl CA monomer in Figure 7–11) are introduced to latent fingerprints and quickly bond with initiators in the residue. In the second stage, the monomer on the fingerprint residue reacts with another CA monomer in the vapor phase to form a dimer on the print. This reacts with yet another monomer, and another, eventually forming a polymer, a long chain of CA molecules. The final phase is when the polymer chain reaction is terminated. The overall development time is fast, especially when volatilization of the liquid glue is accelerated (Lewis et al., 2001, pp 241–246). The polymerization process may, however, be restarted later if fingerprints prove to be underdeveloped with the first exposure to fumes.

Fully developed CA prints are a white three-dimensional matrix, often visible to the unaided eye, and can be further enhanced with a variety of techniques. CA-developed impressions are generally more durable than untreated fingerprints because of the plasticization of the print. Because of this, some authorities recommend CA treatment in the field before evidence packaging to protect otherwise fragile fingerprints during transportation and storage (Perkins and Thomas, 1991, pp 157–162).

For normal eccrine sweat fingerprints, CA polymerized under ambient laboratory environmental conditions appears as noodlelike, fibrous structures when viewed with a scanning electron microscope (SEM) (Figure 7–12). These polymer morphologies change, however, when variables such as the age of the latent print, the residue composition, and environmental conditions are altered.

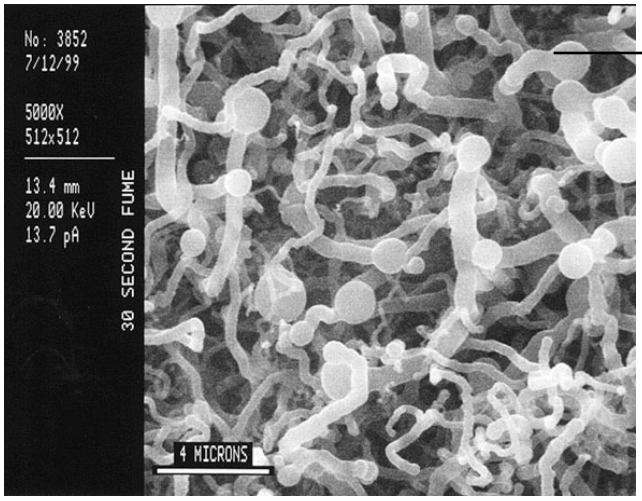


FIGURE 7–12

Scanning electron microscopy of cyanoacrylate polymerized eccrine residue. (Reprinted, with permission from the Journal of Forensic Sciences, *46 (2), copyright ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428.)*

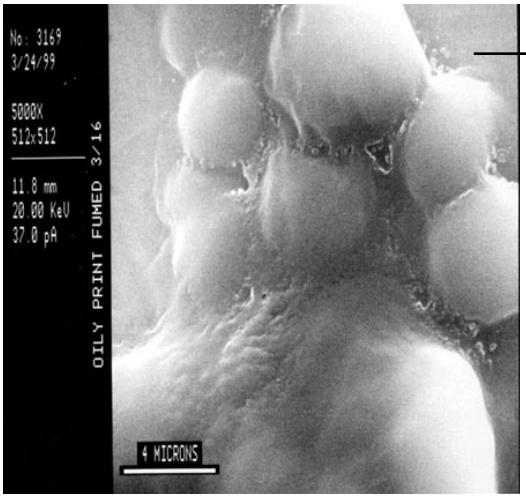


FIGURE 7–13

Scanning electron microscopy of cyanoacrylate polymerized oily residue. (Reprinted with permission from the Journal of Forensic Sciences, 46 *(2), copyright ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428.)*

Lewis et al. (2001, pp 241–246) observed differences between clean and oily latent print residues and the effects of aging on each. Latent prints lacking sebum (clean prints) tended to suffer from the effects of aging to a far greater extent than prints containing sebum (oily prints, Figure 7–13). After 1 day of aging, clean prints showed a trend away from the previously mentioned fibrous morphology toward polymer structures that appeared rounded under SEM. Clean prints also became difficult, if not impossible, to develop after a period of only 2 weeks, whereas prints contaminated with sebum produced distinguishable polymer growth for periods of up to 6 months. Lewis et al. (2001, pp 241–246) also observed that a low-humidity environment during latent print aging had a noticeable and adverse impact on development with CA, whereas prints aged under high humidity lasted longer and produced higher quality polymerization.

Interestingly, latent prints developed in a vacuum chamber also produce smooth spherical or capsule-type formations observed with SEM and tend to be more translucent to the unaided eye (Watkin et al., 1994, pp 545–554). This may be due in part to exposing the print to the near zero-humidity environment of the vacuum, presumably removing moisture from the fingerprints. The role of humidity in CA development of latent prints is not understood at this time. During the mid-1990s, Kent empirically observed that humid environments outperformed vacuum environments in the CA development of latent prints (Kent and Winfield, 1995; Kent, 2005, pp 681–683), whereas Lewis et al. (2001, pp 241–246) observed that humidity during the latent print aging process had a greater effect than during polymerization. Clearly, the role of humidity during aging and polymerization must be examined further.

The actual initiators that cause latent print polymerization are just recently being understood. Originally, it was believed that CA primarily reacted with the water in fingerprint residue (Jueneman, 1982, p 15). However, current research indicates that water-soluble amines and carboxylic

groups in latent print residue are the primary initiators of CA polymerization. These two groups each produce significantly higher molecular weights of polymer growth than water alone. Furthermore, amines and carboxylic acid will polymerize in the absence of any water, leaving the role of water during the aging and development process unclear (Wargacki et al., 2005).

The pH of the humidity to which the latent prints are exposed prior to CA treatment may also play an important role by rejuvenating latent prints prior to the polymerization process. Latent prints that are exposed to acetic acid vapors and then CA fumed have shown higher molecular weights than those not exposed. Conversely, basic humidity produced with ammonia vapors also appears to enhance CA development. Present research makes it clear that acidic and basic humidity environments will both individually enhance latent print polymer growth, with acidic enhancement proving more effective. Although the actual mechanism is not fully understood, it is currently thought that exposure to ammonia vapors primarily enhances the functionality of the amine groups, whereas acetic acid vapors favorably influence the more robust carboxylic initiators (Wargacki et al., 2005).

7.9.3 Application

It is important to mention that liquid CA and its fumes can cause acute damage to skin, eyes, and mucous membranes, and the long-term effects of exposure are not fully known. The user must take care to use appropriate ventilation and personal protective equipment and to always practice safe handling. All manufacturer's warnings, including those given in material safety data sheets, must be heeded during use.

The ideal result of CA development is polymerization on the latent print that sufficiently scatters light and does not coat the background, making the white impression slightly visible against the substrate. This type of "minimal" development produces the greatest amount of detail, especially when used in conjunction with fluorescent dye stains (Figure 7–14). Overfuming will leave prints appearing "frosty" with a lack of edge detail, making them difficult to differentiate from a background also coated with CA polymer.

Sometimes, depending on latent composition and environmental conditions, developed impressions will appear translucent or glassy in nature and will be very difficult to detect without specific lighting or fluorescent dye staining. In fact, most impressions will be aided by some form of enhancement before recording.

Fuming with CA can be as simple and inexpensive as vaporizing the glue in a fish tank with a tight-fitting lid or as elaborate as using a commercially designed chamber with dynamic temperature and humidity controls. Both systems are intended to achieve the same result: vaporizing liquid glue in an environment suitable for polymerization of CA on latent prints.

A common and effective approach to the volatilization of CA is to warm a small amount of liquid glue (approximately 0.5 g or less) in an aluminum evaporation dish on a heating block or coffee cup warmer. An aluminum dish is preferred because it inhibits polymerization (Olenik, 1983, pp 9–10). The warm fumes rise but soon fall to the bottom of the chamber as cooling sets in. Therefore, a circulation fan is often used during fuming to keep the vapors evenly dispersed around the evidence at all levels of the tank. Prints that are later determined to be underfumed can be fumed again, in effect restarting the polymerization process.

A second approach to vaporizing CA utilizes a commercially available fuming wand. These wands typically use butane fuel to heat a small brass cartridge containing ethyl CA (Weaver and Clary, 1993, pp 481–492). Fumes from the heated cartridge on the end of the wand can be directed toward the evidence or used to fill a chamber. The disadvantage of using a fuming wand in an open environment is that air currents easily sweep the CA vapors away from the evidence, making development difficult to control. The use of a fuming wand outside a fume hood also presents some health and safety challenges that must be considered (Froude, 1996, pp 19–31).

Vaporization can also be achieved without an external heat source. Instead, chemical acceleration is produced by the exothermic reaction that can be achieved by pouring liquid glue on a pad of high cellulose content pretreated with sodium hydroxide. Pretreatment simply involves a cotton ball prepared with a few drops of NaOH solution.

CA fuming without acceleration can be achieved by increasing the total surface area of the liquid glue, thereby increasing the rate of evaporation. One way to achieve this is to sandwich a bead of liquid glue between two sheets of aluminum foil (Olenik, 1989, pp 302–304). The sheets are then pressed together and an ink roller is used to evenly disperse the glue into a thin layer across the entire inside

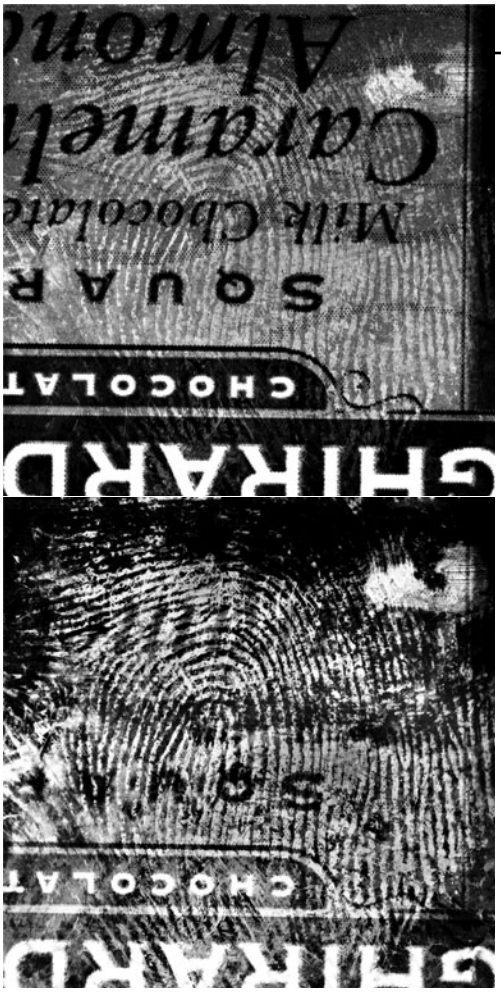


FIGURE 7–14

(A) Cyanoacrylate (CA) polymerized print on a plastic wrapper. (B) CA print stained with RAM and viewed at 475 nm with an orange barrier filter.*

*RAM is a fluorescent stain mixture of rhodamine 6G, Ardrox, and 7-(*p*-methoxybenzylamino)-4-nitrobenz-2-oxa-1,3-diazole (MBD).

of the foil surfaces. These sheets are then opened and placed inside a chamber, exposing the relatively volatile layers of glue to the air. CA development time using this method will vary significantly with the size of the chamber.

Fuming in a vacuum chamber has also been suggested as a method of increasing the volatility of CA (Campbell, 1991, pp 12–16; Yamashita, 1994, pp 149–158; Harvey et al., 2000, pp 29–31; Bessman et al., 2005, pp 10–27). The reduced atmospheric pressure lowers the boiling point of the liquid glue and may vaporize it more rapidly at room temperature. The negative pressure also eliminates humidity in the tank, affecting the overall appearance of the developed impressions. Prints developed in a vacuum environment often appear translucent, making them hard to detect without liquid dye stains (Watkin et al., 1994, pp 545–554). Some researchers have found, however, that this practice is less effective overall than the use of controlled humidity environments (Kent and Winfield, 1995; Kent, 2005, pp 681–683).

Although CA development in a laboratory chamber is preferred, makeshift chambers in the field can also be easily created. Chambers include cardboard boxes, small frames with clear plastic sheeting, large tents, vehicle interiors, and even entire rooms (Weaver, 1993, pp 135–137; Bandey and Kent, 2003). The most common of these field chambers is probably the automobile interior. One method of fuming involves placing a hot plate (reaching approximately 60 °C) in the center of the vehicle, with approximately 1 gram of glue in an evaporation dish. The interior is then sealed off by closing all the doors and windows. The fumes from the heated glue rapidly fill the vehicle interior, developing impressions throughout. This process takes approximately 10–30 minutes, although the length of time is variable. In some cases, so as not to destroy the entire vehicle, parts of the vehicle may be removed and fumed separately (e.g., steering wheel, mirror).

In some instances, CA fuming of a firearm may interfere with subsequent firearms examinations. The firearms

examiners may have to be consulted before any CA processing (Rosati, 2005, pp 3–6).

Fuming times depend on the size of the chamber, the quantity of glue, the temperature of the heat source, and the nature of the substrate and latent print residue. Under all conditions, fuming should be terminated shortly after the first signs of the appearance of fingerprints. Some examiners will place a test strip with fingerprints in the chamber to watch for the development of prints. This not only helps to determine when processing should cease but also acts to ensure that the equipment is functioning properly. Fuming can be restarted later if impressions appear underdeveloped.

7.9.4 Enhancement

Once prints have been developed, they can be enhanced optically with oblique, axial, reflected, and transmitted lighting techniques; chemically enhanced with fluorescent dye stains; and physically enhanced with the application of fingerprint powder, in that order. Fluorescent dye staining and examination with a laser or forensic light source usually produces the most dramatic results; however, not all CA-polymerized prints will accept dye stains.

Dye staining simply requires preparing a commercially available fluorescent stain in solution and applying it to the polymerized fingerprints. For a comprehensive reference of fluorescent dye stain recipes, see the FBI *Processing Guide for Developing Latent Prints* (Trozzi et al., 2000) or the Home Office manual (Kent, 1998, 2004). Once a dye solution is chosen, it is applied to the nonporous surfaces treated with CA fumes by dipping or using a wash bottle to spray it. It is thought that dye-staining polymerized prints works like a molecular sieve, where the dye molecules get stuck in the polymer by filling voids in the compound (Menzel, 1999, p 162). For this reason, it is important to adequately rinse the surface bearing the fingerprints with the dye stain. The result is a print that produces intense fluorescence when viewed with a forensic light source or laser (Figure 7–14). At this stage, proper photography can go beyond simply documenting the image to enhance the visibility of the fluorescing print by recording detail imperceptible to the unaided eye.

Powdering is also a good way to visualize and document polymerized impressions. Oftentimes, impressions are durable enough that they may be repeatedly brushed with fingerprint powder and lifted with tape until the right contrast is achieved in the lift (Illsley, 1984, p 15).

7.9.5 Conclusion

CA fuming is a proven and effective method of developing latent print impressions containing eccrine and sebaceous residues that has been in use since the late 1970s. The CA molecules bond to residue via polymerization to form a visible and durable compound that can be enhanced and recorded by fluorescence, photography, and lifting. Research is ongoing into the actual chemistry and mechanics of the CA reaction. Currently, the heat-accelerated technique in controlled high humidity (60–80% relative humidity) is most often the suggested method of application. It is also recommended that CA development be done shortly after fingerprint deposition for maximum results. Although CA fuming has proven effective for considerable durations of time after deposition, CA fuming prior to evidence packaging can also be an effective means of stabilizing fragile latent impressions during storage and transportation.

7.10 Fluorescence Examination

7.10.1 Background

As early as 1933, fluorescence examination with UV light was suggested as a method of visualizing latent prints dusted with anthracene powder on multicolored surfaces (Inbau, 1934, p 4). Before the late 1970s, UV fluorescent powder was used occasionally and appears to have been the only credible fluorescent method of latent print detection. In 1976, researchers at the Xerox Research Centre of Canada discovered inherent latent print fluorescence via continuous wave argon ion laser illumination. Shortly thereafter, the first latent print in a criminal case was identified, using inherent luminescence via laser excitation (fingerprint on black electrical tape) (Menzel and Duff, 1979, p 96).

Since the late 1970s, advancements in the technology of fluorescence detection have greatly aided the hunt for many types of forensic evidence. Today, evidence that would be barely perceptible or even invisible under normal lighting is routinely intensified by fluorescence. Bloodstains, semen, bruises, bone fragments, questioned documents, flammable residues, fibers, and fingerprints all merit examination with a forensic light source or laser.

7.10.2 Theory

Visible light consists of electromagnetic radiation of different colors and wavelengths. When light passes through a prism, it is separated spatially according to wavelengths, resulting in the classic colors of the rainbow. Violet light has the highest energy and the shortest wavelength (approximately 400 nm, where a nanometer is one-billionth of a meter), whereas red light has the lowest energy and the longest wavelength (approximately 700 nm), with green, yellow, and orange being intermediate in energy and wavelength (Champod et al., 2004, pp 41–76).

Atoms and molecules have different unique arrangements of electrons around their nuclei, corresponding to different discrete "energy levels". When light falls on a surface, a photon of light is absorbed if the energy of the photon exactly matches the difference in energy between two of the energy levels of the molecules of the surface substance. If light of a particular color or energy does not match the difference in energy, it is reflected. The color of the surface is made up of the colors of light that are reflected and is not the color corresponding to the wavelengths of light that are absorbed. Objects that are different colors are absorbing and reflecting different wavelengths of light. For example, chlorophyll, which gives leaves their green color, absorbs strongly at the red and blue ends of the visible spectrum, but reflects green light. We see the world by observing the wavelengths of light reflecting off objects all around us.

After a molecule absorbs light and is raised to a higher energy level, it tends to relax back to the lowest level or "ground state" by giving off energy as heat, usually through collisions with other molecules. In some molecules, however, the excess absorbed energy is given off in the form of light. This is photoluminescence. If the emission is immediate, it is termed fluorescence. If it is long-lived, it is phosphorescence. Fluorescence stops within nanoseconds when the forensic light source is turned off, whereas phosphorescence will continue. The glowing numbers of a darkroom timer are an example of phosphorescence.

The excited molecule will lose some of its energy before it emits light as photoluminescence. As a result, the emitted light is of a different color or wavelength than the excitation light (Figure 7–15). The fluorescence is said to be "red-shifted", meaning that it is to the red side of the electromagnetic spectrum in relation to the incident light from the forensic light source. The difference in the wavelengths of the exciting and emitted light is called the Stokes shift. When using fluorescence to view a fingerprint, the viewing or barrier filter blocks the reflected wavelengths of light from the light source while allowing the fluorescent wavelengths to pass through.

Fluorescence examination of latent prints is extremely sensitive (Menzel, 1999, p 5). By using the correct barrier filters that will block out the light from the forensic light source being used, but not the fluorescence, a very high signal-to-noise ratio may be observed. If there is fluorescent chemical only on the fingerprint, the background will give off no signal, and the print will be easily seen glowing against a black background.

Fingerprint examinations may produce fluorescence from four sources:

- Native constituents in latent print residue
- Foreign substances picked up by the hand and transferred through deposition
- Intentional chemical enhancement
- Substrate (background) fluorescence

Some research has been aimed at identifying "native" or inherent luminescence within fingerprint residue. This fluorescence is typically weak and is thought to come from compounds such as riboflavin and pyridoxin (Dalrymple et al., 1977, p 106). Foreign contaminants in fingerprint residue, such as food or drug residue, also may appear luminescent. Treatment by chemical and physical means designed to produce fluorescence, however, is generally considered to be the most productive. Dramatic results are routinely achieved through the use of fluorescent powders, dye stains, and chemical reagents.

7.10.3 Application

The use of lasers and forensic light sources pose real and sometimes irreversible health hazards. Lasers can generate enough intensity that even incidental or reflected light may damage the unprotected eye. Filtered lamps also produce intense light and, in addition, some will generate hazardous UV radiation. The appropriate eye protection must be used in coordination with the excitation wavelengths being employed. Please read all manufacturer warnings before using any forensic light source.

FIGURE 7–15

Absorption and emission spectra for rhodamine 6G.

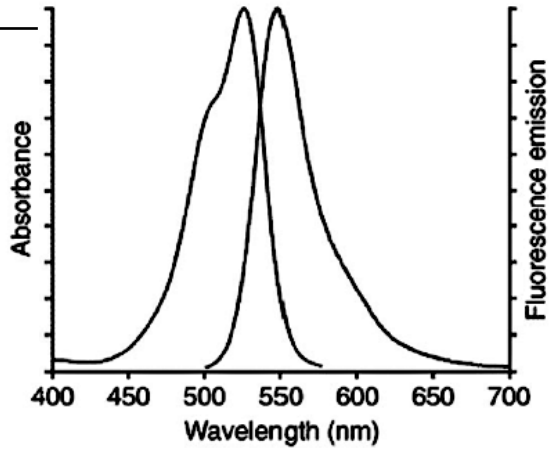
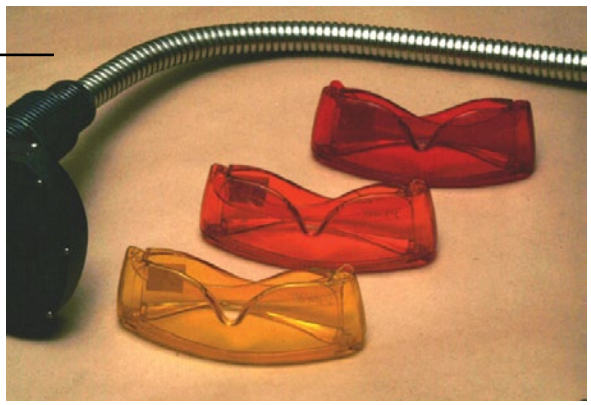


FIGURE 7–16

Goggles.



To visualize latent prints via fluorescence, a specific bandwidth of radiation must be shone on either an untreated latent print or one treated with a fluorescent chemical. The wavelengths chosen will be determined by the chemical involved and the luminescent nature of the substrate. The evidence is then examined through viewing goggles (Figure 7–16) or filter plates that block the incident light from the forensic light source. These goggles act as a barrier filter and are fundamental in separating the incident light generated by the light source and the weak fluorescing signal emitted by the latent print. This separation of incident and emitted light signals gives fluorescence examination its sensitivity. It is important to use the correct goggles to get the optimum results as well as for health and safety considerations.

UV-only excitation does not necessarily require viewing goggles because of the invisibility to the human eye of the incident lighting; however, protective goggles, which can include clear polycarbonate lenses, should be worn during evidence examination to protect the eyes from reflected UV radiation. Not all UV light sources produce pure UV, and a yellow viewing filter will be required if visible light is present. Photography of UV-only excited fluorescence may also require the correct UV barrier filter on the camera because some films and digital media may be sensitive to the incident lighting even when the human eye is not. Protective clothing should be worn to minimize skin exposure to UV radiation.

In general, yellow filters are used for incident light wavelengths from UV to 445 nm, orange filters for light sources of 445–515 nm, and red filters for 515–550 nm. Specific goggles and filters will vary in transmission values and should be matched to the light source being used. Viewing goggles are available through laser and forensic light source companies and most forensic supply houses.

Once a fluorescing image is observed, it can sometimes be "tuned" by adjusting the excitation wavelengths emitted by the light source, and the barrier filter used for viewing, to minimize background fluorescence and maximize contrast. The resulting image must be photographed using a photographic filter that transmits the same wavelengths as the filter used for viewing.

7.10.4 Light Sources

The light sources used to generate these narrow bandwidths come in several different varieties, including UV lamps, filtered lamps, and lasers. Each of these light sources has advantages and disadvantages, depending on the intended purpose and one's budget.

Recently, "alternate" or "forensic" light sources (filtered lamps) have become heavily relied on in laboratories and at crime scenes because of improvements in power output, versatility, portability, and affordability when compared to lasers. These high-intensity lamps use long-pass, short-pass, and band-pass filters in front of a metal halide or xenon bulb to produce the desired wavelength ranges for examining evidence (Wilkinson and Watkin, 1994, pp 632–651; Wilkinson et al., 2002, pp 5–15). Recently, handheld forensic "flashlights" have been introduced, many based on light-emitting diode (LED) technology (Wilansky et al., 2006).

Lasers, on the other hand, have in the past been less portable and affordable but generated considerably more power than filtered lamps. Lasers are desirable when only very weak fluorescence is observed. Some examples of weak fluorescence include the inherent fluorescence of latent fingerprint residue or fingerprints developed with reagents such as crystal violet that emit a very weak fluorescent signal. New lasers (532 nm), which are air-cooled and portable, have recently come on the market. For a more comprehensive discussion of laser types, functionality, uses, and theory, see Menzel's *Fingerprint Detection with Lasers* (Menzel, 1999, pp 3–21) or the Home Office publication, *Fingerprint Detection by Fluorescence Examination* (Hardwick et al., 1990).

Besides simply detecting evidence, a forensic light source or laser is often an effective means of image enhancement as well. This enhancement may come from intentionally causing a background to fluoresce to increase the contrast between a fingerprint and its substrate, or from muting a background pattern by selecting a wavelength range that reduces the background color.

Bloody impressions are a good example of enhancement through absorption at a discrete wavelength. The maximum absorption wavelength for dried blood is approximately 420 nm. Illumination at this wavelength makes the blood-stained ridges appear darker. If the background fluoresces in this wavelength range, the bloody impression will be significantly enhanced (Figure 7–17) (Stoilovic, 1991, pp 289–296; Vandenberg and van Oorschot, 2006, pp 361–370).

7.10.5 Fluorescent Powders, Dye Stains, and Reagents

Many fluorescent processes have been developed to aid the forensic examiner with tools that go far beyond using a light source alone. Fluorescent powders are abundant and widely available at forensic supply companies, with most companies marketing their own particular brand name.

Dye stains such as MBD [7-(*p*-methoxybenzylamino)-4-nitrobenz-2-oxa-1,3-diazole], rhodamine 6G (R6G), Ardrox, basic yellow, and basic red can be prepared in the lab and are extremely effective for enhancing fingerprints developed with cyanoacrylate. Some of these dye stains can be combined to produce a stain that will fluoresce across a broad spectrum. One such stain is RAM, a combination of R6G, Ardrox, and MBD. Because RAM can be used at various wavelengths, the practitioner can often "tune out" problematic backgrounds by selecting a wavelength that maximizes fingerprint fluorescence and suppresses background fluorescence.

Treatments for paper are equally effective as those used on nonporous surfaces and include ninhydrin toned with zinc chloride and the ninhydrin analogues: DFO, 1,2-indanedione, and 5-MTN (5-methylthioninhydrin).

Four excellent references containing recipes and instructions for fluorescent reagents are the FBI *Processing Guide for Developing Latent Prints* (Trozzi et al., 2000), the Home Office *Manual of Fingerprint Development Techniques* (Kent, 1998, 2004), *Fingerprints and Other Ridge Skin Impressions* (Champod et al., 2004, pp 142–145, 228–229), and *Advances in Fingerprint Technology* (Lee and Gaensslen, 2001, pp 105–175).

Table 7–3 is a list of common reagents and their corresponding wavelengths of peak absorption and emission. Precise adherence to a peak excitation and absorption wavelength is not always possible (depending on the available light source) and not always advisable because many substrates may interfere with visibility at these wavelengths. Because the absorption bands are generally quite wide, the excitation wavelength can differ from the absorption maximum and still induce significant fluorescence.

FIGURE 7–17

Composite image of a bloody fingerprint on copy paper. The left side was photographed at 415 nm. The right side was photographed under daylight-balanced photographic lighting.

7.10.6 Time- and Phase-Resolved Imaging

As mentioned earlier, background fluorescence may be generated intentionally to better visualize a fingerprint that is faintly absorbing light but not fluorescing. This condition will increase contrast by brightening the background, making the darker fingerprint stand out. However, background fluorescence is more often a hindrance, competing with a fluorescing fingerprint for visualization.

Time-resolved imaging has been advocated as one possible means to solve this problem. This technique takes advantage of the difference between the time of emission of the substrate and the fluorescing fingerprint (Menzel, 1999, p 126). Early devices utilized a light source with a gated, rotating wheel that "chops" the light to exploit these differences in emissions. The light shines on the fingerprint and substrate when an opening in the wheel is in front of the light. The light source is then effectively turned off when a blade in the wheel passes in front of the light source. Shortly thereafter, an opening in the wheel passes in front of a detector. The size of the openings in the wheel, and the speed with which it turns, will determine the length of time that the print is exposed and the delay between excitation and detection. If the background fluorescence decays faster than the fluorescence of the chemical on the latent fingerprint, the background can be eliminated by adjusting the delay time (Menzel, 2001, p 216; Campbell, 1993, pp 368–377).

Later designs proved more practical by using an electronic light chopper in conjunction with a gateable charge-coupled device (CCD) camera, each component controlled by a computer with the image displayed on a monitor (Menzel, 1999, p 126). Time-resolved imaging is still considered impractical for widespread application. Phase-resolved imaging stands to be the next technological advance and is currently used in other fields of spectroscopy (Menzel, 2001, p 216).

7.10.7 Conclusion

Fluorescence examination is firmly grounded in everyday latent print detection and imaging techniques. The sensitivity of this technique warrants application on all forms of forensic evidence. Specific bandwidths of radiation are shone on untreated prints as well as prints treated with powders and chemical reagents. When viewed with the appropriate barrier filters, sensitivity via photoluminescence detection may be achieved down to nearly the single photon. Absorption at discrete wavelengths, absent fluorescence, is also a beneficial enhancement technique on substances such as the purple impressions from ninhydrin or dried blood, rendering them darker and easier to view and photograph. Because background fluorescence is the biggest hindrance to fluorescence examination, experimental concepts such as time- and phase-resolved imaging have been proposed to address this problem.

Table 7–3

Common reagents and their wavelengths of peak absorption and emission.

Reagent/Substance	Absorption Maximum (nm)	Emission Maximum (nm)
DFO	**560** (Champod et al., 2004, pp 129–130)	**580** (Champod et al., 2004, pp 129–130)
1,2-Indanedione	**515**	
5-MTN	**550** (Wallace-Kunkel et al., 2006, pp 4–13)	
Ninhydrin	**415–560*** (Champod et al., 2004, pp 117–118)	
Ninhydrin/ZnCl	**490**	**540** (Champod et al., 2004, pp 120–124)
Ardrox	**380**	**500** (Lee and Gaennslen, 2001, p 124)
Basic Yellow 40	**445** (Champod et al., 2004, pp 142–145, 228–229) **440** (Lee and Gaennslen, 2001, p 124)	**495** (Champod et al., 2004, pp 142–145, 228–229) **490** (Lee and Gaennslen, 2001, p 124)
MBD	**465**	**515** (Lee and Gaennslen, 2001, p 124)
Basic Red 28	**495**	**585** (Champod et al., 2004, pp 142–145, 228–229; Lee and Gaennslen, 2001, p 124)
Rhodamine 6G	**490–530** (Champod et al., 2004, pp 142–145, 228–229) **525** (Lee and Gaennslen, 2001, p 124)	**565** (Champod et al., 2004, pp 142–145, 228–229) **555** (Lee and Gaensslen, 2001, p 124)
Crystal Violet	**532****	
Acid Yellow 7	**527**	**550** (Sears et al., 2005, pp 741–763)
Acid Yellow 7 + Blood	**445–480**	**485–500** (Sears et al., 2005, pp 741–763)
Untreated Dried Blood	**415*** (Champod et al., 2004, p 168)	

* Does not fluoresce but appears dark.

** Weak fluorescence requiring laser illumination.

7.11 Vacuum Metal Deposition

7.11.1 History

Vacuum metal deposition (VMD) is a long-established industrial technique for the application of metal coatings to components such as glass to form a mirror. In 1964, physics professor Samuel Tolansky (Royal Holloway College, University of London) noted that the deposition of silver in a vacuum system developed latent fingerprints accidentally deposited on a glass component. An investigation into the process as a fingerprint development technique was proposed. However, this was not pursued at the time by the U.K. Home Office because other techniques for fingerprint detection on glass were considered cheaper, easier to use, and sufficiently effective.

In 1968, French workers reported (Theys et al., 1968, p 106) that VMD of a mixture of zinc, antimony, and copper powder was capable of developing latent prints on paper. As a consequence of this article, interest in the technique was revived in the United Kingdom, and Tolansky initiated a research program to investigate the optimum conditions and the potential applications for VMD. One of the early objectives of the research was to establish why the French composition was effective. Closer examination of deposited metal coatings produced by the French laboratory indicated that the coating was almost entirely zinc, the presence of antimony and copper not being necessary to develop prints (Hambley, 1972).

The research program initiated by Tolansky (Hambley, 1972) investigated the deposition characteristics of a range of metals on paper substrates, identifying single metals and metal combinations giving the optimum print development. Research was also conducted into the ability of the technique to detect latent prints on fabrics. These experiments showed that although some print development was obtained by the use of single metals (e.g., gold, silver, copper, zinc, and cadmium), in general, the best results were obtained by the use of a combination of metals, typically gold or silver followed by cadmium or zinc. Initially, the gold and cadmium combination was selected as the optimum, although subsequent health and safety issues have resulted in the gold and zinc combination being recommended instead. Gold was preferred over silver as the initial deposition metal because silver can be degraded by fingerprint secretions and atmospheric pollutants.

The early experimental work was carried out on small-scale equipment with a bell jar, but research continued to develop larger equipment suitable for use in a fingerprint laboratory. By the mid-1970s, systems modified from standard industrial equipment had been developed (Kent, 1982) and were in use in several police forces and forensic providers within the United Kingdom. Later, manufacturers made refinements, increasing the size of the vacuum chamber and adding controls specific to the fingerprint development process. In the 1990s, the technique made its way from Europe to North America (Murphy, 1991, pp 318–320; Misner, 1992, pp 26–33; Masters and DeHaan, 1996, pp 32–45). Specially constructed VMD equipment is now supplied by several manufacturers worldwide.

Although VMD was originally investigated as a fingerprint development technique for use on paper and fabrics, it was established that other processes are capable of giving better results on paper. However, VMD was found to give excellent results on nonporous substrates and in comparative studies was found to outperform all other techniques in developing marks on plastic bags (Misner, 1992, pp 26–33; Kent et al., 1975, 1978; Reynoldson and Reed, 1979). The process was also found to develop marks on substrates exposed to water and conditions of high humidity, giving substantial advantages over techniques such as CA fuming for articles that have been exposed to these conditions.

Few modifications have been made to the process itself since the change in the second deposition metal from cadmium to zinc in the late 1970s. Recently, there has been further research on VMD in Australia, looking in detail at the various print development regimes that can be followed on different grades of polyethylene (Jones et al., 2001c, pp 73–88) and how the surfaces could be "reactivated" to develop prints if excess metal deposition had occurred initially (Jones et al., 2001d, pp 5–12). The work was extended to investigate other polymer substrates, including polypropylene, polyvinylchloride, and polyethylene terephthalate (Jones et al., 2001b, pp 167–177), and different deposition conditions were recommended for each class of polymer, in particular the amount of gold deposited prior to zinc deposition (polyethylene terephthalate and polyvinylchloride require significantly more gold to develop prints than polymer or polypropylene).

However, there are situations where the performance of VMD leaves much to be desired. It is believed that the effectiveness of VMD can be detrimentally affected by the

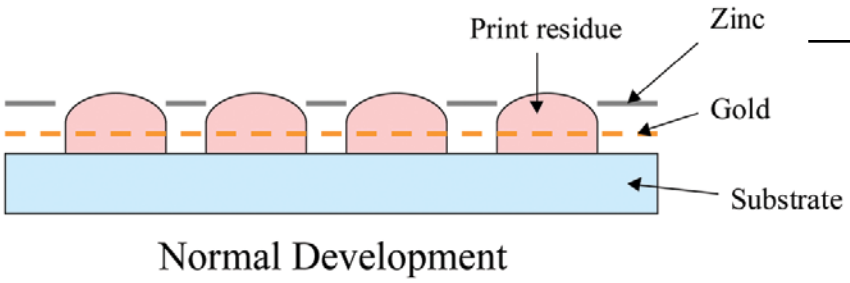


FIGURE 7–18

Schematic diagram of normal development, showing zinc depositing where gold nuclei are available on the surface.

presence of body fluids (Batey et al., 1998, pp 165–175) and drug residues (Magora et al., 2002, pp 159–165). It has also been difficult to develop prints on heavily plasticized polymers (such as clingfilm and plasticized vinyl) using the VMD process. Recent work has indicated that deposition of silver as a single metal may give improved detection rates over the gold and zinc combination for these types of substrates, and the silver deposition process has now been published for operational use (Home Office Scientific Development Branch, 2005, pp 8–9).

7.11.2 Theory

There is general agreement on the theory associated with normal development of prints by the VMD method. The reason that the metal combinations are postulated to work well is due to the condensation characteristics of zinc (and cadmium). These metals will not condense on grease, such as that found in fingerprint residues, even when the oily residues are present only as a monolayer. However, zinc will deposit on small nuclei of metal, and this is the reason that gold or silver deposition is carried out first. Gold and silver can be deposited over the entire surface and begin to form nuclei, the morphology of which depends on the nature of the surface (surface energy, chemical species present) upon which they are being deposited. The resultant gold coating is very thin (several nanometers only) and discontinuous. However, in the regions coated with the fatty residues of the latent fingerprint, the gold diffuses into the fat and hence there are no gold nuclei close to the surface. As a consequence, when zinc is subsequently deposited, it will condense on the regions of gold nuclei (i.e., the background substrate) but not on the regions of the fatty deposit (i.e., the fingerprint ridges). This theory of nucleation was discussed in more detail by Stroud (1971, 1972). The normal development process is depicted schematically in Figure 7–18, and a photograph of a mark produced by normal development is shown in Figure 7–19.

Tests carried out to determine which components of the latent print were most likely to be responsible for inhibiting metal deposition identified several substances, including stearic acid, palmitic acid, cholesterol oleate, glycerol trioleate, and amino acids L-arginine monohydrochloride, L-leucine, and DL-threonine. Most of these substances are non-water-soluble or long-chain fats or acids with low vapor pressure, which determines their stability and non-migration over the surface during the VMD process. These findings were in accord with the observation that VMD was capable of developing prints on substrates exposed to water. Experiments to study the diffusion of gold into thin films of stearic acid (Thomas, 1978, pp 722–730) demonstrated that 60% of the gold penetrated the stearic acid to a depth greater than the detection depth of the electron spectroscopy for the chemical analysis (ESCA) surface analysis technique and hence would probably not be sufficiently close to the surface for zinc to nucleate on it.

Electron microscopy has also been used to confirm that the size and distribution of gold nuclei formed during the deposition process varied greatly according to the substrate and the chemical species present (Kent, 1981, p 15). It was this difference in nuclei size and distribution, coupled with diffusion of gold into the fatty deposits, that subsequently delineated the print during VMD.

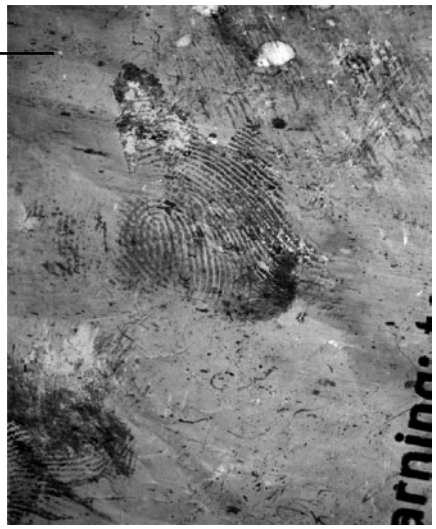
In practice, many prints developed using VMD may be "reverse developed" (i.e., zinc preferentially deposits on the fingerprint ridges rather than the background). There are differences in opinion as to why this arises (Jones et al., 2001b, pp 167–177; 2001c, 73–78; Kent et al., 1976, p 93), but none of the theories have been categorically proven, and in some cases reverse and normal development may be observed on the same substrate (although it is stated that this is most common for [if not exclusive to] low-density polyethylene substrates). Figure 7–20 shows a reverse-developed mark on a polyethylene bag.

FIGURE 7–19

Photograph of a normally developed mark on a polyethylene bag.

FIGURE 7–20

Photograph of a reverse-developed mark on a polyethylene bag.



7.11.3 Application

The equipment used for VMD may vary according to manufacturer, but the essential elements of the system are the same. The equipment consists of a vacuum chamber capable of being pumped down to very low pressure (< 3 x 10^{-4} mbar), filaments for evaporation of gold and zinc, and a viewing window so that the deposition of zinc can be monitored. The chamber may also contain a "cold finger", chilled to low temperature to help reduce pump downtimes by condensing some of the vapor in the chamber. Articles to be coated are attached to the inside circumference of the vacuum chamber, above the coating filaments. A typical system is illustrated in Figure 7–21.

The filaments ("boats") used for deposition of gold and zinc are typically formed from thin sheets of molybdenum. The gold filament usually consists of a shallow dimple in a thin strip of molybdenum. Gold deposition takes place when the chamber has reached a pressure of 3 x 10^{-4} mbar or lower, and the current to the filament is increased until the filament reaches a yellow-to-white heat. Deposition of gold should be complete within 10 seconds, but if any residue is observed on the filament as the current is reduced, the temperature should be increased again until all gold has been evaporated.

Once gold deposition is completed, the pressure in the chamber is increased to ~5 x 10^{-4} mbar and the current to the zinc deposition filament(s) is turned on. The reason for increasing the pressure in the chamber is to reduce the speed of zinc deposition by introducing more air molecules with which the zinc may collide. Some substrates can coat very quickly, so the slower deposition process gives the operator more control. The zinc deposition filaments are larger and significantly deeper than the gold filament, and the quantity of zinc added is greater, typically 1 g per run. For zinc deposition, the current is increased until the

FIGURE 7–21

Typical vacuum metal deposition equipment.

filament glows a cherry-red to dull orange color. Once this occurs, the operator should observe the deposition process through the viewing window, ceasing deposition as soon as marks become visible on the substrate. After zinc deposition, the gold filament should be briefly heated to yellow-to-white heat to burn off any zinc contamination. The process is described in more detail elsewhere (Kent, 2004).

There is great variability in the speed at which different substrates coat, and it may take more than 10 minutes to obtain a suitable coating on some types of material. In some cases, it may be necessary to carry out multiple deposition runs in order to obtain satisfactory results or to develop all the marks present. The presence of surface contamination, release agents, or plasticizers may mean that it is not possible to obtain a zinc coating at all; in these circumstances, the deposition of 60 mg of silver, using the same deposition conditions for gold, may yield additional marks.

The VMD technique was initially adopted as an operational technique for the detection of latent prints on thin polyethylene sheets, and it was shown to be superior to other processes developed subsequent to the initial comparison trials. VMD has now been used operationally for many years and has been shown to be an effective technique for a wider range of materials than polyethylene. Recent results have shown VMD to produce results on a range of substrates (e.g., a ticket coated with ferromagnetic ink, and on expanded polystyrene) (Suzuki et al., 2002, pp 573–578). The use of the technique has also begun to increase in North America, and successful results have been obtained from plastic bags, in some cases several years old and exposed to moisture (Batey et al., 1998, pp 165–175).

The range of specimens that have been successfully treated using VMD is extensive and includes:

- Plastic bags and packaging.
- Glass and plastic bottles.
- Firearms.
- Glossy card, photographic paper, and magazine covers.
- Clean leather items (including handbags and shoes).
- Adhesive tapes (nonsticky side).

It is evident that there is much overlap between the types of articles that can be treated with VMD and those that are treated using CA fuming. In many cases, the deciding factor as to which technique is to be used is whether the article has been wetted because VMD remains effective on wetted items, whereas CA fuming does not. In practice, it is possible to use the two processes in sequence, and more marks may be detected in this way because the two processes work on different fingerprint constituents. However, at present, there still seems to be some debate as to which of the two techniques should be done first.

7.12 Blood Enhancement Techniques

7.12.1 History

Blood is one of the most common known contaminants of fingerprints found at scenes of crime. The use of blood evidence in the history of forensic investigation dates back

over 150 years. The earliest tests were of two types, both relying on the presence of the heme group: those that produced crystals and those that relied on its catalytic nature.

The crystal or confirmatory tests were formulated by Teichmann in 1850 (Thorwald, 1966, p 23) and Takayama in 1912 (Gerber and Saferstein, 1997, pp 18–19). However, these tests require the blood to be scraped from the surface and, therefore, give no regard to the forms of physical evidence such as fingerprints, footwear impressions, or spatter patterns.

Catalytic or presumptive tests that attempted to keep much of the physical evidence intact were produced by Van Deen and Day in 1862 and were based on guaiacol (Gerber and Saferstein, 1997, pp 18–19); by Schönbein in 1863, using hydrogen peroxide; and by Adler and Adler around 1900, using benzidine (Thorwald, 1966, p 23). Adler and Adler pioneered the use of leucomalachite green in 1904 (Eckert and James, 1989, p 2); Medinger modified their method in 1931 to make it more sensitive (Söderman and O'Connell, 1935, p 226).

Other presumptive tests for blood were developed by Kastle and Sheed in 1901 and Kastle and Meyer in 1903, using phenolphthalein; by Ruttan and Hardisty in 1912, using o-tolidine; by Specht in 1937, using luminol (3-aminophthalhydrazide); and by Gershenfeld in 1939, using o-toluidine (Eckert and James, 1989, p 2).

In 1911, Abderhalden and Schmidt (1911, p 37) reported the development of fingerprints on the bottle label of triketohydrindene hydrate (ninhydrin). This discovery was not exploited for the detection of fingerprints or blood until 1954, when Odén (Odén and von Hofsten, 1954, p 449) produced his ninhydrin formulation based on acetone. The use of this method for the enhancement of fingerprints in blood revolutionized thinking in this area of forensic investigation. The emphasis was shifted away from presumptive tests for heme, which generally require expert opinion to interpret the test results correctly, and onto easier-to-use reagents that produce intensely colored products with other components of blood, usually protein or its breakdown products.

Use of the protein dye, amido black (acid black 1), quickly became popular with forensic investigators. Its use by the Metropolitan Police Laboratory, in a solvent base of methanol and acetic acid, was discussed at a forensic science symposium in 1961 by Godsell (1963, p 79). This formulation, with a change in the method for fixing blood— from the use of heat to immersion in methanol (Faragher and Summerscales, 1981), along with a water-based formulation of the same dye (Hussain and Pounds, 1989a)— continued to be recommended for the enhancement of fingerprints in blood by the U.K. Home Office until 2004 (Kent, 2004), when a new formulation by Sears and Prizeman (2000, p 470) was adopted.

Many other protein stains for the enhancement of both fingerprints and footwear impressions in blood have also been proposed: coomassie blue (acid blue 83) and Crowle's double stain (acid blue 83 and acid red 71) by Norkus and Noppinger in 1986 (Norkus and Noppinger, 1986, p 5); fuchsin acid (acid violet 19, Hungarian Red), patent blue V (acid blue 1), and tartrazine (acid yellow 23) by Barnett and colleagues in 1988 (Barnett et al., 1988); benzoxanthene yellow and acid violet 17 by Sears and colleagues in 2001 (Sears et al., 2001, p 28); and acid yellow 7 by Sears and colleagues in 2005 (Sears et al., 2005, p 741).

Although the use of protein dyes became most popular for enhancing fingerprints in blood, research on presumptive enhancement methods continued and, in 1976, Garner et al. (1976, p 816) proposed the use of tetramethylbenzidene (TMB) as safer and just as reliable as benzidine. Suggestions for other presumptive tests continue: tetraamino-biphenyl (TAB) and diaminobenzidine (DAB) in 1989 by Hussain and Pounds (1989b); fluorescein in 1995 by Cheeseman and DiMeo (1995, p 631); and leucocrystal violet (LCV) in 1996 by Bodziak (1996, p 45).

In addition, many modifications have been made to ninhydrin formulations to increase its effectiveness and safety: by Crown in 1969 (Crown, 1969, p 258) and Morris and Goode in 1974 (Morris and Goode, 1974, p 45). Further changes were forced on the fingerprint community because of "The Montreal Protocol on Substances That Deplete the Ozone Layer" (United Nations Environ Programme, 1999), and new formulations were proposed by Watling and Smith in 1993 (Watling and Smith, 1993, p 131) and Hewlett and colleagues in 1997 (Hewlett et al., 1997, p 300). The use of transition metal toners to change the color or make the reaction product between amines and ninhydrin fluoresce has also been proposed by Morris in 1978 (Morris, 1978), Everse and Menzel in 1986 (Everse and Menzel, 1986, p 446), and Stoilovic and colleagues in 1986 (Stoilovic et al., 1986, p 432).

It was also suggested that the use of one of several ninhydrin analogues would improve sensitivity, and many have been proposed: benzo(f)ninhydrin in 1982 by Almog

et al. (1982, p 912), 5-methoxyninhydrin in 1988 by Almog and Hirshfeld (1988, p 1027), DFO in 1990 by Grigg et al. (1990, p 7215), and indanedione in 1997 by Ramotowski et al. (1997, p 131).

In the late 1970s and early 1980s, those developing high-intensity light sources observed that shorter wavelengths of light in the UV and violet regions of the spectrum make surfaces fluoresce strongly. This can give extra detail if a fingerprint is in a strongly light-absorbing material such as blood (Hardwick et al., 1990). This is an especially valuable method for the enhancement of fingerprints in blood, as the heme group absorbs light throughout much of the visible part of the spectrum (Kotowski and Grieve, 1986, p 1079).

All these developments meant that by the late 1990s, there were so many reagents and formulations for the enhancement of blood-contaminated fingerprints and footwear impressions, with little or no comparative data, that it was causing immense confusion amongst practitioners. Also, the emergence of DNA analysis heaped even more uncertainty onto which techniques could or should be used for the enhancement of blood, such that vital evidence was likely to be lost by the wrong choices. Therefore, the U.K. Home Office set out to clarify the situation and began a program of work to review and compare the most commonly used of these techniques (Sears and Prizeman, 2000, p 470; Sears et al., 2001, p 28; 2005, p 741). Resulting from this colossal task were a number of key findings that were incorporated in a comprehensive update to *The Manual of Fingerprint Development Techniques* in 2004 (Kent, 2004).

7.12.2 Theory

Blood consists of red cells (erythrocytes), white cells (leukocytes), and platelets (thrombocytes) in a proteinaceous fluid called plasma, which makes up roughly 55% of whole blood volume. The red cells principally contain the hemoglobin protein but also have specific surface proteins (agglutinogens) that determine blood group. The white cells, which form part of the immune system, have a nucleus that contains DNA.

Hemoglobin makes up roughly 95% of red cells' protein content and is made of four protein subunits, each containing a heme group. The heme group is made of a flat porphyrin ring and a conjugated ferrous ion.

Chemical blood enhancement methods fall broadly into two types—those that use the heme grouping to prove or infer the presence of blood and those that react with proteins or their breakdown products. The latter are not at all specific for blood; however, because of the high content in blood of protein and protein breakdown products, these techniques are the most sensitive available to the forensic investigator (Sears et al., 2005, p 741).

7.12.3 Tests for Heme

Two kinds of tests use the heme group in hemoglobin: crystal tests and catalytic tests.

Crystal tests are specific or confirmatory for the presence of heme, but not whether the blood is human or not. The two best-known crystal tests are those formulated by Teichmann and Takayama. The Teichmann test results in the formation of brown rhombohedral crystals of hematin, and the Takayama test results in red-pink crystals of pyridine hemochromogen (Palenik, 2000, p 1115; Ballantyne, 2000, p 1324). Both these tests have to be carried out ex situ so are of no use for fingerprint enhancement.

The catalytic tests are only presumptive or infer the presence of heme because they are subject to false-positive and false-negative reactions caused by a variety of nonblood substances. Consequently, individual results require careful interpretation by experts. These tests all rely on the peroxidase activity of the heme group (i.e., the ability to reduce hydrogen peroxide to water and oxygen). This reaction may then be coupled to the oxidation of colorless reduced dyes (e.g., phenolphthalein, leucocrystal violet, tetramethylbenzidine, and fluorescein) that, when oxidized, form their colored counterparts (Ballantyne, 2000, p 1324).

$$H_2O_2 + \underset{\text{reduced dye}}{\text{colorless}} \rightarrow H_2O + \underset{\text{oxidized dye}}{\text{colored}}$$

(Lee and Pagliaro, 2000, p 1333).

The luminol test also relies on the peroxidase activity of the heme group but uses sodium perborate instead of hydrogen peroxide. This then produces a product that luminesces in the presence of blood. The bluish-white chemiluminescence is faint and must be viewed in the dark by an operator who is fully dark-adapted to gain the best from this test. Even with careful application of luminol, it is all too easy to damage the fine detail of blood-contaminated fingerprints. This technique should be used only when fine detail is not required and when other techniques might be

compromised by surface type or impracticality, such as dark or patterned carpets (Sears et al., 2005, p 741).

The major concern with the catalytic tests for blood is that they can produce false-positive results in the presence of chemical oxidants and catalysts; salts of heavy metals such as copper, nickel, and iron; and plant peroxidases such as those found in horseradish, citrus fruits, and numerous root vegetables (Lee and Pagliaro, 2000, p 1334). A two-stage test can obviate this. The reduced colorless dye is applied initially and if no color change is observed, then the hydrogen peroxide is added. A color change at this point is more likely to indicate the presence of blood.

It is generally accepted that a negative result with a catalytic test proves the absence of blood; however, strong reducing agents, such as ascorbic acid, may inhibit such tests (Eckert and James, 1989, p 121).

7.12.4 Tests for Protein and Its Breakdown Products

There are two types of techniques for proteins—those that stain proteinaceous material and those that react with amines. Blood contains more protein than any other material, so these techniques are inherently more sensitive than those for heme, although they are not at all specific for blood.

The most effective protein dyes for the enhancement of fingerprints in blood are a group known as acid dyes. They are often characterized by the presence of one or more sulphonate ($-SO_3^-$) groups, usually the sodium (Na^+) salt. These groups function in two ways: first, they provide for solubility in water or alcohol, the favored major solvents from which to apply these dyes; and, second, they assist the reaction by virtue of their negative charge (anionic). If acidic conditions are used (acetic acid being the favored option), the blood protein molecules acquire a positive charge (cationic) and this attracts the acid dye anions. Also, hydrogen bonding and other physical forces, such as van der Waals, may play a part in the affinity of acid dyes to protein molecules (Christie et al., 2000, pp 19–20). The presence of a short-chain alcohol in the dyeing solution helps to prevent the blood from diffusing during the dyeing stage (Sears and Prizeman, 2000, p 470). Ethanol is preferred because this offers lower toxicity and flammability than methanol. The use of water as the major solvent gives the solution a flash point of around 30 °C, enabling this formulation (containing water, ethanol, and acetic acid) to be used at crime scenes with a few simple precautions (Kent, 2004).

If acid dye formulations are applied directly to fingerprints in blood without a fixing stage, the blood will solubilize and ridges will diffuse or be completely washed away. A number of different fixing agents have been used, but the most effective are 5-sulphosalicylic acid and methanol. Which one is used depends on the major solvent used in the dyeing process: if water is the main solvent, then a solution of 5-sulphosalicylic acid is most effective, whereas if the main dyeing solvent is methanol, then methanol is the best fixing agent (Sears and Prizeman, 2000, p 470). These fixing agents act in different ways; the 5-sulphosalicylic acid precipitates basic proteins, and methanol dehydrates the blood.

The use of solutions based on methanol has waned for a number of reasons, including its toxicity, flammability, and tendency to cause damage to surfaces (e.g., paints, varnishes, and some plastics), which has a negative effect on fingerprint development. This fixing stage gives the protein dyes another advantage over the presumptive tests for blood: as well as being a more sensitive test, it often produces more sharply defined fingerprint ridges and the detail is clearer.

A washing stage is required post-dyeing. On nonporous surfaces, this just removes excess dye; however, on porous surfaces, this also acts as a destainer, removing dye that has been absorbed by the background surface. The wash solution has to be carefully constructed so that it solubilizes the dye, does not diffuse or wash away the dyed fingerprint, and retains the intensity of color of the dye in the fingerprint. For this reason, the same solvent mix as that used for the dyeing process, or some small variation of it, is generally most effective (Sears and Prizeman, 2000, p 470).

Ninhydrin and DFO react with amines and are the two most widely used techniques to develop latent fingerprints on porous surfaces (Figure 7–22). They are also very effective for the enhancement of blood (Sears et al., 2005, p 741). They both react with amino acids similarly to form products that contain two deoxygenated molecules of the starting product, bridged by a nitrogen atom that is donated from the amine (McCaldin, 1960, p 39; Wilkinson, 2000a, p 87).

Although the reaction mechanisms and products have similarities, the method of their visualization is entirely different. Ninhydrin, under the right conditions, produces an intensely colored product (Ruhemann's purple), and DFO

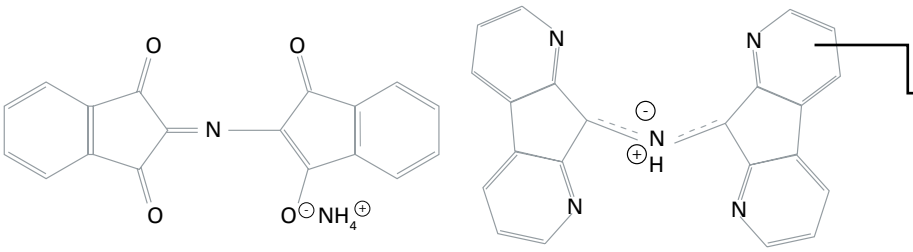


FIGURE 7–22

The reaction products for the reaction of ninhydrin (left) and DFO (right) with amines.

produces a pale pink, extremely fluorescent product. Ruhemann's purple can be made to fluoresce by complexing it with metal salts, but this additional process is still not as sensitive as DFO (Stoilovic, 1993, p 141). DFO requires heat for the reaction to proceed (Hardwick et al., 1993, p 65), whereas ninhydrin will react at room temperature, provided moisture is available, although the process proceeds much faster at elevated temperatures and humidities.

7.12.5 Fluorescence

The use of fluorescence to enhance fingerprints in blood can be extremely effective. There are two ways this may be achieved: (1) by exciting fluorescence in the background surface on which the blood is deposited or (2) by treatment with a chemical that either breaks the heme group or turns the blood into a fluorescent species, or does both of these.

Many materials fluoresce when excited by high-intensity light in the UV and violet regions of the spectrum. This is coincidentally where the heme group is most absorbent, with a peak around 421 nm (known as the Soret Band) (Kotowski and Grieve, 1986, p 1079). This absorbency is why blood-contaminated fingerprints will appear dark against a light background. Fluorescence examination may be used before any other fingerprint enhancement techniques because it is nondestructive, and if long-wave UV or violet light (350–450 nm) (Hardwick et al., 1990) is used, then DNA typing is also unaffected (Kent, 2004). The use of ninhydrin, acid black 1, or acid violet 17 can further intensify the contrast between fingerprint and background by increasing the light absorption properties of the blood.

The use of a strong organic acid in conjunction with hydrogen peroxide breaks up the heme group so that it is no longer as effective at absorbing light. Then, when excited by green (500–550 nm) light, it will fluoresce orange. This effect has also been noted as blood ages.

DFO and acid yellow 7 both produce fluorescent species with blood that can be excited by green (510–570 nm) and blue (420–485 nm) light, respectively. Both can be less effective on heavy deposits of blood because the heme group retains its ability to absorb both the excitation light and that emitted as fluorescence.

7.12.5.1 Application. Currently it is considered that fluorescence examination, two amino acid reagents, and three acid dyes are the most effective means of enhancing fingerprints in blood (Sears et al., 2005, p 741). The most appropriate techniques to use for maximum effectiveness, either individually or in sequential order, depend on the porosity of the surface to be treated. This applies to both latent fingerprint development and enhancement of blood-contaminated fingerprints.

Testing of the surface for fluorescence should always be carried out before any other technique. High-intensity light sources with outputs between 350 and 450 nm are most effective. When the blood-contaminated or latent fingerprints are on porous surfaces, the most effective sequence of techniques is DFO, ninhydrin, either acid black 1 or acid violet 17 (after carrying out a spot test to see which is most suitable), and then finally physical developer (Sears et al., 2005, p 741).

When the blood-contaminated or latent fingerprints are on nonporous surfaces, the most effective sequence of techniques is VMD, powders, acid yellow 7, acid violet 17, then finally either physical developer or solvent black 3 (sudan black). Superglue may be used instead of VMD or powders, but this will inhibit the dyeing process for blood by preventing the dye from reaching the blood (Sears et al., 2005, p 741).

DFO and ninhydrin working solution should be applied by dipping or by brushing with a soft brush on larger articles or surfaces. It is recommended that DFO be heated to 100 °C for 20 minutes; however, when this is not possible, temperatures as low as 50 °C may be used, but the rate of reaction is much slower (Hardwick et al., 1993, p 65). It is recommended that ninhydrin-treated articles or surfaces be heated to 80 °C and humidified to 65% RH. However, the reaction will proceed at room temperature and humidity, but more slowly.

High-intensity light sources capable of emitting wavelengths between 510 and 570 nm must be used to excite fluorescence from blood reacted with DFO. The fluorescence emitted is between 550 and 650 nm. Benefit may also be gained by using shorter wavelengths, between 350 and 450 nm, to excite background fluorescence after ninhydrin treatment.

The three recommended acid dyes, acid black 1 (CI 20470), acid violet 17 (CI 42650), and acid yellow 7 (CI 56205), should all be applied to blood fixed for at least 5 minutes with a solution of 5-sulphosalicylic acid. Dyeing of fixed blood is most effective if the area of interest is immersed in the dyeing solution for at least 3 minutes for acid black 1 and acid violet 17 and for at least 5 minutes in the case of acid yellow 7. Areas heavily contaminated with blood require longer dyeing times. If it is not possible to immerse the bloodied fingerprints, then the dyeing solution should be applied above the area of interest and allowed to flow down over it, keeping the area damp for the specified time. A well may be constructed around the area of interest on horizontal surfaces, which may be flooded and drained as appropriate.

Areas of interest will then need to be washed or destained to remove excess dye. The most effective solution for doing this is the same solvent composition as the dye solution, washing as required to remove excess dye or destain the background.

High-intensity light sources capable of emitting wavelengths between 420 and 485 nm must be used to excite fluorescence from blood dyed with acid yellow 7. The fluorescence emitted is between 480 and 550 nm. The use of shorter wavelengths between 350 and 450 nm, to excite background fluorescence after acid black 1 or acid violet 17 treatment, may be beneficial.

Work carried out by the U.K. Home Office has demonstrated that positive DNA identification may be made after fluorescence examination and any single chemical treatment, provided that simple guidelines are followed. If more than one fingerprint development technique is used in sequence, then the chances of successfully carrying out DNA identification are much reduced (Kent, 2004).

The U.K. work has shown that the most effective formulation for the acid dyes is as follows (Sears et al., 2005, p 741):

Fixing Solution—46 g 5-sulphosalicylic acid dehydrate dissolved in 1 L water.

Staining Solution—1 g acid dye dissolved in 700 mL distilled water, 250 mL ethanol, and 50 mL acetic acid.

Washing Solution—700 mL water, 250 mL ethanol, and 50 mL acetic acid.

The staining and washing solutions are flammable. Safety precautions must be taken if these solutions are used outside a fume cupboard with ambient temperatures above 28 °C (Kent, 2004).

7.13 Aqueous Techniques

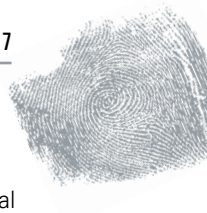
This section covers four commonly used aqueous metal deposition methods: those involving silver nitrate reagents, silver physical developers, multimetal deposition processes, and gun blueing reagents. Each of these methods involves reagents with metal salts dissolved in an aqueous carrier (or an alcohol, as in the case of some silver nitrate reagents). These reagents reveal water-resistant latent prints such as sebaceous prints (except for the silver nitrate reagents used on porous surfaces that target salt). Here, the metal ions are reduced to metal particles on the latent print residue (except for the case of latent prints on metal, where the print residue resists the deposition).

7.13.1 Silver Nitrate Reagents

7.13.1.1 History and Background. One of the first reagents used for developing latent prints on porous surfaces was a 1–3% aqueous solution of silver nitrate, $AgNO_3$. It was used as early as 1891 for this purpose (Forgeot, 1891; Rhodes, 1940, p 10). Most formulations now include an alcohol to hasten drying and to increase the wetness (reduce the surface tension) (Lee and Gaensslen, 2001, pp 105–175). The silver ions in silver nitrate react with the chloride ions in salt (sodium chloride, NaCl) contained in the latent print residue to form silver chloride (AgCl), a highly insoluble salt (Ksp = 1.8×10^{-10}) (Dean, 1985).

$$Ag^+ + Cl^- \rightleftharpoons AgCl \rightarrow K_{formation} = 1/K_{dissociation} = 1/K_{sp} = 5.6 \times 10^7$$

There are at least two reasons the silver nitrate treatment works well on porous surfaces. One is that the precipitation process is much faster than the dissolution process; that is, the reaction to form the insoluble AgCl is quicker



than the ability of the aqueous carrier to dissolve away the soluble NaCl salt. The second reason is that the insoluble AgCl gets trapped within the structure or "micro-roughness" (Kerr et al., 1981, pp 209–214) of the porous surface; that is, the fresh latent print residue is in an aqueous or semiaqueous form that soaks into the porous surface, carrying its constituents with it.

An ethanol-based 3% (w/v) silver nitrate reagent (90% ethanol and 10% water) develops prints on water-repelling surfaces such as waxed paper, cardboard with a wax finish, and Styrofoam (Trozzi et al., 2000). Here, the ethanol is used to reduce the dissolution of the NaCl in the fingerprint residue, to better wet the surface (because these surfaces are usually water-repellent), and to give faster evaporation. As expected, because of the low porosity of such surfaces, developed prints on these surfaces are more fragile than those on porous surfaces like paper and wood.

Under ordinary room light, the silver chloride gradually converts by photo-reduction to elemental silver; however, this is hastened with UV radiation. The most efficient development occurs with short-wavelength UV radiation (254 nm); however, the safer, long-wavelength UV radiation (366 nm) also develops prints, but less efficiently (Goode and Morris, 1983).

$$AgCl + h\nu \rightarrow Ag + \tfrac{1}{2}Cl_2$$

The elemental silver formed is colored dark brown to black (not a silver color). The reason for this is that the silver deposits as an aggregate of tiny (colloidal-size) silver particles, which makes for a highly porous surface that traps much of the light that strikes it. The formation of dark, light-trapping silver happens because the silver ions are reduced very quickly.

7.13.1.2 Application (Porous and Water-Repelling Surfaces). The silver nitrate reagent is usually applied to specimens by dipping them in the solution or by spraying the solution on the specimens. The FBI (Trozzi et al., 2000, pp 38–39) recommends the 3% $AgNO_3$ water-based formulation for porous surfaces and the 3% $AgNO_3$ ethanol-based formulation for water-repellent surfaces.

Champod et al. (2004, pp 153–154) recommend the 2% $AgNO_3$ methanol-based reagent for porous surfaces. After drying, the specimens are exposed to a high-intensity light source, UV light, or sunlight to develop the prints. As soon as the prints develop, they are photographed and the specimens are stored in the dark. Over time, the background darkens because of the gradual reduction of any residual silver nitrate in the specimens (this reduction is accelerated if exposed to light). Rinsing the specimens after development and then drying them in the dark does little to slow down the background development.

Goode and Morris (1983) reported in 1983 that immersing specimens in disodium ethylenediaminetetracetic acid (Na_2EDTA) complexes excess silver ions, which are then easily rinsed away with water. Their modified silver nitrate (MSN) procedure uses a 1% aqueous silver nitrate solution that also contains 5% Na_2EDTA and 3% K_2CO_3. The MSN procedure involves (1) treating the specimens with this modified reagent for just enough time to wet the surface, (2) transferring them to a 1% (w/v) Na_2EDTA solution and leaving them in for 1 minute, (3) removing and washing thoroughly with distilled water, and finally (4) placing this in a 5% thiourea solution containing 1% KOH for about 30 seconds to 2 minutes. The first step creates the silver chloride from the chloride ions in the latent print, and the last step converts this to black silver sulfide. Later in 1998, Price and Stow (1998, pp 107–110) recommended dipping the specimens in a "stopping solution" consisting of an aqueous solution of 40% methanol, 20% acetic acid, and 2% glycerol to suppress the further development of the background.

7.13.1.3 Enhancement. According to Lennard and Margot (1988, pp 197–210), weakly developed prints could be enhanced by treating the specimens with a diluted silver physical developer solution. The dilution factor is 1:10. Goode and Morris (1983) discuss a radioactive enhancing method that converts a silver print to a radioactive, β-emitting silver sulfide print, which is then imaged using radiographic film (this image-recording process is sometimes called autoradiography or β-radiography). If the original silver nitrate treatment did not significantly stain the background with silver, then this method will bring out only the developed prints with little or no interfering background. The process, described by Goode and Morris (1983) and reviewed by Cantu (2001, pp 29–64), involves converting the silver in the silver image to silver bromide (AgBr), using brominating (bleaching) methods, and then treating this with either sodium sulfide or thiourea (where the sulfur is radioactive ^{35}S) to convert AgBr to $Ag_2{}^{35}S$. The process is called radioactive toning. If the MSN procedure is used, which yields a silver sulfide print, then radioactive thiourea is used to form $Ag_2{}^{35}S$.

7.13.1.4 Limitation. The major drawback of the silver nitrate method is that the chloride ions in the latent print residue diffuse over time, and humidity accelerates this diffusion. This will affect the resolution (ridge detail) of the developed print. Normally, prints no older than 1 week will develop well; however, one should attempt to examine the evidence as soon as possible to avoid this diffusion. According to Goode and Morris (1983), in an indoor environment in the United Kingdom, prints on porous surfaces last longer (months) in the winter than in the summer (days to weeks). However, they state that these effects depend on factors such as the type of surface (prints last longer on paper than on raw wood) and, of course, the relative humidity. For this reason, the silver nitrate reagent is used now in special cases. The silver physical developer and multimetal deposition methods are more commonly used for water-insoluble components but do not target chlorine ions.

7.13.1.5 Use of Silver Nitrate on Metals. The discussion so far has been on the use of silver nitrate on porous and certain glossy (water-repellent) surfaces to develop chloride-bearing prints. Silver nitrate has also been used on certain metal surfaces, such as cartridge cases, to develop prints by depositing silver everywhere (giving a gray-to-black metallic appearance) except where the latent print sits (Olsen, 1978; Cantu et al., 1998, pp 294–298). That is, the silver nitrate brings out "lipid-bearing prints" because such prints protect the metal surface on which they lie from reacting with the silver nitrate. (If chloride ions are present in the latent print residue, silver chloride is formed; however, the contrast of the print against the background remains and may even be enhanced upon the reduction of silver chloride to dark silver.) The usual reaction of the silver ions (Ag^+) with the metal surface is

$$nAg^+ + M \rightleftharpoons nAg + M^{n+}$$

Here, M represents the metal and M^{n+} is a corresponding ion. Silver is said to displace the metal M. By observing the placement of the silver in the electromotive series (see Table 7–4), we see that silver can displace copper, iron, nickel, zinc, lead, and aluminum.

$$2Ag^+ + Cu \rightleftharpoons 2Ag + Cu^{2+} \qquad E^\circ_{redox} = 458 \text{ mV}$$

$$3Ag^+ + Al \rightleftharpoons 3Ag + Al^{3+} \qquad E^\circ_{redox} = 2461.6 \text{ mV}$$

Here, for example, E°_{redox} (Ag^+/Ag; Cu/Cu^{2+}) = E°_{red} ($Ag^+ + e^- \rightleftharpoons Ag$) + E°_{ox} ($Cu \rightleftharpoons Cu^{2+} + 2e^-$) is computed from the standard reduction potentials (Table 7–4) (Dean, 1985; Weast, 1986). A positive value of E°_{redox} indicates that the redox reaction is thermodynamically favorable but does not say anything about the rate or speed of the reaction. Another consideration is that these metals oxidize, some more readily than others, and this creates an oxide film on the metal surface. If a print was placed before the metal oxidized, the print may naturally show up, given enough time (some refer to this as the "print getting etched" on the metal). However, if it was placed after the oxide film formed, it is often difficult for the silver nitrate reagent to further oxidize the metal in this oxide film; thus, the deposition of silver and subsequent development of the print may occur but not as readily. The formation of a protective, impermeable oxide layer is called passivation (Atkins, 1990, p 927).

Table 7–4

Standard reduction potentials of several ionic and molecular species.

(E°) in mV	Half Reaction Standard Potential
$H_2O_2 + 2H^+ + 2e^- \rightleftharpoons 2H_2O$	+ 1776
$Au^{3+} + 3e^- \rightleftharpoons Au$	+ 1498
$2Cl_2 + 2e^- \rightleftharpoons 2Cl^-$	+ 1358
$OCl^- + H_2O + 2e^- \rightleftharpoons Cl^- + 2OH^-$	+ 810
$Ag^+ + e^- \rightleftharpoons Ag$	+ 799.6
$Fe^{3+} + e^- \rightleftharpoons Fe^{2+}$	+ 771
$H_2SeO_3 + 4H^+ + 4e^- \rightleftharpoons Se + 3H_2O$	+ 740
$Ag(NH_3)_2^+ + e^- \rightleftharpoons Ag + 2NH_3$	+ 373
$Cu^{2+} + 2e^- \rightleftharpoons Cu$	+ 341.9
$Ag_2O + H_2O + 2e^- \rightleftharpoons 2Ag + 2OH^-$	+ 342
$2H^+ + 2e^- \rightleftharpoons H_2$	0.0
$Fe^{3+} + 3e^- \rightleftharpoons Fe$	– 37
$Pb^{2+} + 2e^- \rightleftharpoons Pb$	– 126.2
$Ni^{2+} + 2e^- \rightleftharpoons Ni$	– 257
$Fe^{2+} + 2e^- \rightleftharpoons Fe$	– 447
$Zn^{2+} + 2e^- \rightleftharpoons Zn$	– 761.8
$Al^{3+} + 3e^- \rightleftharpoons Al$	– 1662

7.13.2 Silver Physical Developers

7.13.2.1 History and Background. The silver physical developer originated in photographic chemistry as an alternate method to the chemical developer for developing film (Cantu, 2001, pp 29–64; Bunting, 1987, p 85; Cantu and Johnson, 2001, pp 242–247). Exposing silver bromide or silver iodide crystals to light causes specks of silver to form on the crystal surface (Walls and Attridge, 1977, pp 104–108). These become "developing centers" (or "triggering sites") for either chemical or physical development. A silver physical developer deposits silver on exposed silver bromide crystals, whereas a chemical developer reduces the exposed silver bromide to silver. The fixing bath, in the former case, removes the unexposed silver bromide crystals and also the exposed silver bromide crystals (leaving behind the silver deposited on them) whereas, in the latter case, it only removes the unexposed silver bromide because the exposed silver bromide has been converted to silver.

Because of this process, the silver physical developer soon became known as one of the most sensitive reagents for detecting trace amounts of silver (Feigl and Anger, 1972, pp 423–424). Latent print examiners (Collins and Thomas) in the United Kingdom recognized this during the early 1970s (Goode and Morris, 1983) and applied it first to prints submitted to vacuum metal deposition. Then they expanded its use to other substances like fabrics and paper. It was found early on that the silver physical developer works better on porous than nonporous surfaces. Also, no one really knew which substances in latent print residue were responsible for causing the silver physical developer to work. That is, no one knew what was in fingerprint residue that acted as a developing center or triggering site (like the silver specks). It was not until recently that some plausible or reasonable explanations emerged.

A silver physical developer is an aqueous solution containing silver ions and a reducing agent that reduces the silver ions to silver, but it also contains two other sets of chemicals: one set keeps the reducing agent from reducing the silver ions to elemental silver unless a "triggering substance" is present (e.g., exposed silver bromide crystals in photographic film), and the other set keeps the solution stable. The first set suppresses the reducing ability of the reducing agent to the point that reduction occurs only when triggering sites are present. It, therefore, *suppresses the formation* of elemental silver in solution. However, due to this delicate balance, some spontaneous reduction occurs whereby colloidal-sized silver particles (nanoparticles) are formed in solution and, because these are triggering sites (i.e., they are silver specks), they grow. They grow in an autocatalytic way; that is, the silver that is formed triggers the reduction of more silver. Thus, the second set of chemicals suppresses this growth.

The silver physical developer currently used for latent print development on porous surfaces contains silver ions (silver nitrate) and ferrous ions (ferrous ammonium sulfate) as the principal components; citric acid and ferric ions (ferric nitrate) as the set of chemicals that *suppress the formation* of spontaneously formed colloidal silver particles; and a cationic and non-ionic surfactant as the set of chemicals that suppress the growth of such particles.

The net equation for the silver-deposition reaction is

$$Ag^+ + Fe^{2+} \rightleftharpoons Ag + Fe^{3+} \quad \rightarrow \rightarrow \quad E^\circ_{redox} = 28.6 \text{ mV}$$

E°_{redox} is computed from Table 7–4.

Adding citric acid reduces the concentration of ferric ions through the formation of ferric citrate and shifts the equilibrium of $Ag^+ + Fe^{2+} \rightleftharpoons Ag + Fe^{3+}$ to the right (forming elemental silver),

$$Fe^{3+} + H_3Cit \rightleftharpoons FeCit + 3H^+ \rightarrow \rightarrow \quad K_{formation} = 0.398 \text{ at } 25\ ^\circ C$$

However, for every ferric citrate molecule formed, three protons are released and these drive the equilibrium to the left (suppression of the formation of elemental silver). The overall reaction is

$$Ag^+ + Fe^{2+} + H_3Cit \rightleftharpoons Ag + FeCit + 3H^+ \quad E^\circ_{redox} = 5.0 \text{ mV}$$

Thus, adding citric acid reduces the E°_{redox} from 28.6 mV to 5 mV. This reduction facilitates adjusting the concentrations of the components (citric acid and the ferric, ferrous, and silver salts) so that the reduction of silver ions to elemental silver nanoparticles occurs only on the triggering sites and not in solution. However, even with this suppression of their formation rate, those that do form become nucleating (triggering) sites for further deposition of silver (the process is autocatalytic) and consequently grow until they precipitate. This will eventually deplete most of the silver ion solution (depending on the concentration of ferrous ions initially present).

To bring stability to the solution, the silver particles formed must somehow have their triggering ability blocked. This is where surfactants become important.

When silver nanoparticles are spontaneously formed, they get surrounded by citrate ions (each of which carries three negative charges) in solution and thus acquire a negative charge. The main surfactant used to suppress the growth of any spontaneously formed silver nanoparticles is a positively charged cationic surfactant, n-dodecylamine acetate. The reason for choosing a cationic surfactant is that it helps suppress the negative charge of the silver nanoparticles formed. This will then reduce the attraction of positive silver ions toward the particles and thus reduce the possibility of their growth (by the reduction of silver on their surface). The cationic surfactant surrounds the negatively charged silver particle in a staggered way, with as many positive ends pointing toward the particle as pointing away from the particle (Cantu, 2001, pp 29–64; Cantu and Johnson, 2001, pp 242–247; Jonker et al., 1969, pp 38–44). This surfactant-encapsulated particle is said to be encased in a *micelle*. A non-ionic surfactant, Synperonic-N, is used in conjunction with n-dodecylamine acetate to aid the dissolution of the latter.

On exposed photographic film or paper, the silver physical developer works by reducing its silver ions on the silver specks (nucleating sites) found on the surface of exposed silver bromide crystals and nowhere else. Being an autocatalytic process, the deposition of silver on the nucleating sites continues until it is stopped, for example, by removing the sample from the solution and rinsing it with water. The surfactant-stabilized silver physical developer remains stable and active for several weeks. If silver ions come in contact with hydroxyl ions, insoluble silver hydroxide (AgOH) is formed, which converts to brownish-black silver oxide (Ag_2O). Today, most paper is alkaline (basic) because it contains calcium carbonate ($CaCO_3$) as filler. When wet, it is basic and will turn black when dipped in a silver nitrate solution and will dry to a brownish-black color. Consequently, alkaline paper must be neutralized before submitting it to silver physical development. Any acid that does not furnish chlorides (which react with silver to form insoluble AgCl) will neutralize the $CaCO_3$. The neutralization reaction involves the release of carbon dioxide bubbles:

$$CaCO_3 + 2H^+ \rightarrow Ca^{2+} + H_2O + CO_2 \text{ (gas)}$$

The following are the rudiments of some concepts that help explain how the silver physical developer visualizes latent prints on porous surfaces.

The Charge of Latent Print Residue (at low pH). It is fortuitous that the silver physical developer is acidic, with a pH of about 1.38 because this helps explain why it works in developing latent prints on porous surfaces. It is known (Saunders, 1989) that when latent print residue (on a porous or nonporous substrate) is immersed in a colloidal gold solution of pH < 3, colloidal gold nanoparticles selectively deposit on the residue. This suggests that at pH < 3, the latent print residue acquires a positive charge. It is also known that colloidal gold at low pH is used to "stain" proteins and this happens because, at low pH, the amine groups (e.g., $R\text{-}NH_2$) in proteins acquire a positive charge upon protonation ($R\text{-}NH_3^+$). Therefore, one possibility is that latent print residue contains proteins that initially were dispersed in latent print residue but, after drying, became nondispersible. It is also known that alkenes (olefins) can acquire a positive charge in an acidic environment (either a carbonium ion or a protonated alcohol is formed). Therefore, another possibility is that latent print residue contains olefins.

The Surface Area of Proteins in Porous Surfaces and Their Binding to Cellulose. It was recognized early on that the silver physical developer works best on porous surfaces, particularly cellulose-based surfaces such as paper and cardboard. When latent print residue is placed on such surfaces, the surfaces' porosity causes the residue to penetrate and thus cover a large surface area; this then exposes more nucleating (triggering) sites for silver physical development than if it did not spread out (as in a nonporous surface). Furthermore, if amines are present (as in proteins), they can form hydrogen bonds with the hydroxyl groups in the cellulose.

The Deposition of Silver Particles on Latent Print Residue. In the silver physical developer, a newly formed silver nanoparticle is negatively charged (due to adhered citric acid ions) and attracts the positive amine "head" ($R\text{-}NH_3^+$) of several surfactant molecules, which eventually envelop the entire particle (in the staggered configuration mentioned above). However, the nanoparticle also gets attracted to the positively charged latent print residue. Once one of these nanoparticles reaches the residue, it gets neutralized. The avalanche of silver particle deposition occurs on this initial particle (because each silver particle is now a nucleating site—the autocatalytic effect) and it grows. The final result is an agglomeration of numerous "grown" particles (about 10–40 μm in diameter) along the latent print residue.

Formulation. The formulation of the silver physical developer reagent provided in Section 7.14 is the original British

formulation (Kent, 1998), which is very close to the Dutch formulation (Jonker et al., 1969, pp 38–44) used in the photofabrication of circuit boards. The procedure for visualizing prints on porous surfaces, however, involves three steps: a pretreatment step, the silver physical development step, and a post-treatment step.

Other formulations for the silver physical developers exist (Cantu, 2001, pp 29–64), but one that is currently used by many is based on using high-purity water, such as that produced by water purification units that use reverse osmosis and deionizing technologies (the water is referred to as RO/DI water). By using such water, less detergent is needed (2.8 g of each, instead of 4.0 g) (Kent, 1998), although the performance is adversely affected. However, Burow et al. (2003, pp 1094–1100) showed that one can also reduce the amount of several other components and end up with a reagent that performs as well or better than the traditional reagent (it does, however, involve adding malic acid to the reagent); the cost reduction is about 16%. Seifert, Burow, and Ramotowski (from the U.S. Secret Service forensic laboratory) showed (unpublished results) that Tween 20 can be used instead of Synperonic-N.

The hypochlorite step is an enhancing step. It does two things: it lightens (bleaches) the paper and darkens the silver print. The print becomes darker through the formation of silver oxide ($OCl^- + 2Ag \rightarrow Ag_2O + Cl^-$ $E^\circ_{redox} = 550$ mV; see Table 7–4). Other enhancement methods are treated by Cantu (2001, pp 29–64) as well as bleaching methods. One bleaching method, used successfully in bringing out a developed print found on highly patterned printing, converts the silver print to a whitish silver iodide print and darkens the background through the starch–iodine reaction (Cantu et al., 2003, pp 164–168).

7.13.2.2 Application. The procedure for using the silver physical developer involves three treatments in sequence and *in the same glass tray.* The latter point is important in that it simplifies the process and saves time. It is based on the fact that residual reagent from one treatment does not affect the performance of the next treatment. The three treatments are the acid pretreatment, the silver physical developer treatment, and the hypochlorite post-treatment. Occasionally, a distilled water pretreatment precedes these to remove any dirt or soil from the specimens. This, as well as the other treatments, removes any prints developed with ninhydrin, and writing or printing made with water-soluble inks (e.g., some roller ball pen inks and inkjet printing inks). Also, a tap water post-treatment is done between the silver physical development and the hypochlorite post-treatment. Again, all this is done in one glass tray.

Water Pretreatment—This is designed to remove dirt and soil, if present.

Acid Pretreatment—This reacts with calcium carbonate in alkaline paper, causing release of carbon dioxide as bubbles, and neutralizes the paper.

Silver Physical Developer Treatment—This is done in subdued light to avoid the photo-reduction of silver ions to elemental silver (which results in background development and weakens the reagent). The tray is rocked back and forth; within 10 minutes, prints begin to develop and continue with increasing contrast. Good development occurs within 10–30 minutes.

Water Post-Treatment—This is done with running tap water for about 5 minutes to remove excess silver physical developer (and any silver chloride that may form).

Hypochlorite Post-Treatment—The treatment time is about 2–3 minutes. This lightens the background and darkens the silver print.

Washing and Drying—The specimens are washed in tap water and dried (e.g., by using a photodryer or by air-drying on blotter paper).

7.13.3 Multimetal Deposition Methods

7.13.3.1 History and Background. In the late 1980s, Dr. George Saunders, then with the Los Alamos National Laboratory in Los Alamos, NM, visited the U.S. Secret Service forensic laboratory to share ideas about techniques for latent fingerprint development. He presented a novel idea that he initially called a Universal Process for Fingerprint Detection and later, because it involved the deposition of two metals, the multimetal deposition (MMD) method. He based his idea on an existing method used for staining proteins, antibodies, and other macromolecules (e.g., proteins separated on membranes or gels). This method involved staining with colloidal gold (whereby colloidal gold binds to the macromolecule) and enhancing (or amplifying) this gold "signal" or stain with a silver physical developer.

The binding of colloidal gold to proteins was first observed in 1939 by Kausche and Ruska (1939, pp 21–24). In 1971, Faulk and Taylor (1971, pp 1081–1083) used this property

to bind rabbit anti-Salmonella antiserum, and the resulting coated colloid was then used to label the surface of Salmonella bacteria. The labeling mechanism was detectable through the electron microscopic image of the gold. In 1983, DeMey (1983, pp 82–112) used uncoated gold to directly stain proteins on membrane surfaces. Also in 1983, Holgate et al. (1983, pp 938–944) showed that a gold stain can be intensified with silver staining. They basically recognized that gold colloids are (1) highly negatively charged particles that bind to many macromolecules and (2) activation (triggering) sites for silver physical development. The colloidal gold particles acquire their negative charge through the adsorption of citrate ions (each carries three negative charges) on their surface (the citrate ions come from the sodium citrate used in the formulation).

Saunders knew that fingerprint residue contains macromolecules like proteins and lipoproteins and, therefore, should be able to be visualized through the staining and enhancing ability of the colloidal gold and physical developer technique. He formulated his own colloidal gold solution using the Frens method (Frens, 1973, pp 20–22) and silver physical developer. He called the latter the *modified* physical developer to distinguish it from the traditional silver physical developer used to visualize latent prints on porous surfaces. By formulating his own reagents, he was able to optimize them. The process was soon found to visualize latent prints on porous and nonporous surfaces; the latter includes surfaces like glass, metal, ceramic, and plastic, whether they are dark or light.

Thus, the MMD process is basically a silver physical development process that is preceded by a colloidal gold treatment; the gold treatment provides the latent print residue with the nucleating sites (gold colloids) for silver physical development. Like the silver physical developer, the MMD process develops the water-insoluble components of latent print residue (e.g., the sebaceous portion of the residue).

For visualizing latent prints on porous surfaces with the MMD process, Saunders provided two important comments for the users. One is that, on porous surfaces, extensive rinsing must be done after the colloidal gold treatment to reduce possible background development. This is because colloidal gold particles get trapped in the pores and become triggering sites for silver physical development. The other is that the zinc salt treatment, sometimes done after the ninhydrin process, should be avoided. Divalent ions such as Zn^{2+} have a tendency to bind to colloidal gold and, therefore, trapped divalent ions in the surface's pores attract the colloidal gold particles and the entire surface is subject to silver physical development.

7.13.3.2 Formulation (MMD). The MMD process involves two reagents used in sequence: the colloidal gold solution and the modified silver physical developer.

There are two points of note regarding the modified silver physical developer: One is that Tween 20, a non-ionic surfactant, is used instead of a more stabilizing cationic surfactant. Cantu and Johnson (2001, pp 242–247) speculate that this may be because a cationic surfactant would surround bound gold particles (that still carry some negative charge) and therefore hinder the physical development process on them. The second point is that the silver ion concentration of the working solution is only 0.2%, and this is apparently low enough that no "blackening" (formation of silver oxide) occurs on the surface of alkaline (basic) paper. Thus, no acid pretreatment is needed to neutralize such paper (which normally contains calcium carbonate). The colloidal gold solution has a pH of about 2.8 and, therefore, causes some neutralization of such paper, but the divalent calcium ions that are generated apparently do not significantly destabilize the gold solution (they may on the surface where they are formed). Examples of latent prints developed (on a variety of surfaces) using the MMD process are found in Figure 7–23.

7.13.3.3 Formulation (MMD II). In 1993, Dr. Bernard Schnetz presented his work, carried out at the Institut de Police Scientifique et de Criminologie of the University of Lausanne, on biochemical techniques for amplifying colloidal gold-treated latent prints. He treated latent prints with colloidal gold, attached a protein to the colloidal gold particles (already bound to latent print residue), and amplified these with enzymes or stains that form colored or fluorescent products (Schnetz, 1993). In 1997, he reported on an update to this work and also on his variation of the multimetal deposition (MMD II) process (Schnetz, 1997), and in 2001, he and Margot published their work on its optimization (Schnetz and Margot, 2001, pp 21–28). Like the MMD process, this is a two-step process, but it uses siliconized glassware, colloidal gold with a particle size of 14 nm diameter (compared to 30 nm for the Saunders colloidal gold), and a silver physical developer quite different from the Saunders modified silver physical developer.

Dr. John Brennan, recently retired from the Forensic Science Service (London, U.K.), has successfully used the

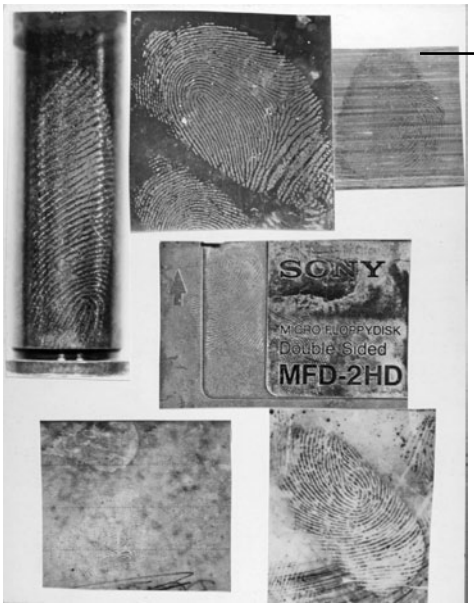


FIGURE 7–23

Latent prints visualized by the MMD process on a variety of surfaces. Top left: revolver cartridge case. Top middle: adhesive side of black Mylar tape. Top right: adhesive side of heavy-duty strapping tape. Middle: plastic and metal surfaces of a computer disk. Bottom left: paper label of computer disk. Bottom right: plastic credit card. Notice that the developed prints appear dark on light-colored surfaces and light on dark-colored surfaces.

MMD and MMD II processes on several evidence types and tends to favor the MMD II (J. Brennan, private communication). Dr. Naomi Jones presented her doctoral thesis several metal deposition methods; she also found that the MMD II process surpassed the MMD process in performance (Jones, 2002).

7.13.4 Gun Blueing Reagents

7.13.4.1 History and Background. Gun blueing is used to refinish gun barrels with a bluish sheen. One is warned not to leave fingerprints on the barrel because the gun blueing solution will not work there (Angier, 1936, p 6). The Bundeskriminalamt (BKA) in Germany discovered that this was also true on bullet cartridges (Cantu et al., 1998, pp 294–298). Thus was the birth of gun blueing solutions for visualizing latent prints on metal surfaces, particularly those of bullet cartridges.

7.13.4.2 Metal Deposition and Etching. Gun blueing of metals involves the simultaneous deposition of two metals, selenium and copper, on a metal surface. The bimetal deposited is blue-black in color.

As discussed previously for silver nitrate, the sebaceous print resists the deposition, and silver deposits (as a gray-to-black metal) everywhere, except where the fingerprint exists. To be more precise about what is occurring, we should note that the deposition process is always accompanied by an etching process. For silver on copper, silver ions deposit (the deposition or reduction process) as cupric ions are removed (the etching or oxidation process). There are, however, etching processes that do not involve metal deposition (e.g., etching with acidified hydrogen peroxide) (Cantu et al., 1998, pp 294–298), and these processes are also hindered by sebaceous material.

Other one-metal deposition methods for revealing latent prints on cartridge cases include the use of palladium (Migron and Mandler, 1997, pp 986–992) and selenium (Bentsen et al., 1996, pp 3–8). Besides showing that palladium can reveal sebaceous prints on metal, Migron and Mandler did an extensive analytical study of how the deposition process works on brass surfaces containing sebaceous prints. The work by Bentsen and colleagues on the deposition of selenium is similar to what gun blueing does and is, therefore, discussed below, along with gun blueing.

7.13.4.3 General Composition. There are several manufacturers of gun blueing solutions, and no two solutions have exactly the same formulation, but all contain the three necessary active ingredients: selenious acid, a cupric salt, and an acid. An acidified solution of selenious acid is a relatively strong etching (oxidizing) reagent, as noted by the oxidation potential (Table 7–4):

$$H_2SeO_3 + 4H^+ + 4e^- \rightleftharpoons Se + 3H_2O \qquad E^\circ = +740 \text{ mV}$$

Note that acid (H^+) is needed, and this is why the blueing solution also contains an acid. Table 7–4 shows that an acidic solution of selenious acid can oxidize and etch copper, lead, nickel, zinc, and aluminum. A solution of cupric ions is also a strong etching (oxidizing) reagent capable of oxidizing lead, nickel, zinc, and aluminum.

As *each* of these reagents etches, the metal ions get deposited on what is etched. For example, on aluminum, the oxidation and reduction (etching and deposition) reaction is

$$3H_2SeO_3 + 12H^+ + 4Al \rightleftharpoons 3Se + 9H_2O + 4Al^{3+}$$

$$E^o_{redox} = 2402 \text{ mV}$$

$$3Cu^{2+} + 2Al \rightleftharpoons 3Cu + 2Al^{3+} \quad E^o_{redox} = 2003.9 \text{ mV}$$

If *both* reagents are present together, as in the gun blueing solution, then the ratio of Cu to Se that deposits depends on the speed (kinetics) of each of the two competing reactions; it is possible that a 1:1 Cu–Se alloy is formed, but it is not certain. The final result is a blue-black metallic coating (everywhere except where a sebaceous latent print exists).

The composition of some gun blueing solutions is given in Table 7–5. Note that all involve selenious acid (one involves selenium dioxide, which is the anhydrous form of selenious acid), a cupric salt, and an acid. Interestingly, Bentsen et al. (1996, pp 3–8) used a 0.4% selenious acid solution (without cupric ions or acid) to develop prints on metal surfaces such as spent cartridge cases. This deposits selenium metal on the metal being treated and this solution, along with the vacuum cyanoacrylate ester treatment, was rated highly among other methods tested.

7.13.4.4 Formulations. Cantu et al. (1998, pp 294–298) recommend a 1:80 dilution of a commercial gun blueing solution. Leben and Ramotowski (1996, pp 8, 10) recommend a stronger solution (a 1:40 dilution) and indicate that an improvement over just using the diluted gun blueing reagent is to treat the metallic specimens first (e.g., cartridge cases) with CA fumes. Table 7–6 is a summary of their recommendations.

7.13.4.5 Application. Since its introduction by the BKA, gun blueing is now used in several laboratories because of its ease. As mentioned above, a CA ester treatment prior to gun blueing improves the detection of latent prints on metal. After treatment, there is a tendency for the gun blueing solution to continue its deposition, and several arresting methods have been proposed (Cantu et al., 1998, pp 294–298). These include dipping in a sodium bicarbonate solution, dipping in clear varnish (Bentsen et al., 1996, pp 3–8), applying a lacquer spray (private communication from Anton Theeuwen and Josita Limborgh, Netherlands Ministry of Justice, Forensic Science Laboratory), and using fingernail polish (private communication from Vici Inlow, U.S. Secret Service forensic laboratory).

If overdevelopment occurs, then acidified hydrogen peroxide is recommended for removing excess gun blue deposit (Cantu et al., 1998, pp 294–298). If we assume the copper–selenium alloy is a 1:1 adduct, then the net reaction for its removal is

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

A suggested composition for the acidified hydrogen peroxide solution is 5 volumes of household vinegar (5% acetic acid) and 7 volumes of household hydrogen peroxide (3% hydrogen peroxide). This is based on stoichiometry of the above equation. However, a 1:1 mixture also works well.

7.13.4.6 Comments on Etching. Etching without metal deposition can also reveal prints on metal surfaces by the contrast formed between the etched background and the unetched latent print. From Table 7–4 it can be seen that acid can displace iron, lead, nickel, zinc, and aluminum. Acidified hydrogen peroxide, however, will also displace copper. Cantu et al. (1998, pp 294–298) noted that acidified hydrogen peroxide visualizes prints rather well on many metal surfaces. They provide a lengthy discussion and explanation of why the etching process should be carefully watched: the metals that are etched out as ions can redeposit as the process continues.

Schütz et al. (2000, pp 65–68) compared etching and gun blueing methods with the multimetal deposition method on their ability to develop latent prints on cartridge cases. They found that (1) gun blueing excelled in visualizing sebaceous prints on brass cartridge cases, (2) for aluminum cartridge cases, MMD worked best (the modified physical developer step, performed after the colloidal gold step, brought out the print contrast), and (3) nothing worked well for lacquered steel cartridge cases. For the latter, they recommend CA fuming.

7.13.5 Sudan Black B

7.13.5.1 History and Background. Sudan black B (herein referred to as Sudan black) was initially used in laboratories for biological testing or chemical screening for fatty components (Figure 7–24). The reaction produces a blue-black product or image. Sudan black was initially reported for use as a friction ridge development technique in 1980 by Mitsui, Katho, Shimada, and Wakasugi of the Criminal Science Laboratory in Nagoya-shi, Japan (Mitsui et al., 1980, pp 9–10; 1981, pp 84–85).

Table 7–5

Composition of some gun blueing solutions*

	Manufacturer	Birchwood-Casey						Brownells	Outers	E. Kettner
	Trade name	Perma Blue Liquid Gun Blue PB22	Perma Blue Immersion Blue PBIM	Perma Blue Paste Gun Blue SBP 2	Super Blue Extra Strength	Brass Black Metal Touch Up BB2	Aluminum Black PAB 17	Formula 44/40 Instant Gun Blue	Gunslick Gun Blue	Waffen-Brünierung
	Used for	Steel	Steel	Steel	Steel	Brass, Bronze, Copper	Aluminum	Steel	Steel	Steel
Selenium Compounds	Selenious Acid	3	4	2	5	3	4	6		
	Selenium Dioxide								3	
Cupric Salt	Cupric Chloride			3						
	Cupric Nitrate				4					
	Cupric Sulfate	3	4			4	8	8	2	
Zinc Salt	Zinc Sulfate					5				
Nickel Salt	Nickel Sulfate						1		2	
Acid	Hydrochloric									
	Nitric	3	2		4					
	Phosphoric		4	4	4	8	3			
	Fluoboric						2			
	Amido sulfonic							12		
Solution pH (dilution factor)		2.3 (1/40)	N/A(1/40)	1.5	2.3 (1/80)	2.3 (1/40)	2.3 (1/80)	1.8 (1/80)	3.3 (1/40)	2.3 (1/80)
Other	Polyethylene Stearyl Ether			15						
	Octylphenoxy Polyethoxyethanol				1					
	Ammonium Molybdate					4				
	Ammonium Bifluoride			1						

*All concentrations are as maximum percent. Except for pH, all information is obtained from Material Safety Data Sheets.

Table 7–6

Summary of recommended protocols for treating cartridge cases.

Nickel Plated Brass	Brass Black	1 mL GB in 40 mL distilled water	Other solutions also worked well on these casings.
Lacquered Steel	Super Glue Only		None of the gun blue solutions produced identifiable detail.

Sudan black is a dye stain used for the detection of sebaceous components of friction ridge skin residue on nonporous and some semiporous substrates (e.g., latex gloves and some ceramics) (Figure 7–25). This dye stain also detects friction ridge skin detail where the friction ridge skin or the substrate has been contaminated with grease, food residue, or dried deposits of soda or sweetened (e.g., by fructose or sucrose) drinks. It has also been used to enhance friction ridge detail previously treated by the CA fuming technique.

The color and porosity of the substrate will need to be considered. Porous substrates tend to absorb the dye, resulting in a lack of contrast between the friction ridge detail and the item background. Because of the blue-black color of the dye stain, there will be a lack of contrast between the friction ridge detail and dark-color items. It is recommended that other processing techniques be used on these items (Stone and Metzger, 1981, pp 13–14).

Dried Sudan black-processed prints have been lifted using conventional lifting tape (as used with the powder processing technique). It has been successful on waxy coated, glossy, and smooth substrates. However, it has been less successful on heavily contaminated, uneven, and semiporous substrates.

7.13.5.2 Validation of Reagent and Application Technique. As in the application of all reagents and processes, it is suggested that the Sudan black solution be validated before use. To validate the solution, contaminate a nonporous substrate with the targeted matrix (e.g., sebaceous-, grease-, fructose-, sucrose-, or food-contaminated friction ridge detail or material), then apply the Sudan black solution to the substrate in the manner noted below. If no reaction is observed, the solution or the validation matrix will require further evaluation. It is sound practice to be familiar with the application technique and the reaction(s) with the substrate and matrix before applying them to evidence.

Review the material safety data sheets for safety, handling, and storage information.

7.13.5.3 Reagent Solution. The reagent solution consists of 15 g of Sudan black dissolved in 1 L of ethanol or methanol, creating a blue-black color solution, which is

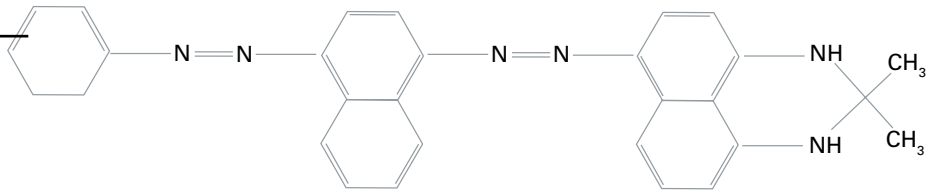


FIGURE 7–24

The chemical structure of sudan black.

FIGURE 7–25

Sebaceous friction ridge detail on a plastic substrate processed with sudan black.

then added to 500 mL of distilled water and stirred until completely mixed. The shelf life of the solution is indefinite.

7.13.5.4 Sequential Methodology and Processing Technique. Before processing with sudan black, view the item with a forensic light source to detect any inherent fluorescence of the friction ridge residue or the substrate. Photograph any visible detail.

- Place the Sudan black solution in a clean glass or metal dish, pouring in a sufficient amount to submerge the item being processed. The solution can also be applied by spraying. It is recommended that the immersion technique be used to prevent inhalation of airborne particulate spray.
- Allow item to be immersed in the solution for approximately 2 minutes. If the item has been previously processed using the CA fuming technique, the item may require longer immersion time in the solution.
- Rinse item under cool or cold, slow-running tap water, or place item in a clean dish containing cool or cold water until excess dye is removed from the background.
- Allow item to dry at room temperature.
- View the developed blue-black image; faint images have been improved by a second treatment with the Sudan black solution (follow the previous application steps). It is suggested that the item be viewed with a forensic light source after Sudan black processing because the background may fluoresce, creating enhanced contrast.
- Any developed images will need to be photographed for comparison, documentation, and archival purposes.

7.14 Formulations for Chemical Solutions

7.14.1 Ninhydrin (Kent, 1998; Champod et al., 2004, p 239)

Stock Solution: 25 g ninhydrin dissolved in 225 mL absolute ethanol, 10 mL ethyl acetate, 25 mL glacial acetic acid.

Working Solution: 52 mL of stock solution diluted to 1000 mL with HFE 7100.

7.14.2 Zinc Chloride Solution (Champod et al., 2004, p 240)

Stock Solution: 8 g zinc chloride dissolved in 180 mL ethanol, 20 mL glacial acetic acid.

Working Solution: 6 mL of stock solution diluted to 100 mL with carrier solvent (e.g., HFE 7100).

7.14.3 1,8-Diazafluoren-9-one (DFO) (Kent, 1998; Champod et al., 2004, p 230)

0.25 g of DFO dissolved in 30 mL methanol and 20 mL glacial acetic acid. Add this to 725 mL HFE 7100 and 275 mL HFE 71DE.

7.14.4 Nonpolar Ninhydrin (Stimac, 2003a, pp 185–197)

Stock Solution: 1.5 g ninhydrin dissolved in 100 mL HFE 71IPA (may require refluxing at low temperature).

Working Solution: 15 mL of stock solution diluted with 100 mL HFE 7100.

7.14.5 1,2-Indanedione

0.25 g 1,2-indanedione dissolved in 90 mL ethyl acetate and 10 mL glacial acetic acid. Add this to 1 L of HFE 7100 (Merrick et al., 2002, pp 595–605).

or

2 g 1,2-indanedione dissolved in 70 mL ethyl acetate. Add this to 1 L HFE 7100 (Almog et al., 1999, pp 114–118).

7.14.6 5-Methylthioninhydrin (5-MTN) (Wallace-Kunkel et al., 2006, pp 4–13)

1.7 g 5-MTN dissolved in 52.5 mL ethyl acetate, 50 mL methyl tert-butyl ether, 12.5 mL absolute ethanol, and 5 mL glacial acetic acid. Add this to 360 mL HFE 7100.

7.14.7 2-Isononylninhydrin (INON, Thermanin) (Al Mandhri and Khanmy-Vital, 2005)

4–5 g INON dissolved in 15 mL ethyl acetate, 5 mL isopropanol, and 980 mL HFE 7100.

7.14.8 Silver Nitrate (Trozzi et al., 2000, pp 38–39)

Dissolve 30 g silver nitrate in 1 L distilled water.

or

Dissolve 30 g silver nitrate in 100 mL distilled water and add to 1 L ethanol.

7.14.9 Physical Developer (Kent, 1998)

Acid Pretreatment: 30 g of maleic acid is dissolved in 1 L distilled water. Indefinite shelf life.

Stock Solution #1: 30 g ferric nitrate nonahydrate dissolved in 900 mL distilled water. 80 g ferrous ammonium sulfate hexahydrate dissolved in this solution. 20 g anhydrous citric acid dissolved in this solution. Shelf life may be several months.

Stock Solution #2: 4 g n-Dodecylamine acetate dissolved in 1 L distilled water. 4 g (4 mL) Synperonic N added to this solution. Indefinite shelf life.

Stock Solution #3: 20 g silver nitrate dissolved in 100 mL distilled water. Indefinite shelf life.

Working Solution: 900 mL stock solution #1. Add 40 mL of stock solution #2 and stir for 5 minutes. Add 50 mL of stock solution #3 and stir for 5 minutes. Shelf life is 1–2 weeks.

Hypochlorite Post-Treatment Solution: 100 mL of household chlorine bleach (~6% NaOCl) is mixed with 100 mL water.

7.14.10 Multimetal Deposition (Saunders, 1989, 1996, 1997)

Colloidal Gold Solution

Stock Solution #1: 10% (w/v) tetrachlorauric acid ($HAuCl_4.3H_2O$) in high-purity (RO/DI) water.

Stock Solution #2: 1% (w/v) trisodium citrate ($Na_3Cit.2H_2O$) in high-purity water.

Stock Solution #3: 0.5 M (10.5% w/v) citric acid ($H_3Cit.H_2O$) in high-purity water.

Stock Solution #4: 1% Polyethylene glycol.

Working Solution: Add 1 mL of stock solution #1 to 1 L of high-purity water and bring to a boil. Rapidly add 10 mL of stock solution #2 and boil gently for 10 minutes. Add 5 mL of Tween 20 (or Tween 80) and mix well. Add 10 mL of stock solution #4 to the cooled solution and adjust the pH to 2.7 using stock solution #3. Restore total volume to 1 L with high-purity water. Shelf life is 3 months.

Modified Silver Physical Developer Solution

Stock Solution #1: Dissolve 33 g ferric nitrate nonahydrate in 1 L of high-purity water. Add 89 g of ferrous ammonium sulfate hexahydrate to the solution. Add 22 g of citric acid to the solution. Add 1 mL of Tween 20 to the solution.

Stock Solution #2: 20% (w/v) silver nitrate in high-purity water.

Working Solution: Add 1 part of stock solution #2 to 99 parts of stock solution #1. Only stable for 15 minutes.

Application

Prewashing: Porous items should be washed several times in high-purity water.

Colloidal Gold: Soak items in colloidal gold solution for 30–120 minutes, but avoid overdevelopment.

In-Between Rinsing: Rinse items in high-purity water. For porous items, use several water changes for 15 minutes or more.

Silver Physical Developer: Place items into freshly made solution. Silver amplification occurs within 10–15 minutes.

Postwashing: Rinse with tap water. Air dry.

7.14.11 MMD II (Schnetz and Margot, 2001, pp 21–28)

Silanization of Glassware

Soak glassware for 8 hours in 10% Extran MA 01 alkaline liquid (Merck). Rinse with high-purity hot water, then high-purity cold water. Dry in an oven at 100 °C. Soak for 5 seconds in 2% (v/v) 3-aminopropyltriethoxysilane in acetone. Rinse twice with acetone, then water. Dry in an oven at 42 °C for 8 hours.

Colloidal Gold Solution

Stock Solution #1: 10% (w/v) tetrachlorauric acid in high-purity water.

Stock Solution #2: 1% (w/v) sodium citrate in high-purity water.

Stock Solution #3: 0.5 M citric acid in high-purity water.

Stock Solution #4: 1% (w/v) tannic acid in high-purity water.

Working Solution #1: Add 0.5 mL of stock solution #1 to 400 mL of high-purity water. Heat to 60 °C.

Working Solution #2: Add 20 mL of stock solution #2 and 0.1 mL of stock solution #4 to 75 mL of high-purity water. Heat to 60 °C.

Once both solutions reach 60 °C, rapidly add working solution #2 to working solution #1 and mix vigorously. Heat the mixture to boiling, cool, and adjust to 500 mL with high-purity water. Solution can be stored in a plastic bottle at 4 °C. Before use, bring to room temperature, add 0.5 mL Tween 20 (or Tween 80), and adjust pH to 2.7 with stock solution #3.

Silver Physical Developer Solution

Stock Solution #1: 24 parts 25.5% (w/v) citric acid solution, 22 parts 23.5% sodium citrate solution, and 50 parts high-purity water. Adjust to pH 3.8 with additional citric acid or sodium citrate solution.

Stock Solution #2: 0.2% (w/v) silver acetate solution.

Stock Solution #3: 0.5% (w/v) hydroquinone in stock solution #1.

Rinsing Solution: 0.25% (w/v) hydroquinone (1 part stock solution #3 and 1 part high-purity water).

Working Solution: One part stock solution #2 and one part stock solution #3. Unstable, so prepare just before use.

Application

Prewashing: Porous items should be washed with high-purity water for 2 minutes. Nonporous items need only brief washing in high-purity water.

Colloidal Gold: Soak items in colloidal gold solution for 5–15 minutes with mild agitation.

In-Between Rinsing: Rinse briefly in high-purity water.

In-Between Hydroquinone Rinsing: Rinse for 2–5 minutes in hydroquinone rinsing solution.

Silver Physical Development: Place items in silver physical developer for about 18 minutes.

Postwashing: Rinse with high-purity water.

Fixing: Fix with 1:9 dilution of photographic fixer for 2–5 minutes, rinse with tap water, air dry.

7.15 Reviewers

The reviewers critiquing this chapter were Christophe Champod, Sue Manci Coppejans, Christine L. Craig, Robert J. Garrett, Deborah Leben, Bridget Lewis, Jon T. Stimac, Juliet H. Wood, and Rodolfo R. Zamora.

7.16 References

Abderhalden, E.; Schmidt, H. Utilization of Triketohydrindene Hydrate for the Detection of Proteins and Their Cleavage Products. *Z. Physiologische Chem.* 1911, *72,* 37.

Al Mandhri, A.; Khanmy-Vital, A. Detection of Fingerprints on Paper with 2-Isononyl-Ninhydrin. Presented at the 17th Meeting of the International Association of the Forensic Sciences. Hong Kong, 2005.

Almog, J. Fingerprint Development by Ninhydrin and Its Analogues. In *Advances in Fingerprint Technology;* Lee, H. C., Gaensslen, R. E., Eds.; CRC Press: Boca Raton, FL, 2001.

Almog, J.; Hirshfeld, A. 5-Methoxyninhydrin: A Reagent for the Chemical Development of Latent Fingerprints That Is Compatible with the Copper Vapour Laser. *J. Forensic Sci.* 1988, *33* (4), 1027–1030.

Almog, J.; Hirshfeld, A.; Klug, J. T. Reagents for the Chemical Development of Latent Fingerprints: Synthesis and Properties of Some Ninhydrin Analogues. *J. Forensic Sci.* 1982, *27* (4), 912–917.

Almog, J.; Hirshfeld, A.; Frank, A.; Grant, H.; Harel, Z.; Ittah, Y. 5-Methylthio Ninhydrin and Related Compounds: A Novel Class of Fluorogenic Fingerprint Reagents. *J. Forensic Sci.* 1992, *37* (3), 688–694.

Almog, J.; Springer, E.; Wiesner, S.; Frank, A.; Khodzhaev, O.; Lidor, R.; Bahar, E.; Varkony, H.; Dayan, S.; Razen, S. Latent Fingerprint Visualization by 1,2-Indanedione and Related Compounds: Preliminary Results. *J. Forensic Sci.* 1999, *44* (1), 114–118.

Anderson, K. N.; Anderson, L. E.; Glanze, W. D., Eds. *Mosby's Medical, Nursing, and Allied Health Dictionary,* 5th ed.; Mosby, Inc.: St. Louis, MO, 1998.

Angier, R. H. *Firearm Blueing and Browning;* Arms and Armour: London, 1936.

Atkins, P. W. *Physical Chemistry,* 4th ed.; W. H. Freeman and Co.: New York, 1990.

Ballantyne, J. Serology: Overview. In *Encyclopedia of Forensic Science;* Siegel, J., Saukko, P., Knupfer, G., Eds.; Academic Press: London, 2000; pp 1322–1331.

Bandey, H. L. *The Powder Process, Study 1: Evaluation of Fingerprint Brushes for Use with Aluminum Powder;* HOPSDB Report 54/2004; Home Office Police Scientific Development Branch: Sandridge, U.K., 2004.

Bandey, H. L.; Kent, T. *Superglue Treatment of Crime Scenes—A Trial of the Effectiveness of the Mason Vactron SUPERfume Process;* HOPSDB Report 30/2003; Home Office Police Scientific Development Branch: Sandridge, U.K., 2003.

Barnett, K. G.; Bone, R. G.; Hall, P. W.; Ide, R. H. *The Use of Water Soluble Protein Dye for the Enhancement of Footwear Impressions in Blood on Non-Porous Surfaces—Part I;* Technical Note 629; Forensic Science Service: Birmingham, U.K., 1988.

Batey, G.; Copeland, J.; Donnelly, D.; Hill, C.; Laturnus, P.; McDiarmid, C.; Miller, K.; Misner, A. H.; Tario, A.; Yamashita, A. B. Metal Deposition for Latent Print Development. *J. Forensic Ident.* 1998, *48* (2), 165–176.

Bayford, F. Sweat. *Fingerprint Whorld* 1976, *1,* 42–43.

Bentsen, R. K.; Brown, J. K.; Dinsmore, A.; Harvey, K. K.; Kee, T. G. Post Firing Visualization of Fingerprints on Spent Cartridge Cases. *Sci. Justice* 1996, *36* (1), 3–8.

Bernier, U. R.; Booth, M. M.; Yost, R. A. Analysis of Human Skin Emanations by Gas Chromatography/Mass Spectrometry: 1. Thermal Desorption of Attractants for the Yellow Fever Mosquito (Aedes Aegypti) from Handled Glass Beads. *Anal. Chem.* 1999, *71* (1), 1–7.

Bernier, U. R.; Kline, D. L.; Barnard, D. R.; Schreck, C. E.; Yost, R. A. Analysis of Human Skin Emanations by Gas Chromatography/Mass Spectrometry: 2. Identification of Volatile Compounds That Are Candidate Attractants for the Yellow Fever Mosquito (Aedes Aegypti). *Anal. Chem.* 2000, *72* (4), 747–756.

Bessman, C. W.; Nelson, E.; Lipert, R. J.; Coldiron, S.; Herrman, T. R. A Comparison of Cyanoacrylate Fuming in a Vacuum Cabinet to a Humidity Fuming Chamber. *J. Forensic Ident.* 2005, *55* (1), 10–27.

Bodziak, W. J. Use of Leuco-Crystal Violet to Enhance Shoeprints in Blood. *Forensic Sci. Int.* 1996, *82* (1), 45–52.

Bottom, C. B.; Hanna, S. S.; Siehr, D. J. Mechanism of the Ninhydrin Reaction. *Biochem. Educ.* 1978, *6* (1), 4–5.

Bowman, V., Sears, V., Bandey, H., Hart, A., Bleay, S., Gibson, A., Fitzgerald, L., Eds. What Is the Oldest Fingerprint You Have Developed? In *Fingerprint Development and Imaging Update.* Home Office Scientific Development Branch: Sandridge, U.K., 2003; pp 2–3.

Boysen, T. C.; Yanagawa, S.; Sato, F.; Sato, K. A Modified Anaerobic Method of Sweat Collection. *J. Applied Physiol.* 1984, *56* (5), 1302–1307.

Bramble, S. K.; Brennan, J. S. Fingerprints (Dactyloscopy): Chemistry of Print Residue. In *Encyclopedia of Forensic Science;* Siegel, J., Saukko, P., Knupfer, G., Eds.; Academic Press: London, 2000; pp 862–869.

Bratton, R. M.; Juhala, J. A. DFO-Dry. *J. Forensic Ident.* 1995, *45* (2), 169–172.

Bratton, R. M.; Gregus, J.; Juhala, J. A. A Black Powder Method to Process Adhesive Tapes. *Fingerprint Whorld* 1996, *22* (83), 28.

Brusilow, S. W.; Gordes, E. H. Ammonia Secretion in Sweat. *American J. Physiol.* 1968, *214* (3), 513–517.

Bunting, R. K. *The Chemistry of Photography;* Photoglass Press: Normal, IL, 1987.

Bureau voor Dactyloscopische Artikelen (BVDA). Product Information for 5-Methylthioninhydrin and Thermanin. http://www.bvda.com (accessed Jan 5, 2010).

Burns, D. S. Sticky-Side Powder: The Japanese Solution. *J. Forensic Ident.* 1994, *44* (2), 133–138.

Burow, D.; Seifert, D.; Cantu, A. A. Modifications to the Silver Physical Developer. *J. Forensic Sci.* 2003, *48* (5), 1094–1100.

Campbell, B. M. Vacuum Chamber Cyanoacrylate Technique Evolution. *RCMP Gazette* 1991, *53* (12), 12–16.

Campbell, B. M. Time-Resolved Photography of Latent Prints on Fluorescent Backgrounds. *J. Forensic Ident.* 1993, *43* (4), 368–377.

Cantu, A. A. Silver Physical Developers for the Visualization of Latent Prints on Paper. *Forensic Sci. Rev.* 2001, *13* (1), 29–64.

Cantu, A. A.; Johnson, J. L. Silver Physical Development of Latent Prints. In *Advances in Fingerprint Technology;* Lee, H. C., Gaensslen, R. E., Eds.; CRC Press: Boca Raton, FL, 2001; pp 242–247.

Cantu, A. A.; Leben, D. A.; Joullié, M. M.; Heffner, R. J.; Hark, R. R. A Comparative Examination of Several Amino Acid Reagents for Visualizing Amino Acid (Glycine) on Paper. *J. Forensic Ident.* 1993, *43* (1), 44–66.

Cantu, A. A.; Leben, D. A.; Ramotowski, R.; Kopera, J.; Simms, J. R. Use of Acidified Hydrogen Peroxide to Remove Excess Gun Blue from Gun Blue-Treated Cartridge Cases and to Develop Latent Prints on Untreated Cartridge Cases. *J. Forensic Sci.* 1998, *43* (2), 294–298.

Cantu, A. A.; Leben, D. A.; Kelley, W. Some Advances in the Silver Physical Development of Latent Prints on Paper. *Int. Soc. Opt. Eng. (SPIE)* 2003, *5071,* 164–168.

Cava, M. P.; Little, R. L.; Napier, D. R. Condensed Cyclobutane Aromatic Systems: V. The Synthesis of Some a-Diazo-Indanones: Ring Contraction in the Indane Series. *J. Am. Chem. Soc.* 1958, *80* (9), 2257–2263.

Champod, C.; Lennard, C.; Margot, P.; Stoilovic, M. *Fingerprints and Other Ridge Skin Impressions;* CRC Press: Boca Raton, FL, 2004.

Cheeseman, R.; DiMeo, L. A. Fluorescein as a Field-Worthy Latent Bloodstain Detection System. *J. Forensic Ident.* 1995, *45* (6), 631–646.

Christie, R. M.; Mather, R. R.; Wardman, R. H. *The Chemistry of Colour Application;* Blackwell Science, Ltd.: Oxford, 2000.

Clay, W. E. Fluorisol: The Solvent of Choice for Ninhydrin Detection of Latent Fingerprints. *Ident. News* 1981, *31* (4), 12–13.

Conn, C.; Ramsay, G.; Roux, C.; Lennard, C. The Effect of Metal Salt Treatment on the Photoluminescence of DFO-Treated Fingerprints. *Forensic Sci. Int.* 2001, *116* (2), 117–123.

Cowger, J. F. *Friction Ridge Skin, Comparison and Identification of Fingerprints;* Elsevier Science: New York, 1983.

Crown, D. A. The Development of Latent Fingerprints with Ninhydrin. *J. Crim. Law Criminol. Police Sci.* 1969, *60* (2), 258–264.

Cuthbertson, F. *The Chemistry of Fingerprints;* AWRE Report No. 013/69; United Kingdom Atomic Weapons Establishment: Aldermaston, U.K., 1969.

Dalrymple, B.; Duff, J. M.; Menzel, E. R. Inherent Fingerprint Fluorescence-Detection by Laser. *J. Forensic Sci.* 1977, *22* (1), 106–115.

Davies, P. J.; Kobus, H. J.; Taylor, M. R.; Wainwright, K. P. Synthesis and Structure of the Zinc(II) and Cadmium(II)

Complexes Produced in the Photoluminescent Enhancement of Ninhydrin Developed Fingerprints Using Group 12 Metal Salts. *J. Forensic Sci.* 1995a, *40* (4), 565–569.

Davies, P. J.; Taylor, M. R.; Wainwright, K. P.; Kobus, H. J. Zinc(II) Chloride-Methanol Complex of 2-[(1,3-Dihydro-1,3-dioxo-2H-inden-2-ylidene)amino]-1H-indene-1,3(2H)- dionate(1-) Sodium Salt: A Complex of Ruhemann's Purple. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* 1995b, *51* (9), 1802–1805.

Dayan, S.; Almog, J.; Khodzhaev, O.; Rozen, S. A Novel Synthesis of Indanediones Using the HOF.CH3CN Complex. *J. Org. Chem.* 1998, *63* (8), 2752–2754.

Dean, J. A. *Lange's Handbook of Chemistry,* 13th ed.; McGraw-Hill: New York, 1985.

Della, E. W.; Janowski, W. K.; Pigou, P. E.; Taylor, B. Synthesis of Fingerprint Reagents: Aromatic Nucleophilic Substitution as a Route to 5-Substituted Ninhydrins. *Synthesis* 1999, *12,* 2119–2123.

DeMey, J. Colloidal Gold Probes in Immunocytochemistry. In *Immunohistochemistry: Practical Applications in Pathology and Biology;* Polak, J. M., Van Noorden, S., Eds.; John Wright and Sons Ltd.: London, 1983; pp 82–112.

Didierjean, C.; Debart, M.; Crispino, F. New Formulations of DFO in HFE7100. *Fingerprint Whorld* 1998, *24* (94), 163–167.

Druey, J.; Schmidt, P. Phenanthrolinchinone und Diazafluorene. *Helv. Chim. Acta* 1950, *33* (4), 1080–1087.

Eckert, W. G.; James, S. H. *Interpretation of Bloodstain Evidence at Crime Scenes;* Elsevier Science: New York, 1989.

Elber, R.; Frank, A.; Almog, J. Chemical Development of Latent Fingerprints: Computational Design of Ninhydrin Analogues. *J. Forensic Sci.* 2000, *45* (4), 757–760.

Everse, K. E.; Menzel, E. R. Sensitivity Enhancement of Ninhydrin-Treated Latent Fingerprints by Enzymes and Metal Salts. *J. Forensic Sci.* 1986, *31* (2), 446–454.

Faragher, A.; Summerscales, L. *Fingerprint Enhancement Using the Amido Black Technique after Chemical Fixation;* Technical Note 240; Forensic Science Service: Birmingham, U.K., 1981.

Faulk, W. P.; Taylor, G. M. An Immunocolloid Method for the Electron Microscope. *Immunochemistry* 1971, *8* (11), 1081–1083.

Feigl, F.; Anger, V. *Spot Tests in Inorganic Analysis;* Elsevier: Amsterdam, 1972.

Fitzgerald, L. Development of a GC-MS Method for the Analysis of Latent Fingerprint Components. Presented at the International Fingerprint Research Group Meeting, St. Albans, U.K., 2003.

Forgeot, R. Etude medico-legale des empreintes peu visibles ou invisibles et revelees par des procedes speciaux. *Archives d'anthropologie criminelle et des sciences penales* 1891, *6,* 387–404.

Förström, L.; Goldyne, M. E.; Winkelmann, R. K. IgE in Human Eccrine Sweat. *J. Investigative Derm.* 1975, *64* (3), 156–157.

Frank, A.; Almog, J. Modified SPR for Latent Fingerprint Development on Wet, Dark Objects. *J. Forensic Ident.* 1993, *43* (3), 240–244.

Frens, G. Controlled Nucleation for the Regulation of the Particle Size in Monodisperse Gold Suspensions. *Nature* 1973, *241* (105), 20–22.

Friedman, M.; Williams, L. D. Stoichiometry of Formation of Ruhemann's Purple in the Ninhydrin Reaction. *Bioorg. Chem.* 1974, *3,* 267–280.

Froude, Jr. J. H. The Super Glue Fuming Wand: A Preliminary Evaluation. *J. Forensic Ident.* 1996, *46* (1), 19–31.

Garner, D. D.; Cano, K. M.; Peimer, R. S.; Yeshion, T. E. An Evaluation of Tetramethylbenzidine as a Presumptive Test for Blood. *J. Forensic Sci.* 1976, *21* (4), 816–821.

Gerber, S. M.; Saferstein, R. Eds. *More Chemistry and Crime: From Marsh Arsenic Test to DNA Profile;* American Chemical Society: Washington, DC, 1997.

German, E. R. You Are Missing Ninhydrin Developed Prints. *Ident. News* 1981, *31* (9), 3–4.

German, E. R. Cyanoacrylate (Superglue) Fuming Tips. *2005.* http://www.onin.com/fp (accessed Jan 5, 2010).

Godsell, J. Fingerprint Techniques. *J. Forensic Sci.* Soc. 1963, *3* (2), 79.

Goode, G. C.; Morris, J. R. *Latent Fingerprints: A Review of Their Origin, Composition, and Methods for Detection;* AWRE Report No. 022/83; United Kingdom Atomic Weapons Research Establishment: Aldermaston, U.K., 1983.

Green, S. C.; Stewart, M. E.; Downing, D. Variation in Sebum Fatty Acid Composition Among Adult Humans. *J. Investigative Dermatol.* 1984, *83* (2), 114–117.

Grigg, R.; Malone, J. F.; Mongkolaussavaratana, T.; Thianpatanagul, S. Cycloaddition Reactions Relevant to the Mechanism of the Ninhydrin Reaction. X-Ray Crystal Structure of Protonated Ruhemann's Purple, a Stable 1,3-Dipole. *J. Chem. Soc., Chem. Commun.* 1986, 421–422.

Grigg, R.; Malone, J. F.; Mongkolaussavaratana, T.; Thianpatanagul, S. X=Y-ZH Compounds as Potential 1,3 Dipoles. Part 23 Mechanisms of the Reactions of Ninhydrin and Phenalene Trione with a-Amino Acids. X-Ray Crystal Structure of Protonated Ruhemann's Purple, a Stable Azomethine Ylide. *Tetrahedron* 1989, *45* (12), 3849–3862.

Grigg, R.; Mongkolaussavaratana, T.; Pounds, C. A.; Sivagnanam, S. 1,8-Diazafluorenone and Related Compounds. A New Reagent for the Detection of a-Amino Acids and Latent Fingerprints. *Tetrahedron Lett.* 1990, *31* (49), 7215–7218.

Guerrero, M. B. L. The Transparent, Liquid Adhesive, Latent Print Lifter. *J. Forensic Ident.* 1992, *42* (2), 101–105.

Hadorn, B.; Hanimann, F.; Anders, P.; Curtius, H.; Halverson, R. Free Amino Acids in Human Sweat from Different Parts of the Body. *Nature* 1967, *215* (99), 416–417.

Hall, M. M. Ridge Detail Through Latex Gloves. *J. Forensic Ident.* 1991, *41* (6), 415–416.

Hambley, D. S. The Physics of Vacuum Evaporation Development of Latent Fingerprints. Ph.D. Thesis, The Royal Holloway College, University of London, 1972.

Hamilton, P. B. Amino-Acids on Hands. *Nature* 1965, *205,* 284–285.

Hansen, D. B.; Joullié, M. M. The Development of Novel Ninhydrin Analogues. *Chem. Soc. Rev.* 2005, *34,* 408–417.

Hardwick, S. A.; Kent, T.; Sears, V. *Fingerprint Detection by Fluorescence Examination: A Guide to Operational Implementation;* White Crescent Press, Ltd.: Luton, U.K., 1990.

Hardwick, S. A.; Kent, T.; Sears, V.; Winfield, P. Improvements to the Formulation of DFO and the Effects of Heat on the Reaction with Latent Fingerprints. *Fingerprint Whorld* 1993, *19* (73), 65–69.

Harvey, K. K.; Dinsmore, A.; Brown, J. K.; Burns, D. S. Detection of Latent Fingerprints by Vacuum Cyanoacrylate Fuming—An Improved System. *Fingerprint Whorld* 2000, *26,* 29–31.

Hauze, D. B.; Petrovskaia, O.; Taylor, B.; Joullie, M. M.; Ramotowski, R.; Cantu, A. A. 1,2-Indanediones: New Reagents for Visualizing the Amino Acid Components of Latent Prints. *J. Forensic Sci.* 1998, *43* (4), 744–747.

Hawthorne, J. S.; Wojcik, M. H. Alcohol Measurement: A Review of the Literature. *Can. Soc. Forensic Sci. J.* 2006, *39* (2), 65–71.

Heffner, R. J.; Joullié, M. M. Synthetic Routes to Ninhydrins. Preparation of Ninhydrin, 5-Methoxyninhydrin, and 5-(Methylthio)ninhydrin. *Synth. Commun.* 1991, *21* (21), 2231–2256.

Herod, D. W.; Menzel, E. R. Laser Detection of Latent Fingerprints: Ninhydrin. *J. Forensic Sci.* 1982a, *27* (1), 200–204.

Herod, D. W.; Menzel, E. R. Laser Detection of Latent Fingerprints: Ninhydrin Followed by Zinc Chloride. *J. Forensic Sci.* 1982b, *27* (3), 513–518.

Hewlett, D. F.; Sears, V.; Suzuki, S. Replacements for CFC113 in the Ninhydrin Process: Part 2. *J. Forensic Ident.* 1997, *47* (3), 300–305.

Hier, S. W.; Cornbleet, T.; Bergeim, O. The Amino Acids of Human Sweat. *J. Biol. Chem.* 1946, *166* (1), 327–333.

Holgate, C. S.; Jackson, P.; Cowen, P. N.; Bird, C. C. Immunogold–Silver Staining: New Method of Immunostaining with Enhanced Sensitivity. *J. Histochem. and Cytochem.* 1983, *31* (7), 938–944.

Home Office Scientific Development Branch. Silver VMD. *HOSDB Fingerprint Development and Imaging Newsletter,* Oct 2005, *47/05,* 8–9.

Hussain, J. I.; Pounds, C. A. T*he Enhancement of Fingerprints in Blood, Part II: A Modified Amido Black Staining Technique;* HOCRE Report 649; Forensic Science Service: Birmingham, U.K., 1989a.

Hussain, J. I.; Pounds, C. A. *The Enhancement of Marks Made in Blood with 3,3',4,4'-Tetraaminobiphenyl;* CRSE Report 653; Forensic Science Service: Birmingham, U.K., 1989b.

Illsley, C. P. Superglue Fuming and Multiple Lifts. *Ident. News* 1984, *34* (1), 6, 15.

Inbau, F. E. Scientific Evidence in Criminal Cases, Part III: Finger-Prints and Palm-Prints. *Sparks From the Anvil* 1934, *2* (12), 4.

Jacquat, A. *Evolution des substances grasses des empreintes digitales au cours du temps. Analyse par TLC et GC-MS.;* University of Lausanne: Lausanne, Switzerland, 1999.

James, J. D.; Wilshire, B.; Cleaver, D. Laboratory Simulation of Commercial Brass Flake Manufacture. *Powder Metal.* 1990, *33* (3), 247–252.

James, J. D.; Pounds, C. A.; Wilshire, B. Flake Metal Powders for Revealing Latent Fingerprints. *J. Forensic Sci.* 1991, *36* (5), 1368–1375.

James, J. D.; Pounds, C. A.; Phil, M.; Wilshire, B. Magnetic Flake Powders for Fingerprint Development. *J. Forensic Sci.* 1993, *38* (2), 391–401.

Johnson, H. L.; Maibach, H. I. Drug Excretion in Human Eccrine Sweat. *J. Investigative Dermatol.* 1971, *56* (3), 182–188.

Jones, N. Metal Deposition Techniques for the Detection and Enhancement of Latent Fingerprints on Semi-Porous Surfaces. Ph.D. Thesis, University of Technology, Sydney, NSW, Australia, 2002.

Jones, N. E.; Jickells, S.; Charles, Y.; Elliott, J. *Chemical Analysis of Latent Print Residue;* Forensic Science Service: London, 2001a.

Jones, N.; Mansour, D.; Stoilovic, M.; Lennard, C.; Roux, C. The Influence of Polymer Type, Print Donor and Age on the Quality of Fingerprints Developed on Plastic Substrates Using Vacuum Metal Deposition. *Forensic Sci. Int.* 2001b, *124* (2–3), 167–177.

Jones, N.; Stoilovic, M.; Lennard, C.; Roux, C. Vacuum Metal Deposition: Factors Affecting Normal and Reverse Development of Latent Fingerprints on Polyethylene Substrates. *Forensic Sci. Int.* 2001c, *115* (1–2), 73–88.

Jones, N.; Stoilovic, M.; Lennard, C.; Roux, C. Vacuum Metal Deposition: Developing Latent Fingerprints on Polyethylene Substrates After the Deposition of Excess Gold. *Forensic Sci. Int.* 2001d, *123* (1), 5–12.

Jonker, H.; Molenaar, A.; Dippel, C. J. Physical Development Recording System: III. Physical Development. *Photographic Sci. and Eng.* 1969, *13* (2), 38–44.

Joullié, M. M. New Reagents for the Development of Latent Fingerprints. U.S. Department of Justice Grant Report 92-IJ-CX-K0154, Jan 17, 2000. http://www.ncjrs.gov/pdffiles1/nij/grants/179287.pdf.

Joullié, M. M.; Petrovskaia, O. A Better Way to Develop Fingerprints. *CHEMTECH* 1998, *28* (8), 41–44.

Joullié, M. M.; Thompson, T. R.; Nemeroff, N. H. Ninhydrin and Ninhydrin Analogs: Syntheses and Applications. *Tetrahedron* 1991, *47* (42), 8791–8830.

Jueneman, F. B. Stick It to 'Em. *Ident. News* 1982, *32* (6), 5,15.

Kaiser, D.; Drack, E. Diminished Excretion of Bicarbonate from the Single Sweat Gland of Patients with Cystic Fibrosis of the Pancreas. *Eur. J. Clin. Investigation* 1974, *4* (4), 261–265.

Kasper, S. P.; Minnillo, D. J.; Rockhold, A. M. Validating IND (1,2-Indanedione). *Forensic Sci. Commun.* 2002, *4* (4).

Kausche, G. A.; Ruska, H. Die Sichtbarmachung der Adsorption von Metallkolloiden an Eiweißkörper: I. Die Reaktion kolloides Gold—Tabakmosaikvirus. *Kolloid-Z.* 1939, 89 (1), 21–26.

Kawerau, E.; Wieland, T. Conservation of Amino-Acid Chromatograms. *Nature* 1951, *168* (4263), 77–78.

Kelly, G.; Bird, D.; Burt, D.; Massey, S.; Morhart, B.; Swiderski, D. Use of Children's Glue as an Aid in Fingerprint Lifting. *Ident. Canada* 2001, *24* (4), 7–12.

Kent, T. Latent Fingerprints and Their Detection. *J. Forensic Sci. Soc.* 1981, *21* (1), 15–22.

Kent, T. *User Guide to the Metal Deposition Process for the Development of Latent Fingerprints;* HOPSDB Publication No. 24; Home Office Police Scientific Development Branch: Sandridge, U.K., 1982.

Kent, T., Ed. *Manual of Fingerprint Development Techniques,* 2nd ed.; Home Office Police Scientific Development Branch: Sandridge, U.K., 1998.

Kent, T., Ed. *Manual of Fingerprint Development Techniques,* 2004 update; Home Office Police Scientific Development Branch: Sandridge, U.K., 2004.

Kent, T. [Letter to the Editor]. A Comparison of Cyanoacrylate Fuming in a Vacuum Cabinet to a Humidity Fuming Chamber. *J. Forensic Ident.* 2005, *55* (6), 681–683.

Kent, T.; Winfield, P. Superglue Fingerprint Development—Atmospheric Pressure and High Humidity, or Vacuum Evaporation? In *Proceedings of the International Symposium on Fingerprint Detection and Identification;* Ne'urim, Israel, 1995.

Kent, T.; Thomas, G. L.; East, H. W. *Application of the Metal Deposition Technique to the Development of Fingerprints on Polythene;* HOPSDB Technical Note; Home Office Scientific Development Branch: Sandridge, U.K.,1975.

Kent, T.; Thomas, G. L.; Reynoldson, T. E.; East, H. W. A Vacuum Coating Technique for the Development of Latent Fingerprints on Polythene. *J. Forensic Sci. Soc.* 1976, *16* (2), 93–101.

Kent, T.; Gillett, P. C.; Lee, D. *A Comparative Study of Three Techniques: Aluminium Powdering, Lead Powdering and Metal Deposition for the Development of Latent Fingerprints on Polythene;* HOPSDB Technical Memo; Home Office Police Scientific Development Branch: Sandridge, U.K., 1978.

Kerr, F. M.; Westland, A. D.; Haque, F. Observations on the Use of Silver Compounds for Fingerprint Visualization. *Forensic Sci. Int.* 1981, *18,* 209–214.

Knaap, W.; Adach, E. The Knaap Process: Lifting Two-Dimensional Footwear and Fingerprint Impressions Using Dental Stone. *J. Forensic Ident.* 2002, *52* (5), 561–571.

Knowles, A. M. Aspects of Physicochemical Methods for the Detection of Latent Fingerprints. *J. Phys. E: Sci. Instrum.* 1978, *11* (8), 713–721.

Kobus, H. J.; Stoilovic, M.; Warrener, R. N. A Simple Lumi-nescent Post-Ninhydrin Treatment for the Improved Visualisation of Fingerprints on Documents in Cases Where Ninhydrin Alone Gives Poor Results. *Forensic Sci. Int.* 1983, *22* (2–3), 161–170.

Kotowski, T. M.; Grieve, M. C. The Use of Microspectrophotometry to Characterize Microscopic Amounts of Blood. *J. Forensic Sci.* 1986, *31* (3), 1079–1085.

Labows, J. N.; Preti, G.; Hoelzle, E.; Leyden, J.; Kligman, A. Steroid Analysis of Human Apocrine Secretion. *Steroids* 1979, *34* (3), 249–258.

Leben, D. A.; Ramotowski, R. Evaluation of Gun Blueing Solutions and Their Ability to Develop Latent Fingerprints on Cartridge Casings. *Chesapeake Examiner* 1996, *34* (2), 8–10.

Lee, H. C.; Gaensslen, R. E. Methods of Latent Fingerprint Development. In *Advances in Fingerprint Technology,* 2nd ed.; Lee, H. C., Gaensslen, R. E., Eds.; CRC Press: Boca Raton, FL, 2001; pp 105–175.

Lee, H.C.; Pagliaro, E.M. Serology: Blood Identification. In *Encyclopedia of Forensic Science;* Siegel, J., Saukko, P., Knupfer, G., Eds.; Academic Press: London, 2000; pp 1331–1338.

Lennard, C. J.; Margot, P. A. Sequencing of Reagents for the Improved Visualization of Latent Fingerprints. *J. Forensic Ident.* 1988, *38* (5), 197–210.

Lennard, C. J.; Margot, P.; Stoilovic, M.; Warrener, R. N. Synthesis of Ninhydrin Analogues and Their Application to Fingerprint Development: Preliminary Results. *J. Forensic Sci. Soc.* 1986, *26* (5), 323–328.

Lennard, C. J.; Margot, P.; Sterns, M.; Warrener, R. N. Photoluminescent Enhancement of Ninhydrin Developed Fingerprints by Metal Complexation: Structural Studies of Complexes Formed Between Ruhemann's Purple and Group IIB Metal Salts. *J. Forensic Sci.* 1987, *32* (3), 597–605.

Lennard, C. J.; Wallace-Kunkel, C.; Roux, C.; Stoilovic, M. B25: 1,2-Indanedione: Is it a Useful Fingerprint Reagent? In *Proceedings of the 2005 American Academy of Forensic Science Meeting;* New Orleans, LA, 2005; p 43.

Lewis, L. A.; Smithwick, R.; Devault, G.; Bolinger, B.; Lewis, S. A. Processes Involved in the Development of Latent Fingerprints Using the Cyanoacrylate Fuming Method. *J. Forensic Sci.* 2001, *46* (2), 241–246.

Lightning Powder Inc. Dusting for Latents. *Minutiae*, Jan–Feb 2002, *70,* 2–3.

Lobitz, W. C.; Mason, H. L. Chemistry of Palmar Sweat: VII. Discussion of Studies on Chloride, Urea, Glucose, Uric Acid, Ammonia–Nitrogen, and Creatinine. *Arch. Dermatol. Syphilol.* 1948, *57,* 907–915.

MacDonell, H. L. Bristleless Brush Development of Latent Fingerprints. *Ident. News* 1961, *11* (3), 7–15.

Magora, A.; Azoury, M.; Geller, B. Treatment of Cocaine Contaminated Polythene Bags Prior to Fingerprint Development by Cyanoacrylate Fuming. *J. Forensic Ident.* 2002, *52* (2), 159–165.

Marshall, T. Analysis of Human Sweat Proteins by Two-Dimensional Electrophoresis and Ultrasensitive Silver Staining. *Anal. Biochem.* 1984, *139* (2), 506–509.

Masters, N. *Safety for the Forensic Identification Specialist,* 2nd ed.; Lightning Powder Inc.: Salem, OR, 2002.

Masters, N.; DeHaan, J. D. Vacuum Metal Deposition and Cyanoacrylate Detection of Older Latent Prints. *J. Forensic Ident.* 1996, *46* (1), 32–45.

McCaldin, D. J. The Chemistry of Ninhydrin. *Chem. Rev.* 1960, *60* (1), 39–51.

McDiarmid, C. Does Haste Make Waste? *RCMP Gazette* 1992, *54* (6), 21–24.

McMahon, P. Procedure to Develop Latent Prints on Thermal Paper. *Ident. Canada* 1996, *19* (3), 4–5.

Menzel, E. R. *Fingerprint Detection with Lasers,* 2nd ed.; Marcel Dekker, Inc.: New York, 1999.

Menzel, E. R. *Fingerprint Detection with Photoluminescent Nanoparticles,* 2nd ed.; Lee, H. C., Gaensslen, R. E., Eds.; CRC Press: Boca Raton, FL, 2001.

Menzel, E. R.; Duff, J. M. Laser Detection of Latent Fingerprints—Treatment with Fluorescers. *J. Forensic Sci.* 1979, *24* (1), 96–100.

Merrick, S.; Gardner, S. J.; Sears, V.; Hewlett, D. F. An Operational Trial of Ozone-Friendly DFO and 1,2-Indanedione Formulations for Latent Fingerprint Detection. *J. Forensic Ident.* 2002, *52* (5), 595–605.

Migron, Y.; Mandler, D. Development of Latent Fingerprints on Unfired Cartridges by Palladium Deposition: A Surface Study. *J. Forensic Sci.* 1997, *42* (6), 986–992.

Misner, A. Latent Fingerprint Detection on Low Density Polyethylene Comparing Vacuum Metal Deposition to Cyanoacrylate Fuming and Fluorescence. *J. Forensic Ident.* 1992, *42* (1), 26–33.

Mitchell, H. H.; Hamilton, T. S. The Dermal Excretion under Controlled Environmental Conditions of Nitrogen and Minerals in Human Subjects, with Particular Reference to Calcium and Iron. *J. Biol. Chem.* 1949, *178* (1), 345–361.

Mitsui, T.; Katho, H.; Shimada, K.; Wakasugi, Y. Development of Latent Prints Using a Sudan Black B Solution. *Ident. News* 1980, *30* (8), 9–10.

Mitsui, T.; Katho, H.; Shimada, K.; Wakasugi, Y. Development of Latent Prints Using a Sudan Black B Solution. *Fingerprint Whorld* 1981, *6* (24), 84–85.

Moenssens, A. A. *Fingerprint Techniques;* Chilton Book Company: Philadelphia, 1971.

Mong, G. M.; Petersen, C. E.; Clauss, T. R. W. *Advanced Fingerprint Analysis Project. Fingerprint Constituents;* PNNL Report No. 3019; Pacific Northwest National Laboratory: Richland, WA, 1999.

Morris, J. R. *Extensions to the NFN Reagent for the Development of Latent Fingerprints;* AWRE Report; Atomic Weapons Research Establishment: Aldermaston, U.K., February 1978.

Morris, J. R.; Goode, G. C. NFN an Improved Ninhydrin Reagent for the Detection of Latent Fingerprints. *Police Research Bull.* 1974, *24,* 45–53.

Murphy, M. A Vacuum Metal Identification. *J. Forensic Ident.* 1991, *41* (5), 318–320.

Naitoh, K.; Inai, Y.; Hirabayashi, T. Direct Temperature-Controlled Trapping System and Its Use for the Gas Chromatographic Determination of Organic Vapor Released from Human Skin. *Anal. Chem.* 2000, *72* (13), 2797–2801.

Nakayashiki, N. Sweat Protein Components Tested by SDS-Polyacrylamide Gel Electrophoresis Followed by Immunoblotting. *The Tohoku J. Exp. Med.* 1990, *161* (1), 25–31.

Nicolaides, N.; Ansari, M. N. A. The Dienoic Fatty Acids of Human Skin Surface Lipid. *Lipids* 1968, *4* (1), 79–81.

Nicolaides, N.; Fu, H. C.; Ansari, M. N. A.; Rice, G. R. The Fatty Acids of Wax Esters and Sterol Esters from Vernix

Caseosa and from Human Skin Surface Lipid. *Lipids* 1972, *7* (8), 506–517.

Norkus, P.; Noppinger, K. New Reagents for the Enhancement of Fingerprints in Blood. *Ident. News* 1986, *36* (4), 5,15.

Odén, S.; von Hofsten, B. Detection of Fingerprints by the Ninhydrin Reaction. *Nature* 1954, *173* (4401), 449–450.

Odland, G. F. Structure of the Skin. In *Physiology, Biochemistry, and Molecular Biology of the Skin,* 2nd ed.; Goldsmith, L., Ed.; Oxford University Press: New York, 1991.

Olenik, J. Super Glue, A Modified Method. *Ident. News* 1983, *33* (1), 9–10.

Olenik, J. H. Cyanoacrylate Fuming: An Alternative Non-Heat Method. *J. Forensic Ident.* 1989, *39* (5), 302–304.

Olsen, Sr., R. D. The Chemical Composition of Palmar Sweat. *Fingerprint and Ident. Magazine* 1972, 3–23.

Olsen, Sr., R. D. *Scott's Fingerprint Mechanics;* Charles C Thomas: Springfield, IL, 1978.

Oro, J.; Skewes, H. B. Free Amino-Acids on Human Fingers: The Question of Contamination in Microanalysis. *Nature* 1965, *207* (1), 1042–1045.

Palenik, S. Microchemistry. In *Encyclopedia of Forensic Science;* Siegel, J., Saukko, P., Knupfer, G., Eds.; Academic Press: London, 2000; pp 1111–1116.

Perkins, D. G.; Thomas, W. M. Cyanoacrylate Fuming Prior to Submission of Evidence to the Laboratory. *J. Forensic Ident.* 1991, *41* (3), 157–162.

Petrovskaia, O.; Taylor, B.; Hauze, D. B.; Carroll, P.; Joullié, M. M. Investigations of the Reaction Mechanisms of 1,2-Indanediones with Amino Acids. *J. Org. Chem.* 2001, *66* (23), 7666–7675.

Pounds, C. A.; Jones, R. J. Physicochemical Techniques in the Development of Latent Fingerprints. *Trends in Anal. Chem.* 1983, *2* (8), 180–183.

Pounds, C. A.; Grigg, R.; Mongkolaussavaratana, T. The Use of 1,8-Diazafluoren-9-one (DFO) for the Fluorescent Detection of Latent Fingerprints on Paper. A Preliminary Evaluation. *J. Forensic Sci.* 1990, *35* (1), 169–175.

Price, D.; Stow, K. A Method for Stopping Overdevelopment of Silver Nitrate Treated Finger and Footwear Marks. *Fingerprint Whorld* 1998, *24,* 107–110.

Puhvel, S. M. Esterification of (4-14C) Cholesterol by Cutaneous Bacteria (Staphylococcus Epidermis, Propionibacterium Acnes, and Propionibacterium Granulosum). *J. Investigative Dermatol.* 1975, *64* (6), 397–400.

Ramasastry, P.; Downing, D.; Pochi, P. E.; Strauss, J. S. Chemical Composition of Human Surface Lipids from Birth to Puberty. *J. Investigative Dermatol.* 1970, *54* (2), 139–144.

Ramotowski, R. Composition of Latent Print Residue. In *Advances in Fingerprint Technology*, Lee, H. C., Gaensslen, R. E., Eds.; CRC Press: Boca Raton, FL, 2001; pp 63–104.

Ramotowski, R.; Cantu, A. A.; Joullié, M. M.; Petrovskaia, O. 1,2-Indanediones: A Preliminary Evaluation of a New Class of Amino Acid Visualizing Compounds. *Fingerprint Whorld* 1997, *23* (90), 131–139.

Retinger, J. M. The Mechanism of the Ninhydrin Reaction. A Contribution to the Theory of Color of Salts of Alloxantine-Like Compounds. *J. Am. Chem. Soc.* 1917, *39* (5), 1059–1066.

Reynoldson, T. E.; Reed, F. A. *Operational Trial Comparing Metal Deposition with Small Particle Reagent for the Development of Latent Prints on Polyethylene;* SRDB Report; Home Office Scientific Development Branch: Sandridge, U.K., 1979.

Rhodes, H. *Forensic Chemistry;* Chapman & Hall, Ltd.: London, 1940.

Robertshaw, D. Apocrine Sweat Glands. In *Physiology, Biochemistry, and Molecular Biology of the Skin,* 2nd ed.; Goldsmith, L., Ed.; Oxford University Press: New York, 1991.

Rosati, B. B. Does Superglue Hinder Traditional Firearms Identification? *AFTE J.* 2005, *37,* 3–6.

Roux, C.; Jones, N.; Lennard, C.; Stoilovic, M. Evaluation of 1,2-Indanedione and 5,6-Dimethoxy-1,2-Indanedione for the Detection of Latent Fingerprints on Porous Surfaces. *J. Forensic Sci.* 2000, *45* (4), 761–769.

Ruhemann, S. Cyclic Di- and Tri-Ketones. *J. Chem. Soc. Trans.* 1910a, *97,* 1438–1449.

Ruhemann, S. Triketohydrindene Hydrate. *J. Chem. Soc. Trans.* 1910b, *97,* 2025–2031.

Ruhemann, S. Triketohydrindene Hydrate, Part III: Its Relation to Alloxan. *J. Chem. Soc. Trans.* 1911a, *99,* 792–800.

Ruhemann, S. Triketohydrindene Hydrate, Part IV: Hydrindantin and Its Analogues. *J. Chem. Soc. Trans.* 1911b, *99,* 1306–1310.

Ruhemann, S. Triketohydrindene Hydrate, Part V: The Analogues of Uramil and Purpuric Acid. *J. Chem. Soc. Trans.* 1911c, *99,* 1486–1492.

Sato, K. The Physiology, Pharmacology, and Biochemistry of the Eccrine Sweat Gland. *Rev. Physiol., Biochem. Pharmacol.* 1979, *79,* 51–131.

Saunders, G. Multimetal Deposition Method for Latent Fingerprint Development. Presented at the 74th Conference of the International Association for Identification, Pensacola, FL, 1989.

Saunders, G. *Fingerprint Chemistry I, Final Report to U.S. Secret Service;* U.S. Secret Service: Washington, DC, 1996.

Saunders, G. *Fingerprint Chemistry II, Final Report to the U.S. Secret Service;* U.S. Secret Service: Washington, DC, 1997.

Schertz, T. D.; Reiter, R. C.; Stevenson, C. D. Zwitterion Radicals and Anion Radicals from Electron Transfer and Solvent Condensation with the Fingerprint Developing Agent Ninhydrin. *J. Org. Chem.* 2001, *66* (23), 7596–7603.

Schnetz, B. Latent Fingerprint and Colloidal Gold: New Reinforcement Procedures. Abstract for the International Association of Forensic Science Conference, Dusseldorf, Germany, 1993.

Schnetz, B. Latent Fingerprint, Colloidal Gold, and Biochemical Techniques. Abstract for the European Network of Forensic Science Institutes (ENFSI) Meeting, Lausanne, Switzerland, 1997.

Schnetz, B.; Margot, P. Latent Fingerprints, Colloidal Gold and Multimetal Deposition (MMD). Optimisation of the Method. *Forensic Sci. Int.* 2001, *118,* 21–28.

Schütz, F.; Bonfanti, M.; Champod, C. La révélation des traces papillaires sur les douilles par les techniques de etching et de blueing et comparaison avec la déposition multimétallique. *Can. Soc. Forensic Sci. J.* 2000, *33* (2), 65–81.

Schwarz, L.; Frerichs, I. Advanced Solvent-Free Application of Ninhydrin for Detection of Latent Fingerprints on Thermal Paper and Other Surfaces. *J. Forensic Sci.* 2002, *47* (6), 1274–1277.

Scruton, B.; Robins, B. W.; Blott, B. H. The Deposition of Fingerprint Films. *J. Phys. D: Appl. Phys.* 1975, *8* (6), 714–723.

Sears, V.; Prizeman, T. M. The Enhancement of Fingerprints in Blood, Part I: The Optimization of Amido Black. *J. Forensic Ident.* 2000, *50* (5), 470–480.

Sears, V.; Butcher, C.; Prizeman, T. M. The Enhancement of Fingerprints in Blood, Part II: Protein Dyes. *J. Forensic Ident.* 2001, *51* (1), 28–38.

Sears, V.; Butcher, C.; Fitzgerald, L. A. Enhancement of Fingerprints in Blood, Part III: Reactive Techniques, Acid Yellow 7, and Process Sequences. *J. Forensic Ident.* 2005, *55* (6), 741–763.

Shelley, W. B. Apocrine Sweat. *J. Investigative Dermatol.* 1951, *17,* 255.

Smith, A. M.; Agiza, A. H. The Determination of Amino-Acids Colorimetrically by the Ninhydrin Reaction. *Analyst* 1951, *76,* 623–627.

Söderman, H.; O'Connell, J. J. *Modern Criminal Investigation;* Funk & Wagnalls Co.: New York, 1935.

Sodhi, G. S.; Kaur, J. Powder Method for Detecting Latent Fingerprints: A Review. *Forensic Sci. Int.* 2001, *120* (3), 172–176.

Speaks, H. A. The Use of Ninhydrin in the Development of Latent Fingerprints. *Finger Print and Ident. Magazine* 1964, *45* (3), 11–13, 23.

Speaks, H. A. Ninhydrin Prints from Rubber Gloves. *Finger Print and Ident. Magazine* 1966, *48* (3), 3–5.

Speaks, H. A. Ninhydrin Development of Latent Prints. *Finger Print and Ident. Magazine* 1970, *52* (2),14–17.

St-Amand, F. Fingerprints from Latex Gloves. *Ident. Canada* 1994, *17* (4), 11–13.

Stimac, J. Thermal and Carbonless Papers—A Fundamental Understanding for Latent Friction Ridge Development. *J. Forensic Ident.* 2003a, *53* (2), 185–197.

Stimac, J. Thermal Paper: Latent Friction Ridge Development via 1,2-Indanedione. *J. Forensic Ident.* 2003b, *53* (3), 265–271.

Stoilovic, M. Detection of Semen and Blood Stains Using Polilight as a Light Source. *Forensic Sci. Int.* 1991, *51,* 289–296.

Stoilovic, M. Improved Method for DFO Development of Latent Fingerprints. *Forensic Sci. Int.* 1993, *60* (3), 141–153.

Stoilovic, M.; Kobus, H. J.; Margot, P.; Warrener, R. N. Improved Enhancement of Ninhydrin Developed Fingerprints by Cadmium Complexation Using Low Temperature Photoluminescence Techniques. *J. Forensic Sci.* 1986, *31* (2), 432–445.

Stone, R. S.; Metzger, R. A. Comparison of Development Techniques Sudan Black B Solution/Black Magna Powder for Water-Soaked Porous Items. *Ident. News* 1981, *31* (1), 13–14.

Stroud, P. T. *Some Comments on Finger Print Development by Vacuum Deposition;* AWRE Nuclear Research Note 5/71; Atomic Weapons Establishment (AWRE): Aldermaston, U.K., 1971.

Stroud, P. T. *Further Comments on Finger Print Development by Vacuum Deposition;* AWRE Nuclear 10/72; Atomic Weapons Establishment (AWRE): Aldermaston, U.K., 1972.

Suzuki, S.; Suzuki, Y.; Ohta, H. Detection of Latent Fingerprints on Newly Developed Substances Using the Vacuum Metal Deposition Method. *J. Forensic Ident.* 2002, *52* (5), 573–578.

Takatsu, M.; Kageyama, H.; Hirata, K.; Akashi, S.; Yoko Ta, T.; Shiitani, M.; Kobayashi, A. Development of a New Method to Detect Latent Fingerprints on Thermal Paper with o-Alkyl Derivative of Ninhydrin. *Rep. Natl. Res. Inst. Police Sci.* 1991, *44* (1), 1–6.

Theys, P.; Lepareux, A.; Chevet, G.; Ceccaldi, P. F. New Technique for Bringing Out Latent Fingerprints on Paper: Vacuum Metallisation. *Int. Criminal Police Rev.* 1968, *217,* 106–109.

Thomas, G. L. The Physics of Fingerprints and Their Detection. *J. Phys. E: Sci. Instrum.* 1978, *11* (8), 722–730.

Thornton, J. I. Modification of Fingerprint Powder with Coumarin 6 Laser Dye. *J. Forensic Sci.* 1978, *23* (3), 536–538.

Thorwald, J. *Crime and Science: The New Frontier in Criminology;* Brace and World, Inc: New York, 1966.

Toth, I.; Faredin, I. Steroid Excreted by Human Skin: II. C 19-Steroid Sulphates in Human Axillary Sweat. *Acta Medica Hungarica* 1985, *42* (1–2), 21–28.

Trozzi, T.; Schwartz, R.; Hollars, M. *Processing Guide for Developing Latent Prints;* Federal Bureau of Investigation, Laboratory Division, U.S. Department of Justice, U.S. Government Printing Office: Washington, DC, 2000.

United Nations Environ Programme. *The Montreal Protocol on Substances That Deplete the Ozone Layer;* United Nations Environ Programme: Nairobi, Kenya, 1999. http://www.un-documents.net/mpsdol.htm (accessed Jan 5, 2010).

Uyttendaele, K.; De Groote, M.; Blaton, V.; Peeters, H. Analysis of the Proteins in Sweat and Urine by Agarose-Gel Isotachophoresis. *J. Chromatogr.* 1977, *132* (2), 261–266.

Valussi, S. Microfluidics Systems for the Chemical Analysis of Fingerprint Residues. Presented at the International Fingerprint Research Group Meeting, St. Albans, U.K., 2003.

van Oorschot, R. A. H.; Treadwell, S.; Beaurepaire, J.; Holding, N. L.; Mitchell, R. J. Beware of the Possibility of Fingerprinting Techniques Transferring DNA. *J. Forensic Sci.* 2005, *50* (6), 1417–1422.

Vandenberg, N.; van Oorschot, R. A. H. The Use of Polilight in the Detection of Seminal Fluid, Saliva, and Bloodstains and Comparison with Conventional Chemical-Based Screening Tests. *J. Forensic Sci.* 2006, *51* (2), 361–370.

Vree, T. B.; Muskens, A.; Van Rossum, J. M. Excretion of Amphetamines in Human Sweat. *Archives Internationales de Pharmacodynamie et de Therapie* 1972, *199* (2), 311–317.

Wade, D. C. Development of Latent Prints with Titanium Dioxide (TiO_2). *J. Forensic Ident.* 2002, *52* (5), 551–559.

Wakefield, M. A.; Armitage, S. Canberra Institute of Technology, Canberra, Australia. Unpublished Results, 2005.

Wallace-Kunkel, C.; Stoilovic, M.; Lennard, C.; Roux, C. A0571: 1,2-Indanedione as a Finger Mark Reagent Optimisation and Characterisation. Presented at the 17th Meeting of the International Association of the Forensic Sciences, Hong Kong, 2005.

Wallace-Kunkel, C.; Lennard, C. J.; Stoilovic, M.; Roux, C. Evaluation of 5-Methylthioninhydrin for the Detection of Fingermarks on Porous Surfaces and Comparison. *Ident. Canada* 2006, *29* (1), 4–13.

Walls, H. J.; Attridge, G. G. *Basic Photo Science: How Photography Works;* Focal Press: London, 1977.

Walter, S. *Latent Print Visualization of Aged Lipid Residues;* Status Report, April 1999; Savannah River Technical Center: Aiken, SC, 1999.

Wargacki, S.; Dadmun, M. D.; Lewis, L. Identifying the True Initiator in the Cyanoacrylate Fuming Method. Presented at the 2005 International Association for Identification Conference, Dallas, TX, 2005.

Watkin, J. E.; Wilkinson, D.; Misner, A. H.; Yamashita, A. B. Cyanoacrylate Fuming of Latent Prints: Vacuum Versus Heat/Humidity. *J. Forensic Ident.* 1994, *4* (5), 545–554.

Watling, W. J.; Smith, K. O. Heptane: An Alternative to the Freon Ninhydrin Mixture. *J. Forensic Ident.* 1993, *43* (2), 131–134.

Weaver, D. E. Large Scale Cyanoacrylate Fuming. *J. Forensic Ident.* 1993, *43* (2), 135–137.

Weaver, D. E.; Clary, E. J. A One-Step Fluorescent Cyanoacrylate Fingerprint Development Technology. *J. Forensic Ident.* 1993, *43* (5), 481–492.

Weast, R. C., Ed. *CRC Handbook of Chemistry and Physics,* 67th ed.; CRC Press: Boca Raton, FL, 1986.

Wiesner, S.; Springer, E.; Sasson, Y.; Almog, J. Chemical Development of Latent Fingerprints: 1,2-Indanedione Has Come of Age. *J. Forensic Sci.* 2001, *46* (5), 1082–1084.

Wilansky, M.; Jomphe, A.; Kovacs, G.; Lawrence, J.; LeBlanc, D.; St-Pierre, M.; Wilkinson, D.; Yamashita, A. B. *A Comparison of Hand-Held Forensic Light Sources;* FIRS Technical Report #16; Royal Canadian Mounted Police: Ottawa, 2006.

Wilkinson, D. Study of the Reaction Mechanism of 1,8-Diazafluoren-9-one with the Amino Acid, L-Alanine. F*orensic Sci. Int.* 2000a, *109* (2), 87–103.

Wilkinson, D. Spectroscopic Study of 1,2-Indanedione. *Forensic Sci. Int.* 2000b, *114,* 123–132.

Wilkinson, D.; Watkin, J. E. A Comparison of the Forensic Light Sources: Polilight, Luma-Lite, and Spectrum 9000. *J. Forensic Ident.* 1994, *44* (6), 632–651.

Wilkinson, D.; Yamashita, A. B.; Annis, K. A Comparison of Forensic Light Sources: Luma-Lite, Mini-Crimescope 400, Omniprint 1000A, Polilight PL500, and Quaser 2000/30. *Ident. Canada* 2002, *25* (1), 5–15.

Wilkinson, D.; Mackenzie, E.; Leech, C.; Mayowski, D.; Bertrand, S.; Walker, T. The Results from a Canadian National Field Trial Comparing Two Formulations of 1,8-Diazafluoren-9-one (DFO) with 1,2-Indanedione. *Ident. Canada* 2003, *26* (2), 8–18.

Wilkinson, D.; Rumsby, D.; Babin, B.; Merrit, M.; Marsh, J. *The Results from a Canadian National Field Trial Comparing 1,8-Diazafluoren-9-one (DFO) with Ninhydrin and the Sequence DFO Followed by Ninhydrin;* Technical Report TR-03-2005; Canadian Police Research Centre: Ontario, 2005.

Willinski, G. Permeation of Fingerprints Through Laboratory Gloves. *J. Forensic Sci.* 1980, *25* (3), 682–685.

Wilson, J. D.; Darke, D. J. *The Results of Analyses of the Mixtures of Fatty Acids on the Skin: Part I. Commentary;* AERE Report No. G-1154; Atomic Energy Research Establishment: ca 1978.

Yamashita, A. B. Use of a Benchtop Desiccator for Vacuum Cyanoacrylate Treatment of Latent Prints. *J. Forensic Ident.* 1994, *44* (2), 149–158.

Yemm, E. W.; Cocking, E. C; Ricketts, R. E. The Determination of Amino-Acids with Ninhydrin. *Analyst* 1955, *80,* 209–214.

7.17 Additional Information

Almog, J.; Gabay, A. A Modified Super Glue Technique—The Use of Polycyanoacrylate for Fingerprint Development. *J. Forensic Sci.* 1986, *31* (1), 250–253.

Beecroft, W. Enhancement of PD Prints. *RCMP Gazette,* 1989, *21,* 17.

Boniforti, L.; Passi, S.; Caprilli, F.; Nazzaro-Porro, M. Skin Surface Lipids. Identification and Determination by Thin-Layer Chromatography and Gas-Liquid Chromatography. *Clin. Chim. Acta* 1973, *47* (2), 223–231.

Burns, T. D.; Brown, J. K.; Dinsmore, A.; Harvey, K. K. Base-Activated Latent Fingerprints Fumed with a Cyanoacrylate Monomer: A Quantitative Study Using Fourier-Transform Infra-Red Spectroscopy. *Anal. Chim. Acta* 1998, *362* (2), 171–176.

Champod, C.; Egli, N.; Margot, P. A. Fingermarks, Shoesole and Footprint Impressions, Tire Impressions, Ear Impressions, Toolmarks, Lipmarks, Bitemarks—A Review: 2001 to 2004. In *Proceedings of the 14th Interpol Forensic Science Symposium;* Lyon, France, 2004; pp 227–244.

Forgeot, R. *Des empreintes digitales étudiées au point de vue médico-judiciaire;* A. Storck: Lyon, France, 1891.

Hamm, E. D. Chemical Developers in Footwear Prints. *Fingerprint Whorld* 1984, *9,* 117–118.

Loveridge, F. H. Shoe Print Development by Silver Nitrate. *Fingerprint Whorld* 1984, *10* (38), 58.

Morton, S. Shoe Print Development by PD Treatment. *Fingerprint Whorld* 1983, *9* (34), 60–61.

Siegel, J., Saukko, P., Knupfer, G., Eds.; *Encyclopedia of Forensic Sciences;* Academic Press: London, 2000.

Yong, A. S. J. *Detection of Latent Fingerprints with Cyanoacrylates: New Techniques Involving Coloured and Photoluminescent Compounds;* Australian National University: Canberra, ACT, Australia, 1986.