An Investigation into the Use of Surfactants in Powder Suspension Formulations for Fingermark Development

By

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Certificate of original authorship

I, Lumikki Clover Ree, declare that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Mathematical and Physical Sciences at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Abbreviations

ASPD	Adhesive-side powder dark
BY40	Basic yellow 40
CA	Cyanoacrylate fuming
CAST	Centre for Applied Science and Technology
CMC	Critical micelle concentration
Cryo-TEM	Cryogenic Transmission Electron Microscopy
CTAB	Cetyltrimethylammonium bromide
DLS	Dynamic light scattering
DNA	Deoxyribonucleic acid
DOSS	Dioctyl sulfosuccinate
DSLR	Digital single-lens reflex
DSTL	Defence Science and Technology Laboratories
FePS	Iron oxide powder suspension
FVM	Fingermark Visualisation Manual
GC-MS	Gas chromatography/ mass spectrometry
HOSDB	Home Office Scientific Development Branch
HDPE	High density polyethylene
HLB	Hydrophilic-lipophilic balance
IFRG	International Fingerprint Research Group
IND-Zn	Indanedione-Zinc
KP	Kodak Photo-Flo
LN	Liqui-Nox
MALDI MS	Matrix assisted laser desorption/ ionization mass spectrometry
MMD	Multi-metal deposition

NaCl	Sodium chloride
n-DDAA	n-dodecylamine acetate
NIN	Ninhydrin
NMR	Nuclear Magnetic Resonance
PE	Polyethylene
PD	Physical developer
PdI	Polydispersity index
R6G	Rhodamine 6G
SANS	Small-Angle Neutron Scattering
SAXS	Small-Angle X-ray Scattering
SDS	Sodium dodecyl sulfate
SMD	Single metal deposition
SP80/T80	Span 80/ Tween 80 mixture
ST	Surface tension
T20	Tween 20
T80	Tween 80
TX100	Triton X-100
UC	University of Canberra
UK	United Kingdom
VMD	Vacuum metal deposition

Research communication

Publications

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Portions of this article and additional analysis are found in ***Chapter 2: Comparison of surfactant effectiveness in FePS***

Conferences

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Abstract

Significant research has been conducted to explore and expand the boundaries of fingermark detection capabilities, resulting in many techniques that may be used for different surfaces and conditions. However, historical focus on such investigations has produced limited understanding of the mechanisms involved in these techniques. Improving our understanding of the fundamental interactions involved in fingermark development will aid in improving the efficacy and value of future research efforts. One such fingermark detection technique with little mechanistic understanding is iron oxide powder suspensions (FePS). FePS is recommended for use on wetted non-porous surfaces, however ongoing research suggests it may have wider operational applications in the future. This research aimed to investigate the role of the surfactant in FePS to improve fundamental understanding of the mechanism responsible for fingermark development.

Firstly, a thorough investigation into the effect of different surfactant types, concentrations and ratios was performed to gain a broad understanding of how surfactants impact development on non-porous surfaces. Surfactant efficacy was variable and not solely dependent on ionic nature, however non-ionic surfactants Tween 20 and Kodak Photo-Flo were the most effective with a powder to surfactant ratio of 1:2. Application of formulations made only with water and powder resulted in heavy background development, suggesting that surfactants are paying a vital role in the selectivity of powder deposition. Variation in development was also noted across different non-porous substrates, with poorer powder adherence on plastic, suggesting surface chemistry influences FePS efficacy. Surfactants were found to have variable effectiveness over a range of concentrations and had a greater role in controlling the contrast of a developed fingermark than previously suggested.

The importance of micelle presence and structure was then investigated and shown to be vital for successful fingermark development. Continuing variation between surfactants demonstrated that micelle structure also influences development quality. Effective surfactants were sufficiently stable to prevent background deposition yet still be sensitive enough to fingermark residue components to allow for on-ridge deposition. Analysis of the chemical structures of assessed surfactants determined that optimal surfactants contained between 8 to 12 carbons in the surfactant tail, however the size and complexity of these structures did not appear to influence development. Importantly, surfactants had specific concentrations at which they were effective in FePS and therefore cannot be used interchangeably at the same concentrations.

Artificial fingermark simulants were used to explore target residue components. Although simulants did not fully replicate the performance of natural fingermark residue, they demonstrated that different surfactants affect sensitivity to particular residue compound classes. Development success was not solely tied to the presence of either eccrine or sebaceous residue, but rather a complex interaction of both, modulated by the surfactant used. An investigation into residue removal confirmed that development could still occur even when no visible residue was apparent under phase contrast microscopy, particularly for eccrine-rich deposits. This suggests that formulation stability, rather than residue absence, was responsible for previous development failures**.**

This research provided important information about the interactions between surfactants, substrates and fingermark residue components in the FePS technique. As surfactants are used in a range of other development techniques, the results may inform future research efforts on a wider scale. This research has emphasized the importance of continuing to build upon fundamental understanding of fingermark residue composition and development.

Chapter 1: Introduction

1. *Introduction*

1.1 Fingermarks

Fingermarks are widely used in criminal investigations as a means of identifying individuals through the transfer of patterns present within friction ridge skin found on the fingertips. Fingermarks are created when contact is made between the skin on the fingertips and a substrate, resulting in the transfer of fingermark residue. These impressions are deposited as the mirror image of the patterns in the skin which can be used for identification purposes due to their polymorphous and persistent nature [1].

1.1.1 Friction ridge skin

Friction ridge skin refers to the skin present along the fingers, palms and soles of the feet. It is made up of raised ridges and recessed furrows and enhances the ability to grasp or grip surfaces. The ridges present on the tip of the finger form patterns and persistent features that can be used for identification [2-5].

Fingerprint patterns are fully developed at approximately seven months of foetal development in the womb. At 7-8 weeks gestation, volar pads appear on the fingertips which are covered by the epidermis as the foetus grows. Friction ridge skin begins to develop over these volar pads at 10-12 weeks gestation [2, 3]. The unique patterns are influenced by the shape and size of volar pads, speed of the friction skin growth, bone morphology as well as genetics. Because of this, the friction ridge skin pattern can be used to identify and individual, and even identical twins can be differentiated by their fingerprints [2, 3, 5]. Fingerprints are unchanging throughout a person's life except from permanent alterations deep enough to penetrate the epidermis and cause permanent scarring. This includes mechanical damage such as cuts and burns, as well as some medical conditions, which often provide even more unique and identifiable features to the fingermark [2, 4, 5].

1.1.2 Composition of fingermark residue

Fingermark residue is made up of a mixture of endogenous and exogenous compounds. The endogenous compounds primarily come from the eccrine, sebaceous and apocrine glands [6-8]. Compounds from these glands are either secreted from pores on the fingertip or transferred to the friction ridge skin from other areas of the body such as the forehead and underarms [7, 9].

Eccrine glands are present all over the body and is the only kind of gland present in friction ridge skin on the hands and soles of the feet and therefore is present in all fingermarks. Eccrine secretions are

generally considered to be made up of 98% water with its primary function to regulate temperature cooling through evaporation from the skin [7, 8, 10]. The rest of the sweat is comprised of minerals, organic acids, urea and sugars [6, 11]. Recent studies suggest that the eccrine components of fingermark secretions do not contain as much water as previously assumed, likely less than 20%. This is due to reabsorption of water by the skin before deposition, as well as evaporation from the substrate immediately after deposition [12, 13].

Sebaceous glands are associated with hair follicles and are located everywhere on the body except the hands and soles of the feet. They secrete an oil called sebum, which is produced to protect the skin and hair from water, act as a lubricant and help absorb fat soluble substances [6]. Sebaceous secretions are often found in fingermarks due to natural grooming actions such as touching the face and brushing the hair. The sebum contains organic constituents such as glycerides, fatty acids, wax esters, squalene, and sterol esters, and is not water-soluble [6, 8, 14]. Apocrine glands are located in the coarse hairs of the groin and armpit areas and consist of water, proteins, carbohydrates, cholesterol and steroids. Sweat from these glands are found more infrequently in fingermark secretions than eccrine and sebaceous due to their location, except in cases of a sexual nature [6, 7]. The locations and compositions of eccrine, sebaceous and apocrine secretions are summarised in Table 1.1.

Table 1.1 Summary of main chemical constituents found in glandular secretions [3]

Source	Location	Inorganic Constituents	Organic Constituents
Eccrine glands	All over body, only glands on palms of hands and soles of feet	Chloride Metal ions (Na^+, K^+, Ca^{2+}) Sulphate Phosphate Bicarbonate Ammonia Water (>98%)	Amino acids Proteins Urea Uric acid Lactic acid Sugars Creatinine Choline
Apocrine glands	Groin and armpits; associated with hair follicles around genitals and mammary glands	Iron Water (>98%)	Proteins Carbohydrates Sterols
Sebaceous glands	All over body except palms of hands and soles of feet; highest concentration on the forehead and back	n/a	Glycerides (30-40%) Fatty acids (15-25%) Wax esters (20-25%) Squalene (10-12%) Sterol esters (2-3%) Sterols (103%)

1.1.3 Deposition factors

For fingermark development, it is important to understand not only the initial composition of fingermark residue but also factors influencing it, both pre- and post- deposition. There are many variables involved in the deposition of fingermarks which influence their composition and quality. The deposition process can be separated into three stages: pre-transfer, transfer, and post-transfer [7]. At each of these stages, various conditions affect the resulting fingermark and are important to consider when determining what detection method should be employed [8].

Pre-transfer conditions are mostly related to individual donors. This includes parameters such as health and condition of the donor's friction ridge skin as well as the amount and type residues present. They are influenced by internal factors such as age, health, and sex, as well as external factors such as contaminants that were touched prior to deposition [7, 10, 15].

Transfer conditions involve both the donor and the receiving surface and will influence the suitability of the resulting impression. Surface conditions may vary by factors including texture, shape, temperature and residues or contaminants present. The interaction between friction ridge skin and a surface will also vary with angle, force, and duration of contact [7, 15].

Post-transfer conditions are also known as environmental factors and affect the quality of marks after deposition [7, 16]. This can include physical contact, for example wiping or smudging, as well as ambient conditions such as humidity and temperature. Post-transfer conditions affect the fingermark until it is recovered, so the composition of aged marks is often very different to freshly deposited ones. Volatile components such as moisture in eccrine secretions evaporate from the surface over time, and this ageing process dehydrates the fingermark. Similarly, exposure of the mark to high humidity or wetting can result in the diffusion of water-soluble components [8, 10]. These conditions all significantly affect the composition of the fingermark and knowledge of the conditions under which a surface has been exposed to will aid in determining the most suitable detection technique or sequence.

1.2 Latent fingermark detection and enhancement

Latent fingermarks are the most common types of marks found at a crime scene. There are a wide range of optical, physical, and chemical processes that may be employed to visualise these latent marks [17, 18]. Fingermarks can be developed in a sequence, sometimes requiring multiple enhancement processes which target different types of compounds. The methods in a sequence moves from least to most destructive and are documented at every step due to the fragile nature of fingermarks. The first stage is optical enhancement, in which contrast between the mark and surface

in increased with lighting techniques such as diffused reflection and absorption [6, 8, 10]. Optical enhancements are non-destructive processes which precede physical or chemical developments.

It is often unknown what contaminants or environmental conditions may have affected a latent mark found at a scene, and therefore surface type is often the primary factor in determining what technique should be employed for development [19]. Surface characteristics play a vital role in transfer and post-transfer conditions and no technique is applicable on every surface type. Important characteristics are the porosity of a surface and its capacity to retain transferred fingermark constituents. Fingermark detection techniques are often separated into three categories based on what surfaces they can develop marks on: porous, non-porous and semi-porous[6]. Examples of each type of substrate is shown in Table 1.2.

Table 1.2 Summary of different substrate types and their fingermark residue absorption characteristics [15]

Substrate	Characteristics	Examples
Porous	Eccrine compounds rapidly absorbed Sebaceous compounds absorbed but more slowly	Paper, cotton, wood
Semi-porous	Eccrine compounds absorbed but more slowly than on porous substrates Sebaceous compounds very slowly absorbed, more slowly than eccrine compounds	Varnished wood, waxy surfaces, glossy papers
Non-porous	Eccrine and sebaceous compounds stay on substrate until physical, chemical, or biological degradation occurs	Glass, metal, paint, plastics

1.2.1 Porous substrates

A porous substrate is one that tends to absorb latent fingermark deposits very quickly, such as paper, cardboard and untreated wood. As water evaporates, water-soluble residual components such as amino acids, urea and chlorides are left behind as a latent fingermark that cannot be rubbed away. However, upon contact with water, these compounds are easily lost. The non-water soluble components remain longer on the substrate and small amounts can be detected after a significant period of time under normal conditions [6].

On porous substrates, enhancement techniques usually work by detecting amino acids found in eccrine secretions. On average, a latent fingermark contains 250 ng of amino acids. Although this amount varies based on donor, amino acids are always present in eccrine secretions to some degree

[7]. Upon contact with porous substrates such as paper, the amino acids are retained in the substrate and do not migrate to a significant extent due to their affinity for cellulose, as shown in Figure 1.1**.** They therefore persist in fingermark residue on porous substrates and have been detected in latent marks up to 80 years old [6, 7, 20, 21].

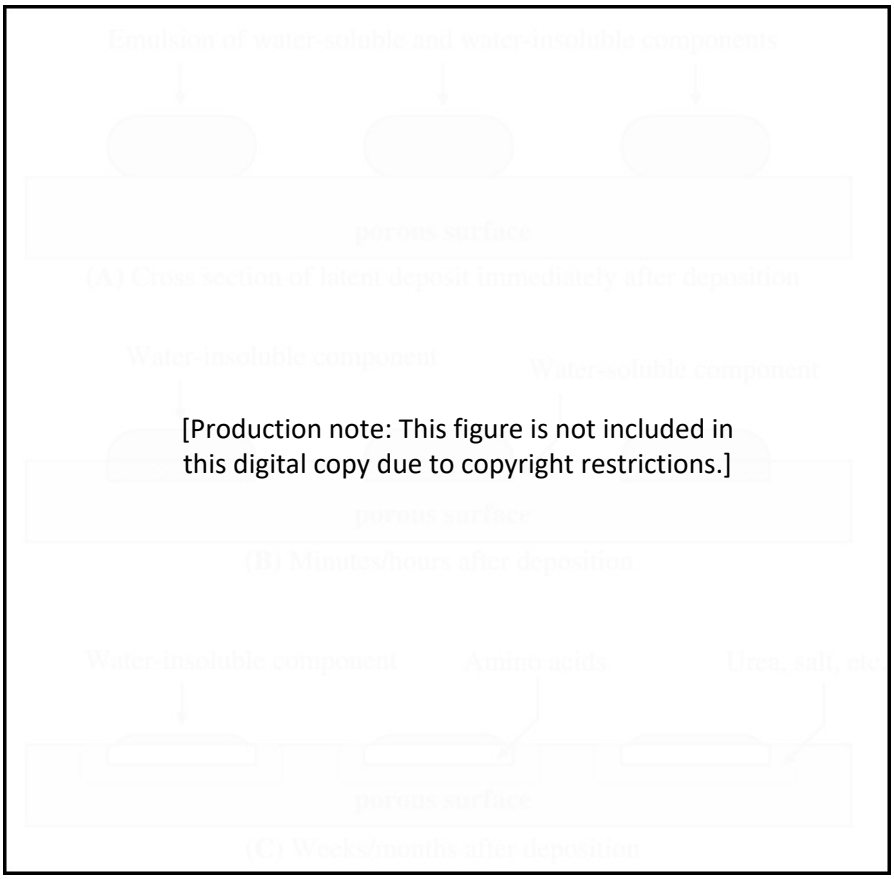


Figure 1.1 Interaction of fingermark residue with porous substrates [6]

Indanedione-Zinc (IND-Zn) and Ninhydrin (NIN) are the most common amino acid reagents used for latent mark enhancement on porous substrates and have a similar reaction mechanism. NIN reacts with amino acids to produce a dark purple product known as Ruhemann's purple [8, 22]. IND-Zn develops visible marks that are also strongly fluorescent to increase contrast on a range of coloured porous substrates. The effectiveness of IND-Zn is notably influenced by the relative humidity the mark has been exposed to prior to treatment [6]. 1,8-Diazafluoren-9-one (DFO) is another reagent which produces visible and luminescent fingermarks. DFO is considered to be a more sensitive reagent than NIN as it reacts with more specific amino acids, and the luminescence creates better contrast [6-8].

Alternate techniques are required on porous substrates that have been wet or exposed to high humidity, as the water-soluble constituents which NIN, IND-Zn and DFO react with are easily lost or tend to diffuse upon contact with water. Physical developer (PD) is an aqueous technique which reacts with an emulsion of the water-insoluble and water-soluble fractions of fingermark residue. It involves

a complex oxidation-reduction reaction that uses an iron-based developer to reduce silver nitrate to a grey-coloured silver metal which deposits on the latent reside [6, 7, 23]. It is an effective method for substrates on which amino acid reagents are ineffective and may also be used on dry porous substrates where other techniques have failed as a final step due to its destructive nature [6, 8]. Lipid stains are also effective on wetted porous substrates, such as Oil Red O (ORO) and Nile Red. These stains interact with the lipid components in latent marks and may be used on both wet and dry porous substrates in a sequence with other detection techniques [6, 7, 18, 22].

1.2.2 Semi-porous substrates

Semi-porous substrates are those which do not fit within the porous or non-porous categories and are typically comprised of features from both. Examples of this kind of substrate include painted substrates, polymer banknotes and glossy paper. On these substrates, water-soluble components are absorbed more slowly than on porous substrates, however some diffusion will occur over time [6].

1.2.3 Non-porous substrates

A substrate is classified as non-porous if it does not absorb any component of latent fingermark residue. Some common examples of non-porous substrates include plastic bags, glass, glazed ceramic, and shiny metal substrates. Latent mark residue can persist on non-porous substrates for a very long time unless it is rubbed off or suffers from environmental degradation, as illustrated in Figure 1.2. Contact with water or high humidity can cause the water-soluble components to be washed away, however water-insoluble components will persist [6]. A range of chemical and physical detection techniques are applicable on non-porous substrates.

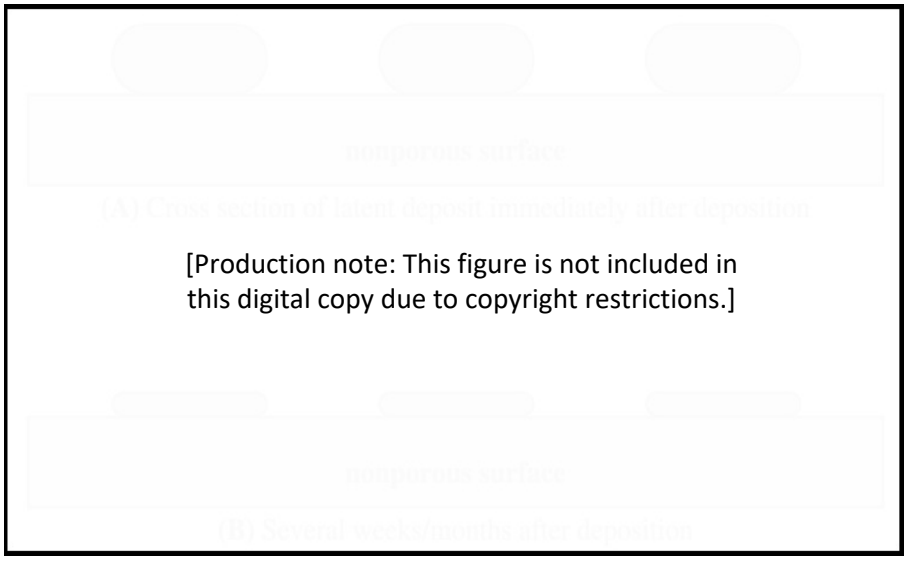


Figure 1.2 Interaction of fingermark residue on non-porous substrates [6]

Chemical development methods include Cyanoacrylate (CA) fuming and different metal deposition techniques. CA fuming is a versatile and effective development technique on almost all non-porous substrates [10, 24]. It involves a vaporised cyanoacrylate ester which reacts with eccrine components of latent fingermarks and polymerises to form a stable, colourless resin along the fingermark ridges. The process occurs in a fume chamber and therefore marks at a scene cannot be treated without highly specialised equipment. However, CA fuming is widely used as the first step in a development sequence on non-porous items due to the development of stable marks which prevents further mechanical destruction of latent marks during handling. CA is very sensitive to fingermark residues, however as it deposits preferentially on water-soluble compounds, the effectiveness of this technique is drastically reduced on marks that have been in contact with water. Marks developed with CA can also be further enhanced with luminescent dyes such as rhodamine 6G (R6G) or basic yellow 40 (BY40) to increase contrast on a range of substrates [7, 8, 11, 25].

Metal deposition methods are another kind of chemical enhancement technique used on non-porous substrates. Vacuum metal deposition (VMD) is a highly sensitive technique that may be used on a wide range of substrates and is notably effective on packaging films and glass. This process requires a vacuum chamber in which a thin layer of gold is vaporised evenly onto a surface. Where fingermark residue is present, the gold diffuses into the fatty constituents. A layer of zinc is then vaporised on the same surface where it condenses onto the gold, leaving behind transparent ridges where the gold has diffused [6, 7, 23]. The marks developed are known as negative marks as the background substrate is developed instead of the fingermark ridges. As VMD relies on the non-water-soluble deposits for development, it is an effective technique on wet or aged marks. However, the process is lengthy, requires highly specialised equipment and must be closely controlled as no specific or consistent set of conditions will result in good development on every type of item [6, 7].

Multi-metal deposition (MMD) and single-metal deposition (SMD) are also applicable on a large range of non-porous substrates and are very sensitive to fingermark residue. MMD is an aqueous development method that is similar to silver PD but is preceded by a colloidal gold treatment that aids the silver in developing on the fingermark ridges [6, 7, 23, 25]. MMD is applicable on a range of substrates including those that have been wet, however the formulations are complex and require a high level of control over parameters such as temperature and pH [26]. These limitations led to the development of SMD, a simpler alternative which replaces the silver enhancement of gold nanoparticles with a gold enhancement procedure. This reduces the number of reagents involved and performs comparatively to MMD. MMD and SMD both rely on the non-water-soluble fractions of

fingermark residue for development and are therefore effective techniques on wetted and aged marks [6, 7, 18, 23].

The most common type of physical fingermark development technique is powdering. Fingerprint powders have been used for over a hundred years and many different types have been developed for use on a range of non-porous substrates. They are popular due to their simple, economical, and portable nature and reply on mechanical adherence to the moist and oily components of fingermark residue. This means they are less effective on aged marks due to drying of the residue and cannot be used on absorbent substrates [8, 27, 28]. The effectiveness of dry powders relies heavily on the size and shape of the powder particles as small, fine particles adhere to the latent mark more easily than large, coarse ones. Most formulations are composed of fine, rounded particles around 1 µm in diameter or fine flake particles around 10 µm in diameter [27]. Issues with dry powders include their lack of sensitivity, which sometimes results in significant background development. They are also most effective on fresh marks which are less commonly found in crime scenes than aged ones [6, 27, 28].

Many dry powders have been developed for enhancement on a range of substrate types and colours, including magnetic and luminescent. Powders are generally applied to a substrate using a brush made of animal hair, fiberglass filaments, feathers or magnetic wands. Powder brushes can sometimes damage fragile marks if incorrect technique is applied, such as too much force or dragging across the substrate [7]. The type of powder chosen depends on the substrate involved. It must provide enough contrast between the substrate and fingermark ridges and not react chemically with the substrate or be physically attracted to it [27, 28]. Carbon black powder is the most common and versatile fingerprint powder, and fluorescent powders can be used on reflective or multicoloured substrates that are more difficult to create contrast on [7, 28].

1.3 Powder suspensions

Powder suspension (PS) is a fingermark development technique made up of an insoluble powder, a surfactant, and water. It is a physio-chemical detection process that involves applying the paint-like mixture to a substrate or item before rinsing it with water to reveal developed fingermarks [25, 29]. This method has been found to detect marks on adhesive tapes, non-porous, and semi-porous substrates. PS is a safe, inexpensive, rapid and simple to use technique that is notably effective in difficult conditions such as marks that have been wet or aged [28, 30, 31]. PS formulations were developed from another fingermark detection technique called small particle reagent (SPR). SPR involves an aqueous solution made up of an insoluble powder and weak surfactant that is sensitive to non-water-soluble fingermark residue. This allows the technique to work on wetted and aged marks. SPR formulations are applied by spraying, soaking, or dipping a substrate in solution before rinsing it

with water to reveal developed fingermarks. This technique is not particularly sensitive and is commonly used on wet substrates that cannot be transported to a laboratory for conventional processing, such as car bodies [6, 8, 9]. SPR is commonly made up of dark grey molybdenum disulfide (MoS_2) particles and Dioctyl sulfosuccinate sodium salt (DOSS) surfactant, however white and fluorescent formulations are also available. PS formulations are much thicker and are generally more sensitive in developing latent marks.

1.3.1 History

PS was first developed in Japan in 1989 as a method of enhancing latent marks on the adhesive side of tape. This method was brought to the United States by Burns, commercially developed by the Lightning Powder Company and branded as Sticky-Side Powder™ in 1994 [18, 25, 29]. This formulation was comprised of a dark powder (composition unknown) and Kodak Photo-Flo surfactant and produced dark grey fingermarks. In late 1996, researchers found Sticky-Side Powder™ to be a superior and less toxic development method to gentian violet, especially on eccrine marks [32, 33]. At this time, researchers Bratton and Gregus looked at different surfactant solutions and found that a mixture of Liqui-Nox detergent solution and Lightning Black powder produced superior fingermark development on adhesive substrates than Sticky-Side Powder™ [31, 32].

The success of this research prompted investigations into other PS formulations, leading to the development of Pink Wop (fluorescent pink) as well as grey and white PS for enhancement on a wider range of adhesive substrates [30, 31]. In the late 1990's the Police Scientific Development Branch (PSDB) proposed a PS formulation made with a precipitated magnetic iron oxide and Kodak Photo-Flo surfactant, which yielded better results than Sticky-Side Powder™ [31]. Research by the United Kingdom (UK) Home Office Scientific Development Branch (HOSDB) into PS's began in 1999 and revealed that a carbon-based powder outperformed other black powders in suspension for use on adhesive tape. This formulation was therefore developed and commercialised [28, 31].

In 2002, a white PS was developed by Wade using titanium dioxide and Kodak Photo-Flo for use on dark coloured adhesives. This formulation was developed by combining Sirchie's Small Particle Reagent White (SPR-W) and adding the surfactant in a 2:1 ratio [34]. The combination of SPR-W and Photo-Flo was also reported as successful on adhesive tapes by Williams *et al.* in 2005, and the authors also noted that immersion was the best application technique despite it being the most time-consuming [35]. Fingermark development on adhesives tapes was considered the only use for PS formulations until the mid-2000's. In 2004, Auld compared powdering, CA fuming, SPR and Stick-side Powder™ for latent fingermark development on wet and dry vehicles. The authors found that Sticky-side Powder™ was found to yield the best results, especially on the wetted items [36]. At the same

time, Strathclyde police department found that PS was effective in developing marks on accelerant-contaminated items from arson scenes [37, 38]. The results from this research prompted further study by the HOSDB (later known as the Centre for Applied Science and Technology (CAST), and now as the Defence Science and Technology Laboratory (DSTL) into the application of PS on non-porous substrates [39]. PS has also been shown to be effective on fingermarks in blood, especially in conjunction with acid dyes on non-porous surfaces [26, 31, 40-42]. Both titanium dioxide based white PS and Sticky-Side Powder™ have been demonstrated to effectively develop blood contaminated fingermarks, however should be used as a final step after DNA collection to overcome the possible loss of DNA cause by physical application of the technique [42, 43].

An iron oxide-based powder suspension formulation (FePS) was developed in 2006 by DSTL which performed at least as well as CA fuming/ dyeing on light coloured, non-porous substrates with the additional ability to enhance marks on wetted substrates. This formulation however could only be used on rubber-based adhesives such as duct tape, as significant background staining was observed on acrylic-based adhesives [29, 31]. Currently, PS is only used operationally on adhesive tapes in Australia, however recent success using these formulations on non-porous substrates suggest its capacity for operational use can be increased [37, 44, 45].

1.3.2 PS formulations

PS formulations are made up of an insoluble base powder and a surfactant solution. There are many different types of powders and surfactants that may be used in PS, some that are sold pre-mixed and others that must be made up before use. DSTL currently recommends three formulations for operational use, as shown in Table 1.3.

Table 1.3 DSTL recommended PS and their uses [41]

Substrate	Powder Suspension
Light adhesive tapes	Carbon-based suspensions, commercially available such as Wet Powder Black (Kjell Carlsson) or WetWop Black (Armor Forensics)
Light, non-porous substrates	Iron oxide-based suspensions made of 50 g iron oxide nanopowder 50-100nm (Sigma Aldrich) and 100 mL surfactant solution (10% Tween 20)
Dark, non-porous substrates and dark, wetted adhesive tapes	Titanium dioxide-based suspensions, commercially available such as Wet Powder White (Kjell Carlsson) or WetWop White (Armor Forensics)

PS formulations are applied by brushing the mixture onto a substrate of interest using a soft animal hair fingerprint brush which is loaded up with suspension to avoid damage caused by dry brushes leading to 'streakiness' in background development [26]. The mixture is left for 10-15 seconds before being rinsed with water. If the developed marks are faint, the suspension can be re-applied however background development may occur if the suspension is left on too long [25, 28]. The temperature of the rinsing water does not affect development, and the substrate may also be wetted before application to avoid the suspension drying out [26].

The recommended formulations have also been optimised for consistency. PS formulations are described to be the optimally "the consistency of paint" and alterations in the consistency can affect the resulting development [31]. On adhesive substrates, thinner formulations produce fainter marks, and thicker ones result in clumps of powder left on the substrate and dry more quickly which can damage the fingermarks. On non-porous substrates, diluted suspensions can still yield good results and are easier to apply [25, 31].

1.3.3 Powder type

There are a range of powders that can be used in PS; however not all powders are suitable. The powder used directly affects what kind of substrate a PS formulation is applicable on, for example white powders are only effective on dark substrates where contrast is created. Operationally, only black and white PS are used as shown in Table 1.3 above. Carbon, titanium, and iron oxide-based formulations appear to have a greater affinity for fingermark residues than other compounds tested in PS formulations [26].

1.3.3.1 Powder particle size

The size of powder particles is an important factor in the development of fingermarks using PS. Early studies by Frank *et al*. in 1993 on white PS found that smaller particles (around 2 µm) were more effective than larger ones (around 6 µm) [28]. In 2010, Jones *et al*. looked at the effect of substrate surface topography on FePS using atomic force microscopy (AFM) and scanning electron microscopy (SEM). The authors found that the iron oxide particles which adhered to the latent mark consisted of cubic crystal clusters ranging from 1 µm to a few hundred nanometres in diameter. It was observed that larger particles present in the dry powder did not adhere to the mark [46]. DSTL recommended that this range of powder particle size is optimal for use in PS formulations, and that compounds with primarily larger or smaller sizes would be less effective [31, 45]. This is largely supported by a study by Downham in 2017 which compared six different iron oxide powders in PS formulations. The results showed that powders with particles within this range were effective and ones predominantly larger (75 µm) were not effective and separated in solution [29]. In 2007 tests were performed by DSTL using

nanopowders in PS formulations, however only some developed fingermarks (such as an iron oxide nanopowder) and they were less effective than traditional formulations [31]. These observations, coupled with research by Downham *et al.* led to the recommendation for use of an iron (II,III) oxide from Fisher Scientific with a mean particle size of <5 µm in the 2014 Fingermark Visualisation Manual (FVM) [31].

In 2018, Downham found notable variation between batches of iron oxide powder used in PS formulations [45]. Particle size analysis between two batches of Fisher Scientific iron oxide powder from 2008 and 2015 showed significant differences between the volume of particles between 5 µm and 1 µm, 13.2% in the 2008 batch and 1.8% in the 2015 batch. The 2008 batch recovered 19% more fingermarks in the study, adding further support that this range of particle size is essential in fingermark development using FePS [45]. This study also investigated the use of iron oxide nanopowders with a 50-100 nm particle size. In a PS formulation made with 10% Tween 20 surfactant, the nanopowder developed 27% more fingermarks than the 2015 batch of Fisher Scientific iron oxide. It is unclear if these results are largely affected by the poor performance of the 2015 batch. The authors suggested the nanopowder would have less batch variation and may be viable for optimisation in a new PS formulation [45].

Iron oxide nanopowder was also used in a recent study by Illston-Baggs *et al.* investigating the detection of fingermarks on eco-friendly soft plastic packaging [47]. Two formulations were initially tested, one using the 2014 recommended DSTL formulation outlined in the FVM, comprised of the Fisher scientific iron oxide powder and Triton X-100 surfactant, and the other using the Sigma iron oxide nanopowder mixed with 10% Tween 20 surfactant solution. Preliminary tests in this study showed that the Fisher powder formulation produced poorer contrast and was more difficult to wash off than the nanopowder, which was therefore chosen to be included in further parts of the study. However, as these results were preliminary to the bulk of the study, details such as substrate type, number of donors and age of fingermarks are not stated.

Issues of inconsistency with the Fisher iron oxide powder used in the 2014 FVM formulation outlined by Downham led to a reformulation of the recommendation in the 2022 edition of the FVM [25, 41]. The updated formulation is shown in Table 1.3. An iron oxide powder from Sigma Aldrich with a mean particle size of 50-100 nm is now recommended for use in FePS, however the authors note that due to the higher price of the nanopowder, the Fisher powder may still be used with consideration of possible batch inconsistencies. The recommended surfactant was also updated to a 10% Tween 20 solution, due to toxicity concerns of Triton X-100. These observations are supported by results produced by Illston-Baggs *et al.* and a 2023 study by Clover Ree *et al.* [47, 48].

1.3.4 Carbon and iron oxide-based formulations

There has been considerable research into the applications of carbon-based PS and FePS formulations, as they both develop dark fingermarks on light-coloured substrates. The main difference between carbon and iron oxide powders in PS is their opposing abilities to develop mark on adhesive and non-porous substrates.

1.3.4.1 Adhesive substrates

PS was first developed for use on adhesive tapes, which is a difficult substrate for fingermark development as both sides often require different treatments. This is due to the varying chemical nature of adhesive coating and backing layer. Adhesives can be made with different products which affect its use such as staying power and strength. For fingermark development, adhesives are categorised as being rubber-based or acrylic-based [49]. Carbon- based PS and FePS perform similarly on rubber-based adhesives, however on acrylic the FePS produces heavy background staining and is therefore ineffective in creating contrast between the mark and substrate [25, 31, 45]. As it is very difficult to determine the reaction of an adhesive with FePS without doing a spot test, carbon-based formulations are recommended for all adhesive substrates. DSTL recommends CA fuming for the non-adhesive side of tape unless it has been wet [25, 31, 49].

In an effort to create a PS formulation that could be used effectively across all adhesive substrates, the Scientific Police Division in Spain developed a new PS called WET UCIO in 2021 [50]. This formulation was made using 20 mL sodium dodecyl sulphate surfactant and 30 g Sirchie Silk Black carbon black powder. In a 2022 study by Claveria *et al.*, this formulation was compared to WetWop and an iron-oxide formulation called ASPD (adhesive-side powder dark) from Sirchie. Eight tapes were used to compare the WET UCIO to Wet Wop on acrylic-based adhesives and to the ASPD formulation on rubber-based adhesives. In all comparisons, the WET UCIO formulation was equal or superior in effectiveness to the commercially available products [50]. However, WetWop was also not used on the rubber-based adhesives despite its common use on all adhesive substrates, so the comparative performance of WET UCIO cannot be properly evaluated on these substrates. The authors acknowledge that further investigation using this formulation is required before it is operationally viable, as variables such as aged fingermarks and depletion sequences were not tested. This study demonstrates the importance of further research to improve the use of PS formulations on tape, as issues associated with common techniques may be overcome.

1.3.4.2 Non-porous substrates

The use of PS to develop fingermarks on non-porous substrates is a far more recent application of the technique. Many techniques are known to be effective on a range of non-porous substrates, so research has focussed on the comparative effectiveness of PS formulations to other techniques. FePS is currently recommended for use on light coloured, non-porous substrates, as carbon-based formulations were less sensitive and more prone to background staining [31].

In 2013, Bacon *et al.* found that carbon-based PS caused overdevelopment when in contact with titania pigment, which is found in many light-coloured plastics. Despite not using an iron-oxide based formulation in this study, the authors referenced a 2010 study by Jones *et al.* and suggested that FePS would likely not have this issue due to larger particle size and partially conducting material [46, 51]. Both studies used the same three plastic substrates and therefore these results are only applicable to plastic substrates.

FePS has been used in several studies investigating environmental effects on fingermarks, such as water, sea salt spray, fire and grease. In 2009, DSTL performed a pseudo-operational trial for the development of fingermarks on flexible plastics. They compared the performance of FePS to VMD and CA/BY40 and found that PS and CA performed comparatively on dry plastics and PS was the most effective on wetted plastics [39]. Bradshaw (2008), Gardner (2016) and Dominick (2011) used FePS to develop fingermarks on items from simulated arson scenes which had been exposed to temperatures up to 600°C [37, 52]. Both studies concluded that PS and CA/BY40 were comparably effective on a range of non-porous substrates, however PS should be used if the item has been wet. In arson scenes, items of interest are often wetted during fire suppression and therefore are an extremely important consideration in these kinds of scenes. PS formulations may also assist the recovery of fingermarks on arson items by removing soot as well as developing fingermarks due to its aqueous nature and use of detergent [31, 37, 53].

In 2013, Gaskell found that on tiles contaminated with various substances commonly found in household environments, VMD developed the most fingermarks, however FePS was a more portable and efficient process [54]. PS was particularly effective on marks contaminated with drinks, sauces, and moisturisers, as well as aged, dried, and heated marks. This study is relevant in an operational context for use of PS on non-porous substrates, however only one donor was used in the study and the author noted that looking at a wider range of substrates and using split marks would have given a more complete and accurate picture [54].

Most recently, in 2023 Claveria *et al.* developed a new FePS formulation named POSME for use on non-porous substrates such as glass, metal and plastic as part of a sequential development process to be used after dry powders [55]. After initial optimisation, the POSME formulation contained a surfactant solution made with Tween 80 and Synox Black 6318 iron oxide powder. Fingermarks which had been exposed to heat and humidity as well as those aged in laboratory conditions were included in this study and it was found that using the POSME formulation after dry powder increased development by 26.5% over all substrates. However, half of the substrates tested were plastic and are prone to background development when treated with dry powder, which likely skewed results. No fingermarks were detected on metal using POSME, and it performed more poorly than dry powder alone on glass. Furthermore, the results of the initial optimisation were not published, and it is therefore not clear how the POSME formulation compares to those previously tested and recommended.

Many of the above studies have been conducted in the Europe and the UK, however FePS have also been involved in Australian studies. Goldstone investigated the effectiveness of 11 kinds of dry powders, PS and SPR to develop salt-spray affected fingermarks in 2015 [44]. FePS was the most effective technique, recovering 96% of affected marks aged 1-week and 67% of marks aged 1-month. This further supports the effectiveness of this PS formulation on non-porous substrates and gives it an Australian context. However, only one substrate was investigated (glass) and the environmental effect of salt spray is not relevant for cases in the city or indoors. Clover Ree *et al.* also compared carbon PS with FePS on a range of adhesive and non-porous substrates, after first optimising FePS formulations based on products available in Australia [48]. It was found that overall, carbon-based formulations were more effective due to heavy background development using iron oxide on some adhesives, however the FePS were notably effective on plastic substrates.

Considerable research has been done into the various applications of different PS formulations. The limitations of each formulation have been identified mainly based on the powder type used, showing that the powder component of PS formulations is an important variable governing the effectiveness to which PS can develop fingermarks. However, powders only make up half of the PS ‘recipe’, and consideration must also be given to surfactants used in each formulation.

1.4 Surfactants

1.4.1 Types of surfactants

Surfactants, or surface active agents, are amphiphilic molecules which are the primary component in cleaning detergents. They are used to reduce the surface tension between two liquids, between a gas

and a liquid, or between a liquid and a solid. For liquids, this increases the spreading and wetting properties. They are also used to disperse aqueous suspensions of insoluble materials such as powder particles [56]. Surfactant molecules consist of two main components: a hydrophobic 'tail' and a hydrophilic 'head'. The hydrophilic group, also known as the functional group, has an affinity for water and its charge largely determines the properties of the surfactant. The hydrophobic group is usually an alkyl chain made up of 8-22 carbons which does not have an affinity for water and may be linear, branched or cyclic [56, 57].

There are many kinds of surfactants, however all can be grouped into two main categories: ionic and nonionic. Ionic surfactants may be sub-classified as cationic and anionic surfactants if the head groups are positively or negatively charged, respectively. Ionic surfactants can also be amphoteric, in which the head group contains both positive and negative charges. Ionic surfactants dissociate into their respective ions when dissolved in an aqueous solution. If the surfactant contains both positive and negative groups, it is zwitterionic, and if the head group has no charge the surfactant is nonionic and will not dissociate in solution [56-58]. Examples of different surfactant and their uses are shown in Table 1.4.

Table 1.4 Examples of different surfactant types [57, 59, 60]

Surfactant type	Example	Use
Anionic	Alkyl sulfates, soaps	50% of overall industrial production, laundry detergent, dishwashing liquids, shampoos
Cationic	Quaternary ammonium salts	Used together with nonionic surfactants but not with anionic, softener in textiles, anti-static additives
Nonionic	Ethoxylated aliphatic alcohol, polyoxyethylene surfactants	45% of overall industrial production, a wetting agent in coatings, food ingredient
Zwitterionic/ Amphoteric	Betaines, amphoacetates	Specialty use (eg. Cosmetics)

A fundamental property of surfactants is their ability to form aggregates called micelles, in a process called micellization. In a micelle, surfactant molecules (or monomers) are clustered together with the hydrophobic tail in the interior and the hydrophilic head group on the exterior, as illustrated in Figure 1.3. Micellization only occurs beyond a point known as the critical micelle concentration (CMC), which differs between surfactants. The properties of surfactants can be very different when micelles are

formed in comparison to monomers in solution, and the CMC can be measured by changes in surface tension, conductance, and solubilization. The CMC is lower for nonionic surfactants than it is for ionic surfactants and also decreases with increasing alkyl chain length and can be affected by parameters such as temperature and pH [56-58, 61-63].

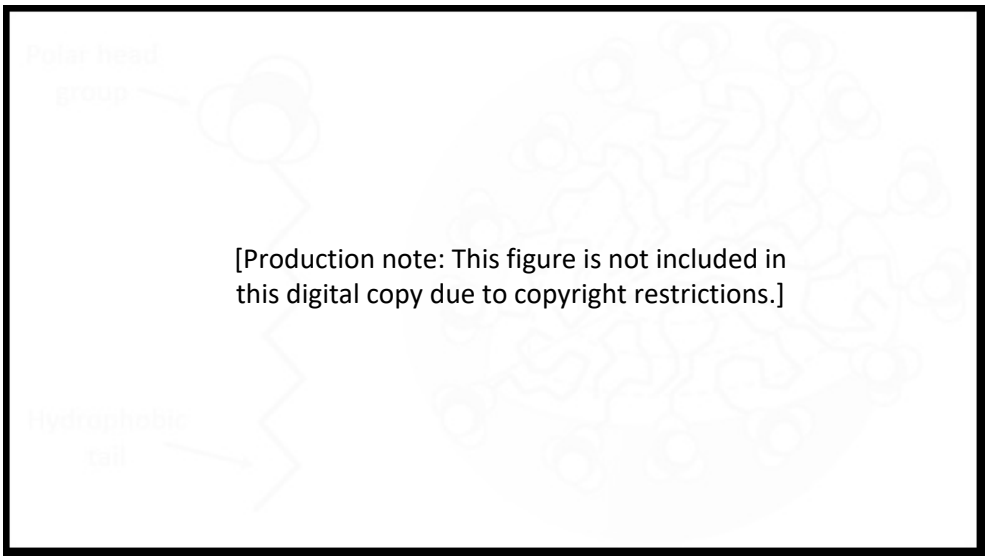


Figure 1.3 Schematic diagram of surfactant micelle structure [56]

Figure 1.3 represents a spherical micelle, however not all micelles assemble in this way. The chemical structure of a surfactant will influence the structure by which micelles are formed. Micelles usually form with the hydrophobic tails pointing inwards while the polar head groups are on the outside, as illustrated in Figure 1.3, and can form as a sphere or as a cylinder. The length of cylindrical micelles is highly variable, and often surfactants with cylindrical micelles are polydisperse [56, 61, 64]. Micelles can also form as bilayers, creating either a sheet or a ring in which hydrophobic tails are sandwiched between two polar heads on either side. It is also possible for micelles to be inverted, in which a water core is surrounded by the polar head group and the hydrophobic tails orient outwards. In these cases, the alkyl chains and organic solvent make up the continuous medium [56, 61, 64, 65].

Several experimental methods can be used to determine the shape of surfactant micelles. Small-Angle X-ray Scattering (SAXS) and Small-Angle Neutron Scattering (SANS) are widely used to observe how micelles scatter light or particles to determine shape or size [66]. Cryogenic Transmission electron Microscopy (cryo-TEM) provides direct visualisation of micelle morphology, allowing differentiation between spherical, rodlike or branched structures [67]. Other methods like Nuclear Magnetic Resonance (NMR) and Dynamic Light Scattering (DLS) techniques measure how micelle move in solution, which may also provide information about their shape [68]. These complementary methods are often used together to comprehensively characterise micellar shape and structure [69].

It is theorised that micellar shape is affected by factors such as concentration, temperature and the presence of added electrolytes [70]. For example, studies by Montes-de-Oca (2022) and de Lera-Garrido (2022) suggest that the cationic surfactant cetyltrimethylammonium bromide (CTAB) forms as spherical micelles around the CMC, however above this concentration, flexible, rod-shaped micelles may be formed at approximately 300 mM [65, 71]. In contrast, cetyltrimethylammonium chloride forms spherical micelles at all concentrations between the CMC and solubility limit [70]. The addition of electrolytes such as sodium chloride can change the shape and size of surfactant micelles [70, 72, 73]. This demonstrates the complexity and fluidity involved with micelle shape determination.

1.4.2 The role of surfactants in fingermark development

For fingermark development, surfactants are often used to suspend insoluble materials in an aqueous solution, which occurs due to the presence of micelles in surfactant concentrations above the CMC. Nonionic and anionic surfactants are more commonly used due to their lower CMC and more abundant nature [56]. There are several fingermark development techniques which involve the selective deposition of solid material onto fingermark ridges from an aqueous medium. These techniques involve the suspension of a solid phase medium in an aqueous solution and have varying degrees of stability which controls their ability to selectively deposit solid particles. This stability is controlled by surfactants as they form micelle structures around solid particles to suspend them [26]. Fingermark detection methods other than PS that utilise surfactants include SPR, PD, MMD and SMD. Despite the widespread use of these techniques for fingermark development, there are still many gaps in our understanding of the role of surfactants which, if filled, may assist in further optimisation of the techniques.

SPR relies heavily on surfactants to preferentially deposit powder on the fingermark residue. This technique involves a suspension of powder particles in surfactant solution which rapidly becomes unstable. This allows the solid particles (which are suspended within a micelle) to settle towards the surface being treated and interact with the fingermark residue. The hydrophobic tail of the surfactant molecules is affected by high molecular weight components of the residue (such as fats and oils). This interaction releases the powder particle and allows it to deposit preferentially on the ridges, as illustrated in Figure 1.4. It is also possible that the hydrophilic head of the surfactant reacts with metal salts to create a black precipitate and therefore increases contrast of the developed mark. Washing the substrate then removes unbound particles and reveals the developed fingermark [6, 26, 31].

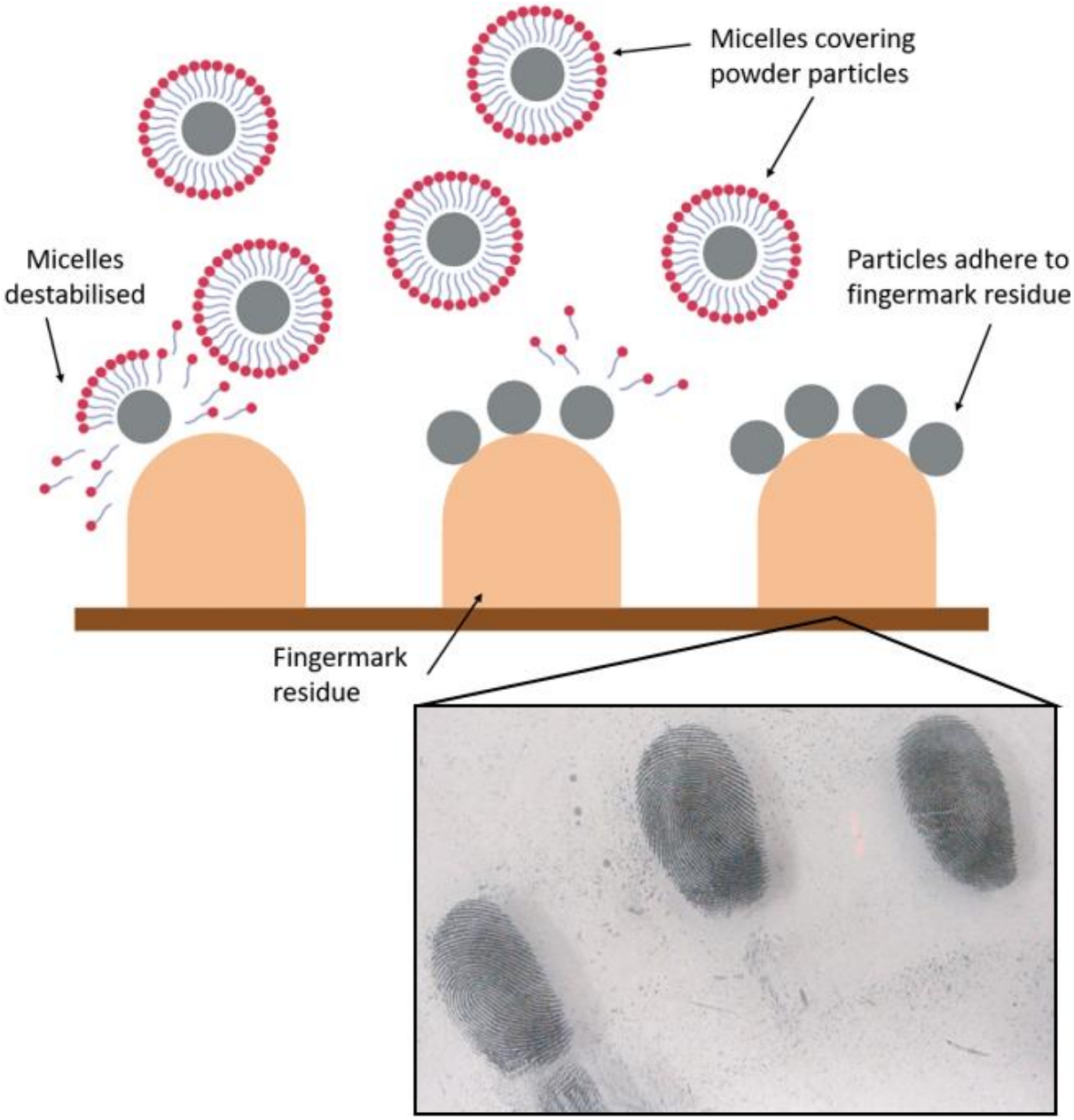


Figure 1.4 Mechanism of SPR in which micelles are destabilised by fingermark constituents. Adapted from Bleay et al. [31]. Image of fingermarks developed with SPR on glass surface adapted from Lennard [8]

Current SPR formulations contain a surfactant called DOSS, which is anionic. Older formulations used Tergitol-7 surfactant (which is nonionic) until it was found to have harmful environmental effects [25, 31]. It is suggested that the nature of surfactant (ionic or nonionic) used is not critical, however surfactants containing molecules with a tail made of less than 8 carbons are ineffective in this technique and the optimum length is between 17 and 20 carbons [26, 28, 31]. Surfactants are a key component in SPR for fingermark development, and the quality of developed marks is affected by concentration and solubility of the surfactant. Too high a concentration prevents the deposition of powder and produces very faintly developed marks, and too low results in background development [9, 28]. Research by Haque in 1989 showed that an SPR formulation using iron oxide powder was

effective on a range of non-porous substrates. However, there was a significant difference in performance depending on which surfactant was used. SPR made with Kodak Photo-Flo surfactant did not develop any fingermarks, however a surfactant solution using Brij 35 and choline chloride was very effective. Both Kodak Photo-Flo and Brij 35 are nonionic surfactants, suggesting there was another difference between the two which is important in the success of fingermark development using SPR. The authors questioned whether the addition of choline chloride was necessary, however no further studies were performed. This study is considered to have led to the development of a FePS formulation [74].

PD is another technique in which the addition of a surfactant is essential for fingermark development. This process involves a working solution containing silver nitrate, a redox solution, and stock detergent in which items of interest are sequentially submerged. The stock detergent requires both a cationic and a nonionic surfactant, without which the PD technique is ineffective. Although the full details of this detection mechanism remain unclear, it is commonly accepted that the presence of surfactants holds the silver nanoparticles in a stable suspension until a destabilising agent (such as fingermark residue) is introduced to the working solution [75]. The silver nanoparticles acquire a negative charge as they are surrounded by citrate ions from the redox solution. The cationic surfactant then supresses the negative charge by surrounding the silver in a micelle structure with positively charged head groups, staggering the placement of these molecules as illustrated in Figure 1.5. This creates a stable suspension by preventing the silver ions from aggregating. The nonionic surfactant is required to aid this stability and assist the cationic surfactant to dissolve in solution [7, 18, 26, 31].

Similarly to the mechanism of SPR, the micelles are destabilised by a part of the fingermark residue to allow the silver colloids to deposit onto the mark. Current PD formulations use Symperonic-N as the nonionic surfactant and n-dodecylamine acetate (n-DDAA) as the cationic surfactant [6, 25, 26]. In a paper by Coulston in 2022, it was noted that Symperonic-N had recently been “environmentally outlawed” after its manufacture was banned in Europe and the UK, however Tween 20 may also be used as an alternative [75]. Houlgrave (2011) suggests that the use of Tween 20 will also increase the shelf life of the PD working solution [76].

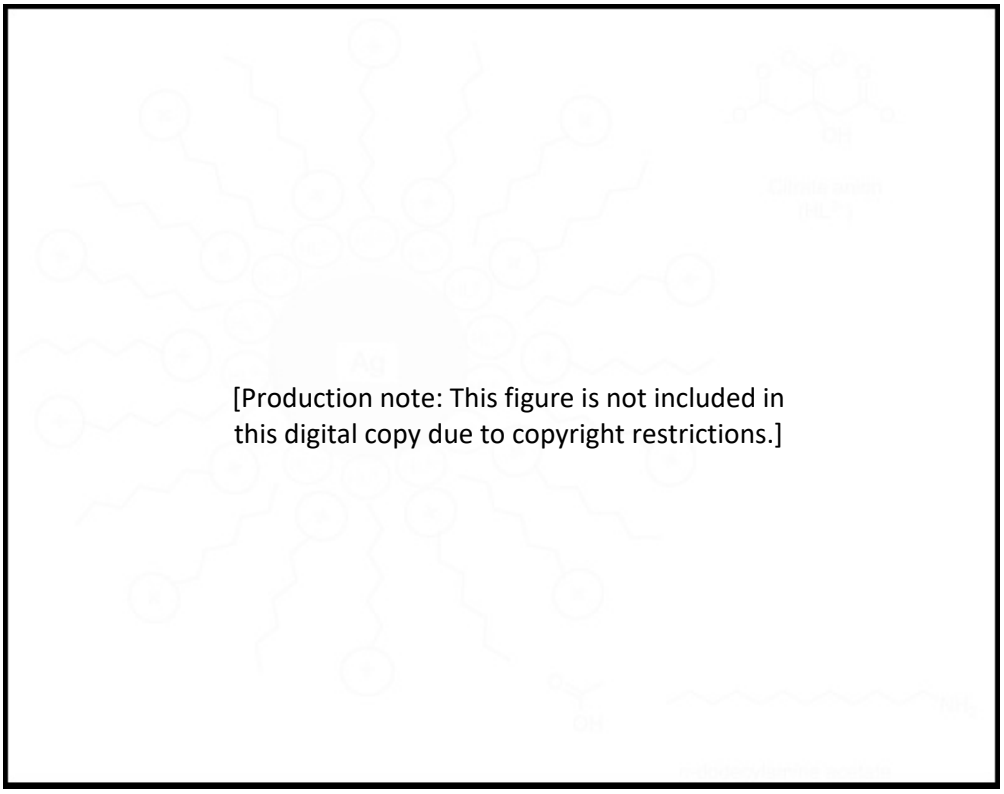


Figure 1.5 Structure of cationic surfactant micelle surrounding a silver particle in PD working solution [26]

The technique of MMD is similar to that of PD but uses a modified silver solution. In this technique, the item of interest is submerged in a colloidal gold working solution, which includes Tween 20 surfactant to stabilise a suspension of the gold nanoparticles. The gold particles are deposited on amino acids, proteins and peptides present within fingermark residue and develop marks of very poor contrast [77]. Due to this poor contrast, a second working solution containing silver nitrate (similar to that used in PD) is required to better enhance the marks. The micelles holding silver particles in the nitrate solution are destabilised by gold particles instead of fingermark residue. For this reason, the nonionic surfactant Tween 20 is used instead of n-DDAA, as the use of a more stable cationic surfactant would hinder the selective deposition of silver particles [7, 25, 26, 77].

1.4.3 The role of surfactants in PS formulations

The exact mechanism behind successful fingermark visualisation using PS is still unknown and remains a subject of debate. It is believed to be similar to SPR, where surfactant micelles surround and suspend powder particles. Some component or property of the fingermark residue then destabilises these micelles to allow the powder to deposit along the ridges [25, 31]. PS has been shown to be effective on aged fingermarks and those affected by heat, processes which act on the latent mark by drying it out and altering the composition of the residue [19, 26, 29, 52]. One possible explanation for this observation is that as the fingermark dries out, the eccrine components which are protected by a

water-insoluble matrix are brought closer to the substrate which allows deposition to occur more readily, as illustrated in Figure 1.6 [26]. This proposed mechanism is further supported by work performed by Bacon *et al.* investigating background development by carbon-based PS on polymer substrates. The authors found that the carbon powder was being preferentially deposited on titania pigment particles within the polymer as well as on fingermark residue. This behaviour was only observed with pigment particles located less than 30 nm below the surface, suggesting the interaction which destabilises micelles and releases the powder is dependent on proximity [26, 51].

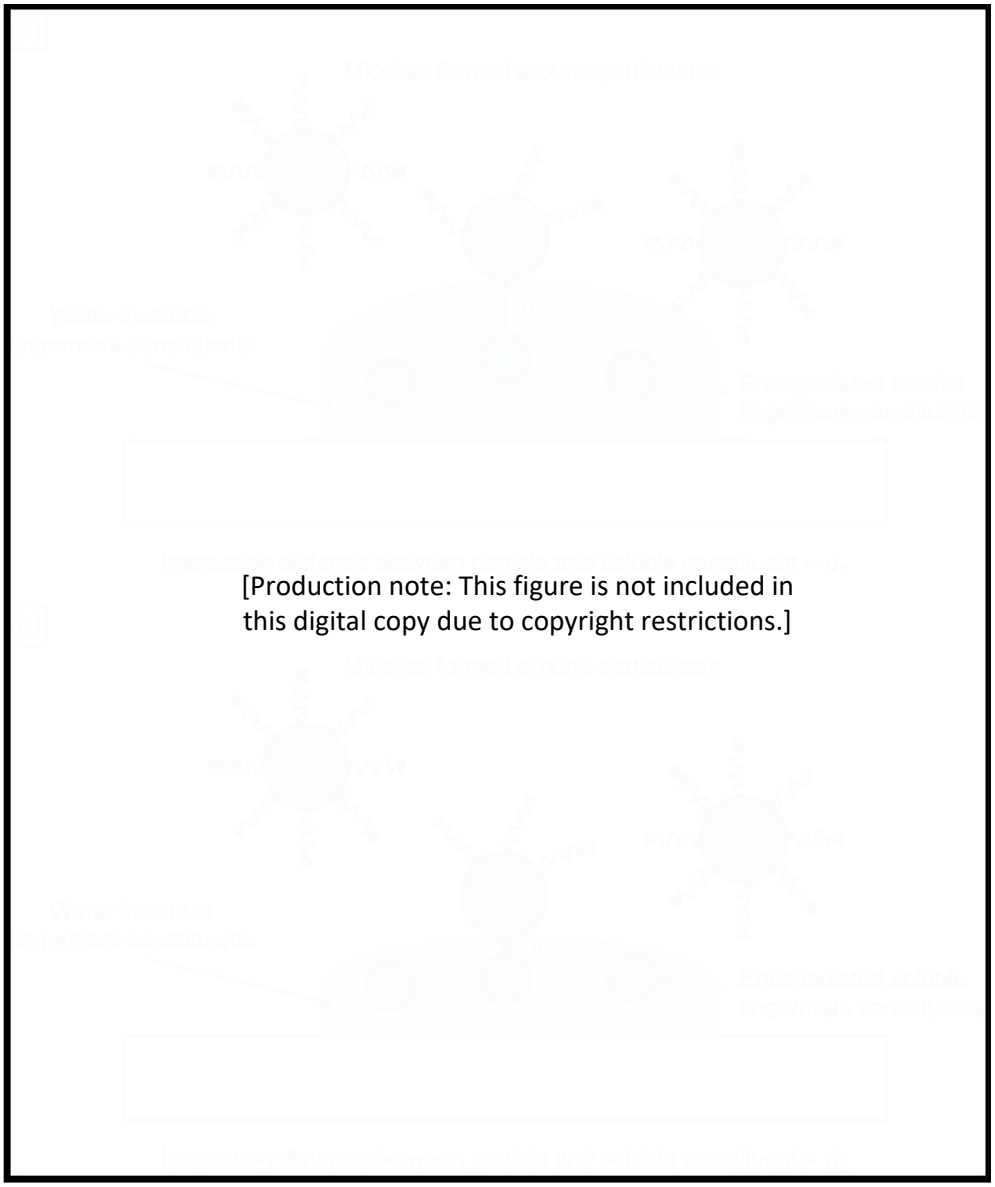


Figure 1.6 Schematic diagram of proposed mechanism for PS, with possible explanation why it is more effective on older, dried marks: (a) large interaction distance d_1 in fresh marks and (b) significantly reduced interaction distance d_2 in aged marks [26]

Surfactant solutions play an important role in the development of fingermarks using PS formulations and research has been conducted into what kinds of surfactants are effective [31, 41, 48, 55]. In

addition to the role of surfactants mentioned in the mechanism above, surfactants also suspend powder particles and decrease surface tension to allow for more homogenous mixing. Research into different kinds of surfactants for use in PS have been focussed on FePS formulations, as other common types are commercially available pre-mixed solutions [31, 78]. Despite surfactant solutions having been broadly tested for use in PS, their more specific role in the PS mechanism remains unclear. Because of this, any changes to surfactant recommendations subsequently require robust testing of multiple variables such as donors, substrates, and environmental conditions to be validated for use in casework. Improving the fundamental understanding of how surfactants actually interact with powder particles, fingermark residue components and substrates will aid in understanding of the intricate mechanisms involved in fingermark development and improve future optimisation attempts.

1.5 Project aims and objectives

The aim of research into fingermark detection techniques is to increase the quantity and quality of fingermark recovery. Due to the focus being primarily on the performance of techniques, our understanding of the exact mechanisms through which these processes can enhance latent marks is often unknown [14, 18, 79]. More recently, focus has been directed to improving our understanding of currently used techniques as it may aid in optimising them. Mechanistic studies will improve fundamental understanding of vital research factors such as fingermark residue composition and changes after deposition, as well as interactions between fingermark residue components, technique application, and substrate types. These studies provide invaluable insight into residue interactions and aid future technique development and optimisation efforts, which in turn will improve the rate and quality of fingermark detection in casework.

The overarching aim of this research project was to contribute to the fundamental understanding of fingermark development using FePS by investigating the role of surfactants in this technique. The current theorised role (as outlined in section 1.4.3) suggests surfactant micelles encapsulate powder particles in suspension before being destabilised by fingermark residue and allowing for preferential powder deposition. However, no current research has explored the more specific factors relating to successful development, such as the effect of different surfactant types and concentrations, the importance of micelle formation, and the interaction between surfactant micelles and fingermark residue.

Guidelines published by the International Fingerprint Research Group (IFRG) outline recommendations for how to carry out different phases of fingermark research [80]. This encompasses research from early technique validation studies (Phase 1) to fully operational trials for casework (Phase 4). However, as first-principles research investigating foundational understandings only has

relatively recent interest, there are currently no guidelines or recommendations available for this kind of work. Because of this, in addition to the exploratory nature of the project, much of the work presented in this thesis involves trialling new methods or further exploring the potential of currently proposed methods to better understand the fundamental interactions occurring in this technique. As such, the objectives of this project involve not only exploring factors relating to surfactant efficacy in fingermark development with FePS but also assessing possible methods that may be used to improve this kind of fundamental research in fingermark detection more broadly. To achieve the aim of the project, the research was split into four main parts to gain a more holistic understanding of the role surfactants may play in fingermark development with FePS.

In the first part (chapter 2), a broad overview of the effect of different surfactants, concentrations, and powder to surfactant ratios are tested on non-porous substrates. This work provided a robust foundation for further work by assessing the impact surfactants have on fingermark development. Ten surfactants representing a range of ionic natures were used to compare the performance of surfactant types and concentrations on glass, plastic and ceramic substrates on natural donor fingermarks. This allowed for an examination of the role of surfactant ionic nature in FePS development, as well as the effect of substrate type on both fingermark and background development. Surfactants were chosen based on their use in PS, other development techniques or common industrial applications. This work builds upon previous research looking at the impact of surfactants on fingermark development, by assessing the widest range of surfactants to date. This was done by examining the relationship between FePS formulation parameters such as surfactant type, concentration and ratio with the development of fingermark ridge detail, contrast and background deposition. Chapter 2 aimed to examine the boundaries of surfactant efficacy and provide a reference for further work of the impact surfactants have in fingermark development on a wide range of variables.

The second part of the study (chapter 3) looked more closely at the mechanism of FePS by assessing the importance of micelle formation. The CMC of each surfactant was determined by measuring surfactant surface tension so formulations could be made above and below this concentration and compare formulations with and without micelles. FePS formulations were made of each surfactant at 0.5x and 50x the CMC, to determine if surfactant performance was more consistent at the same point above the CMC and if the presence of surfactant monomers influenced development. This part of the study also investigated the structural features of surfactant monomers and micelles, to evaluate the possibility of providing recommendations or predictions for surfactant efficacy in FePS based on factors such as surfactant chain length or polar head group complexity. Overall, this work aimed to

determine the importance of surfactant micelles in fingermark development with FePS and investigate what structural factors may be influencing performance.

Chapter 4 details the third part of this research, which focussed on better understanding the interaction between surfactant micelles and the fingermark residue being developed. This was done by attempting to control fingermark residue composition through the use of artificial fingermark simulants which have been explored in previous research [81, 82]. This part of the study first aimed to evaluate the current capabilities of artificial emulsions, by comparing a house-made and commercially available simulant emulsion on porous and non-porous substrates. The emulsions were compared to natural fingermark residue after being developed by commonly used fingermark detection techniques to assess the capabilities of the emulsions to mimic natural fingermark residue. Artificial simulants were then used to examine the residue targets involved in FePS development and the extent to which surfactants influenced development of eccrine and sebaceous residue fractions. The overall aim of this part of the research was to better understand the interactions between surfactants and fingermark residue fractions responsible for the initiation of FePS.

The final part of this research (chapter 5) investigated a new method that may be used to quantify the amount of residue present on a non-porous substrate and determine differences in residue presence before and after application of surfactants. This method utilised visualisation of fingermark residue using microscopy and quantification of the residue present using Image J software. Fingermarks were washed with a surfactant solution (no powder) and imaged before and after this washing step, with the aim of determining if some surfactants may be removing fingermark residue during development and subsequently resulting in lack of ridge detail. Donor fingermarks loaded with eccrine or sebaceous residue were used to evaluate the interaction between surfactant solutions and each residue fraction. The impact of this washing step on subsequent FePS technique development was then investigated using Tween 20 surfactant and establishing the effect of this washing – development sequence using a range of Tween 20 concentrations. This also allowed for an examination of the extent to which FePS formulations could develop fingermarks and underlying substrates that had been washed by a surfactant. This part of the research endeavoured to better understand the interaction between surfactant micelles and fingermark residue overall.

This body of research aims to investigate and better understand the interactions between surfactants and factors relating to fingermark development in FePS, including powder particles, fingermark residue, and substrates. This was done with the goal of improving the fundamental understanding of the FePS technique and improve future research efforts, supporting further optimisation and expanding the capabilities of this technique for fingermark detection.

Chapter 2: Comparison of surfactant effectiveness in FePS

2. *Comparison of surfactant effectiveness in FePS*

Portions of this chapter have been published within Forensic Science International – see ***Research Communication*** *for more information.*

2.1 Introduction

Research into FePS formulations over the last few decades has focussed on optimising formulations by testing different powder and surfactant combinations [40, 45, 46]. Studies have focussed largely on FePS as carbon and titanium dioxide-based formulations are commercially available as pre-mixed formulations, whereas FePS must be made up in the laboratory before use. This allows for a high degree of customisation and investigation of changing properties, while remaining relevant for operational use. Research into FePS formulations have understandably centred around evaluating the effectiveness of various powder and surfactant combinations with the aim of better optimising the technique [45, 48, 55]. However, research into the fundamental understanding of how these components work and why some are more successful than others is very limited. Some studies have investigated the effect of powder particle size and shape on the effectiveness of PS formulations, however research into surfactant properties has been less thorough despite a long history of changing surfactant recommendations for use in FePS [45, 46].

Surfactants are amphiphilic molecules used widely outside of fingermark development in several applications, such as cleansers, cosmetics, biological systems, and food production [57, 83-85]. Surfactants are used to reduce surface and interfacial tension between two or more phases through the formation of self-aggregating micelles which can range in diameter from nanometres to microns. They are also used as stabilisers in the dispersion of nanomaterials and have great potential for further application in nanotechnology [85]. A wide range of surfactants are available for different uses, and the suitability of a surfactant may be determined by physical properties such as ionic nature, chain length and micelle shape/ size. Knowledge of these parameters and their effects in various applications can help identify and tailor useful surfactant types and concentrations. Despite research into surfactant properties being widespread for industrial use, very little investigation has been published into the effect of surfactant properties on fingermark development. Because of this, it is difficult to determine why some surfactants are successful in FePS formulations while others are not.

Despite a lack of understanding of surfactant properties and their effect on FePS development, many surfactants have been tested for use over the last few decades. In 2006, DSTL recommended an FePS formulation that used a surfactant solution of Kodak Photo-Flo and distilled water in a 1:1 ratio,

however this recommendation changed to a surfactant solution containing Triton X-100 and ethylene glycol in 2009 [31]. Triton X-100 is a nonionic surfactant and was recommended for use in FePS formulations from 2014-2022 and has therefore been used in many studies. In 2017, Downham compared Kodak Photo-Flo and Triton X-100 for use in an FePS formulation. There was no notable difference in the performance of the two, and the authors suggested this was partly due to a small sample size. This result was expected, as Kodak Photo-Flo is a commercial mixture which contains Triton X-100 along with propylene glycol. A surfactant solution made using 2-year-old Triton X-100 was also effective in this study, showing that Triton X-100 has a long shelf life however a small sample size was again used and therefore these are only indicative results [29].

In 2018, Downham performed further studies using the Triton X-100 surfactant solution and compared it to Tween 20, another nonionic surfactant that may be used in FePS formulations [45]. This was due to Triton X-100 being put on the Candidate List of Substances of Very High Concern in the EU, which may lead to it requiring authorisation for use in the future and affect the availability and permissible use of the product. In the 2018 study, Downham found that both 40% and 4% Tween 20 surfactant solutions produced comparable results to Triton X-100 in an FePS formulation. As the 4% solution was also significantly easier to apply, it was considered a viable alternative to Triton X-100 [45]. Similar results were obtained by Clover Ree *et al.* in 2023, during optimisation of an FePS formulation which found that diluted Triton X-100 solution and Tween 20 (4%) were similarly effective in developing fingermarks on non-porous and adhesive substrates [48].

Many surfactants used for fingermark development purposes in previous literature have been nonionic or anionic, likely due to their lower CMC and more abundant nature [56]. Bleay *et al.* suggest that ionic nature is not critical to the success of a surfactant when used in SPR, which is thought to have a similar mechanism to that of PS [26]. Commonly used surfactants in PS formulations such as Triton X-100, Tween 20 and Kodak Photo-Flo are nonionic surfactants. Jones *et al.* state that the surfactants used in pre-mixed formulations Wet Powder White and Wet Wop are anionic, however information about the specific surfactant used in these products is not publicly available [40]. The carbon-based WET UCIO formulation developed by Claveria *et al.* described in section 1.3.4.1 uses an anionic surfactant called sodium dodecyl sulphate (SDS) [50]. The research conducted to develop this formulation has not been published and it is therefore unclear what other surfactants were tested. Although this formulation is reported by the authors to be highly effective on adhesive substrates, SDS is a toxic substance that may have health implications for operational use [86].

It is clear from previous work that surfactant solutions play an important role in the ability of FePS formulations to preferentially deposit on fingermark residue, as well as controlling the consistency,

toxicity and shelf life of formulations [26, 29, 78]. The changing of recommendations and restrictions of such chemicals highlights the importance of further understanding of the role surfactants play in FePS to make recommendations for more sustainable formulations. This research aims to thoroughly investigate the interactions of surfactants within FePS formulations and their effect on fingermark development on non-porous substrates, to assist in further understanding and improving the capabilities of this fingermark development technique. This research will investigate the effect of surfactant type, concentration, and powder to surfactant ratio on the development of fingermarks using FePS. To better understand the interactions between surfactant, substrate, and fingermark residue, the parameters of ridge detail, contrast, and background development will be assessed individually to understand how surfactants are impacting different areas of fingermark quality. This will provide insight into how the addition of a surfactant is affecting each parameter in the successful development of fingermarks.

2.2 Materials and method

A range of FePS formulations were used to develop fingermarks on non-porous substrates. To observe the effect of different surfactants, a single source and batch of iron oxide powder was used throughout the study to ensure minimal effects from iron oxide powder batch variation. A magnetic iron oxide powder from Fisher Chemicals (CAS 1317-61-9) was used due to its recommendation in the 2014 FVM for use in FePS [25]. The 2022 FVM recommended powder was not utilised in this study as it was commenced before the updated manual was published, however the updated formulation suggests that due to the high cost of the recommended powder, the iron oxide from Fisher Chemicals could still be used provided initial tests are done to ensure the batch is effective. Previous work has demonstrated inconsistencies within different batches of the iron oxide from Fisher Chemicals which affects the quality of developed fingermarks [45]. Formulations were tested on fingermarks from a range of donors, ethics approval was completed through the University of Technology Sydney (ETH18-252, Appendix A) and participants were required to consent to the collection of their fingermarks prior to deposition.

2.2.1 Surfactants

Ten surfactant solutions were chosen based on previous use in PS research or other fingermark development techniques. Two surfactants chosen (CTAB and SP80/T80) had not been previously investigated for fingermark development and were selected to improve range of ionic and nonionic surfactants. Table 2.1 outlines the types and concentrations selected. Trade names of surfactants are used here in line with previous research, however a detailed analysis of the chemical structures of each surfactant can be found in section 3.4.

Table 2.1 Surfactant stock solutions investigated in this study

Surfactant Code	Stock solution components	CAS Number	Ionic nature
TX100 [41, 45]	25 mL Triton X-100 35 mL Ethylene glycol 40 mL Water	9002-93-1 107-21-1	Nonionic
T20 [41, 45]	10 mL Tween 20 90 mL Water	9005-64-5	
T80 [55]	22 mL Tween 80 6 mL Ethanol 60 mL Water	9005-65-5 64-17-5	
SP80/T80	5 mL Span 80 5 mL Tween 80 90 mL Water	1338-43-8 9005-65-5	
KP [31, 55]	50 mL Kodak Photo-Flo 200 50 mL Water	57-55-6/9036-19-5	
DOSS [26, 41]	1.5 g Dioctyl sodium sulfosuccinate 100 mL Water	577-11-7	Anionic
SDS [50]	5.8 g Sodium dodecyl sulphate 100 mL Water	151-21-3	
LN [87]	25 mL Liqui-Nox 75 mL Water	N/A	
CTAB	7.29 g Hexadecyltrimethylammonium bromide 100 mL Water	57-09-0	Cationic
n-DDAA[25, 88]	4.91 g n-dodecylamine acetate 100 mL Water	2016-56-0	

The concentration of CTAB used was in line with the other cationic formulation (n-DDAA, 200 mM), and the SP80/T80 mixture concentration was consistent with the 10% of T20 used. TX100, T20, T80, DOSS, LN and ethylene glycol were purchased from Sigma Aldrich. KP was purchased from Kodak Alaris. SP80 was obtained from TCI chemicals. CTAB was obtained from Fluka analytical. n-DDAA was obtained from MP biomedicals. SDS was obtained from BDH chemicals. A precipitated magnetic iron (II/III) oxide powder (lot # 2185036) was purchased from Fisher Scientific.

For each surfactant, a range of concentrations and powder to surfactant ratios were tested. The formulations detailed in Table 2.1 made up the stock solution for each surfactant. All surfactant solutions except for T80 were prepared by combining relevant products in a 250 mL Scott bottle, then swirling thoroughly to mix homogenously. T80 was made as recommended by Claveria *et al.* by mixing water and ethanol with a magnetic stirring bar at 800 rpm for 5 minutes before slowly adding in T80 without stopping the stirrer, with further stirring for another 15 minutes [55]. The stock concentration of each surfactant was tested, as well as half and quarter concentrations which were diluted with deionized water. For each concentration, ratios of 1:1 and 1:2 w/v (powder (g): surfactant (mL)) were chosen to investigate differences between consistencies of formulations. This is summarised in Table 2.2. For both ratios, a formulation made with only water and powder was also tested as a control. A total of 62 formulations were investigated in this stage of the study.

2.2.2 Fingermark collection and substrates

Each formulation was tested on fingermarks deposited by four donors of varying deposition quality on three non-porous substrates; glossy white ceramic tiles (Johnson Tiles), resealable clear polyethylene (PE) plastic bags (J. Burrows) and 3 mm thick clear glass squares (see Table 2.2). The glass and tile substrates were cleaned by wiping with acetone and Kimtech™ wipes, then rinsed with deionised water before being air dried. Plastic (PE) samples were used straight from the packaging. For fingermark deposition, donors were asked to wash their hands five minutes before the first deposition and then wait two minutes in between subsequent depositions. Initial investigations into replenishment time of fingermark residue were conducted and it was determined that the times chosen were sufficient to allow for secretion replenishment, while also considering the time constraints of fingermark collection and donor availability. Immediately before each deposition, donors rubbed their fingertips together for homogenisation of fingermark constituents. This methodology resulted in fingermarks which were predominantly eccrine-rich as no sebaceous-loading activities were performed, however this allowed for more controlled consistency of fingermark residue between deposition sessions. As this study focussed on comparing the effect of surfactants on fingermark residue, the chosen methodology aided in reducing variability of development outside of surfactant interaction.

For each substrate, all donors deposited nine fingermarks using their three middle fingers from either hand in a sequence of three depletions (Figure 2.1). A total of 6,696 fingermarks were collected, and all marks were developed on the same day they were deposited. The use of four donors and three depletions is in line with the recommended number of donors for a Phase 1 study in the IFRG guidelines [80]. This allowed for a broad comparison of many formulations, as the aim of this study

was not to determine a single optimal formulation for casework. Further investigations with a wider range of donors, substrates and fingermark ageing periods should be conducted before any of the tested formulations can be considered for operational use.

Table 2.2 Summary of parameters tested for each surfactant

Ratio powder (g): surfactant (mL)	1:1		1:2
Substrates	Ceramic	Glass	Plastic (PE)
Concentration of surfactant	Stock	Half	Quarter

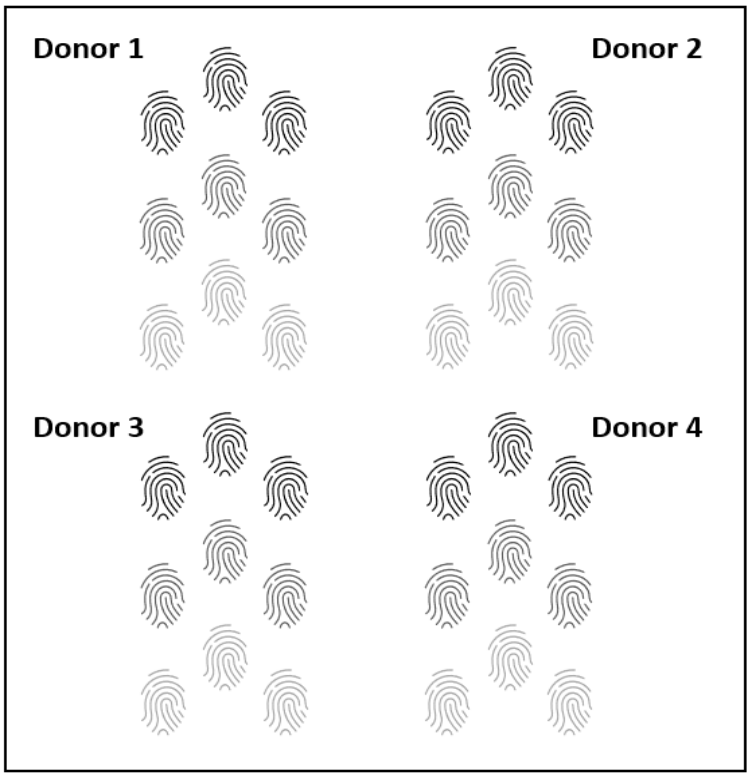


Figure 2.1 Illustration of fingermark deposition placement on each substrate

2.2.3 Development

Fingermarks were developed with FePS formulations using the same method, and all formulations were made fresh before each development by combining the surfactant and powder components and mixing until smooth with a glass stirring rod. A wet powder squirrel hair fingerprint brush from Optimum Technology was then used to gently brush the FePS across the substrate. The surface was not rinsed pre-development (as recommended in the FVM) to better control and compare the amount of water in the suspensions in the context of this specific fundamental research, as this has shown to

be an effective methodology in published literature [24, 41, 48]. The suspension was left on the substrate for approximately 15 seconds before being rinsed gently with tap water. Different brushes were used for each surfactant and rinsed thoroughly between application of different formulations to prevent any potential contamination between formulations. Developed samples were left to air dry overnight and then photographed using a Canon EOS 800D DSLR camera with a Canon EF-S 60mm macro lens. A Rofin Polilight PL500 was used to apply oblique white light for visual enhancement on the plastic samples.

2.2.4 Assessment

Each developed fingermark depletion was graded to determine the effects of surfactant type, concentration, and powder to surfactant ratio on fingermark development. The marks were graded based on three different parameters: ridge detail, contrast, and background development. These parameters were chosen to provide a more detailed assessment of the suspension's interaction with fingermark residue, as well as with the substrate. Representative grades for each parameter are shown in Tables 2.3 to 2.5. More than 6600 grades were produced in this analysis. Data was analysed and graphed using Microsoft Excel. For the purpose and ease of data visualisation, scores of 1 or 2 were combined to indicate 'poor quality' scores, and 3 or 4 combined to indicate 'good quality' scores. Optimal development is produced by higher scores (scores of 3-4), as this represents high quality marks which are more likely to be used for comparison purposes in an operational setting.

Table 2.3 Modified CAST scale used for grading ridge detail present in developed fingermarks [89]

Ridge detail grade	0	1	2	3	4
Criteria	No development	Signs of contact, less than 1/3 continuous ridge detail	Between 1/3 and 2/3 continuous ridge detail	More than 2/3 continuous ridge detail but not quite complete	Full development, whole fingermark, continuous ridges
Example					

Table 2.4 Scale used for grading contrast present in developed fingermarks [89]

Contrast grade	0	1	2	3	4
Criteria	No contrast between ridges and substrate	Poor contrast between ridges and substrate	Moderate contrast between ridges and substrate	Good contrast between ridges and substrate	Very good contrast between ridges and substrate
Example					

Table 2.5 Scale used for grading background development around developed fingermarks [90]

Background Development grade	0	1	2	3	4
Criteria	Very heavy background development	Heavy background development	Moderate background development	Light background development	No background development
Example					

2.3 Results

2.3.1 Overall results

This study found that all formulations, even those made with only water, were able to develop fingermarks. The addition of a surfactant solution did not always improve development compared to formulations made with only water. The ranked order of surfactant efficacy changed based on the parameter considered (ridge detail, contrast, or background development), suggesting that all surfactants interacted differently with fingermark residue and substrates. The combined results of each grading parameter (i.e. all scores of ridge detail, contrast, and background development considered together) for formulations made with surfactants are shown in Figure 2.2. To compare the effect of each surfactant, the combined scores given to marks developed with formulations made with only water (no surfactant) are represented in the background of this graph. This shows that most

surfactants improve the development of fingermarks in FePS, except for T80, SP80/T80 and LN. The surfactants which best aid development of parameters assessed with the highest overall scores of 3 and 4 were T20, TX100 and KP (72%, 76% and 78% respectively). T20 was the only surfactant of all those tested that did not produce scores of 0 across any development parameter. The surfactants with the lowest overall scores of 3 and 4 were SP80/T80, T80 and LN (15%, 27% and 36% respectively).

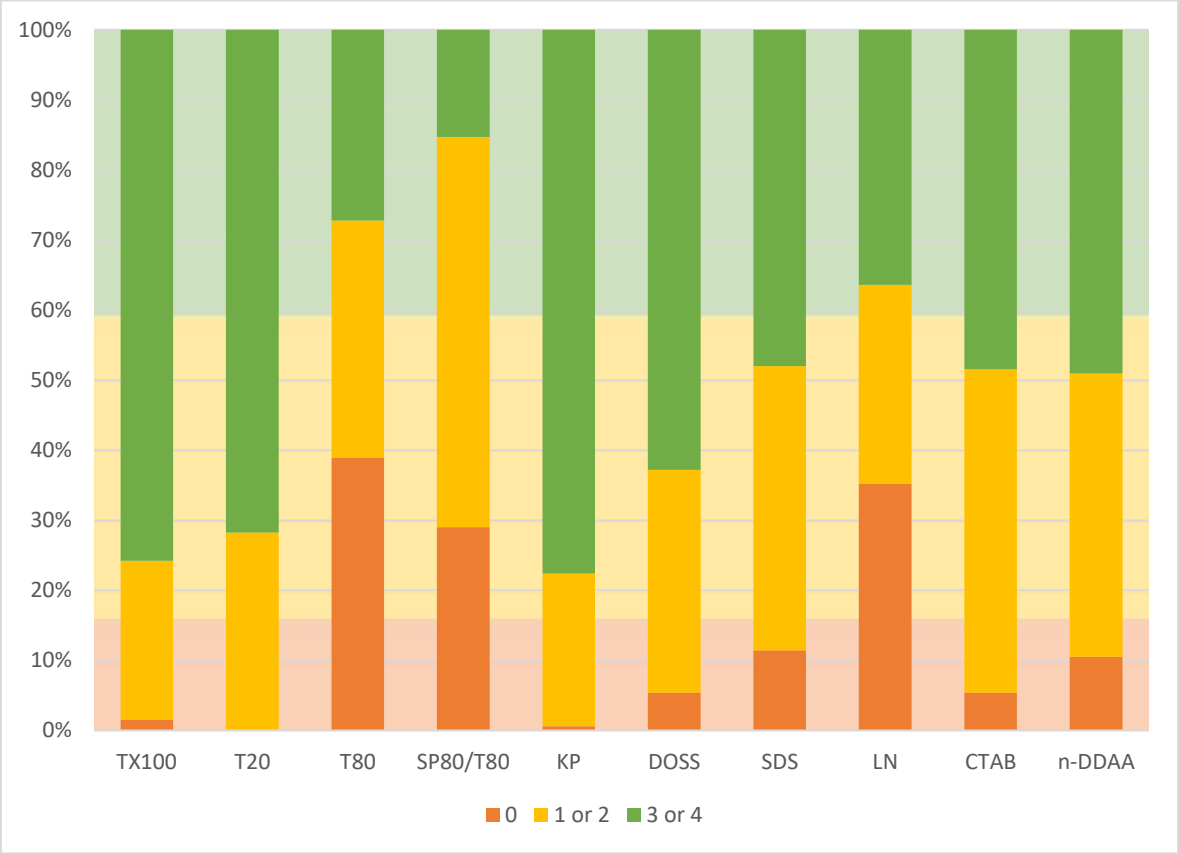


Figure 2.2 Total grades received by each surfactant for all formulations, donors and development parameters. Background colour represents scores of formulations made only with water

Figure 2.2 illustrates an overview of the performance of each surfactant for all concentrations and ratios, however it is important to further analyse these results by separating the scores of each grading criteria. Ridge detail, contrast, and background development are all important and interrelated parameters which contribute to fingermark quality [91]. To better understand how each parameter is influenced by the type of surfactant, further analysis was performed.

To show the relationship between development parameters, surfactants were ranked based on the amount of 'good quality' development (graded 3 or 4) and no development (graded 0). The results of each parameter are shown in Table 2.6. Some surfactants performed consistently for all criteria such as T20, n-DDAA, KP and SP80/T80. However, others were highly variable depending on the criteria that was considered, such as LN and SDS. Interestingly, surfactants of the same ionic nature did not

share similarities across any development parameter, suggesting that the ionic nature of the surfactant does not govern its effectiveness.

Table 2.6 Ranking of surfactants for each development parameter (n=11) considering scores for all formulations

Surfactant	Rank of ridge detail	Rank of contrast	Rank of background development
TX100	1	2	5
T20	3	3	4
T80	11	10	7
SP80/T80	9	8	10
KP	2	1	2
DOSS	4	5	8
SDS	8	9	3
LN	10	11	1
CTAB	5	6	9
n-DDAA	7	7	6
Water	6	4	11

2.3.2 Effect of ratio and concentration

The performance of all surfactants was affected by changes in powder: surfactant ratio and concentration, some to a greater extent than others. Generally, solutions with more dilute surfactants and less powder created thinner suspensions which were more easily brushed onto and washed off the substrates. However, the reduction of powder and decrease in surfactant concentration did not have a consistent effect on the results of all tested surfactants and was occasionally detrimental to fingermark development quality. An example of the variable effect of changing powder to surfactant ratio as well as concentration is shown in Figure 2.3.

As an example, DOSS received a high number of overall 'good' scores (63%), suggesting some conditions were allowing optimal development with this surfactant, however 5% of marks were also graded 0 (Figure 2.2). These scores were all produced by stock concentrations, suggesting that the more dilute suspensions are better optimised for fingermark development. However, the higher rate of 0 scores compared to T20, KP, and TX100 (0%, 1% and 2% respectively) reduces its overall

effectiveness. This shows that some surfactants have a narrow range in which they can develop high quality fingermarks and may require more specific conditions than others to preferentially deposit powder. This suggests that the changing of surfactant concentrations and powder to surfactant ratios affects the abilities of each surfactant differently.

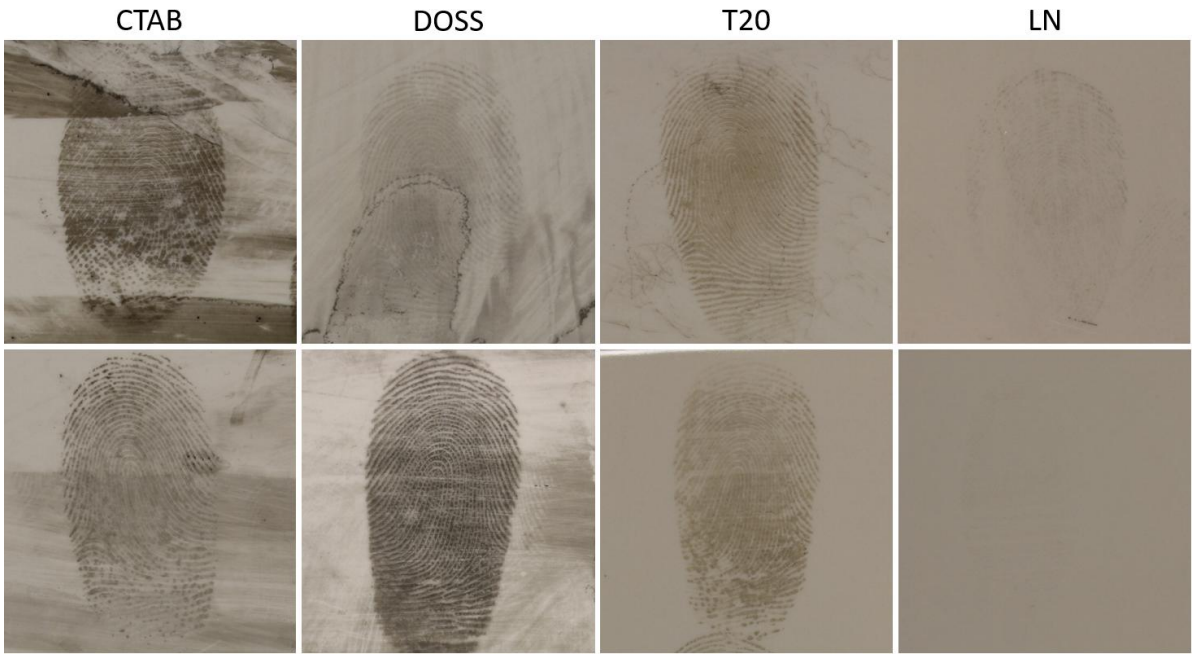


Figure 2.3 Comparison of development of one donor on tile substrate produced by surfactants using different surfactants, ratios and concentrations. Formulations of 1:1 ratio, stock concentration (top row) and 1:2 ratio, quarter concentration (bottom row)

The graphs shown in Figures 2.4 and 2.5 compare the spread of ridge detail scores given to each formulation made with DOSS and n-DDAA. The stock concentrations of DOSS (Figure 2.4) range from 0-4 and have median scores of 1 or 2. The more dilute half and quarter concentrations however have a much narrower range between 2 and 4 with median scores of 4. This demonstrates a surfactant that is sensitive to changing concentrations. The ridge detail results for n-DDAA formulations (Figure 2.5) instead show a surfactant which has consistent results for each formulation, however the spread of all these results is very wide. All suspensions except for the quarter 1:1 formulation span the range of scores from 0-4, with all medians between 1 and 3. This suggests that variation in ridge detail development using n-DDAA is more dependent on substrate or donor rather than changing concentrations and ratios.

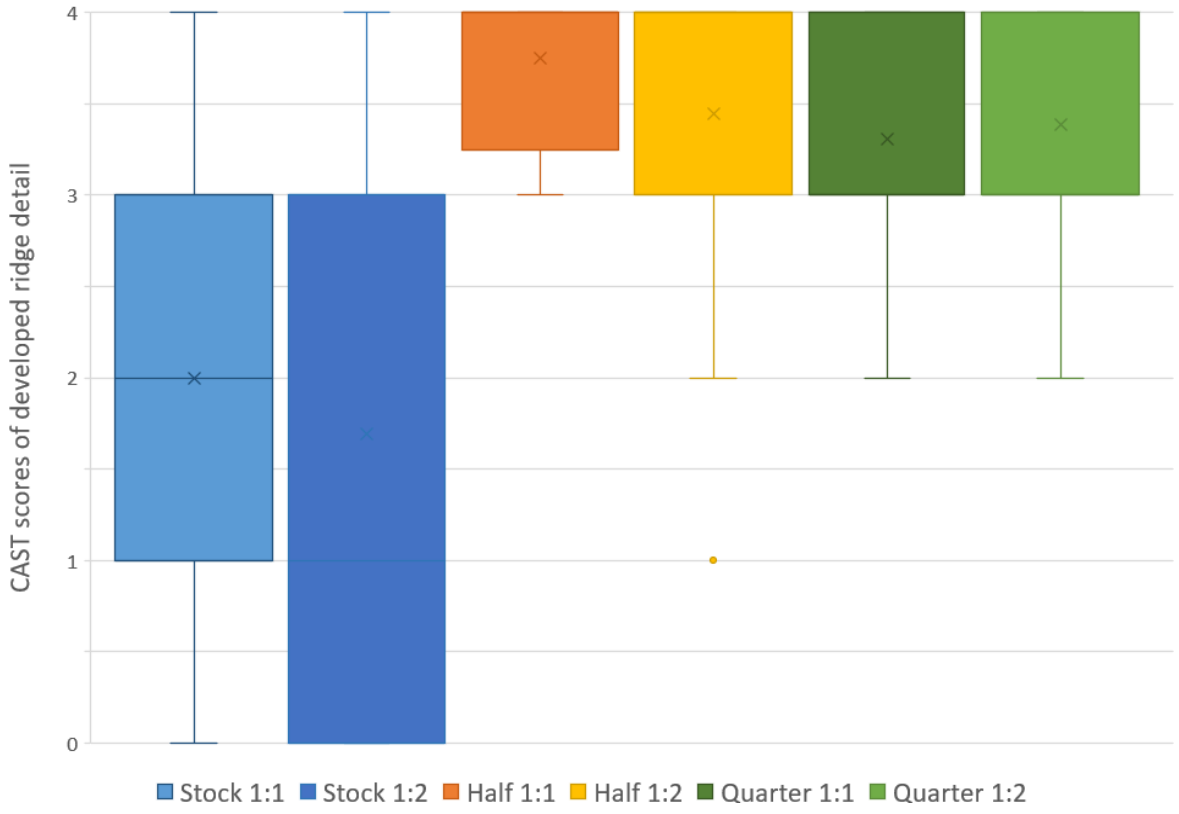


Figure 2.4 Box plot showing the spread of all donor ridge detail scores given to each formulation made with DOSS

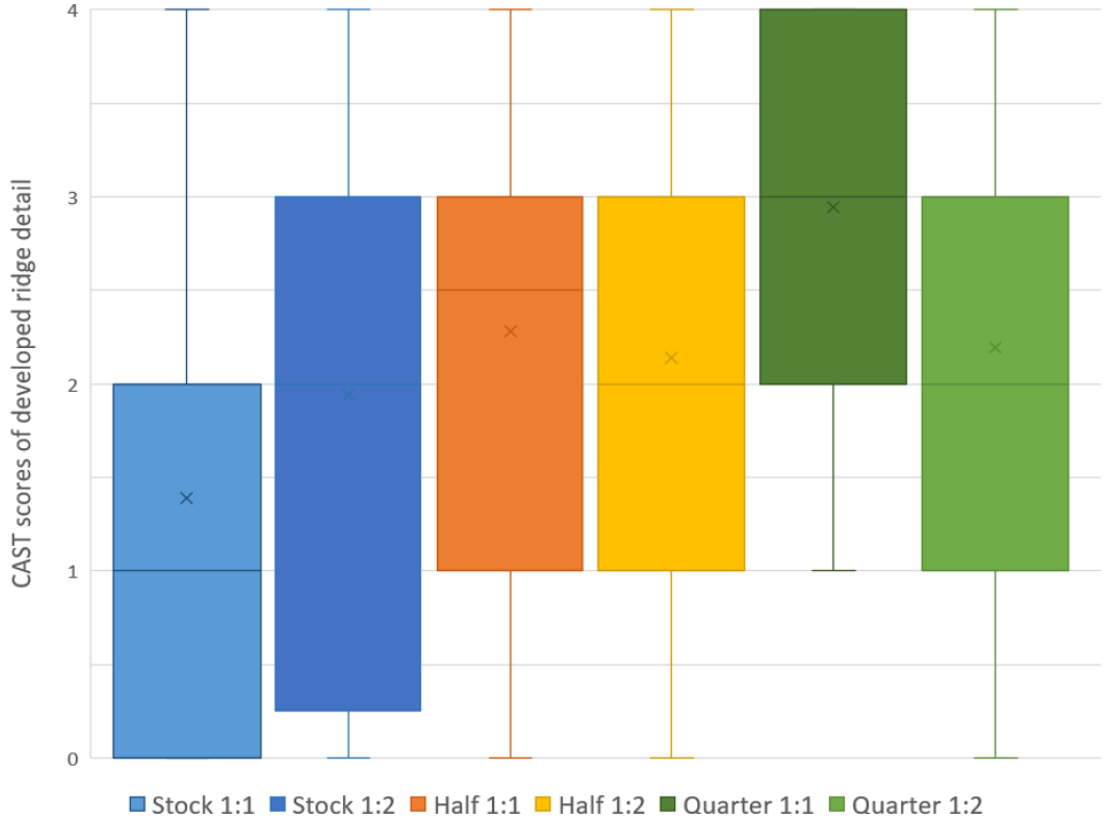


Figure 2.5 Box plot showing the spread of all donor ridge detail scores given to each formulation made with n-DDAA

The most effective formulations for each surfactant, determined by the highest amount of 'good' development and lowest amount of no development grades for all quality parameters pooled together, are outlined in Table 2.7. All surfactants except for LN benefited in diluting the surfactant concentration, however improvements made by each powder to surfactant ratio was almost evenly split between surfactants. This demonstrates that thinning the formulations by reducing powder or diluting surfactant does not increase effectiveness for all surfactants, and that surfactant type influences this efficacy more than consistency.

Table 2.7 Most effective formulations produced by each surfactant

Surfactant	Powder: Surfactant ratio	Concentration
TX100	1:1	Half
T20	1:2	Quarter
T80	1:1	Quarter
SP80/T80	1:1	Quarter
KP	1:2	Quarter
DOSS	1:2	Half
SDS	1:1	Quarter
LN	1:2	Stock
CTAB	1:2	Quarter
n-DDAA	1:2	Quarter
Water	1:1	n/a

2.3.3 Optimal solution results

The rank of each development parameter for these best formulations are shown in Table 2.8. The order of most effective surfactants does not change when considering only these optimal formulations, except for DOSS. DOSS has the highest percentage of marks graded 3 or 4 in ridge detail, and second highest in contrast. However, it is outperformed by many other surfactants when considering the background development. For all surfactants, the ridge detail and contrast scores follow similar patterns as these parameters consider the quality of developed ridges. Half of the optimised surfactants tested received only scores of 3 and 4 for background development. As these formulations have been optimised for powder to surfactant ratio and surfactant concentration, scores of 0 for any of the assessed parameters makes the surfactant considered unsuitable for practical

fingermark development. Only T80, SP80/T80 and water produced any scores of 0 for background development, while LN and SDS also produced scores of 0 in ridge detail and contrast.

Table 2.8 Ranking of surfactants for each development parameter (n=11) considering scores only for best formulations of each surfactant

Surfactant	Rank of ridge detail	Rank of contrast	Rank of background development
TX100	= 5	3	= 1
T20	2	4	= 1
T80	8	10	5
SP80/T80	9	5	6
KP	3	1	= 1
DOSS	1	2	3
SDS	7	8	= 1
LN	10	11	= 1
CTAB	= 5	7	4
n-DDAA	6	9	2
Water	4	6	7

Formulations made with only water were effective in developing good ridge detail and contrast, especially on plastic substrates. However, across all substrates these formulations also produced the heaviest background development of all tested surfactants, illustrated in Figure 2.6. Changing the powder to water ratio of these formulations did not have a notable effect on ridge detail and contrast, however the 1:2 ratio increased background development.

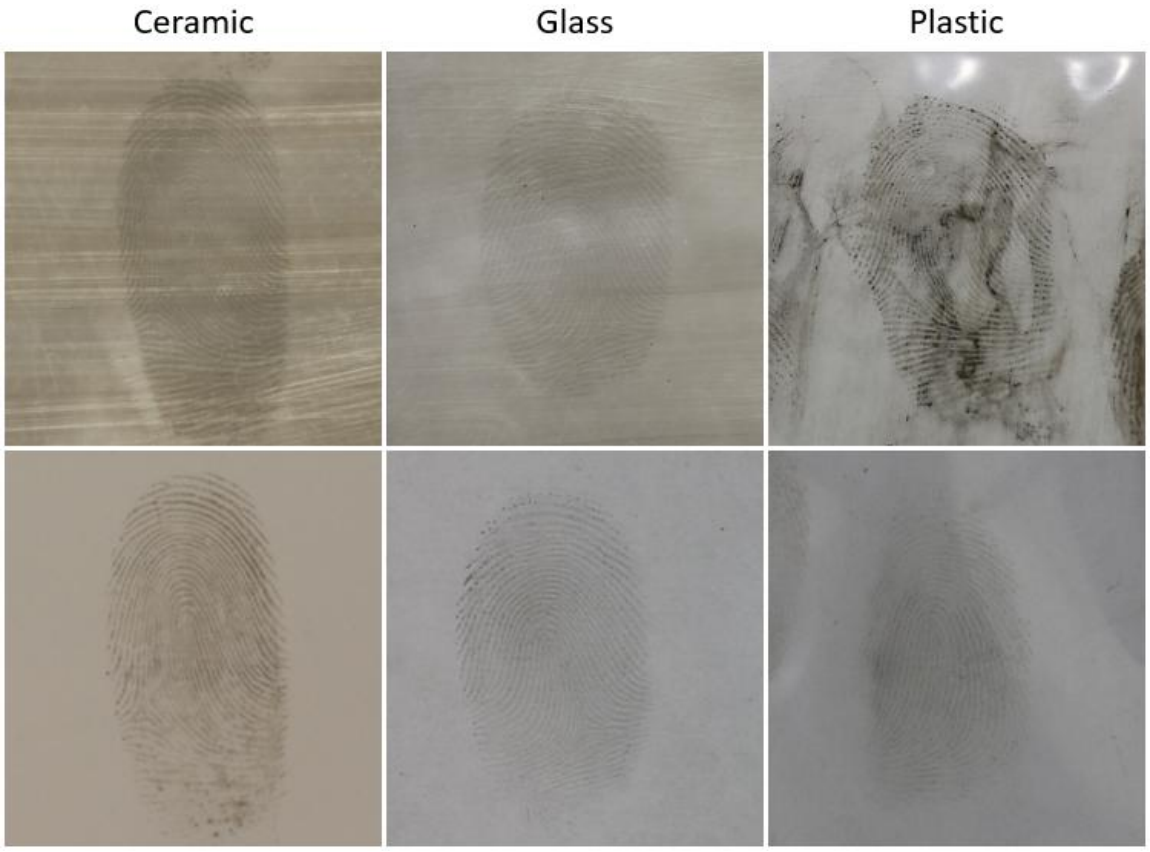


Figure 2.6 Comparison of background development on each substrate caused by FePS made with water only (top row) and KP (bottom row)

2.3.4 Volume of powder deposition

To better understand how surfactants may control the volume or size of powder particles being deposited, the scores of contrast was assessed. For surfactants which performed similarly in developing ridge detail, differences in contrast provided further insight into how effective the surfactants were in depositing sufficient powder.

Formulations made with KP, T20, and TX100 produced very similar scores of ridge detail as illustrated in Figure 2.7. In the contrast scores however, T20 performed more poorly and received 13% of marks graded 3 or 4 less than TX100 and KP. This was due to very light-coloured marks produced by the stock T20 concentration on all substrates (Figure 2.8), despite development of good ridge detail. The 0 grades for ridge detail and contrast produced by KP and TX100 occurred using stock concentrations. When comparing the scores of these surfactants to water (Figure 2.7) only TX100 and KP notably improved the contrast of developed marks while very minimal improvement was seen for the ridge detail scores. Despite similar contrast scores for water and T20 formulations, the resulting marks had different amounts of powder deposition onto the fingermark ridges, as illustrated in Figure 2.8. Marks produced by water formulations had a high level of powder deposition onto the fingermark ridges, but contrast scores were reduced due to background staining, while contrast scores of T20 were

reduced by light coloured ridge development. This comparison demonstrates a relationship between contrast and background development and shows that despite similar scores of ridge detail and background development the addition of T20 does affect how powder is deposited on fingermark ridges using FePS. The addition of all three surfactants had the greatest effect in reducing background development for ideal fingermark development.

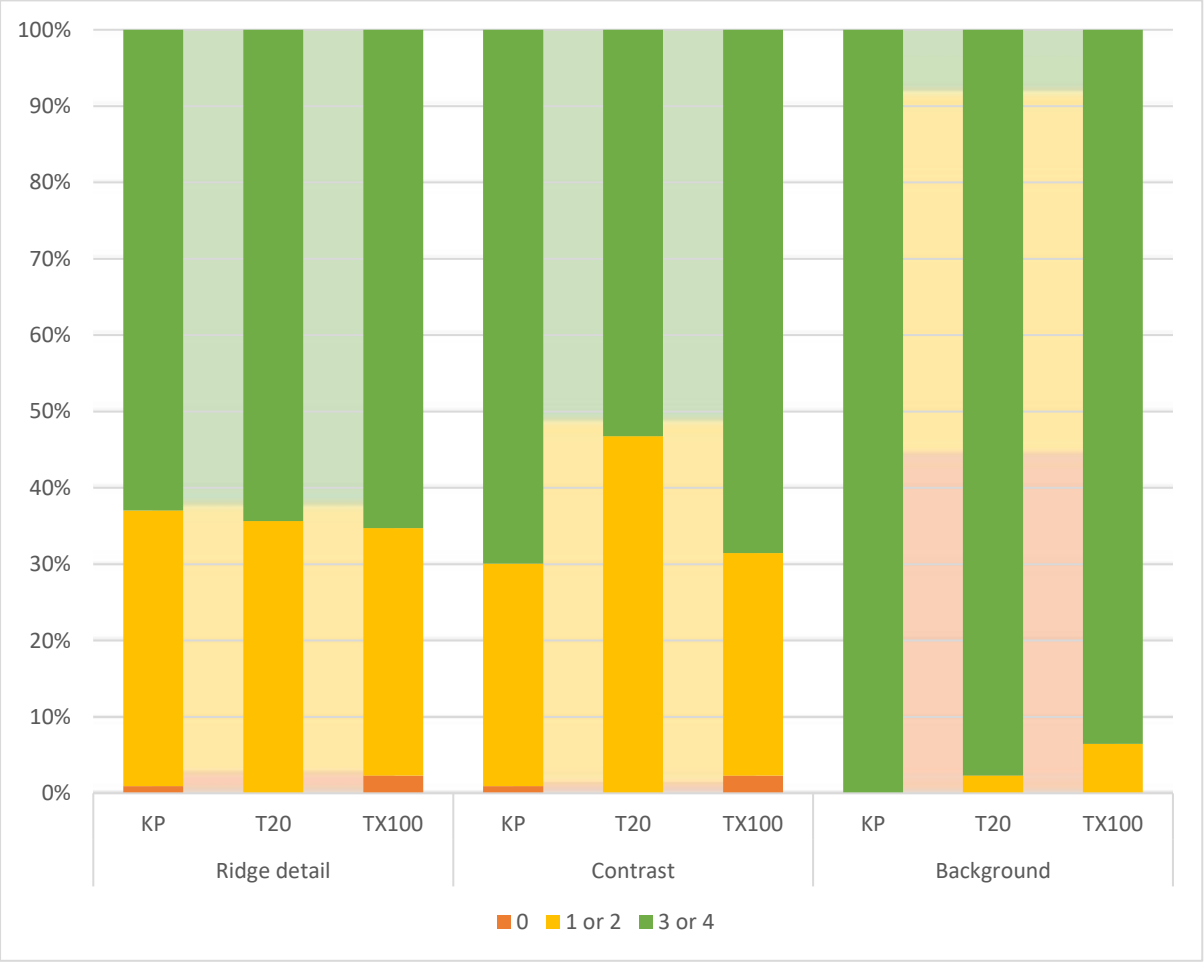


Figure 2.7 Combined donor scores of all KP, T20 and TX100 formulations for each development parameter. Background colour represents scores of formulations made only with water

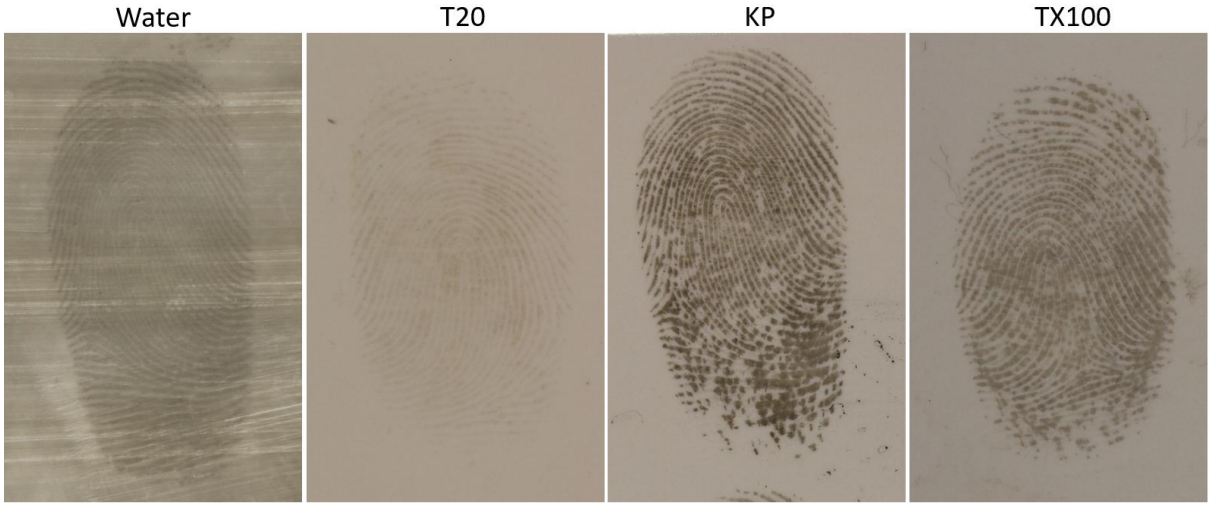


Figure 2.8 Comparison of contrast produced by FePS made with stock concentrations of water, T20, KP and TX100 on ceramic substrate

Another demonstration of surfactants controlling powder deposition can be observed when comparing the scores produced by T80, LN, and SP80/T80. As seen in Figure 2.2, the addition of these surfactants hindered the quality of fingermark development overall, however the relationship between the assessed parameters changed for each surfactant. Poor development of ridge detail and low contrast was produced by these surfactants, as illustrated in Figure 2.9, however the scores of background development varied wildly. LN produced the least background development of any surfactant, with all marks scoring 3 or 4, however this was due to a lack of powder being deposited anywhere on the substrate (Figure 2.10) and resulting in poor ridge detail and contrast scores. SP80/T80 and T80 produced moderate to heavy background development, and this prevented any visible ridge detail or contrast resulting in poor scores shown in Figure 2.9. The background development produced by T80 was most evident on the plastic substrate (Figure 2.10) with 15% of marks on this substrate graded 0 for background development and 65% graded 1 or 2. On ceramic and glass however, notably less background development was present, with no scores of 0 on either substrate and over 90% of marks graded 3 or 4 for both. This demonstrated that volume of powder deposition can also be influenced by substrate type.

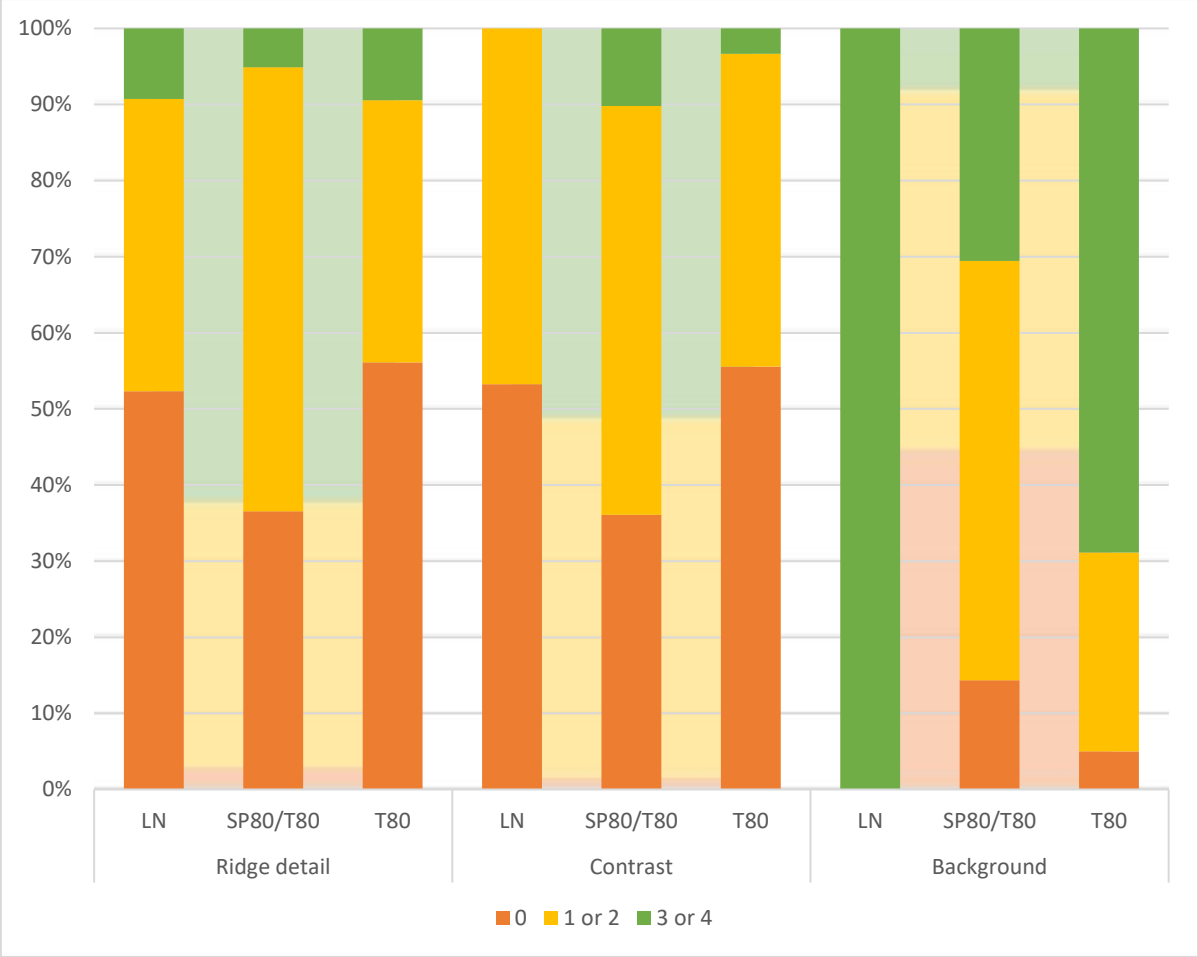


Figure 2.9 Comparison of all donor scores for LN, SP80/T80 and T80 FePS formulations for each development parameter

Figure 2.10 Comparison of background development produced by FePS made with LN, SP80/T80 and T80 on plastic with oblique white light to visualise development

When formulations made with SP80/T80 were diluted, increased background development was observed across all substrate types, however this also resulted in increased ridge detail and contrast. SP80/T80 also produced some cases of reverse development (where the technique develops the substrate surrounding fingermark residue instead of the ridges) or voids where fingermark residue was deposited (Figure 2.11). Interestingly, these observations were not consistent between donors or substrates, and there was no trend observed for a particular donor or substrate causing the unusual development. The lack of development on the fingermark residue in these cases suggests that there is some component of the residue repelling powder particles suspended by this surfactant, or that the mark is being washed away in the development process.

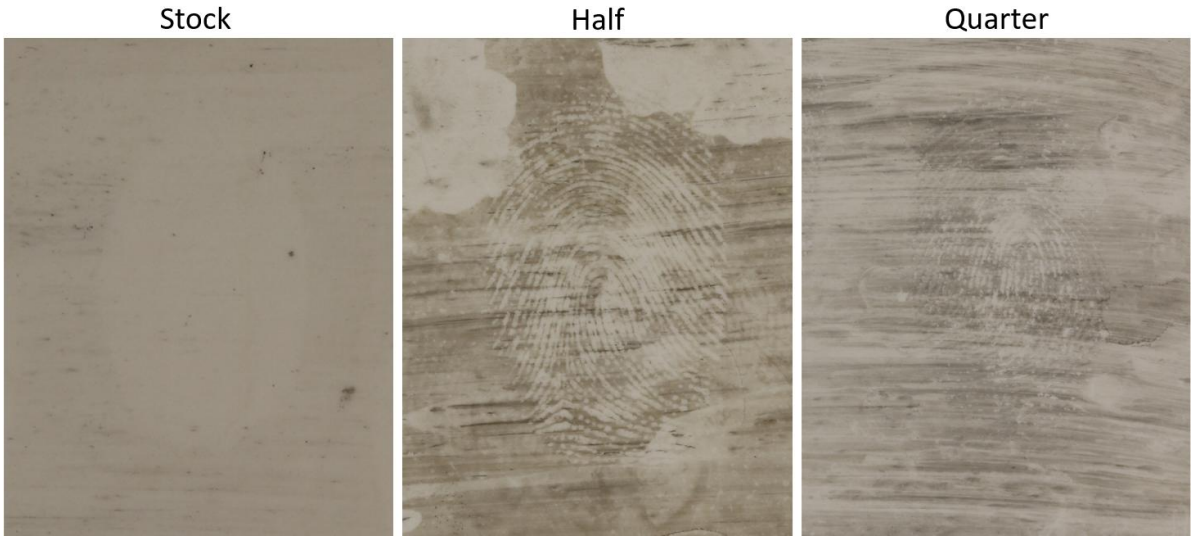


Figure 2.11 Reverse development of fingermarks on ceramic developed using FePS made with SP80/T80 surfactant at stock, half and quarter concentrations

2.3.5 Donor and depletion

When investigating the effect of donor and depletion number on different FePS formulations, only the scores for ridge detail and contrast were considered as they represent interactions between the technique and fingermark residue, while background development involves interaction between the technique and substrate. It was clear from initial observations that the performance of each donor's marks was highly varied. As shown in Figure 2.12, each donor received the same number of marks graded 0 for ridge detail and contrast. This is expected as ridge detail and contrast cannot be present without the other to some degree. Three of the four donors received 10-25% fewer scores of 3 or 4 for contrast compared to ridge detail, the only exception being donor 4 who had a 15% higher rate of good contrast scores compared to ridge detail. Donor 4 also had the highest rate of marks graded 0. This suggests that marks produced by donor 4 are more poorly developed using FePS formulations and there are higher instances of incomplete ridge detail with good contrast.

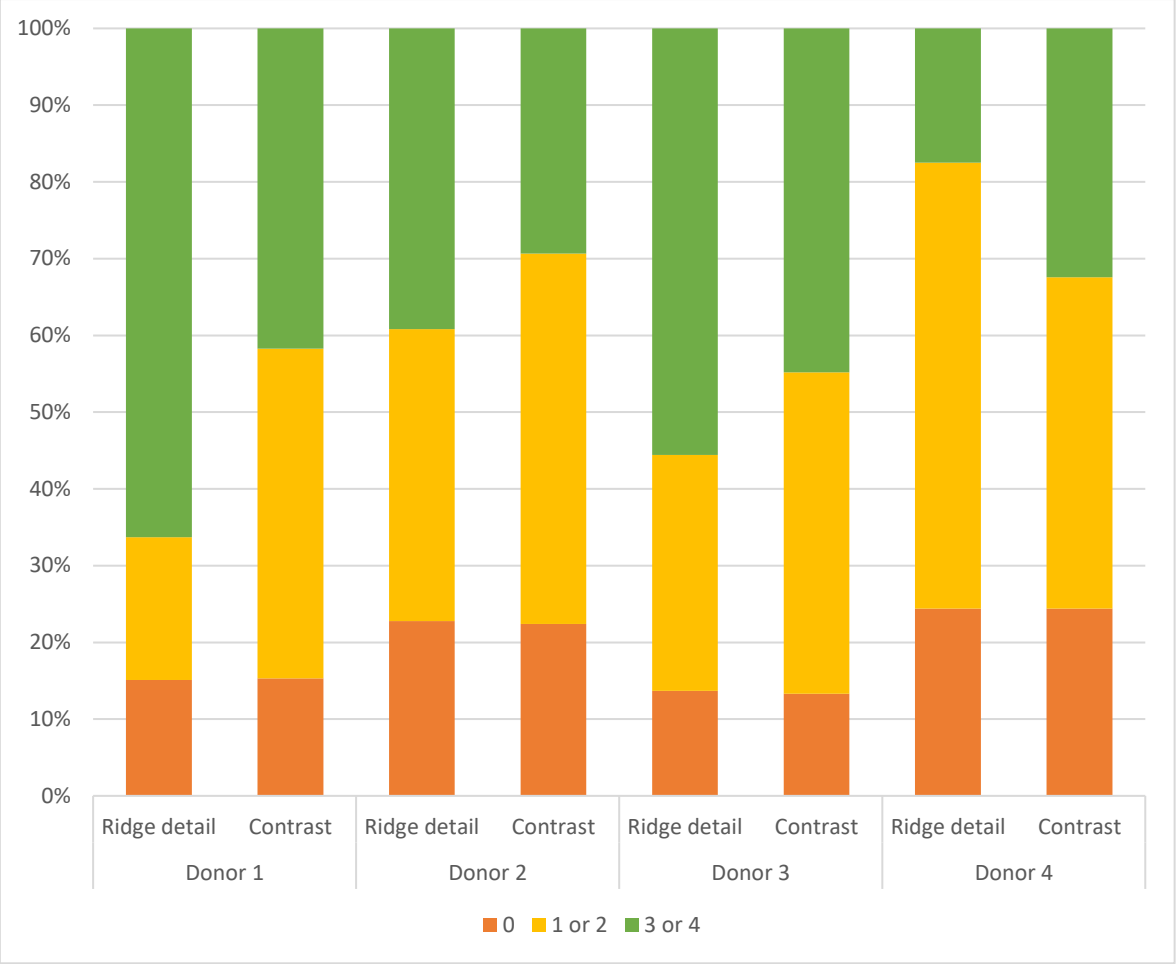


Figure 2.12 Ridge detail and contrast scores given to each donor for all FePS formulations and substrates

Donors 1 and 3 produced the lowest percentages of marks graded 0 for both parameters (15% and 14% respectively), while donor 1 received the highest rate of good ridge detail and donor 3 the highest rate of good contrast. Generally, the donor variability followed similar patterns for each surfactant used, however some developed marks from all donors more consistently than others. DOSS, LN and SP80/T80 had the most consistent results between donors, while formulations made with only water were the most varied. When the scores for ridge detail and contrast were combined, donor 1 produced the highest graded marks except for when developed with CTAB, SP80/T80, and water. For these formulations, donor 3 produced the highest quality marks. These results show that donor variability does influence how FePS formulations are able to develop ridge detail and contrast, and that donor efficacy is influenced by surfactant type.

Depletion number slightly influenced the results of ridge detail and contrast. When results for all donors and formulations are combined, the scores of 3 and 4 in both ridge detail and contrast are reduced around 10% from the first to the third depletion. The percentage of marks graded 0 increased less than 5% for both ridge detail and contrast by the third depletion. This suggests that marks

developed with FePS formulations reduce slightly in quality with each fingermark depletion. This also demonstrates that FePS remains effective on fingermarks with reduced residue volume in later depletions.

2.3.6 Substrate

This research tested different FePS formulations on ceramic, glass, and plastic substrates, all of which are non-porous. Figure 2.13 illustrates how the substrate type influenced development over all the formulations tested. From these results, it is clear that the highest scores of ridge detail and contrast were given to marks developed on ceramic tile, while the lowest ridge detail and contrast were present on plastic. However, when considering background development, the plastic substrate produced the highest rate of ideal background development while ceramic scored the poorest. This suggests that for FePS formulations overall, a higher rate of powder deposition is occurring on ceramic compared to plastic.

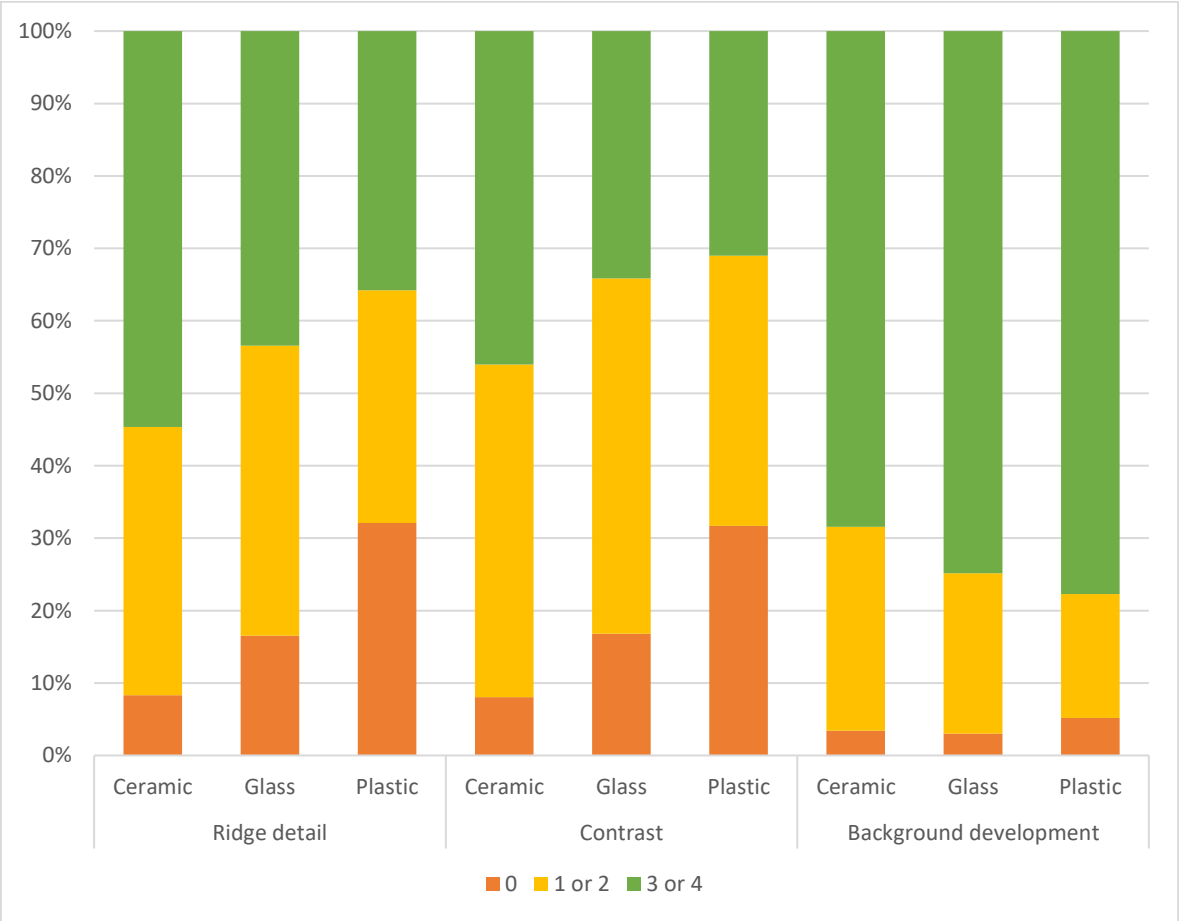


Figure 2.13 Comparison of development parameters with all FePS formulations for each substrate

The plastic substrate developed the highest percentage of marks graded 0 for all parameters, and the ridge detail and contrast scores for almost all surfactants were poorest on plastic substrate. This was

expected as plastic is a notoriously difficult non-porous substrate to develop fingermarks on using powders [92]. The only exception to this was the T20 surfactant, which produced the highest percentage of good development for ridge detail and contrast on plastic (74% and 68% respectively) and the poorest on glass (51% and 35% respectively).

The differences in background development for each substrate was not the same for all surfactants. T80, SP80/T80, and n-DDAA produced the highest rate of 0 scores in background development on the plastic substrate. Examples of the heaviest staining produced by these surfactants is shown in Figure 2.14. These examples were produced using formulations with a 1:1 powder to surfactant ratio, and for n-DDAA and T80 the background development on plastic was notably reduced with a 1:2 ratio. For formulations made with only water, the plastic substrate had less background development than ceramic and glass. The background development for all substrates was also increased with the 1:1 powder to surfactant ratio compared to the 1:2.

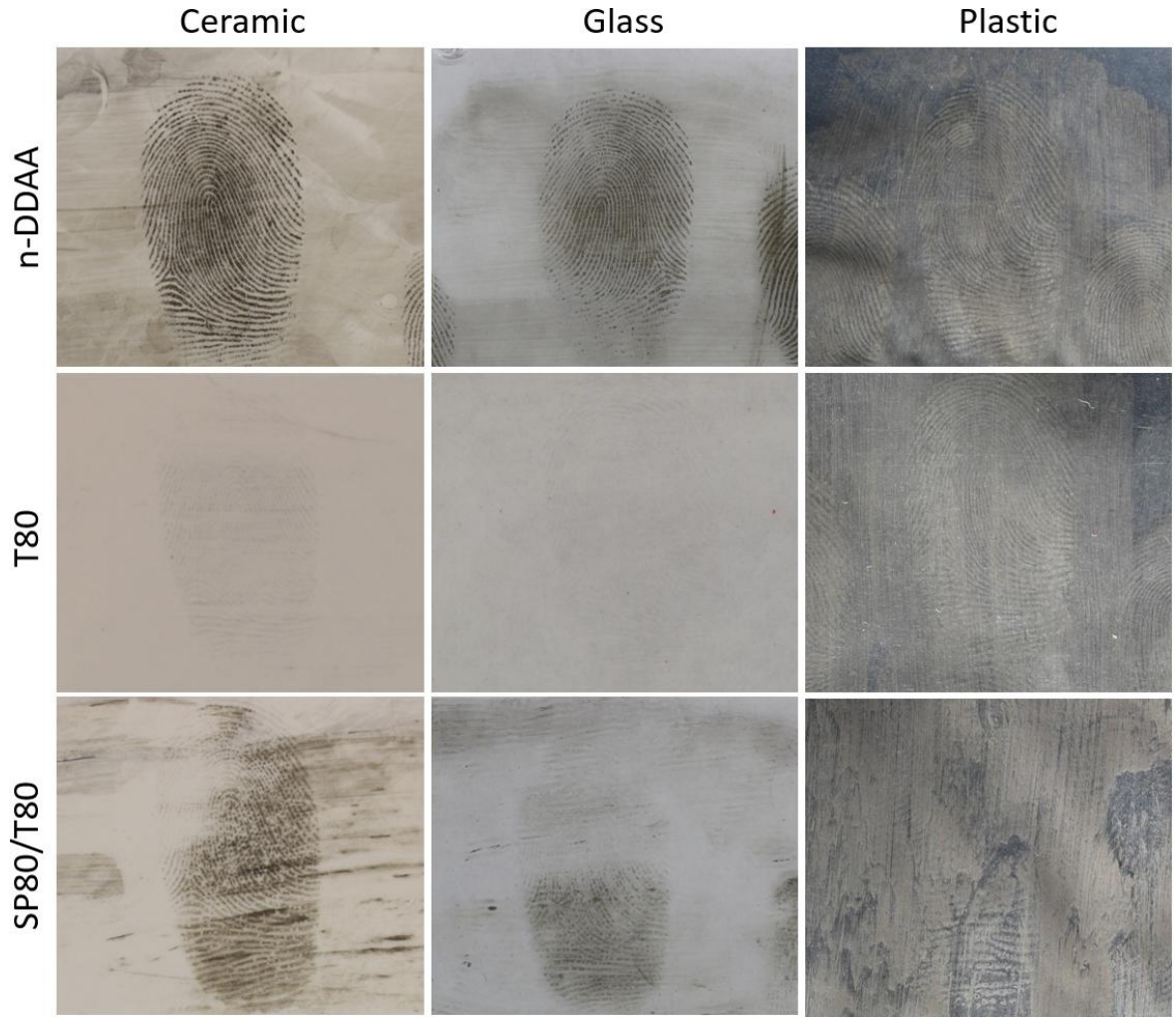


Figure 2.14 Examples of background development produced on each substrate developed using FePS made with n-DDAA, T80 and SP80/T80 using 1:1 powder to surfactant ratios

These results show that the amount of ridge detail, contrast, and background development produced on each of the substrates tested is dependent on surfactant type as well as powder to surfactant ratio used. This indicates that there is some interaction between the substrate and surfactant independent of the presence of fingermark residue.

2.4 Discussion

This investigation showed that not all surfactants are suitable for use in FePS formulations, and that the efficacy of FePS is heavily affected by the type and concentration of surfactant used. It is made clear that the parameters of ridge detail, contrast, and background development all contribute to the effectiveness of FePS formulations in developing fingermarks.

Overall, KP, TX100, and T20 were the most highly graded surfactants across all development parameters, which is consistent with their effectiveness in current literature. These three surfactants are all nonionic surfactants and were chosen based on their use and effectiveness in previous studies [25, 31, 45, 48, 78]. Until 2022, TX100 was the recommended surfactant for use in FePS however it was changed to T20 due to the recent chemical restriction of TX100. Chemical safety and environmental impact are important considerations for fingermark detection techniques, especially for those which are often transported to and used in the field. KP is made up of water and Kodak Photo-Flo, which is a wetting agent used in photographic film development to minimise drying streaks. KP also contains 5-10% TX100 as one of the products along with propylene glycol, meaning its use comes with similar environmental concerns and the production of KP has been discontinued. As T20 is not a toxic product, this research supports T20 as a suitable surfactant for use in fingermark development using FePS, consistent with the 2022 FVM recommendation [41, 45, 47, 48]. These three surfactants all consistently improved fingermark development compared to formulations made with only water, suggesting that their presence is aiding the mechanism by which FePS is able to develop fingermarks.

While the most effective surfactants were all nonionic, the results of this study show that not all nonionic surfactants are effective. Formulations made with T80 and SP80/T80 (both nonionic surfactants) produced some of the lowest scores across all grading parameters. This highlights that the ionic state may play a minor role in a surfactant's suitability for use in PS, but it is not the primary factor. SP80/T80 has not been used in published literature for fingermark development, however this mixture has been used for other pharmaceutical and industrial applications [93, 94]. T80 however has been used in published FePS formulations by Claveria *et al.* in a new FePS formulation called 'POSME' [55]. The stock T80 concentration used in this investigation is the same as the POSME formulation. In this paper, the authors tested T80, T20, and KP with a range of iron oxide powders and determined

that T80 was the most effective surfactant in a PS formulation with Synox Black 6318 iron oxide powder in a 1:1.5 ratio. The Fisher Chemical iron oxide and Sigma nanopowders were not tested. The POSME formulation was used as the second step in a sequential development after dry powder to develop fingermarks on non-porous substrates and is reported to have improved development. The results of this study are not consistent with the observations of Claveria *et al.*, which may be due to a difference in iron oxide powder used or the presence of residual dry powder in the assessed sequence. However, as the paper focussed on the improvement of fingermarks after dry powder application the results of the surfactant comparison were not reported in the published work and can therefore not be readily compared.

The results of this study have shown that the type and amount of surfactant used in FePS does influence the volume of powder deposited on developed marks. Contrast of developed ridges was graded with the aim of indicating any differences between the amount of powder deposited along fingermark ridges with each formulation. This suggests that the role of surfactants in FePS is not only controlling preferential deposition along fingermark ridges, but also the volume of powder deposited. This may be due to differences in micelles size, shape or their affinity for different components of fingermark residue. A clear example of this variability is in the difference in colour of marks produced with stock concentration T20, TX100, and KP formulations. T20 developed very light, yellow-coloured marks while the others resulted in darker black or grey marks, despite use of the same powder type, batch and weight used in all formulations. This difference in colour has been noted in previous studies investigating both powder and surfactant types in FePS [45, 48]. As these studies varied the powders used, it was suggested that differences in colour were caused by powder type rather than surfactant. One study has shown that particles which adhere to fingermark ridges in FePS have a diameter of 0.2 to 1 µm, even if the powder used is predominantly composed of particles above this range [46]. In other studies, iron oxide nanopowders comprised of particles between 50-100 nm have been highly effective and consistent in developing black fingermarks [45, 48]. Due to these findings, the 2022 FVM changed its iron oxide powder recommendation from the Fisher powder used in this study to a nanopowder which seemed to more consistently produce black marks [41, 45, 48]. The FVM suggested that the Fisher iron oxide could still be used, however users are warned that “batch inconsistencies” may result in poor quality marks which are brown or yellow in colour. As this study was completed using the same batch of Fisher powder, it is clear that the surfactant is also playing a role in the amount and size of powder particles deposited, which in turn influences the colour, contrast, and overall quality of developed marks. It is possible that some surfactants are more effective than others at facilitating the interaction between smaller particles and fingermark residue, resulting in higher

volume of particles adhering to fingermark ridges. Further studies involving different powders would improve our understanding of this interaction.

Some surfactants were more affected by changing surfactant concentrations and ratios, while others were able to perform consistently across most formulations. The varying ability of surfactants to perform consistently despite minor formulation changes suggests that there is an optimal range for each surfactant to produce ideal development. This further demonstrates that the interaction involved in the successful development of fingermarks is extremely complex and is influenced by the intrinsic properties of surfactants. With the current understanding of surfactants and their interaction with fingermark residue, there are a range of possible hypotheses to explain this observation. It is possible that increasing surfactant concentration and reducing powder volume may increase the number of 'free' micelles in a formulation that are not suspending powder particles and are therefore free to interact with and possible remove the fingermark reside. The shape and structure of surfactant micelles may also play a role in this variation, as the optimal range varies for each surfactant. The sensitivity of surfactants may be influenced by a range of these intrinsic factors, and further work investigating specific parameters is required and will be explored in the following chapters. Considering the physical application of FePS formulations, it is optimal for a surfactant to be able to perform consistently to minimise risk of prepared batch variations and human error in solution preparation. It is also easier to formulate the suspensions regarding physical properties, allowing for use of thinner suspensions which are more easily applied and washed off without diminishing fingermark quality. This research demonstrated that thinner formulations are not more effective regardless of surfactant used, and that optimal concentration and powder to surfactant ratio changes with surfactant type.

Formulations made only with water were able to produce ridge detail and contrast superior to some surfactants, however it also resulted in the heaviest background development. This suggests that in most formulations, the addition of the surfactant is aiding the selective deposition of powder particles along the fingermark ridges and preventing deposition over the entire substrate surface. This observation is consistent with results produced by Morris *et al*. while investigating effect of surfactant on SPR development in 1978 [95]. The ability of surfactants to deposit powder preferentially on fingermark residue strongly affects its efficacy in PS formulations. This is highlighted by comparing the results of LN, SP80/T80, and T80. The lack of background development caused by LN coupled with poor ridge detail and contrast suggests that the surfactant is preventing powder deposition anywhere on the substrate. However, the poor ridge detail and contrast produced by SP80/T80 is coupled with moderate to heavy background development, suggesting that the surfactant is allowing surface-wide

deposition which is not concentrated on the fingermark ridges. It also may be that some surfactants are removing the fingermark residue during the application process, leaving nothing for the powder to adhere to. It is important to note that the methodology used during deposition likely resulted in fingermarks from all donors which were predominantly eccrine-rich, as after handwashing donors did not perform any tasks to load sebaceous material or other contaminants. This study has demonstrated that the interaction between surfactants and fingermark residue is more complex than previously thought. However, due to natural donor variation in fingermark residue it is difficult to draw any conclusions about these specific interactions. Without a controlled matrix of known chemical composition our understanding of this relationship remains limited, however advances in research investigating artificial residues may allow future work to better explore this interaction [96-98]. This will be further explored in chapter 4.

This investigation also demonstrated that efficacy of a surfactant is influenced by the substrate on which fingermarks are being developed. Fingermarks on the plastic substrate had the highest rate of 0 scores across all parameters, as well as the highest rate of marks graded 3 or 4 in background development. This suggests that powder particles are either not adhering to the plastic substrate as easily as ceramic or glass, or the fingermark residue is more easily removed during development. The FVM currently recommends either CA fuming and subsequent staining or FePS for fingermark development on soft plastics. If the item had been wetted, FePS would be the preferred development technique [7, 8, 25]. T20 surfactant appeared to be the most consistent in developing marks across all substrates and was highly effective on the plastic substrate. These results are in line with the recommendation for use of T20 on wetted plastics in the 2022 FVM [41]. Plastic substrates are a traditionally difficult substrate to develop fingermarks on and dry powders are known to be ineffective on this substrate and often cause heavy background development [25, 92]. The addition of a surfactant to the dry powder in FePS formulations is therefore shown to reduce this background development. However, as the level of background development changes based on surfactant type and concentration, the surfactant plays an important role in influencing powder deposition regardless of the presence of fingermark residue, especially on plastic substrates. Further investigation into substrate characteristics would aid in better understanding these interactions and determining if there are specific physical properties causing these differences.

It has been hypothesised that the role of the surfactant is to suspend the powder particles in solution by surrounding them with micelles, similar to the mechanism involved in SPR as described in section 1.4.3. These micelles are then destabilised by some component of the fingermark residue to allow the selective deposition of powder along the ridges [26, 31, 41]. There is little evidence to support this

theory, however it can be suggested that surfactant micelles have varying ability to allow the optimal suspension and deposition of particles. LN micelles, for example, may not be destabilised sufficiently by the fingermark residue present to allow for powder deposition. However, the SP80/T80 and T80 micelles seem to be releasing the powder regardless of the presence of fingermark residue. Identifying which property of the surfactants is resulting in this difference is crucial to understanding the role of surfactants in producing background development using FePS and will be explored further in future chapters.

This study has shown that the surfactants tested all interact differently with fingermark residue and substrates and further indicated the ways in which surfactants are influencing fingermark quality that have not yet been investigated. To date, there are no published investigations which aim to improve our fundamental understanding of the relationship between surfactants, fingermark residue and substrates. This research therefore provides a strong foundation and direction for further investigations which may determine what properties are responsible for the variable effectiveness of surfactants in this technique and further optimise FePS formulations to increase our fingermark detection capabilities.

2.5 Conclusion

This study assessed a range of surfactant types, concentrations, and ratios in FePS formulations to determine the effect of the surfactant on fingermark development using this technique. The results showed that there is no trend in surfactants of the same ionic nature in developing ridge detail, improving contrast or reducing background development on non-porous substrates. Not all surfactants were able to improve the development of fingermarks in the FePS process compared to water-only formulations. The most highly graded surfactant against the assessed parameters was KP due to the high level of ridge detail and contrast produced from all formulations and lack of background development. The lowest graded surfactant against the assessed parameters was SP80/T80, which developed very poor ridged detail and contrast and had a high rate of background development across all substrates. Formulations made with only water were effective in developing fingermarks but produced heavy background staining which reduced overall quality. This study has demonstrated that optimal surfactants play a vital role in preventing background development using FePS and may have a greater influence on the volume of powder deposited on ridges than previously thought. Using these results as a robust foundation of the qualitative impact of surfactants on FePS development, further work can be conducted to explore whether surfactant efficacy can be predicted by particular physical traits of surfactant monomers or micelles. This will be explored in the following chapter.

Chapter 3: An investigation into the importance of micelles in FePS

3. *An investigation into the importance of micelles in FePS*

3.1 Introduction

3.1.1 Surfactant structure

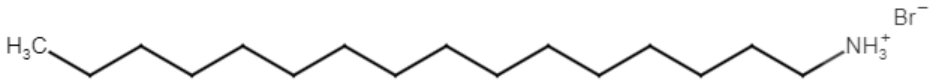
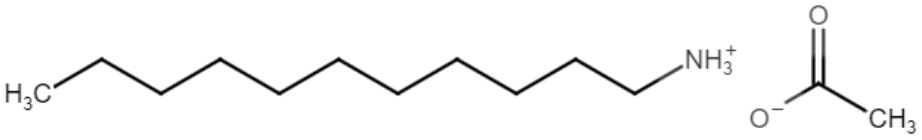
It has been established that the use of different surfactants in FePS formulations impacts the efficacy of the technique. Because of this, it may be suggested that a part of the surfactant monomer or micelle structure is influencing the way FePS suspends powder particles and interacts with fingermark residue. All surfactants are amphiphilic molecules, and the size and structure of both the polar and non-polar regions can vary greatly between surfactants. As discussed in section 1.4.1, the charge of the surfactant head group determines its ionic nature (cationic, anionic, or nonionic). The non-polar carbon chain tail section can vary in chain length and complexity, and the relationship between these polar and non-polar parts will influence the size and shape of a micelle as well as the CMC and solubility of a surfactant. The structure of surfactants must therefore be considered when assessing the role of micelle formation [70, 99, 100].

Cationic surfactants

Two cationic surfactants were assessed in this study (CTAB and n-DDAA), whose structures are shown in Table 3.1. These surfactants are classified as cationic, as the polar head group is positively charged when dissolved in water. Most cationic surfactants are quaternary ammonium compounds and are commonly used as antimicrobial agents, fabric softeners, antistatic agents, and emulsifiers [60, 101, 102]. However, issues arise with the biodegradability of cationic surfactants, and as such they are not as commonly used in household applications [103].

Both CTAB and n-DDAA contain an ammonium ion as the positively charged surfactant head group, however, differ in chain length and type of anion in the surrounding solution. CTAB has a carbon chain length of 16 and produces bromide ions in solution, while n-DDAA has an 11-carbon chain and produces carboxylate ions [65, 104].

Table 3.1 Chemical structure of compounds present in cationic surfactant solutions CTAB and n-DDAA

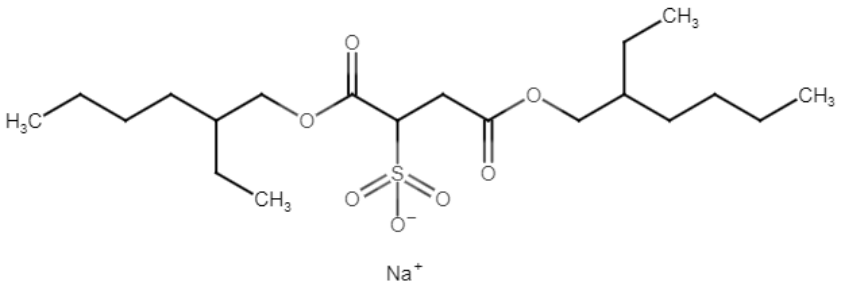
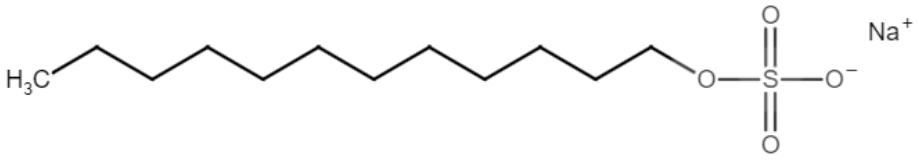
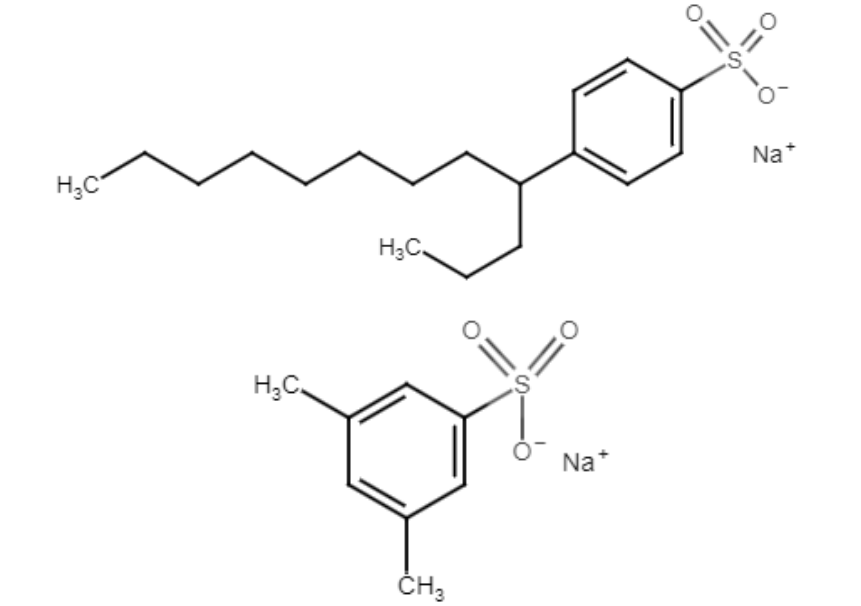
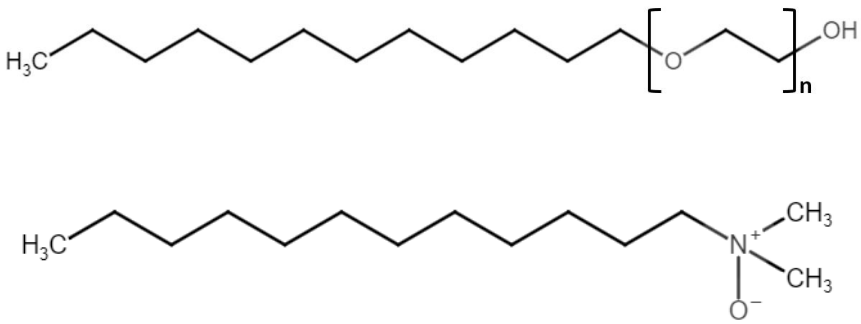
Surfactant	Structure
CTAB	
n-DDAA	

Anionic surfactants

The structures of three anionic surfactants assessed in this study (DOSS, SDS, and LN) are illustrated in Table 3.2. Anionic surfactants contain negatively charged head groups when dissolved in solution and are the most commonly used surfactant class due to their application in laundry and personal care products [60, 105]. They typically contain 12 to 18 carbons within the hydrocarbon chain with head groups consisting of carboxylates, sulfonates, sulfates and phosphate salts [102].

SDS has a carbon chain containing 12 carbon atoms and a sodium sulfate head group. The structure of DOSS orients the polar head group (sodium sulfonate) at the centre of two branched 8 carbon ester groups. LN is a mixed commercial surfactant solution, and the exact quantity of each product is not publicly available, however the two main products also contain sodium sulfate ions in the polar head region. LN contains two anionic molecules with aromatic rings (C13 and C6) as well as a nonpolar alcohol ethoxylate. All three anionic surfactants assessed in this study contain sodium counterions when dissolved in solution.

Table 3.2 Chemical structure of compounds present in anionic surfactant solutions DOSS, SDS and LN

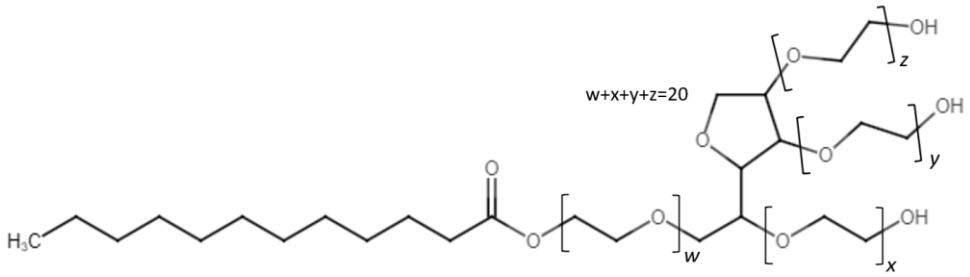
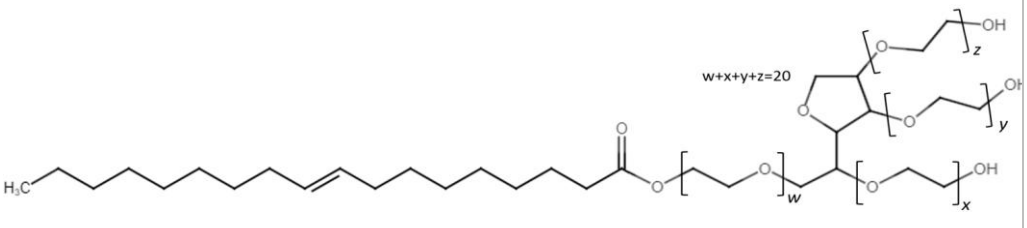
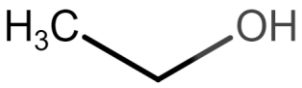
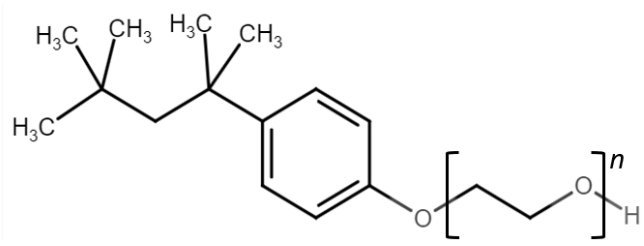
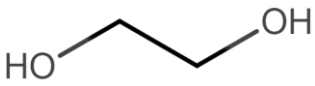
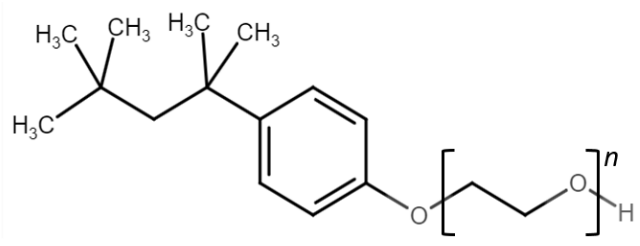
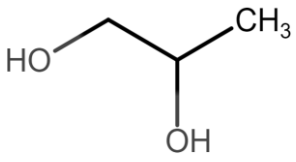
Surfactant	Structure
DOSS	
SDS	
LN Sodium alkylbenzene sulfonate (10-15%) Sodium Xylenesulphonate (2.5-10%) Alcohol ethoxylate (2.5-10%) Lauramine oxide (1-2%)	 

Nonionic surfactants

Most of the surfactants assessed in this study were nonionic, as they have been most frequently used in previous fingermark research. The structures of the nonionic surfactants T20, T80, TX100, and KP are shown in Table 3.3. These surfactants do not have ionic products and do not carry any charge at the head group. Nonionic surfactants are used because they are generally more stable and less toxic than ionic surfactants and are widely used in pharmaceuticals, as a wetting and emulsifying agent and detergent [60, 102, 106]. The hydrophilic head group of nonionic surfactants is usually a polyoxyethylene group, which is true for all nonionic surfactants assessed in this study [57, 100, 106]. Greater number of polyoxyethylene units within a surfactant molecule generally make the surfactant more water soluble [107].

T20 and T80 have very similar chemical structures and differ only in the length of the hydrocarbon chain (T20 = C12, T80 = C18), and T80 contains a double bonded carbon in the centre of the chain. The longer carbon chain (hydrophobic group) present in T80 makes the surfactant less water-soluble, therefore requiring the addition of ethanol to facilitate dissolution in water. TX100 and KP contain the same major product but differ in the additional products used to dissolve the surfactant in water. KP is also a pre-mixed surfactant solution and therefore the exact concentration of each product is not publicly available. The TX100 surfactant contains a hydrophobic branched 8-carbon chain which includes a benzene ring, and 9-10 repeating oxyethylene units as the hydrophilic head [108, 109]. All nonionic surfactants assessed in this study contain a notably larger polar head group that the ionic surfactants.

Table 3.3 Chemical structure of components in nonionic surfactant solutions T20, T80, TX100 and KP

Surfactant	Structure
T20	
T80 Tween 80 Ethanol	 
TX100 Triton X-100 Ethylene glycol	 
KP Triton X-100 Propylene glycol	 

3.1.2 Micelles in FePS

The efficacy of surfactants rely heavily on their physical properties which occur when surfactant micelles are present in solution [57, 59, 62, 102]. As discussed in section 1.4.1, micelles will form above a certain concentration known as the critical micelle concentration (CMC) which changes by surfactant type and can be affected by factors such as temperature, pressure, pH, or the presence of additional products such as salts or alcohols [110, 111]. Above the CMC, the physical properties of a surfactant will change which allows it to carry out its intended function. The most common function of surfactants is to reduce the surface tension of water to increase spreading and wetting properties of a solution. When surfactant molecules are in water, their amphiphilic nature causes them to orient themselves at the air-water interface (adsorption) to ensure the hydrophilic head is in the water and the hydrophobic part in air. Once the surfactant concentration surpasses the CMC, these molecules can self-aggregate into micelles (aggregation) where the hydrophobic tails are protected by the hydrophilic head groups and dispersed in solution [57, 58, 70, 84, 110]. This is illustrated in Figure 3.1.

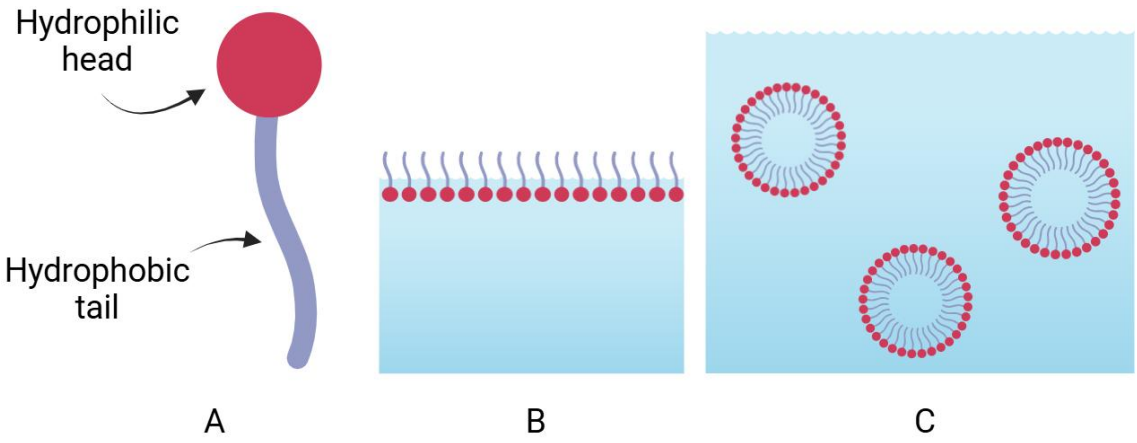


Figure 3.1 A- Amphiphilic molecule B- Surfactant molecules at air-water interface (below CMC) C- Spherical surfactant micelle (above CMC)

Due to the changes caused by micelle formation, the CMC of a pure surfactant can be found by experimentally determining the point of inflection while plotting a physical property of the surfactant, such as surface tension (ST), conductivity, or light scattering intensity, as a function of concentration [65, 70, 73, 112]. Depending on the method chosen and property being measured, the changing of physical properties often occurs over a narrow range rather than at a specific point. Similarly, result variations can occur from different methods depending on what is being measured. For example, ST measurements are sensitive to changes in monomer concentration while light scattering techniques are sensitive to changes in micelle concentration [70, 113]. Because of this, literature values of surfactant CMC can vary or be presented as a range estimated from plotted data.

All recommended FePS formulations contain surfactants which are at a concentration above their CMC. It is suggested that the presence of micelles in FePS formulations is vital to the successful development of fingermarks, however, to date it is only theorised how surfactant micelles are involved in the FePS mechanism. The only published literature examining the effect of CMC on fingermark development using FePS was performed by Downham *et al.* in 2017, in the second part of an in-depth investigation into the effect of TX100 on FePS formulations. This study investigated the effect of changing the concentrations of TX100 and ethylene glycol, developing fingermarks using the FVM 2014 recommended solution (approx. 400x CMC) and diluting this down to 0.5x CMC [25, 78]. The authors estimated from previous literature that the addition of a 40% ethylene glycol solution, used to facilitate dissolution of TX100, raises the CMC of TX100 from 0.2 mM to 0.8 mM [78, 114]. The results showed that the concentration of TX100 used in FePS can be reduced to 0.5x CMC and still effectively develop fingermarks. It was observed that at concentrations of 1x CMC or lower, slightly darker marks were produced than at higher concentrations, suggesting heavier powder deposition. These solutions however also resulted in surface-wide iron oxide background deposition which varied slightly depending on the substrate used. This suggests that the use of surfactants below their CMC allows the solution to more readily deposit powder particles both on and off ridge, and that the addition of more concentrated surfactants controls the selective deposition of powder. Physical differences between 0.5x CMC and 400x CMC were also observed, with the more dilute suspension reticulating across the substrate it was applied to and the more concentrated solution evenly spreading across the substrate.

This study also found that changing the concentration of ethylene glycol did not notably affect the development quality or physical properties of the FePS formulation, however the TX100 took significantly longer to dissolve in water without it [78]. Overall, this study demonstrated the importance of micelles in particle distribution and selective powder deposition of FePS. It is still unknown exactly how the micelles interact with the powder particles in suspension, and the authors of this study suggested three potential models as illustrated in Figure 3.2. The first model (a) represents the simplest orientation by which many spherical micelles are thought to suspend insoluble material, with the hydrophobic surfactant tails pointing inwards to hold the particle. Models b and c show alternate possibilities in which multiple micelles either form individually around the outside of the particle or entangle together to form an outer ‘layer’ of monomers. There are currently no methods that can be used to reliably determine which of these formations are most likely, or the subsequent impact of this interaction on FePS development. The focus of the study on one surfactant allowed a deeper investigation into the components that may be affecting this particular formulation than had previously been investigated. However, this means our understanding of the role of

surfactants is restricted to this context, and this issue is highlighted by the fact that the surfactant investigated in this work is no longer recommended for use in FePS [41]. As several surfactants can be used in FePS, studies aiming to understand the impact of micelles and CMC in a broader context would be valuable in informing and focussing research to further optimise FePS formulations.

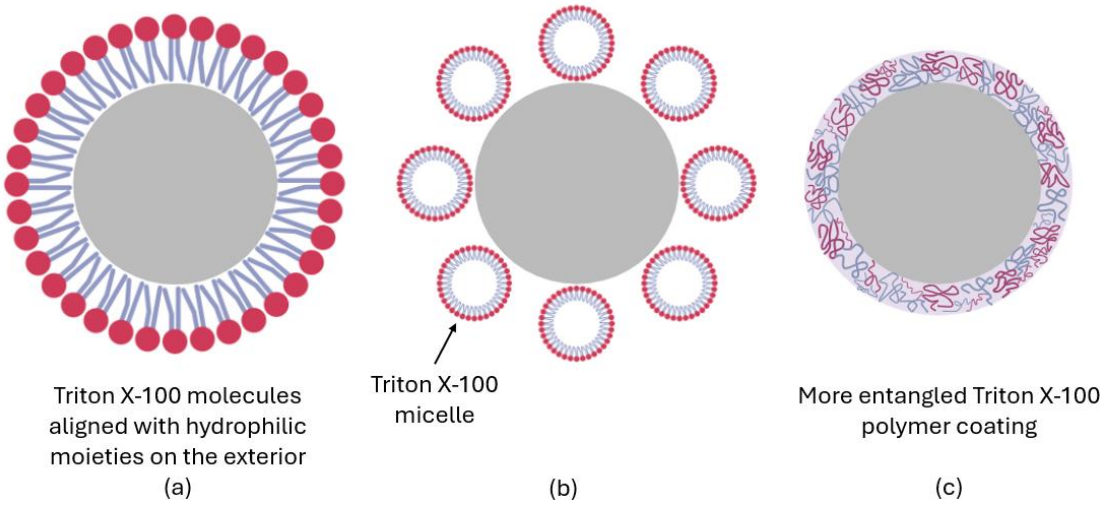


Figure 3.2 Schematics showing iron oxide particles (grey) and their hypothetical association with Triton X-100 molecules (a-c). Adapted from Downham et al. [78]

Despite many theories surrounding the importance of micelle presence in FePS formulations, there have been no published investigations determining the effect of CMC using multiple surfactants on fingermark development in this technique. Investigating this property is key to better understanding the mechanism by which FePS formulations can develop fingermarks and could potentially determine if there are surfactant properties which influence the effect of CMC on fingermark development. This knowledge will therefore improve understanding of the FePS development mechanism and aid in future optimisation recommendations.

3.2 Materials and method

To assess the importance of the presence of micelles in FePS surfactant solutions, natural fingermarks were developed with FePS formulations using surfactants above and below their reported CMC. For surfactants which did not have CMC values readily available in literature, ST experiments were first conducted to determine the CMC. Surfactant solutions were then made of each surfactant tested in previous chapters at approximately 0.5x and 50x their CMC and used to develop fingermarks in FePS on a range of non-porous substrates. To observe the effect of different surfactants, the same batch of Fisher Chemicals magnetic iron oxide powder (CAS 1317-61-9) as used in chapter 2 was used for the duration of the study. Fingermarks were collected from a range of donors; ethics approval was

completed through the University of Technology Sydney (ETH18–2521, Appendix A) and participants were required to consent to the collection of their fingermarks prior to deposition.

3.2.1 CMC determination

The CMC for all surfactants assessed were not available in literature as some contained additional products (for example, the addition of ethylene glycol and ethanol to TX100 and T80 formulations respectively) or were proprietary mixtures, such as KP and LN. ST experiments were therefore conducted to provide an approximate CMC value for KP, LN, TX100 and T80. T20 was also tested, as the CMC of this surfactant is reported in literature and could be used to compare and validate results. This was performed by diluting surfactant solutions and plotting the ST of each dilution, to determine the concentration at which there is a sharp rise towards the ST of water caused by the reduction of micelles (below the CMC).

3.2.1.1 Chemicals and equipment setup

TX100, T20, T80, LN, PCC-54™ and ethylene glycol were purchased from Sigma Aldrich. KP was purchased from Kodak Alaris.

All glassware (Schott bottles, volumetric pipettes, volumetric flasks and glass dishes), lids, magnetic stirring bar and torsion balance ring was surface cleaned by soaking in a bath of PCC-54™ detergent overnight, before being thoroughly rinsed with ultrapure water and left to air dry for 2 days. This was done to remove any potential contamination that may affect results.

3.2.1.2 Solution preparation

Stock solutions of each surfactant were made as per Table 3.4 and stored in clean 250 mL Schott bottes. Twelve dilutions of each stock solution were then prepared in individual 100 mL volumetric flasks. For each surfactant, 100 mL of stock solution was added to the first flask. Using a 50 mL volumetric pipette, half the solution was transferred to a second flask before adding 50 mL deionised water to dilute the surfactant. This was repeated eleven times to produce twelve dilutions of each surfactant. While the solutions were not in use, the flasks were capped to prevent contamination.

As KP and LN are commercial mixtures, the exact ratio of compounds present in solution is not publicly disclosed. Because of this, calculations done with these surfactants used only the weight of added product instead of the molar concentration.

Table 3.4 Formulations of surfactant stock solutions used for dilution in ST experiments

Surfactant	Weight of surfactant (g)	Weight of additional product (g)	Weight of water (g)	Concentration (mM)
T20	1.1	n/a	99.12	4.47
TX100	2.65	Ethylene glycol 3.85	94.06	4.22
T80	2.64	Ethanol 0.69	96.67	2.021
KP	2.5	n/a	97.52	n/a
LN	2.64	n/a	97.55	n/a

3.2.1.3 Surface tension

A torsion balance was used to measure the ST of each surfactant dilution. The instrument was calibrated before testing each new surfactant by measuring the ST of water to ensure results were accurate. Between each dilution, the ring was submerged in water and carefully dabbed dry using a Kimtech™ wipe. To measure the ST, sufficient sample was poured into a circular glass dish, so the solution filled 1 cm of the dish. The metal ring of the torsion balance was then submerged, before being raised until it broke the surface. The force required to break the ST was measured by the instrument in milli-newtons and each solution was tested in triplicate. The average of each triplicate measurement was used for subsequent analysis. The results were graphed using excel and plotted on a logarithmic scale to determine the point at which the surface tension changes, approximating the CMC of that surfactant. The graphed results of the ST experiments can be found in Appendix B. From these graphs, approximate CMC values were determined and shown in Table 3.5.

Table 3.5 Mass and molarity of surfactants tested at the CMC. CMC value of commercial products marked as n/a due to lack of exact product concentrations publically avaiable

Surfactant	Mass of surfactant (g) at CMC in 100 mL water	Approximate CMC
T20	8.580×10^{-3}	0.069 mM
TX100	1.625×10^{-3}	0.026 mM
KP	7.800×10^{-3}	n/a
LN	1.035×10^{-3}	n/a
T80	5.172×10^{-3}	0.016 mM

3.2.2 Surfactant preparation

Solutions were prepared for each surfactant at 50x CMC. The ten surfactants investigated in previous chapters were used in this study, except for the Span 80/Tween 80 (SP80/T80) mixture. This was

excluded as no literature value for the CMC was available and could not be determined using the ST method used in section 3.2.1 as the surfactants did not mix homogenously. For the remaining surfactants, CMC values were determined through literature or experimental work. The CMC of mixed surfactants such as TX100 and T80 were determined by ST experiments as the literature values available were not calculated using the additional products used in these mixtures. The values used in stock solutions containing approximately 50x CMC of each surfactant are shown in Table 3.6. The stock solutions were stored in a cupboard at room temperature in glass Schott bottles.

*Table 3.6 Approximate CMC values of surfactants used and mass of surfactant added to 50x CMC solution. Values marked with * determined from ST experiments*

Surfactant components	CMC of pure surfactant in literature	Mass (g) in 1 L for CMC	Mass (g) in 1 L for 50x CMC
CTAB	0.98 mM [115]	0.357	17.85
DOSS	2.7 mM [116]	1.20	60.02
KP	n/a	0.780*	39.0
LN	n/a	0.104*	5.20
n-DDAA	0.52 mM [88]	0.128	6.40
SDS	8 mM [115]	2.31	115
T20	0.059 mM [88]	0.0858*	4.29
T80 **Ethanol**	0.015 mM [111]	0.00517* 0.0012	0.259 0.06
TX100 **Ethylene glycol**	0.2 mM [114]	0.562 [78] 35	28.1 35

All FePS formulations were made using the same ratio as recommended by the 2022 FVM in a 1:2 w/v (powder (g): surfactant (mL)) ratio [41]. For each surfactant, two formulations with surfactant concentration at 50x and 0.5x the CMC were made. These values were chosen to ensure the formulations were above and below the CMC, especially for surfactants where the CMC was approximated as a wider range. The stock solutions (50x) were diluted to 0.5x using distilled water immediately before development. All FePS formulations were made immediately before development and applied using the same method as outlined in section 2.2.3.

3.2.3 Fingermark deposition and enhancement

Fingermarks were deposited on the same three non-porous substrates used in section 2.2.2 (ceramic tile, clear glass tiles, and PE plastic bags). To best compare the 50x and 0.5x formulations, split marks

from three donors were deposited. The donors were asked to use the same deposition method as outlined in section 2.2.2, and split marks were made by depositing fingermarks as illustrated in Figure 3.3. For ceramic and glass substrates, the middle fingermark was deposited on the seam of two samples so they could be separated and developed with different formulations, while the plastic samples were cut down the centre fingermark before development. All fingermarks were developed on the same day as deposition and imaged using the same methodology as described in section 2.2.3. Three repeats were performed for each of the nine surfactants and a total of 2,187 marks were developed.

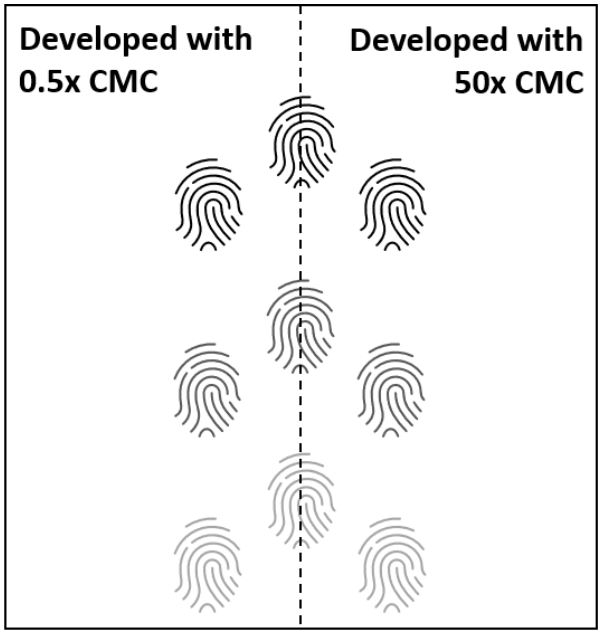


Figure 3.3 Deposition of split marks developed with formulations above and below the CMC on all substrates

Developed fingermarks were then graded for their ridge detail, contrast, and background development to assess the effect of CMC on fingermark development. The whole marks (ring and index fingers) developed by 50x and 0.5x CMC formulations were assessed for ridge detail, contrast, and background development using the same grading scale shown in section 2.2.4 (Tables 2.3 to 2.5) by one assessor, resulting in a total of 6,561 scores. The scores were analysed and graphed using Microsoft Excel.

3.2.4 Micelle size

The mean micelle diameter size and polydispersity index (PdI) of each surfactant was measured using DLS techniques. These two measurements provide information on the size of surfactant micelles and an estimate of the width of size distribution (uniformity of micelle size and aggregation) respectively [72, 112]. DLS is commonly used to measure the intensity of light scattered after being passed through a sample, which gives information about the size distribution profile of particles within the solution

[73, 108, 117]. This data was then used to determine any correlation to the results of fingermark development quality presented in this chapter to assess if micelle size is an influencing factor in FePS development.

Surfactant stock solutions as outlined in Table 2.1 were made fresh using ultrapure water to ensure no contamination was present. The Malvern Zetasizer Pro was then used to measure the micelle size and PdI of each surfactant with DLS at 22°C. Each reading produced data in triplicate, and the mean of this data used in subsequent analysis. Analysis was performed three times for each surfactant at weekly intervals, and a single graph was produced for each surfactant using the averaged data from all tests. In total, each graph represented nine data sets. The results were graphed on a logarithmic scale (Appendix C) and the micelle size of each surfactant determined from the highest point of the graph's peaks. Pearson's correlation coefficient was calculated using Microsoft Excel to determine the relationship between surfactant micelle size and fingermark quality grades (ridge detail, contrast, and background development).

3.3 Results

3.3.1 Comparison of CMC ranges

As the CMC values of surfactants were approximated from either literature or ST experiments, a short preliminary comparison of surfactant concentration ranges was performed. This was done to assess the differences between high and low ends of a CMC range and how much fingermark development with FePS was impacted by variations in approximate CMC. Within this preliminary study, the effect of additional products was also investigated. T20 formulations were employed to investigate the impact of small changes in approximate CMC concentration, while TX100 was used to assess the effect of additional product volume on fingermark development, as the recommended formulation includes ethylene glycol to assist the dissolution of TX100 in water.

3.3.1.1 T20 concentration ranges

ST experiments performed with T20 showed the CMC to be approximately between two dilutions at D7 (0.07 mM) and D8 (0.035 mM), indicated at the point where red lines intersect in Figure 3.4. This concentration is consistent with available literature (CMC 0.059 mM [88]). To assess the impact of variations in CMC concentration on fingermark development, a comparison was performed between both sides of the ranges. To do this, FePS formulations were made at 50x and 0.5x the concentrations of D7 and D8 and compared by developing either side of split fingermarks.

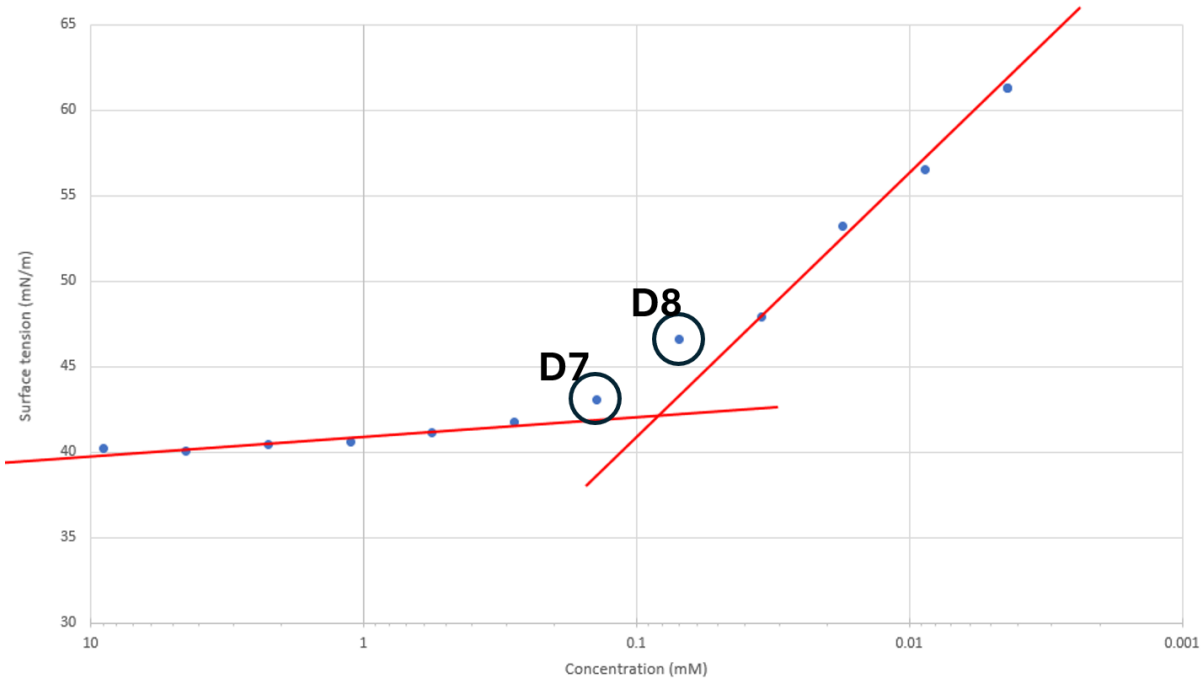


Figure 3.4 Graph of T20 ST experimental data used to determine the approximate CMC (where the red lines intersect). Solutions made at D7 and D8 to assess effect of range around approximate CMC

Samples developed at 50x and 0.5x concentrations of D7 and D8 showed clear differences in developed fingermark quality and levels of background deposition. Figure 3.5 illustrates a comparison between formulations, showing that at 50x concentration there is a large difference between background development produced. However, at 0.5x concentration the fingermark quality and background development appear similar. Despite both D7 and D8 concentrations certainly being above the measured CMC at 50x, the differences in development suggest that there is a threshold at some point between 50x D7 and 50x D8 where enough T20 micelles are present in solution to prevent surface-wide powder deposition. This demonstrates that these small differences in approximate CMC concentration can have a large effect on fingermark development when scaling surfactant concentration. Later experiments comparing CMC development in this chapter utilise the D7 T20 concentration to ensure enough micelles are present in solution for an effective FePS.

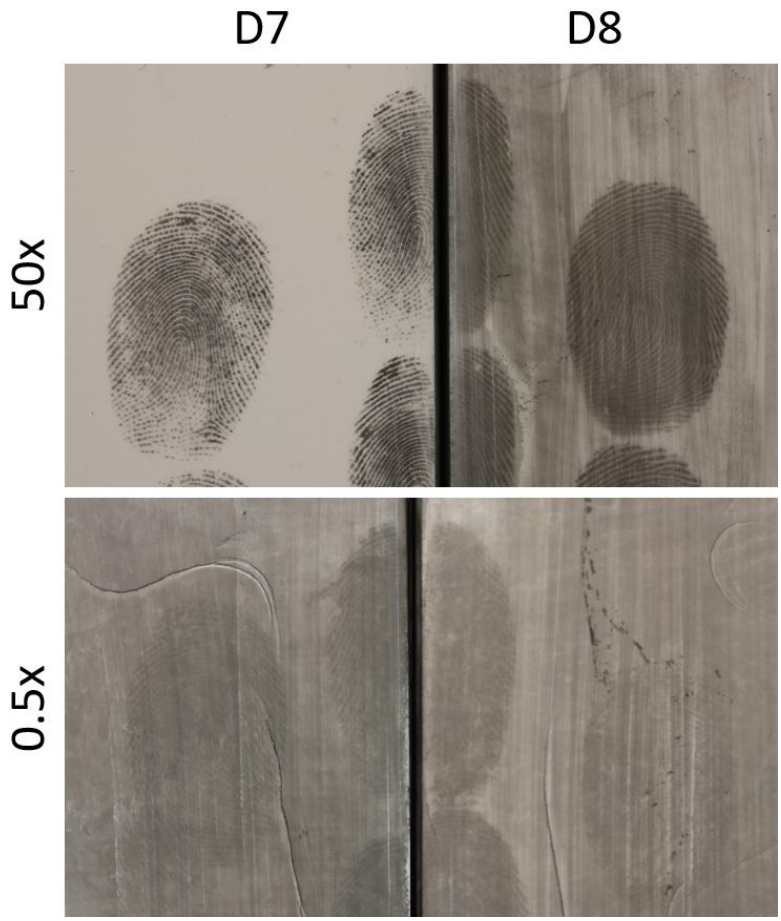


Figure 3.5 Comparison of development of split fingermarks from one donor using FePS made with 50x and 0.5x T20 D7 and D8 concentrations on ceramic

3.3.1.2 TX100 concentration ranges

As two surfactants used in this study (TX100 and T80) incorporate other products in their stock solutions to facilitate surfactant dissolution in water, the effect of these additions on fingermark development were investigated. TX100 was chosen for investigation as its CMC has been researched previously by Downham *et al.* (2017) [78]. The authors reported that TX100 was quoted by suppliers as having a CMC range of 0.2 mM to 0.9 mM, and used the upper range for subsequent calculations to ensure micelle formation as no CMC determination experiments were done in this research. The authors did not explore the effect of changing ethylene glycol on CMC and maintained the concentration of ethylene glycol at 35% for all formulations. The ST experiments described in section 3.2.1.3 involved the dilution of the TX100 formulation and subsequently the concentration of ethylene glycol was proportionally reduced in this experiment. The differences between TX100 mass at CMC measured in ST experiments and reported by Downham are outlined in Table 3.7. There is a large difference between these values, which is likely largely due to Downham selecting the higher end of the reported CMC range for this surfactant.

Table 3.7 Mass of TX100 in 100 mL water for 50x CMC as calculated by ST experiments and reported by Downham (2017)

Source	Surfactant code	Mass of TX100 in 100 mL water for 50x CMC
Downham (2017) **[78]**	A	2.81 g
ST experiments	B	0.052 g

The effect of these differing CMC values and ethylene glycol concentration is shown in Figure 3.6. These images show again that at 50x CMC, the lower approximate CMC concentration (B- from ST experiments) produces much more background development than the higher concentration. The reduction of ethylene glycol does not affect fingermark development, which is consistent with experiments performed by Downham [78]. Similarly to the comparison of T20 concentrations, these results show that despite formulations made at 50x CMC for A and B TX100 concentrations certainly being above the CMC, the presence of more micelles in solution are greatly improving quality of fingermark development. Further CMC experiments in this chapter utilised Downham's CMC calculation and 35% ethylene glycol to ensure the presence of sufficient micelles for subsequent comparison.

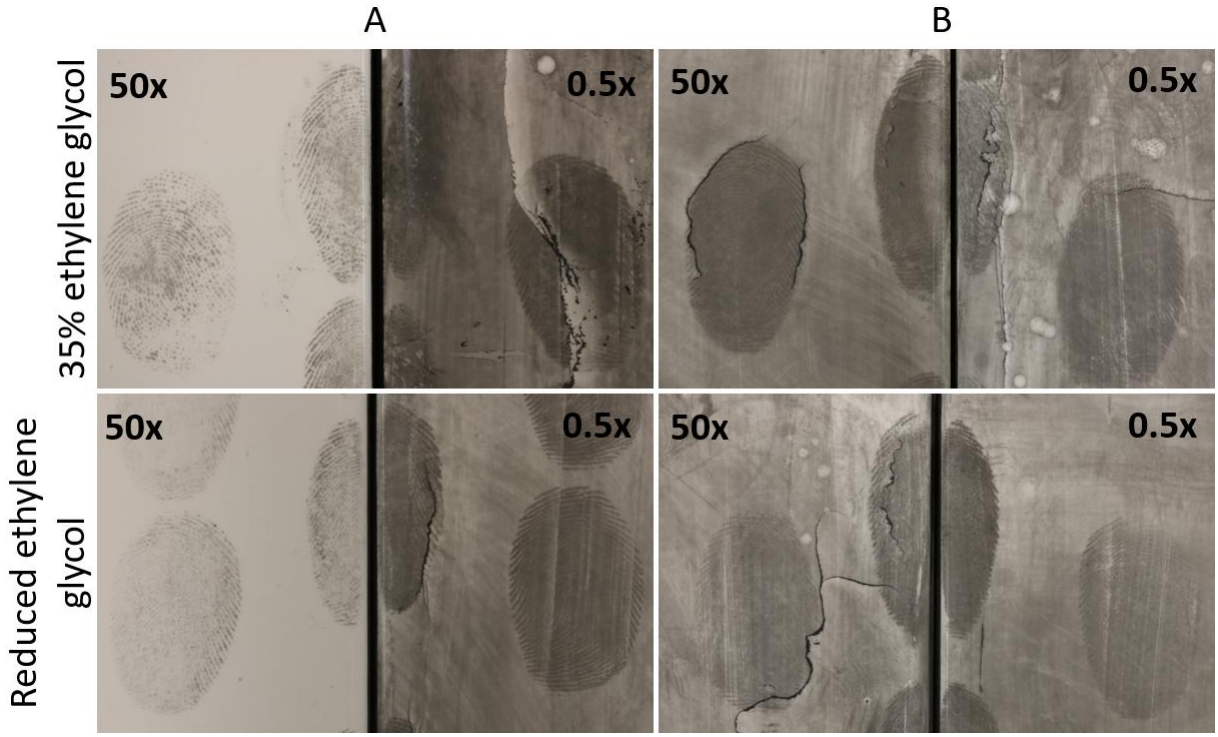


Figure 3.6 Comparison of FePS made with TX100 formulations with changing ethylene glycol and TX100 concentrations (CMC determined by A- Downham, B- ST experiments).

3.3.2 Overall results

The combined results of all surfactant developments on donor fingermarks are shown in Figure 3.7. This graph shows that overall, more scores of 0 were given to the 50x formulations in ridge detail and contrast, however the inverse is true for background development. Despite slightly higher quality developed fingermarks (in terms of ridge detail and contrast) being developed with solutions below the CMC, these formulations also produced much heavier background development. This was expected as the results presented in chapter 2 (section 2.3.3) demonstrated the importance of surfactants in allowing powder to selectively deposit on fingermark ridges rather than all over the substrate. The results of this study show that selectivity is certainly linked to the presence of micelles in solution. This provides support for the generally held belief that iron oxide particles are suspended and subsequently deposited on fingermark residue by surfactant micelles. Previous work reporting these observations have used only one surfactant type, however these results show this is true for all surfactants assessed. An example of development produced by each surfactant above and below their CMC is shown in Appendix D.

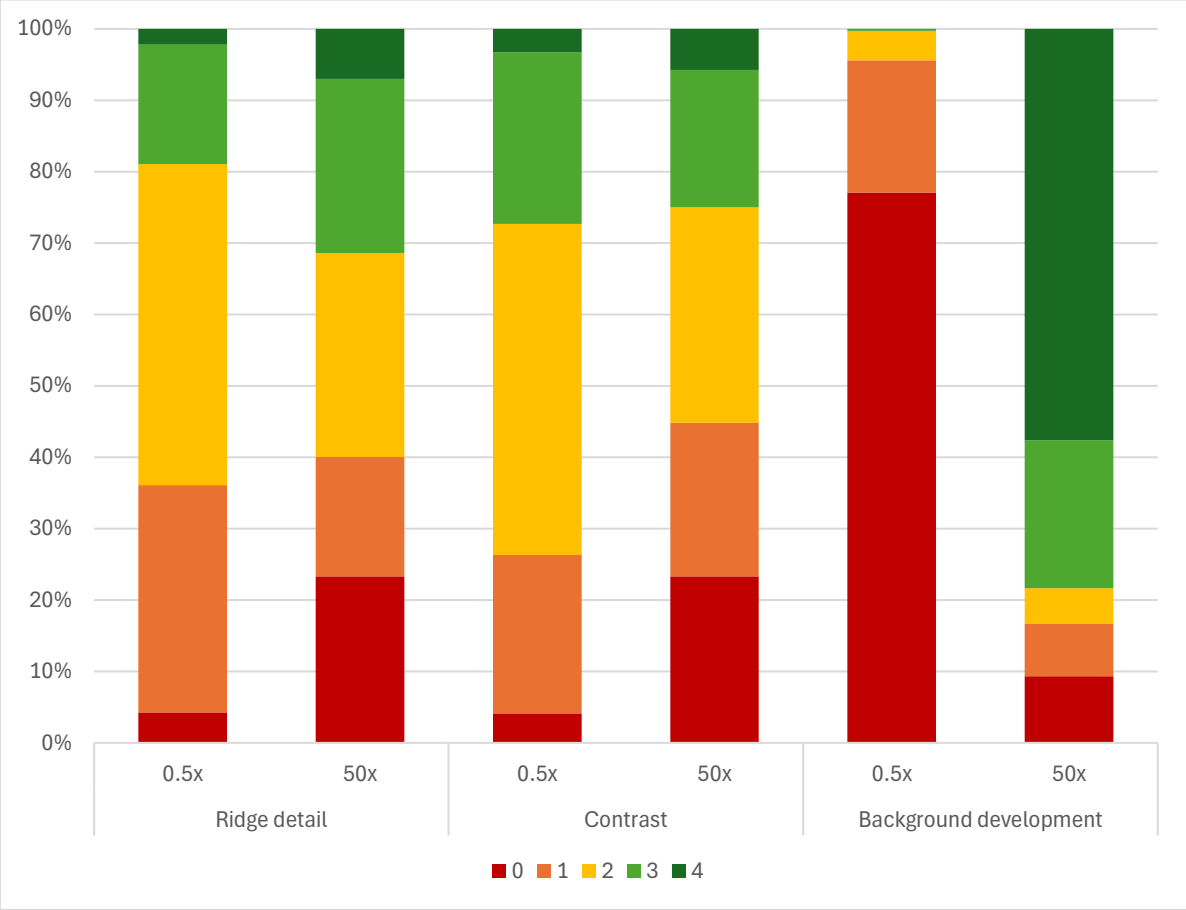


Figure 3.7 Ridge detail, contrast and background development scores for all donor marks developed with FePS made using surfactants at 50x and 0.5x CMC

3.3.3 50x CMC development

The ability of surfactants to enhance fingermark ridge visibility at 50x CMC varied for all surfactants, shown in the ridge detail scores presented in Figure 3.8. This was expected, as the results of chapter 2 showed that surfactant type influenced fingermark quality development in FePS. At 50x CMC, it appears that there is sufficient micelle concentration in the solutions for each surfactant to impact ridge development. The highest scores of ridge detail were produced by T20, T80, and KP. Notably, formulations made at 50x CMC with DOSS surfactant did not develop any fingermarks across any of the repeats. In previous experiments discussed in chapter 2, DOSS produced relatively high-quality ridge detail compared to other surfactants on the same substrates included in this study. Due to the discrepancy between these data sets, the concentration of stock surfactant solutions used in chapter 2 (Table 2.1) relative to their CMC was calculated, shown in Table 3.8. From these calculations, it was found that the stock DOSS concentration previously used was only 12x CMC. Because of this, it can be suggested that the concentration of DOSS was too high at 50x CMC to produce fingermark development, either due to surfactant micelles not allowing powder deposition or the high concentration of surfactant removing fingermark residue. This supports previous observations that there is an optimal concentration above the CMC that achieves fingermark development, however this point changes depending on the surfactant used. SDS was the only other surfactant that the stock solution previously used was below 50x CMC, however SDS was able to produce ridge detail in both investigations.

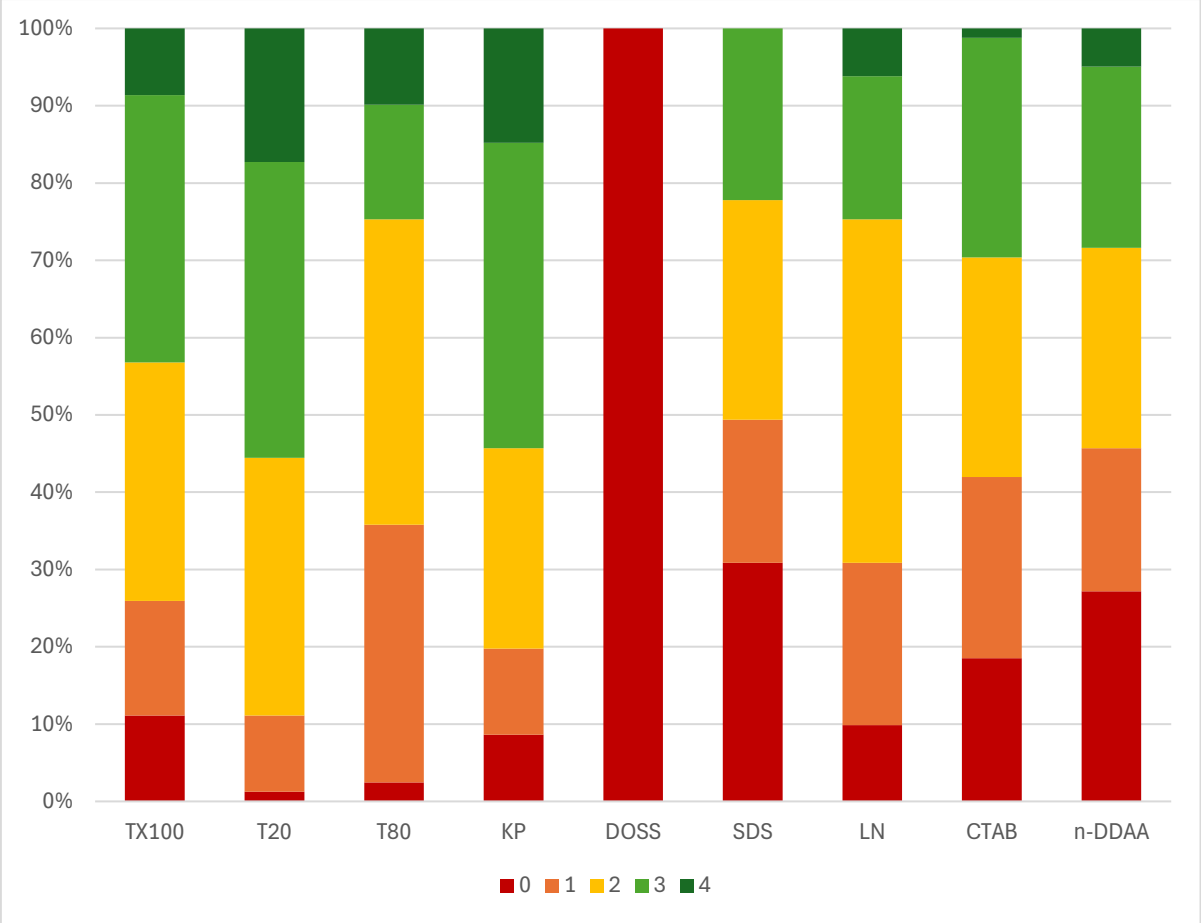


Figure 3.8 Comparison of ridge detail scores given to all donor marks developed with FePS made using each surfactant at 50x CMC

Table 3.8 Approximate CMC concentrations of stock surfactant solutions presented in Table 2.1

Surfactant	Mass (g) for CMC in 1L water	Mass (g) used in stock concentrations in 1L water	Approximate CMC of stock solutions
CTAB	0.357	72.9	204x
DOSS	1.20	15.0	12x
KP	3.90	510	131x
LN	0.525	268	510x
n-DDAA	0.128	49.08	383x
SDS	2.31	58.0	25x
T20	0.0858	110	1282x
T80	0.0206	265	12864x
TX100	0.562	268	479x

The scores of background development at 50x CMC were also affected by surfactant used, however there was less variation than in ridge detail. As shown in Figure 3.9, most surfactants scored 3 or 4 (little/ no background development) for the majority of samples, with the exception of LN and T80 surfactants. LN and T80 formulations at 50x CMC produced heavy surface-wide powder development, with T80 producing the most scores of 0 for this parameter. These surfactants were also two of the three stock formulations investigated in chapter 2 whose concentration was the highest above the CMC, along with T20 as shown in Table 3.8. The T80 stock solution was by far the highest above the CMC (approx. 12864x CMC) followed by T20 (approx. 1282x CMC) and then LN (approx. 510x CMC). However, the T20 formulation did not produce heavy background development like LN and T80, as illustrated in Figure 3.10.

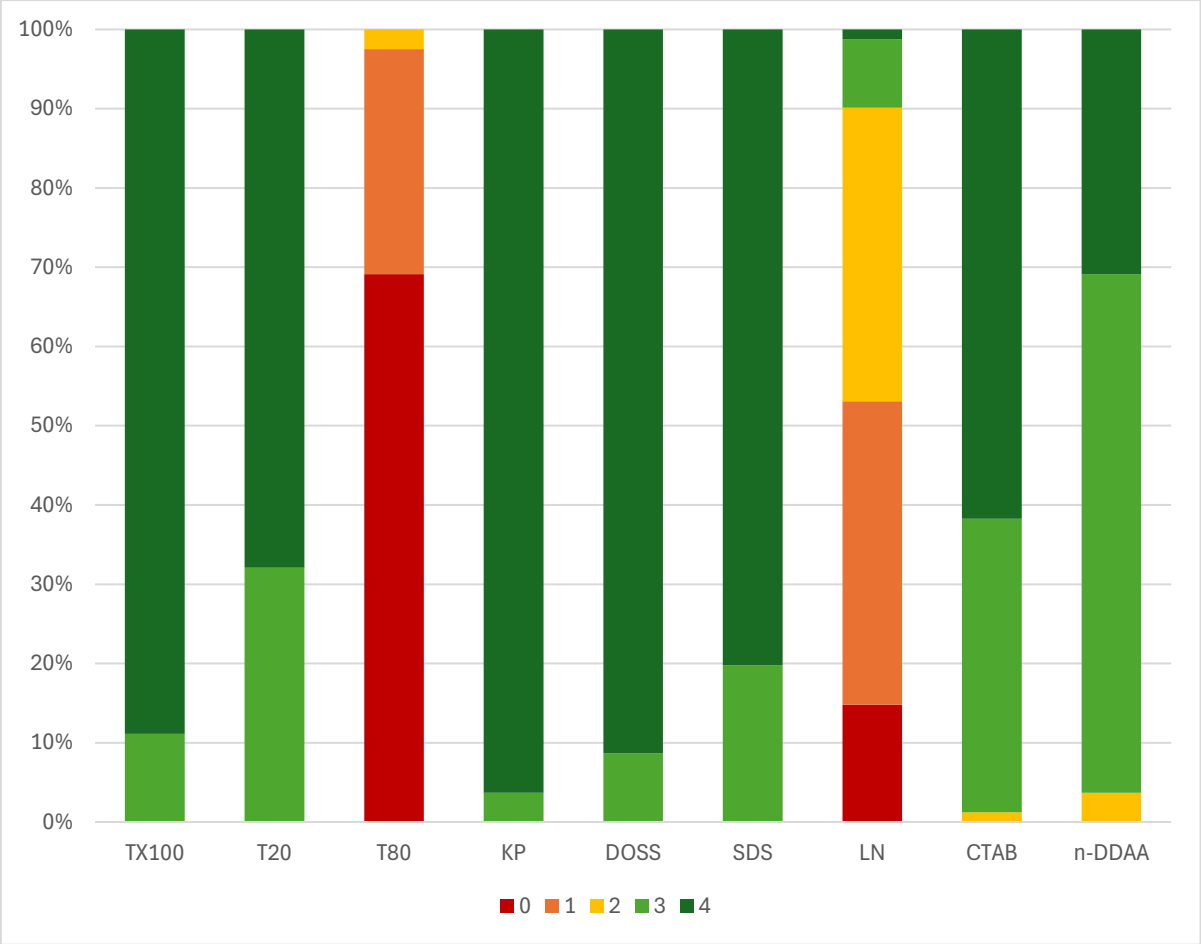


Figure 3.9 Comparison of background development scores given to FePS development with surfactants at 50x CMC on all substrates

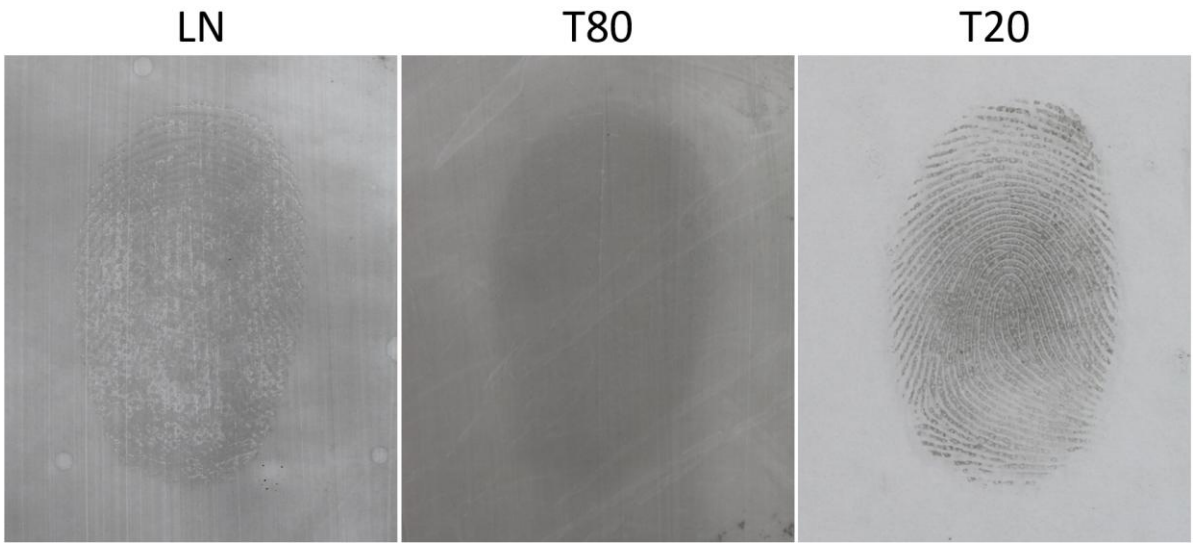


Figure 3.10 Comparison of background development produced by FePS made with 50x CMC formulations of LN, T80 and T20 on glass

The results of background development for LN and T80 using stock solutions in section 2.3.4 (Figure 2.10) were also very different from the scores produced in this investigation. The stock concentration of LN previously did not produce any powder deposition (on ridge or off), while T80 produced variable background development depending on substrate type, occurring mostly on the plastic substrate and very little on ceramic and glass. This suggests that while the stock concentration of LN was too high to deposit any powder particles, at 50x CMC this surfactant's micelles are less stable and therefore facilitate powder deposition. However, the same comparison of T80 background development shows that regardless of micelle concentration, not all surfactants have the same threshold at which no powder is deposited. This is supported further by the way T20 appears to limit background deposition at each concentration tested above 50x CMC while still developing third level ridge detail. There may be a difference in surfactant or micelle structure, leading to differences in the way micelles are suspending the powder particles causing differences in the surfactant's ability to prevent surface-wide powder deposition and selectivity for fingermark residue.

From these results, it can be stated that each surfactant has a different concentration range in which it can be effectively used in FePS. Because of this, future studies investigating the use of new surfactants should ensure to explore a wide range of concentrations to more accurately identify a suitable formulation. It is also clear that surfactants cannot be used interchangeably and "swapped out" at the same concentrations, and any new surfactant tested much be thoroughly evaluated outside the concentration ranges of previously effective surfactants.

3.3.4 0.5x CMC development

The ridge detail scores of all surfactants made at 0.5x CMC were analysed and compared to scores developed with water only formulations to assess the impact of a small amount of surfactant monomers in solution which had not yet formed micelles. As displayed in Figure 3.11, there is only slight variation in ridge detail scores produced by different surfactants. This was expected, as it is hypothesised that the presence of micelles is important to the functioning of surfactants in FePS formulations, and this data supports the hypothesis for all surfactant types tested. Without surfactant concentrations above the CMC, FePS formulations perform very similarly to those made with only water, demonstrated by the background of Figure 3.11.

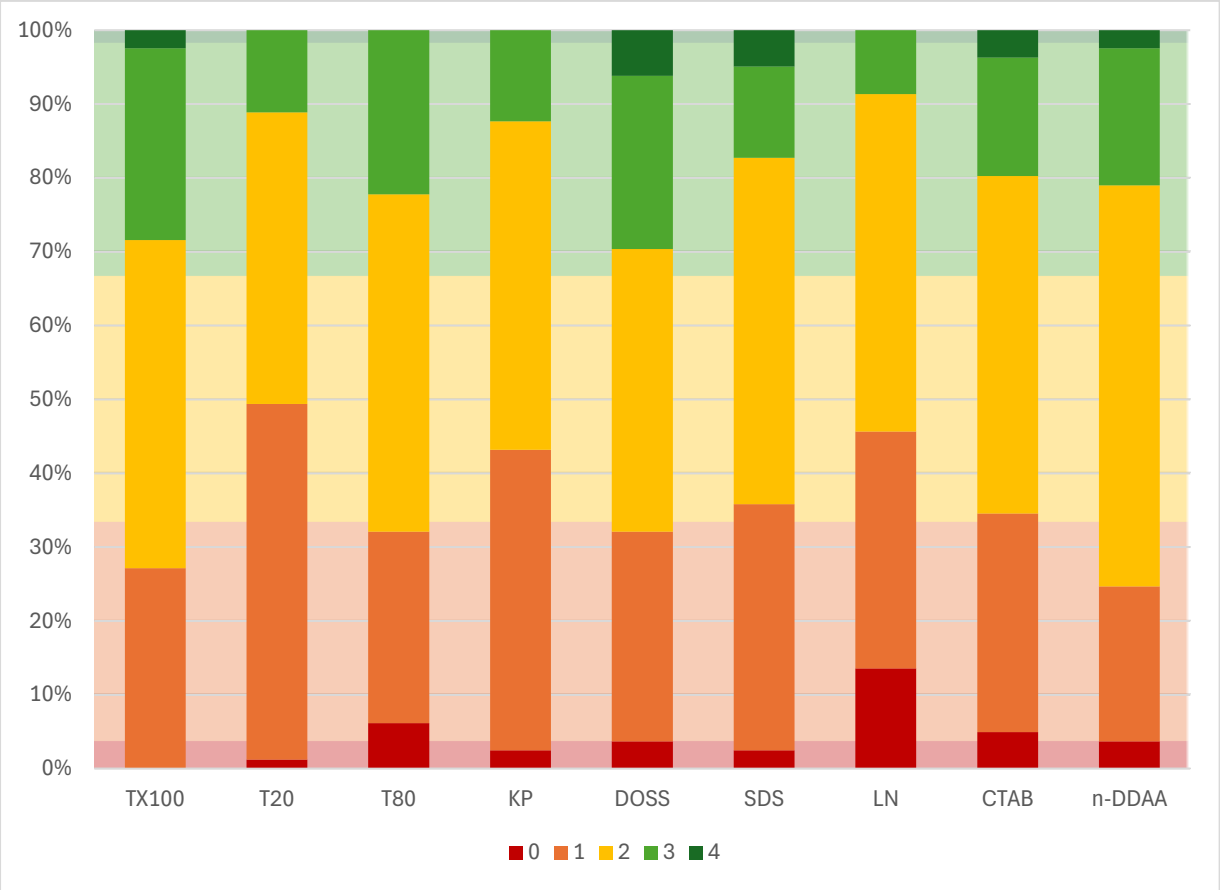


Figure 3.11 Comparison of ridge detail scores for all donors and substrates developed with FePS made using each surfactant at 0.5x CMC. Background colour represents scores of formulations made only with water

The water scores displayed in the background of Figure 3.11 show a higher percentage of donor fingermarks graded 3 or 4 than any surfactant at 0.5x CMC. It is possible that the presence of surfactant monomers is influencing fingermark development, either by interacting with fingermark residue or reducing ST without yet achieving particle suspension within micelles. The highest ridge detail scores produced at 0.5x CMC were DOSS and TX100, however it appears that surfactant selection is not having a great influence on the effect of 0.5x CMC surfactant concentration in FePS. In

a discussion about liquid phase deposition techniques for fingermark development, Bleay (2018) suggests that for SPR development, the concentration of DOSS used in this technique should be between 1/3 – 1x CMC [26]. The 2022 FVM recommendation for the preparation of SPR results in a formulation including approximately 0.75 g DOSS in 5 L tap water, which is mixed with 50 g MoS_2 particles [41]. This results in a surfactant concentration of approximately 1/8x CMC, as the published literature CMC of DOSS is reported to be approximately 2.7 mM [116, 118]. The discrepancies between these values may be due to inconsistencies in reported CMC values being used, however it is evident that the required concentration for DOSS in SPR solutions remains below the CMC.

The 2022 FVM suggests that, in line with the results found in this chapter, concentrations of surfactants in DOSS below 1/3x CMC would result in heavier background development, while concentrations at or above CMC produce less background development but also fainter ridges [41]. This further supports the hypothesis that surfactant micelles are vital in FePS formulations in aiding the selective deposition of powders by reducing background development. Between FePS and SPR there are differences in suspension consistency (SPR is much thinner as it is applied by spraying or dipping) and powder particle type (chemical composition, size, and shape) which will contribute to the success of DOSS in suspending particles and subsequently developing fingermarks. It is interesting however, that two techniques involving the same basic components of surfactant solutions and insoluble powders have such different requirements in micelle formation and stability for successful fingermark development. FePS is generally considered to be more specific to fingermark residue than SPR, and the reason for this may be the low concentration of surfactant used creating a less stable suspension, or the difference in powder type (MoS_2 for SPR, and iron oxide for FePS) [31]. Both powders have similar average particle sizes, with MoS_2 reported to be 1.5 µm and the Fisher iron oxide powder used in this study <5 µm. It is likely that the differences in powder particle size, shape, or density between MoS_2 and iron oxide powders are influencing the suspension and subsequent deposition of particles in a surfactant micelle, and that the micelles may be interacting differently with each powder type. Further investigation into the interaction between surfactant micelles and different powder particles will aid in better understanding these results. This further demonstrates the complexity of the role of surfactants in fingermark development techniques.

3.3.5 Substrate

The effect of substrate on fingermarks developed at 50x and 0.5x CMC was also investigated. As shown in Figure 3.12, very similar ridge detail scores were given to ceramic and glass substrates while the plastic substrate produced the most scores of 0 at both 50x and 0.5x CMC. This was consistent for all individual surfactants. For the scores of background development, ceramic and glass again performed

very similarly while plastic produced slightly higher scores than the other substrates for both 50x and 0.5x CMC formulations. This indicates that regardless of surfactant or surfactant concentration relative to the CMC, the plastic substrate prevents some powder particles from adhering both to the fingermark ridges and around them. This leads to lower ridge detail scores and higher background development scores, which can be seen in Figure 3.13. These results are consistent with observations made in section 2.3.6.

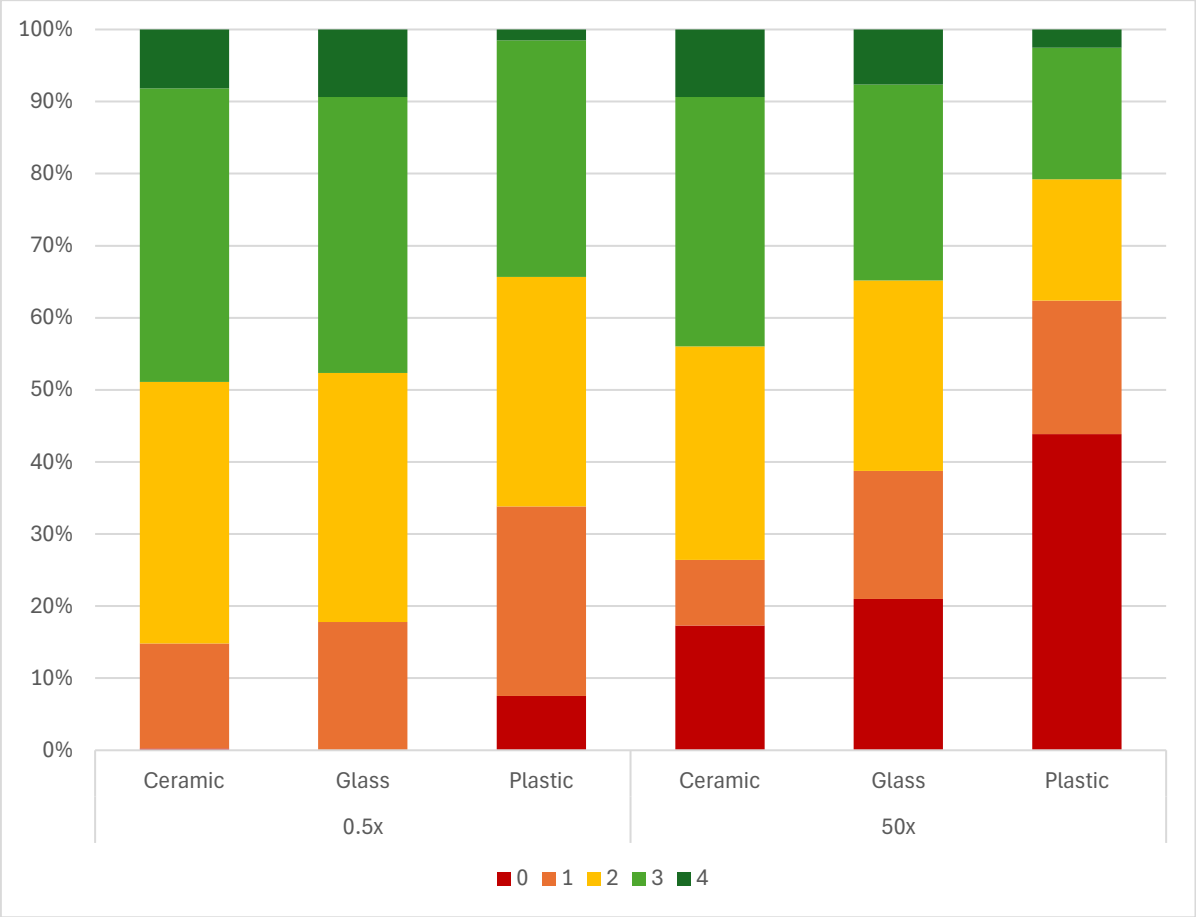


Figure 3.12 Comparison of ridge detail scores given to all donor marks developed with FePS made using surfactants at 50x and 0.5x CMC on each substrate

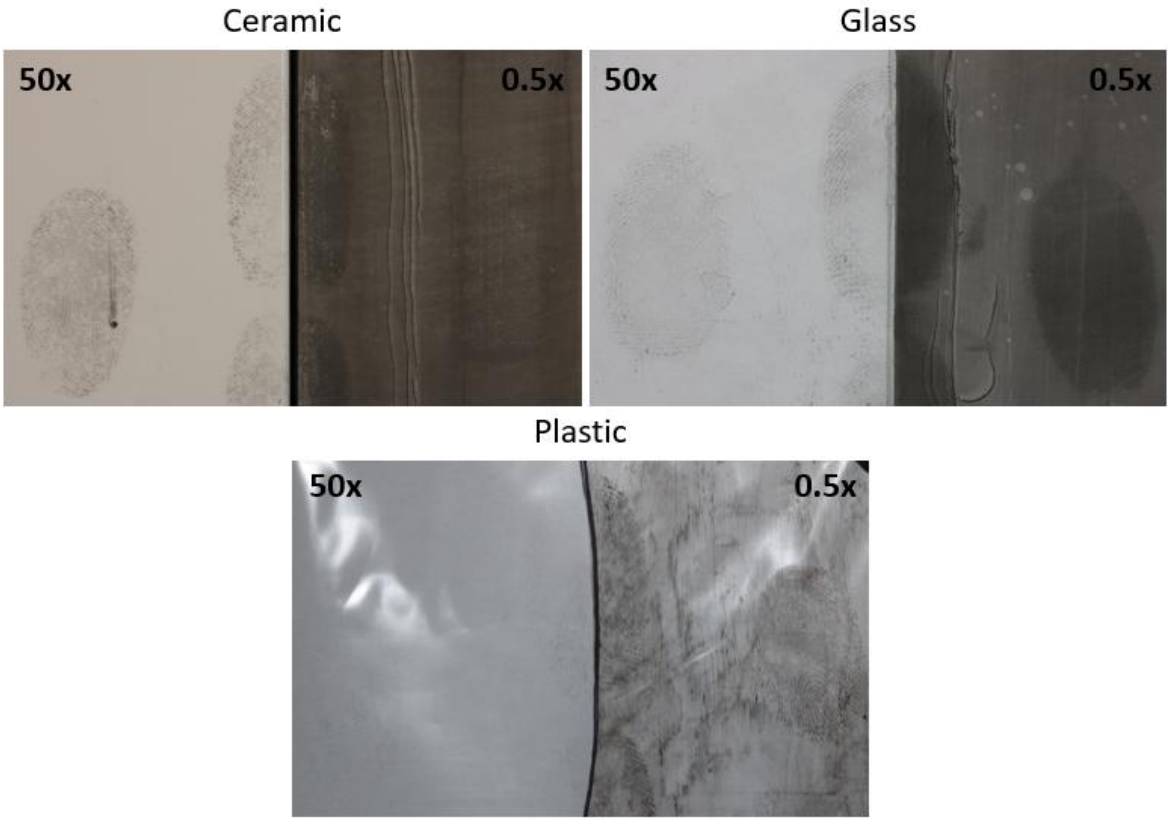


Figure 3.13 Comparison of donor 2 fingermarks developed with FePS made using TX100 50x (left) and 0.5x (right) CMC on each substrate

Heavy background development from FePS formulations made with 0.5x CMC TX100 has been reported by Downham and the authors noted that this off-ridge deposition was also influenced by substrate type [78]. The authors tested TX100 FePS at 0.5x, 1x, 2x and 10x CMC on three substrates; formica laminate, high density polyethylene (HDPE) bags and PE board. This research found that the formica and HDPE produced heavy background development at 0.5x and 1x CMC, while the PE substrate had the heaviest background development at 1x CMC. They found that for all substrates, concentrations at 2x CMC and above did not produce background development. When considering ridge detail produced, the authors only counted the number of 'identifiable' marks (graded 3 or 4) and found a similar number of marks identifiable on the HDPE and PE substrates, and notably less on the formica substrate for all formulations tested. The results of both studies agree that powder deposition both on and off ridge can be influenced by substrate type, however further investigation into the effect of substrate morphology with difference surfactant concentrations is needed. It can also be concluded that at or below the CMC more background development is produced on all substrates, further supporting the importance of micelle concentration on optimal fingermark development.

3.3.6 Micelle size results

The micelle size diameter and PdI of each surfactant was measured from graphs produced with Zetasizer measurements shown in Appendix C. These results are summarised in Table 3.9. Some surfactants produced highly polydisperse readings with multiple peaks in the graphed results. In these cases, the highest peak was used for average size determination.

Table 3.9 Results of micelle diameter size and PdI measurements for each surfactant measured using DLS

Surfactant	Average micelle diameter (nm)	Average PdI (range)
TX100	10.5	0.148 (0.088-0.235)
T20	7.5	0.194 (0.146-0.220)
T80	15	0.211 (0.169-0.255)
SP80/T80	550	0.835 (0.62-0.941)
KP	8	0.141 (0.099-0.208)
DOSS	200	0.272 (0.244-0.364)
SDS	0.85	0.815 (0.387-1)
LN	3.5	0.293 (0.137-0.495)
CTAB	150	0.921 (0.680-1)
n-DDAA	250	0.461 (0.357-0.662

The micelle diameter and PdI of each surfactant was then used to determine any correlation between these measurements and fingermark quality developed with the same surfactant. Pearson correlation coefficients were determined for the relationship between surfactant micelle size and PdI with scores given to fingermarks assessing their ridge detail, contrast, and background development. These results are shown in Table 3.10.

Table 3.10 Correlation coefficients given to relationships between fingermark quality (ridge detail, contrast and background development) scores and DLS measurements (micelle size and PdI of surfactants)

Fingermark quality parameter	Correlation coefficient for micelle diameter size	Correlation coefficient for PdI
Ridge detail	-0.181	-0.171
Contrast	-0.061	-0.181
Background development	-0.543	-0.377

The results show very weak negative linear correlation for each quality parameter. The only score which shows moderate association (-0.4 to -0.6) is that of background development to micelle diameter size, which suggests smaller micelle size leads to more background development. However, it was determined that the score was not significant enough for further statistical analysis. This is consistent with the severe background development produced by water-only formulations discussed in section 2.3.3, where the micelle size was 0 (due to lack of any surfactant present in these formulations).

3.4 Surfactant structure analysis

This study demonstrated the importance of the presence of micelles in a surfactant solution to facilitate the selective deposition of powder on fingermark residue. However, at the same level above the CMC (50x) there were still clear differences between each surfactant. This shows that there is a part of the surfactant structure either influencing how the micelles are constructed or how these micelles are able to interact with iron oxide powder and fingermark residue. The results of chapter 2 demonstrated that ionic nature was not the sole influence on formulation efficacy, however there are currently no recommendations for other features which may suggest if a surfactant will be successful in FePS. Bleay *et al.* describe results from Morris *et al*. (1978) which suggest that in SPR, requirements for an effective surfactant solution include the surfactant having an optimal 'tail' length of 12-17 carbons [26, 31, 95]. Because of this, an analysis of the surfactant and micelle structures was conducted to see if similar recommendations could be made for FePS.

3.4.1 Cationic surfactants

Both cationic surfactants (CTAB and n-DDAA) produced similar results when used to develop fingermarks at 50x and 0.5x CMC. At 50x CMC they were the only surfactants other than LN and T80 to produce background development scores of 2, as all other surfactants had scores of 3 or 4 (Figure 3.9). This is consistent with the results of chapter 2, as the cationic surfactants also produced background development on non-porous substrates.

Some research has been conducted into the possible structure of micelle formation for CTAB, however n-DDAA is a less commonly used surfactant, and as such, less information is available in literature. Research by de Lera-Garrido *et al.* (2020) and Montes-de-Oca *et al.* (2022) agree that the shape of CTAB micelles is likely to change with varying concentrations [65, 71]. They suggest that above 300 mM this surfactant forms elongated spheroid shaped micelles, however closer to the CMC they are spherical. At higher concentrations, new micelle shapes lead to an increase in aggregation number (average number of surfactant monomers in a micelle) which could indicate a 'second CMC' for this

surfactant, at approximately 30% volume [65, 71]. Concentrations this high were not investigated in this study, however the gradual change in micelle shape could explain the highly polydisperse nature of this surfactant as measured in section 3.3.6 (Table 3.9). Both surfactants were found to be more effective when diluted from their stock concentrations in chapter 2 and it is therefore possible that spherical micelles are preferable for FePS. The effect of cationic surfactant chain length on emulsion stability of nanoparticles in CO_2/ water was investigated by Ma *et al.* (2022) [119]. The authors assessed three cationic surfactants with varying chain length (including CTAB) and demonstrated that longer hydrophobic alkyl chains required lower surfactant concentration for stabilisation of the emulsion. This suggests that longer carbon chains may lead to more stable emulsions, however this investigation looked at colloidal stability while FePS contains much larger particle sizes and therefore likely interact differently with formed micelles.

3.4.2 Anionic surfactants

At 50x CMC, DOSS did not produce any ridge detail and SDS produced the second poorest ridge detail scores. LN was able to develop good quality fingermarks (despite heavy background development), and this difference may be due to the addition of extra products in the LN solution. The CMC of DOSS and SDS were the highest of all surfactants tested (Table 3.6), meaning a greater mass of these surfactants were required to achieve the 50x CMC. It is possible that either this higher required concentration or the anionic nature of the surfactants is causing poor ridge detail development, either through removal of fingermark residue or due to surfactant micelles being more stable and not depositing powder particles. LN differs from the other anionic surfactants through the addition of a nonionic product, and as such its different effect on fingermark development (higher ridge detail and more background development) may be attributed to it not being a pure anionic surfactant.

Previous research has found that SDS forms relatively spherical micelles with the hydrophobic tails oriented inwards and the charged head groups on the outer surface of the micelle [105]. Because of this, the surface of SDS micelles contain a large net negative charge. Montes-de-Oca *et al.* suggest that SDS micelle shape may slowly change from spherical to rod-like shapes over a concentration gradient and both shapes co-exist over a wide concentration range [65]. However, the concentrations of SDS used in this study were very low and therefore most micelles were likely spherical. The average micelle diameter of SDS was measured in section 3.3.6 (Table 3.9) to be the smallest of all surfactants tested (approx. 0.85 nm), however this surfactant was also quite polydisperse, which can be explained by this gradual micelle shape change observed by Montes-de-Oca [65].

The structure of DOSS micelles has been well documented when dispersed in non-polar systems such as oil, however has been less thoroughly investigated in water systems. In non-polar mediums, DOSS

micelles commonly form as reverse micelles, where the polar head group is oriented towards the centre of the micelle and the dual hydrophobic tails surrounding the outside (illustrated in Figure 3.14) [120-123]. This is effective in non-polar medium, where the hydrophobic tails are not surrounded by water, however when dispersed in water this micelle shape cannot form. Some research suggests again that the shape of DOSS micelles changes depending on concentration, however a lamellar mesophase orientation is best documented ('sheets' of surfactant monomers with the polar head groups forming repeating layers separated by carbon chains shown in Figure 3.14). At higher concentrations, cubic and hexagonal structures have also been suggested [120, 121, 123].

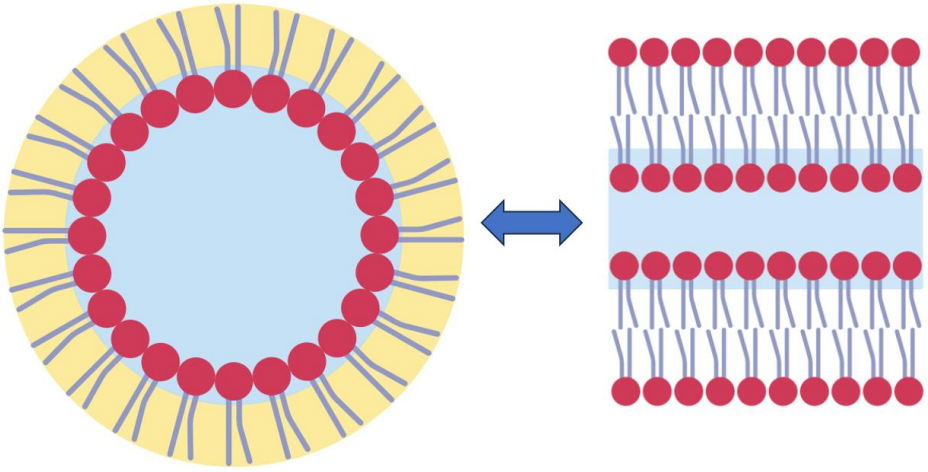


Figure 3.14 Proposed orientation of DOSS reverse micelle (left) in yellow non-polar medium and lamellar structure (right) in blue polar medium (water). Adapted from Moilanen et al. [120]

It is unclear how this lamellar micelle form may impact the way the surfactant can suspend iron oxide or MoS_2 particles in FePS and SPR respectively, however this is possibly the reason why DOSS may be used in SPR below the CMC. The hydrocarbon tails of DOSS monomers are branched unlike other surfactants used in this study, which may also facilitate the stability of insoluble powders in a solution without micelle formation. SDS and DOSS surfactants have a higher CMC than other tested surfactants (Table 3.6) suggesting that they would have more monomers present in solution before micelles started to aggregate. This may also play a role in aiding suspension of powder in solution below CMC, however it can be stated that this does not impact fingermark development due to similar quality grades being obtained for both surfactants at 0.5x CMC compared to water development.

3.4.3 Nonionic surfactants

The polar head groups of all four nonionic surfactants assessed in this study (TX100, T20, T80, and KP) contain repeating ethoxylate units as the polar head group. The two Tween surfactants contain a total of 20 ethoxylate units which branch out to create a large polar head group, while the pure TX100 used in TX100 and KP solutions contains an average of 9.5 repeating linear units [124]. Inversely, while the

Tween surfactants have a linear hydrocarbon chain, the 'tail' of the TX100 monomer is branched and contains a benzene ring. As such, the structures of both the Tween and TX100 surfactants vary in both the side and shape of the hydrophilic and hydrophobic moieties. As measured in section 3.3.6 (Table 3.9), the nonionic surfactants have very similar micelle diameters (between 8 and 15 nm) and are relatively monodisperse, suggesting that these differences do not have a big impact on the size of formed micelles. T20 and T80 micelles are both reported to form as oblate spheroids, there is no literature reporting a change in micelle shape at higher concentrations however Mandal *et al.* suggest that with increasing temperatures the micelles may become less symmetric [99, 125]. TX100 also forms ellipsoidal micelles however they are more asymmetric than Tween micelles, and some literature suggests that TX100 micelles form in a multilayer staggered spherical structure [125-127]. However, there are still some uncertainties about how the carbon chain is arranged within the micelle [126].

Knoch *et al.* (2021) suggest that the published literature CMC values of T20 and T80 should not be used to indicate the lack of micelles below these concentrations [128]. The authors state that the assumption that insufficient surfactant monomers are present in solution below the CMC for micellization is based on "single-component surfactants". They suggest that Tween surfactants, however, are more complex "multi-component surfactants" and do not follow the same model of micellization. They propose that below the CMC, some components of the Tween surfactants already begin to aggregate, with additional products adding to these micelles as is concentration increased (illustrated in Figure 3.15). It is unclear exactly which components are forming the proposed initial micelles; however, this further demonstrates the complexity with which different surfactants form micelles.

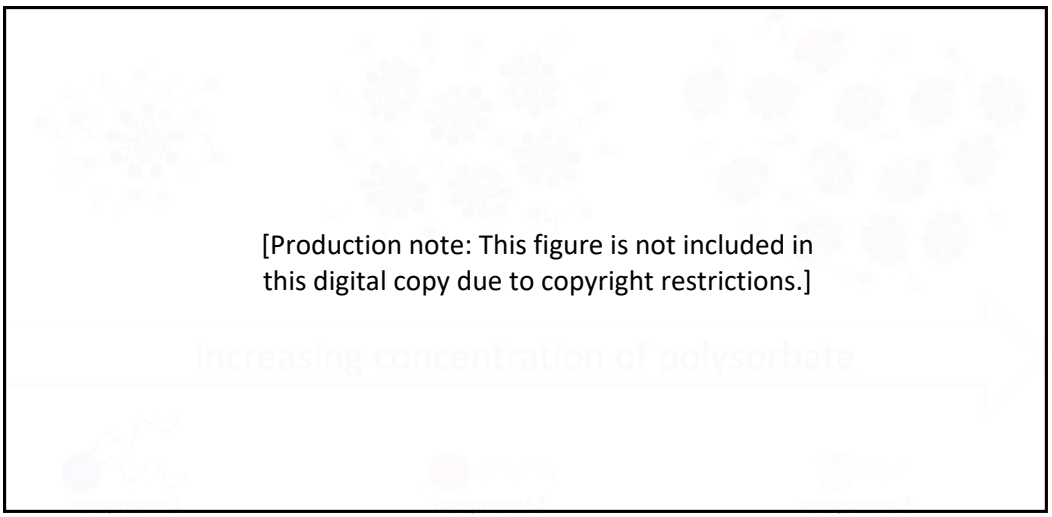


Figure 3.15 Model of complex micellization behaviour of multiple-component Tween surfactants. Adapted from Knoch et al. [128]

The complex micellization behaviour observed by Knoch *et al.* suggests that the simple micelle formation model may not be accurate for all other surfactants. Notably for LN, which has multiple surfactant products, making it more likely that similar multiple-component micelle formation may be occurring with increasing concentration for this surfactant. Especially as LN contains a small amount of nonionic alcohol ethoxylate, which has a polyethoxylate head group similar to Tween surfactants. Similarities can be drawn between the use of T80 and LN surfactants in FePS, as these both produced notably heavier background development than other assessed surfactants at 50x CMC (Figure 3.9). This may be due to requiring a higher concentration of surfactant to initiate the formation of complete micelles that allow the selective deposition of powder with FePS. However, at 0.5x CMC all nonionic surfactants and LN produced similar development scores, with TX100 obtaining the highest grades of 3 or 4. This suggests that regardless of the method in which micelles are formed, at concentrations of 0.5x CMC these surfactants have insufficient monomers present to make a large impact on fingermark development.

Despite similarities in surfactant head group and ionic nature, T20 and T80 produced very different development in FePS formulations. At 50x CMC, T20 produced the highest scores of ridge detail of any surfactant and very little background development. Inversely, T80 produced very heavy background development, and the ridge detail scores were very similar to those achieved at 0.5x CMC with the same surfactant, suggesting that at 50x CMC T80 was not forming micelles which impacted fingermark development in FePS. This may be due to the longer chain length and subsequent impact on T80 solubility in water and it is similarly possible that the addition of ethanol to T80 is preventing selective powder deposition in FePS at 50x CMC. Another possible explanation for the differences between these surfactants is the presence of the double-bonded carbon in the hydrophobic region of T80, whereas T20 only has single-bonded carbons. This double bond may result in a more reactive micelle and therefore produce a less stable suspension which causes indiscriminate powder deposition observed in heavy background development.

3.4.4 General analysis

This research has shown that the presence of micelles in solution is important to facilitate selective deposition of powder with FePS. Analysis of the chemical structures of surfactants tested has shown a range of carbon chain lengths and size as well as complexity of polar head groups. All surfactants used in this study have a hydrophobic tail length of 8 to 18 carbons, with varying levels of effectiveness in FePS. Effective surfactants used in SPR are recommended to have an open carbon tail with no less than 8 carbons, with 12-17 the optimal length as suggested by Bleay [26]. Surfactants shown to be effective in producing selective powder deposition with FePS such as TX100, DOSS and T20 contain

carbon chains between 8-12 carbons, which were aromatic, branched and linear, respectively. This may suggest that a shorter carbon chain is optimal for FePS than is for SPR, however the structure of this chain does not appear to be a defining feature. Research by Morris *et al.* reported poor SPR development was produced with surfactants with fewer than 8 carbons in the 'tail' [31, 95]. This study did not grade the developed fingermarks and therefore it is unclear what the cause of the poor development is (heavy background development or poor ridge detail). No surfactants with less than 8 carbon atoms were tested in this study, and therefore no conclusions can be made as to the viability of using surfactants with shorter chain lengths in FePS.

The size of assessed surfactant micelles were determined to have a range of between 0.85 to 500 nm, determined by DLS measurements. As Jones *et al.* suggest that powder particles which adhere to fingermark reside in FePS measure between 0.2 to 1 µm, this indicates that the iron oxide powder particles used in these studies (<5 µm) cannot be encapsulated by the micelle structure as traditionally accepted [46]. Rather, this lends support to additional hypotheses proposed by Downham et al. as illustrated in Figure 3.2 that the surfactant micelles/ monomers create a layer surrounding the particle and in this way suspend it in solution. Further investigation into alternate theories surrounding surfactant micelle structure around the particle would aid in understanding these observations.

Structure of micelle formation was also investigated, with many surfactants reported to form spherical micelles however these shapes are likely to change with variation in concentration. Many investigations into the possible structure of surfactant micelles can only theorise possible models and are often analysed within the context of emulsifying other products such as nanoparticles within solutions and therefore it is difficult to draw conclusions based on micelle shape. The shape of formed micelles is also reported to vary based on concentration, temperature, and additional products [61, 110, 123]. Surfactants tested are likely to have spherical, ellipsoidal, or lamellar micelle structures and from the available literature, no conclusions can be made about optimal micelle shape or size. It is possible that variations in micelle shape are more likely to influence the stability of FePS formulations in suspending iron oxide particles than affecting the interactions between surfactants and fingermark residue. Better understanding of fingermark residue interactions with surfactant monomers and micelles is limited by inherent donor variability, and without the ability to control fingermark residue composition limited conclusions can be made.

3.5 Conclusion

An investigation into the effect of CMC and micelle formation for different surfactants on fingermark development with FePS has shown that the presence of micelles is vital for selective powder deposition. Formulations made below the CMC developed fingermarks in a similar manner to

formulations made only with water and made differences between surfactants used more consistent, suggesting that surfactant concentrations below the CMC are not having a great impact on fingermark development. This is relevant to both on and off-ridge development, indicating that the presence of micelles in solution is suspending powder particles and preventing surface-wide deposition. This research also found that at 50x CMC, not all surfactants influenced fingermark development the same way, further indicating that surfactant type is vital in optimal fingermark development. Differences in background deposition at 50x CMC for LN and T80 compared to other surfactants suggests that these surfactants do not form stable micelles at this concentration, leading to poor suspension of powder particles and subsequent surface-wide background development. This investigation further supports the role of suspension stability in selective powder deposition, where an optimal surfactant type and concentration must be stable enough to prevent surface-wide deposition yet still be sufficiently destabilised by fingermark residue to deposit powder on-ridge. Further investigation into the possible initiate compounds required for this destabilisation will be explored in the next chapter.

An analysis of the structure of assessed surfactants and their proposed micelle structure formation was completed, suggesting that the optimal surfactants used contained carbon chain lengths of between 8 and 12, however the structure of these tails did not appear to influence fingermark development. Importantly, the results of this study have shown that surfactants have specific concentrations at which they can be effective for use in FePS and cannot be used interchangeably at the same concentrations. The results show that the ideal surfactant concentration cannot be predicted relative to the CMC and therefore any surfactant tested for use in FePS must be individually evaluated across a very wide range of concentrations to best determine an optimal formulation. Overall, this research provided strong support for the importance of micelle presence in surfactant solutions for fingermark development with FePS formulations.

Chapter 4: An evaluation of FePS residue targets using artificial simulants

4. *An evaluation of FePS residue targets using artificial simulants*

4.1 Introduction

Artificial fingermark secretions were first developed by researchers with the aim of controlling the chemical composition of fingermark deposits to assess the efficacy of fingermark reagents [129]. It is well documented that the composition of latent fingermark residue varies between donors, but also for the same donor on different days [7, 15, 129, 130]. The variable nature and composition of fingermark residue makes it challenging to understand and explain interactions between residue and fingermark development techniques. In 2009, Schwartz created a simulant consisting of amino acids and sodium chloride, which were useful in assessing the reactivity of amino acid reagents such as NIN and IND-Zn [129]. The simulants were inexpensive, fast to use, and the concentrations could be adjusted to test the sensitivity of the reagent. However, it was based only on the eccrine fraction of fingermark residue and was therefore a partial representation and only suitable for porous substrates [129, 131]. Some commercially available synthetic residues are available as soaked pads which can be used in a similar manner to ink pads by stamping artificial residues, so they are deposited like a real fingermark. Examples of this include amino acid validation targets from Forster + Freeman® and Arrowhead Forensics [132, 133]. They are used by manufacturers to assess the efficacy of fingermark reagents, however these reference residues are only available as amino acid-based or sebaceous oil secretions and once again, only represent fractions of natural latent residue and poorly mimic the concentrations of compounds found in natural residue [129, 134, 135]. This is because many commonly used techniques are designed to specifically target these compounds and can therefore be used as positive controls. To date, there is no commercial product which has been tested for use as a reliable and realistic representation of the complete fingermark residue.

4.1.1 Emulsions

It is thought that natural fingermark residue is present as an emulsion, a complex mixture of eccrine and sebaceous residue [136, 137]. Few studies have focussed on combining eccrine and sebaceous fractions to create more realistic fingermarks, as it is challenging to emulsify the hydrophobic and hydrophilic fractions [138]. A formulation developed by Sisco *et al.* was shown using mass spectrometry to have a similar chemical composition to real residue from an unspecified number of donors and contained a mixture of eccrine and sebaceous compounds [139]. An emulsifying agent called Steareth-20, sometimes found as an exogenous contaminant on the hands, was also used to

stabilise the emulsion of the two fractions. The simulant was deposited on paper, glass, and packing tape using stamps made of rubber or ballistic gelatine and developed using a range of detection techniques: fingerprint powders, CA fuming, NIN, IND-Zn, and gentian violet. The results showed that this simulant developed similar results to real fingermarks using common detection techniques [82, 129, 139].

A simplified emulsion was developed by de la Hunty, specifically formulated for use with PD as part of a wider investigation into the mechanism involved with this technique [97]. Only endogenous compounds were used, and the properties of oleic acid were used in place of an emulsifying agent. This formulation showed excellent reactivity with PD and IND-Zn, however only porous substrates and techniques were tested. In 2022, Steiner *et al.* compared the two emulsions developed by Sisco and de la Hunty on porous substrates [82]. Spot tests of each simulant were developed using IND-Zn, NIN, ORO and PD. Despite neither formulation being successfully detected using PD, results of development with the other techniques suggested that de la Hunty's formulation was more promising in producing realistic artificial fingermarks. The authors noted that the use of spot tests resulted in misrepresentation of the amount of residue present in a fingermark as well as inconsistencies with how the simulants interacted with the paper fibres [82]. This highlighted a further need for a reproducible deposition method to further control the chemical variability of fingermark residue.

In 2023, Steiner *et al.* published a further study investigating the use of a modified inkjet printer to deposit simulants in more representative fingerprint pattern and quantity [96]. These marks were deposited on both porous (paper) and non-porous (acetate sheets) substrates and subsequently developed using IND-Zn, ORO and PD on porous substrates and CA/R6G, VMD, and black powder on non-porous substrates. The simulant marks were compared to natural marks made by one donor. This study was able to successfully and repeatedly develop artificial simulants using the chosen techniques, except for PD. The simulants appeared to react differently on each substrate type, with higher quality marks produced on paper due to absorbance by the paper fibres, while the acetate sheets did not absorb the simulants, and the artificial marks dried inconsistently which affected the quality of developed marks. The authors note that ideally the emulsion may be optimised to improve the adhesion of artificial fingermarks on non-porous substrates.

The effect of deposition method for artificial fingermarks has not been compared in published work. Arsenault *et al.* (2023) investigated DNA recovery and persistence in fingermarks using the artificial emulsion developed by Sisco *et al*. by loading donor fingermarks with the emulsions, however the method has not been used for fingermark development studies [139, 140]. Previous research has explored different methods of depositing simulants, including stamps, inkjet printing and spot tests.

Recent studies have explored the used of an inkjet printer to deposit artificial secretions and reproduce fingermark patterns [96, 98, 131, 134, 141], however this method requires buying and modifying a printer, as well as making and optimising simulant deposition and was therefore determined to be unsuitable for this investigation. Sisco *et al.* (2015), Staymates *et al.* (2013) and Zadnik *et al.* (2013) have explored using fingermark stamps made of rubber, acrylic and ballistic gel for deposition of simulants [139, 142, 143]. This method is considered simple and effective; however it should be noted that the volume of simulant deposited is difficult to control and no thorough investigations have been performed to determine if interactions are occurring between the simulant and stamp material [98, 131, 141].

Many developed fingermark simulants suffer from the limitation of being only an approximate representation of a real fingermark, that do not wholly account for the complexity and behaviour of latent residue [129]. To incorporate artificial fingermarks into laboratory work, simulants need to be developed which accurately mimic real residue so they may be used in early phase studies and to gain deeper understanding of residue interactions with development techniques.

4.1.2 Use in PS investigations

In a 2018 PhD thesis by Stubbs-Hayes, the interaction of FePS with fingermark components was investigated [144]. The studies involved used natural fingermarks, some of which were groomed to contain predominantly eccrine or sebaceous residue. Spot tests of individual constituents commonly found in eccrine sweat were also tested, both by pipetting the chemical directly onto substrates and by spiking donor fingermarks with the individual chemicals before deposition. As eccrine sweat is composed of water-soluble components and PS is an aqueous technique, some eccrine depositions were chemically fixed to the substrate before development. Non-porous substrates including plastic, glass and metal were included in this study and the FePS formulation used contained KP as the surfactant.

The results of the study indicated that FePS may have an affinity for eccrine residue when they are able to persist in water-insoluble contaminants such as those found in sebaceous residue [144]. However, spot tests such as these are not representative of real residue in quantity or chemical composition. Steiner *et al.* showed that the use of spot tests resulted in heterogenous and unrealistic results on porous substrates due to interactions between simulants and the substrate, however the non-porous nature of substrates used by Stubbs-Hayes may have mitigated this interaction [82]. Despite this, Stubbs-Hayes also noted that substrate type had clearly influenced the quality of development both of natural marks and individual constituents, suggesting that is an important consideration. This investigation utilised only one FePS formulation and therefore the results are only

representative of the workings of this particular solution. As previous research has shown variation in fingermark development using FePS with changing powder and surfactant components, the understanding developed in this research has limited application to any changing recommendations.

Despite these limitations, this research was the first instance of a thorough investigation into the interaction between FePS and fingermark residue. It has highlighted the importance of further research focussing on the fundamental interactions between fingermark residue, substrate, and the FePS technique, rather than focussing on a single formulation. Further research developing the application of artificial fingermarks on non-porous substrates is vital not only for our understanding of PS formulations, but other common or emerging non-porous development techniques. It is important to note that the goal of artificial fingermark investigations is not to replace the use of natural fingermark residue in research, but to provide a matrix of known composition for a range of uses. Possibilities include quality control of detection methods, initial phase testing for new techniques as well as exploration of fingermark residue targets of commonly used techniques that cannot be performed without a known and standardised residue material.

This research aims to explore the use of artificial fingermark simulants on non-porous substrates and investigate the interactions of FePS formulations with these simulants. Two types of artificial fingermark emulsions were compared through development with commonly used fingermark enhancement techniques to better understand the capabilities of artificial emulsions on non-porous substrates. The simulants were then used to investigate the effect of surfactant type in FePS development on different artificial residue fractions. This was done to determine if the use of different surfactants influences what components of fingermark residue are being targeted in FePS development and better understand the interaction between FePS formulations and chemicals present in fingermark residue.

4.2 Materials and method

Before using artificial simulants to investigate the interactions of surfactants with fingermark residue components, the simulants had to be tested to ensure they could be developed by fingermark detection techniques on non-porous substrates. The work presented in this chapter is separated into two parts.

Part 1, a house-made (HM) fingermark simulant emulsion used in previous research by de la Hunty *et al.* [97] and Steiner *et al.* [82] was compared with a commercially available emulsion from Pickering Laboratories (product code 1700-0547) [145]. The emulsion from Pickering Laboratories is marketed for use in cosmetic testing and stated to contain “5% sebum in eccrine perspiration”. The HM emulsion

was created by first making a synthetic eccrine sweat solution and a synthetic sebum, before combining the two fractions through emulsification. To assess the different emulsion's ability to mimic natural fingermarks, emulsions were deposited on porous and non-porous substrates and developed using benchmark fingermark development methods.

Part 2 of the experiments looked at the interaction between the emulsions and FePS with different surfactants. This was done to determine if the use of different surfactants influenced what components of fingermark residues are targeted in this technique. In this investigation, four types of artificial fingermark simulants (the two emulsions used in part 1, as well as the separate eccrine sweat and sebum components used to make the HM emulsion) were developed by a range of optimised FePS formulations made with the surfactants investigated in chapter 2.

4.2.1 Chemicals and products

All fatty acids, as well as stigmasterol and sodium chloride, were purchased from Sigma Aldrich. Cholesterol, maleic acid and silver nitrate were purchased from BDH chemicals. Vegetable oil (Crisco-Coles Australia) and vitamin E oil (Blackmores) were used as a source of triglycerides and α-tocopherol respectively. All amino acids were sourced from Sigma Aldrich, except for Histidine (Fluka). Iron (II/III) oxide nanopowder (≤ 97% purity) was purchased from Sigma Aldrich. T20, ammonium ferrous sulphate, and ferric nitrate nonahydrate was obtained from ChemSupply. n-DDAA was obtained from Optimum technologies.

A commercial artificial eccrine perspiration - sebum emulsion was obtained from Pickering laboratories. This product contained 5% artificial sebum in eccrine perspiration solution and was kept in the fridge at 4°C. The exact composition of this product is unknown due to proprietary formulations. Simulant was removed from the fridge and allowed to come to room temperature before use.

4.2.2 HM artificial emulsion

4.2.2.1 Preparation of synthetic sweat

The synthetic sweat stock solution was prepared as per de la Hunty's formulation (Table 4.1) [97] containing 1.02 g total amino acids per 100 mL deionised water (10.2 g/L). 250 µL of the stock solution was diluted to 100 mL using milliQ water to produce representative concentrations of fingermark amino acids. 2.59 mg/L sodium chloride (NaCl) was also added to represent typical NaCl concentration in sweat. The solution was stored in the fridge at 4°C.

Table 4.1 Molar ratios of compounds present in artificial eccrine simulant

Compound	Brand	Concentration (mM)
Serine	Sigma (reagent grade, ≥ 99%)	33.3
Glycine	Sigma (reagent grade, 98%)	20.0
Ornithine	Sigma (approx. 99%)	13.3
Alanine	Sigma (≥ 98% TLC)	10.0
Threonine	Sigma (reagent grade, ≥ 98%)	5.7
Histidine	Fluka (≥ 99%)	5.0
Leucine	Sigma (reagent grade, ≥ 98%)	3.3
Lysine	SAFC (≥ 97%)	2.3
Phenylalanine	Sigma (reagent grade, ≥ 98%)	2.0

4.2.2.2 Preparation of synthetic sebum

The synthetic sebum was prepared as per de la Hunty's formulation (Table 4.2) [97] by adding the chosen compounds to 45 mL of dichloromethane (DCM) and then mixed with a magnetic stirring rod at 600 rpm for 10 minutes until dissolved. Before use, the DCM was removed from solution using a constant stream of N_2 gas and a heating block set at 40°C for approximately 15 minutes. Heating was necessary as the solution became more viscous as DCM was removed, so complete removal of DCM could only be achieved by heating to reduce viscosity.

Table 4.2 Compounds present in artificial sebaceous simulant (dissolved in 45 mL of DCM)

Class	Representative compound/ mixture	Brand	Quantity (g)
Squalene	Squalene	Sigma	1.6
Triglycerides	Vegetable oil	Crisco	4.8
Fatty Acids	Stearic acid	Sigma (reagent grade, 95%)	1
	Palmitic acid	Sigma (BioXtra, >99%)	1
	Oleic acid	Sigma (technical grade 90%)	2.1
Cholesterol esters	Stigmasterol	Sigma	0.3
Cholesterol	Cholesterol	BDH biochemicals	0.6
α- tocopherol	Vitamin E oil	Blackmores vitamin E 1000 IU	0.1

4.2.2.3 Preparation of synthetic HM emulsion

The HM fingermark simulant emulsion was made by combining sebaceous and eccrine constituents in a 1:4 ratio respectively. DCM was evaporated from 3.35 mL synthetic sebum, leaving approx. 2mL sebaceous fluid. 8 mL diluted eccrine solution was then added to the artificial sebum and sonicated for 10 minutes at 35°C until the product was emulsified into a cloudy white liquid. Solution was stored in the fridge at 4°C and allowed to come to room temperature before use.

4.3 Part 1: Comparison of simulant emulsions

The Pickering and HM emulsions were first compared by developing them using recommended enhancement techniques on porous and non-porous substrates and comparing them to natural fingermarks. As published works investigating artificial fingermark emulsions have primarily used porous substrates and deposition using a modified inkjet printer, a preliminary comparison of different deposition techniques on both substrate types was conducted.

4.3.1 Development techniques

Paper samples were treated with IND-Zn and PD, while glass samples were developed with CA and FePS. All marks were photographed using a Canon EOS 800D DSLR camera with a Canon EF-S 60mm macro lens on the same day they were developed.

Indanedione-Zinc

IND-Zn working solution was prepared and applied as outlined in Champod *et al.* [6]. Treated marks were developed by placing the sample in a Singer Magic Steam ironing press at 165 °C for 10 seconds. Marks developed with IND-Zn were photographed in the luminescence mode with the Rofin Poliview® IV forensic imaging system. The Rofin Polilight PL550XL was used at 505 nm with a 590 nm barrier band-pass filter to visualise the marks.

Physical Developer

PD was prepared and applied as outlined previously by de la Hunty *et al.* [81]. Fingermarks were imaged under standard laboratory ambient lighting.

Cyanoacrylate fuming

Items were placed in a MVC 100 Cyanoacrylate Fuming Cabinet (Foster + Freeman) and treated with CA, with the following parameters for fingermark development. 0.5 g of Loctite™ 406 superglue, fuming set to 20 minutes at 120 °C at 80% relative humidity. No post-treatment staining was applied. Fingermarks were imaged under standard laboratory ambient lighting.

Powder Suspension

An FePS recommended in the 2022 FVM was used in this study [41]. The formulation consisted of a Sigma Aldrich iron oxide nanopowder (10 g, 50-100 nm) and a 10% T20 stock solution (20 mL) in a ratio of 1:2 w/v [41]. The formulation was mixed immediately before development and spread onto the substrate using a wet squirrel-hair brush from Optimum Technologies. The suspension was left on the substrate for approximately 10 seconds before being thoroughly rinsed off with running tap water and left to dry at room temperature. Fingermarks were imaged under standard laboratory ambient lighting.

4.3.2 Comparison of deposition technique

Preliminary investigations were done to determine the most effective deposition method, comparing deposition of artificial emulsions through loading donor fingermarks and using fingerprint stamps to remove any presence of natural residue. The aim of this was to determine the most simple and effective method by which the emulsion could be deposited in a pattern that mimicked natural fingerprints.

4.3.2.1 Donor fingermark deposition

Donor loaded fingermarks were created by asking donors to wash their hands before immediately adding 5 µL of one emulsion onto each of the three middle fingers on one hand before the donor rubbed the fingertips of each hand together to distribute the product across their fingertips, akin to methods applied by Arsenault *et al.* [140]. These loaded marks were then deposited in three depletions with an approximate deposition pressure of 400-500 g (weighed on a digital scale) on both paper and glass substrates, as illustrated in Figure 4.2. Both HM and Pickering emulsions were used to load donor fingermarks, and marks were deposited by two donors to assess whether artificial emulsions were affected by donor variability. A second set of natural marks were also deposited immediately after handwashing, to determine if any residue was able to be detected and potentially interfere with loaded marks. No current research is available detailing the prevalence of fingermark residue on the hands immediately after handwashing.

4.3.2.2 Artificial emulsion stamp deposition

Application of artificial emulsions using fingerprint stamps was also investigated to completely remove the need of a real human fingerprint. Rubber and acrylic stamp materials were investigated, and stamps were purchased from Etsy stores. The chosen rubber stamp measured 14 x 19 mm (PinkPeonyStationary) with a stylised fingermark pattern to mimic the size and complexity of a real fingermark. Two acrylic stamps were purchased (MonicaGiftFinds) in different sizes and complexities

due to difficulty producing small details in this medium. The smaller stamp measured 30 x 20 mm and the larger stamp 40 x 30 mm. Images of all stamps are shown in Figure 4.1. To deposit marks using the stamps, 5 µL of each simulant was pipetted onto a clean glass tile and the stamp rubbed into the simulant to evenly distribute the solution across the ridges, similar to the method applied by Sisco *et al*. [139]. A set of five sequential depletions were deposited for each simulant and an approximate pressure of 400-500 g was applied, shown in Figure 4.1.

Two repeats of each set of marks were deposited. For all deposition methods, simulants were placed in an oven at 50°C for 10 minutes to dry out the residue before being left to cool for 10 minutes and then developed with fingermark enhancement techniques. Preliminary experiments comparing the development of artificial residues being dried out using either heating or natural ageing for 24 hours was conducted and no notable differences observed.

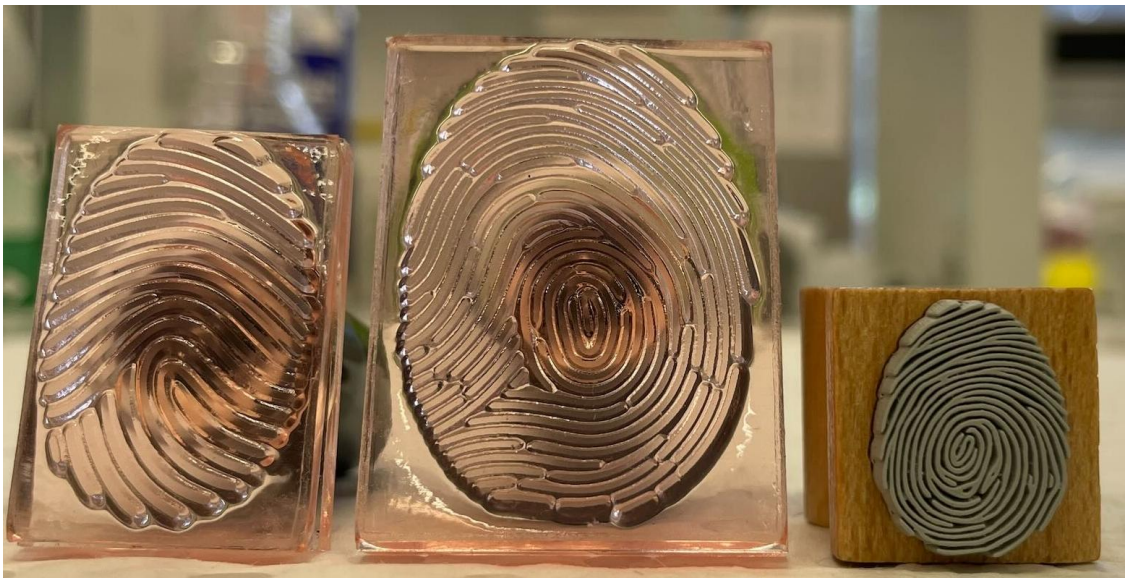


Figure 4.1 Fingermark stamps used to deposit artificial simulants. Small acrylic (left, 30x20 mm), large acrylic (middle, 40x30 mm) and rubber (right, 14x19 mm)

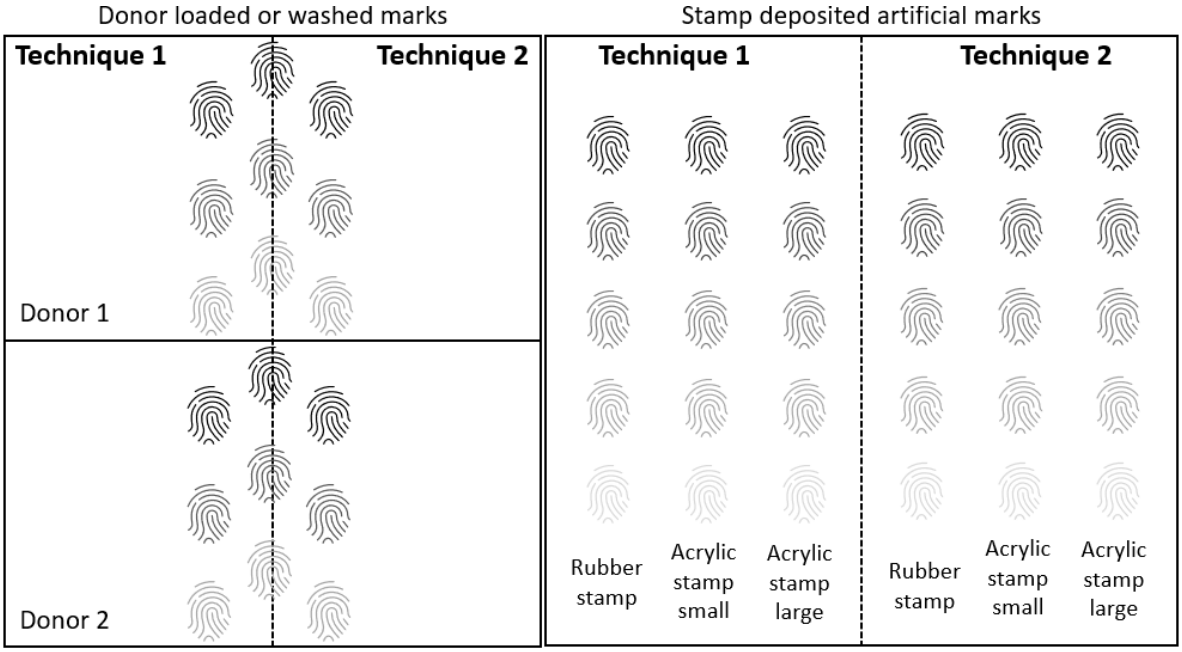


Figure 4.2 Deposition of artificial and donor (loaded or washed) fingermarks deposited on glass and paper substrates

4.3.2.3 Microscopy

Light microscopy was used to observe deposited residues before development on non-porous substrates, as well as marks after treatment with CA fuming and FePS. The Leica FS CB microscope was used in dark field mode. The microscope was equipped with a Leica DFC7000 T camera and controlled using the Leica Application Suite software, version 5.2.

4.3.2.4 Fingermark deposition

To compare with the two emulsions, natural fingermarks from four donors were deposited on paper and glass substrates. Donors were asked to wash their hands approximately an hour before depositing and avoid obvious contaminants such as food grease and dirt but otherwise go about normal daily activities. This extended timeframe allowed for fingermarks to be produced which more closely mimic those encountered in casework. Fingermarks from the three middle fingers of either hand were deposited in a set of three depletions with the middle finger on the seam of two substrates, so the middle fingermark could be split and developed with two different techniques. Ethics approval for collection of donor fingermarks was completed through the University of Technology Sydney (ETH18–2521, Appendix A) and participants were required to consent to the collection of their fingermarks prior to deposition. The rubber stamp was chosen as the most representative way to deposit artificial fingermark emulsions. It was therefore used for further comparison of the HM and Pickering emulsions to natural fingermark development.

HM and Pickering artificial emulsions were deposited using the rubber fingerprint stamp in the same method as described in section 4.3.2.2. Spot tests of the emulsions were also added to the developed samples, by pipetting a 5 µL spot of each simulant onto both substrates and developed in the same way. Three depletions of each simulant were deposited, and all fingermarks were left to age in laboratory conditions for 24 hours before being developed by techniques outlined in section 4.3.1.

4.3.2.5 Fingermark assessment

Three repeats of all tests were completed and whole fingermarks graded using the modified CAST scale assessing ridge detail (shown in Table 2.3) to assess their overall quality. Three assessors were asked to grade the fingermarks, and the median scores were used for subsequent analysis.

4.3.3 Results - Comparison of deposition technique

4.3.3.1 Donor loaded marks

Donor fingermarks loaded with HM and Pickering simulants were able to be developed using the chosen techniques on both substrates. The effect of simulant loading did not overall improve or reduce fingermark quality, but interacted differently depending on what simulant, substrate, technique or donor was used. Importantly, differences between the loaded fingermarks of each donor were observed, an example of which is shown in Figure 4.3. The development quality also varied between the two repeats performed. From these images, loaded marks deposited by donors 1 and 2 did not develop similarly for any of the development techniques. On glass, CA and FePS were less reactive to marks deposited by donor 2 and produced very little development. On paper, IND-Zn developed clear and well-defined ridges for donor 2, however PD produced reverse development. The natural marks of donor 2 were generally poorer than donor 1 and produced little to no development using FePS and PD. Enhancement with these two techniques showed the largest differences between donors. Development of fingermarks deposited straight after handwashing showed some reactivity with IND-Zn and CA on each donor (Figure 4.4), suggesting incomplete removal of natural residue by handwashing and fast replenishment time, possibly triggered by handwashing.

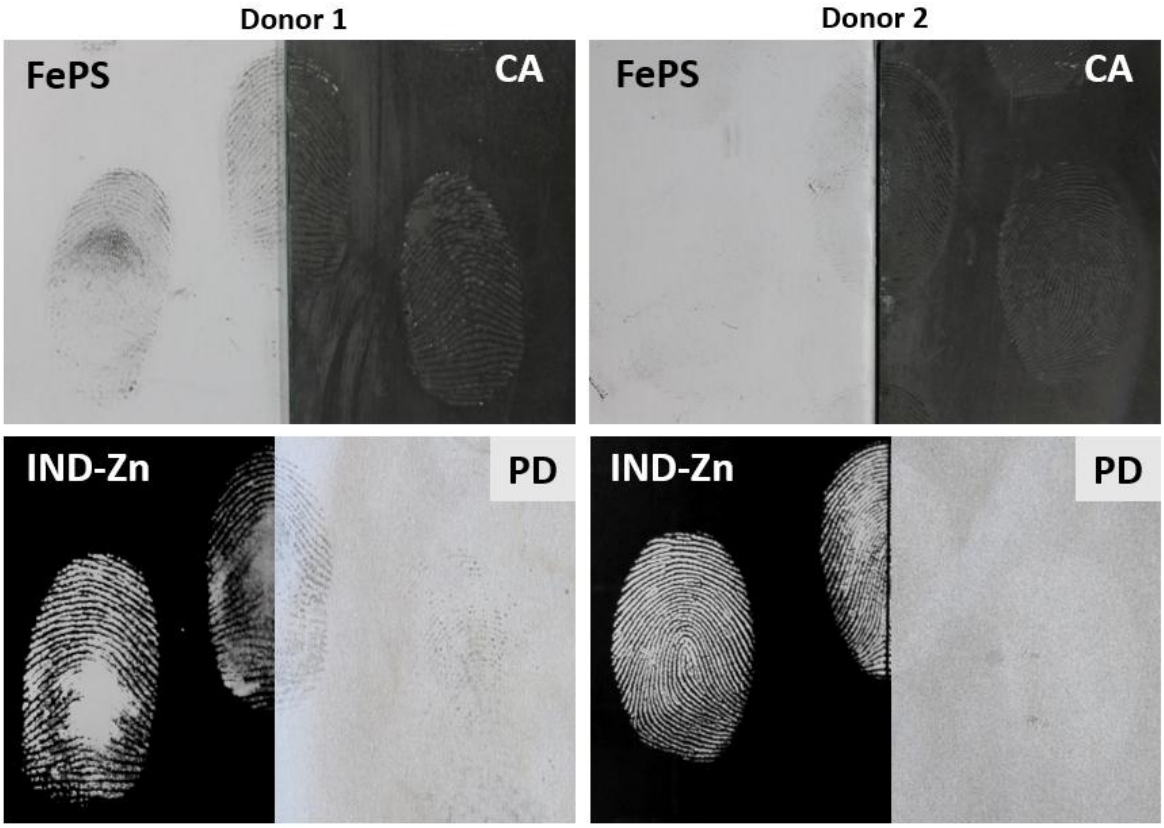


Figure 4.3 Comparison of development produced by each donor loading fingermarks with Pickering emulsion on non-porous (top row) and porous (bottom row) substrates

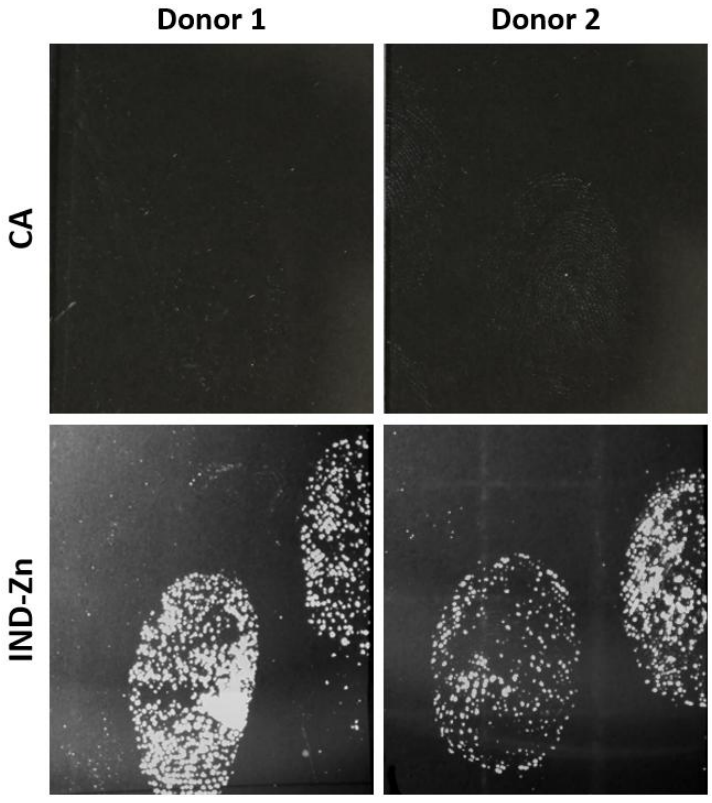


Figure 4.4 Development produced by CA and IND-Zn on marks deposited immediately after handwashing, showing the presence of residual compounds on both donors

It is clear from these results that the addition of artificial simulant to a donor's fingertips does not result in more homogenous or similar development using the chosen techniques. It was hypothesised that the addition of the simulant would minimise the inter and intra-donor fingermark development, as they primarily had the same type and amount of compounds present in deposited marks. However, the inconsistent development quality between donors and repeats disproves this. The donor differences suggest that there is a factor involved in the deposition method that is altering the composition of the simulants. This study also showed that some residual endogenous compounds were still present after handwashing to interact with the simulants which may explain the differences. However, it is also possible that the donor's skin may be absorbing some of the compounds or there are physical skin characteristics altering residue composition. It is suggested that fingermark residue is present as a complex emulsion of endogenous and exogenous compounds, either as water-in-oil (w/o) or oil-in-water (o/w) emulsions depending on the nature of the fingermark [7, 96, 98, 137]. It appears that the addition of artificial emulsions to the donor's fingertips and subsequent contact with a substrate may be changing the dynamic of these emulsions, resulting in variation of development quality. This shows that the method of loading donor fingermarks with artificial emulsions is not an appropriate deposition method for this type of research.

4.3.3.2 Stamped artificial marks

Stamps made of both acrylic and rubber were tested for deposition of the simulants, with designs to mimic the shape and complexity of fingermark ridges. The stamps were custom made; however, the complexity of the designs was restricted by the ability of the manufacturer and as such, only the rubber stamp was small enough to replicate the size of a real fingerprint. The ridges of the acrylic stamps were approximately 700-800 µm wide, while the ridges of the rubber stamp were approximately 200 µm. The width of natural fingermark ridges varies between 100-300 µm [146], so the acrylic stamp ridges were almost double the width of natural ridges which alters the amount of residue present as well as the drying time. The material of the acrylic stamps was very hard and prone to sliding on the glass substrate during deposition, while the rubber stamp was more flexible and better represented the behaviour of friction ridge skin when contacting the substrate.

Artificial emulsions deposited with each stamp could be developed by one or more development techniques, as shown in Figures 4.5 to 4.7. None of the stamped marks could be developed using PD, and the only development produced with FePS was on HM emulsion using the rubber stamp. Otherwise, similar levels of development were produced with each simulant regardless of stamp used for deposition. However, as the rubber stamp was better able to represent the approximate size,

shape and complexity of real fingermarks, it was chosen as the most appropriate deposition tool for artificial emulsions.

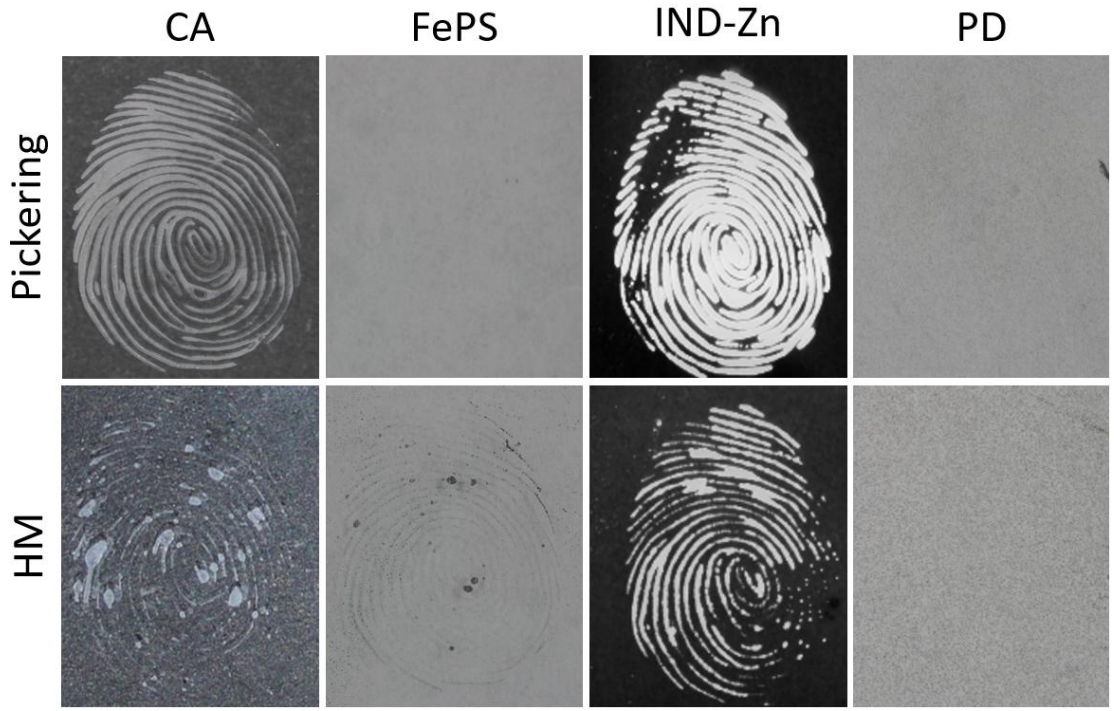


Figure 4.5 Development of HM and Pickering emulsions applied with rubber stamp on non-porous (CA and FePS) and porous (IND-Zn and PD) surfaces

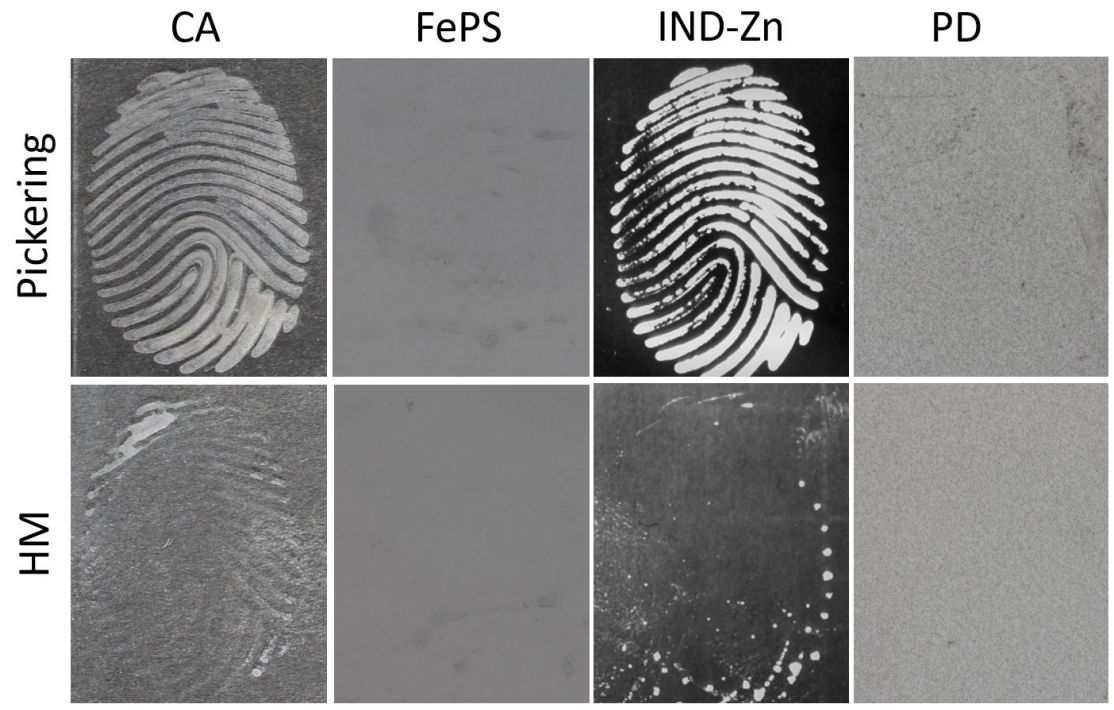


Figure 4.6 Development of HM and Pickering emulsions applied with small acrylic stamp on non-porous (CA and FePS) and porous (IND-Zn and PD) surfaces

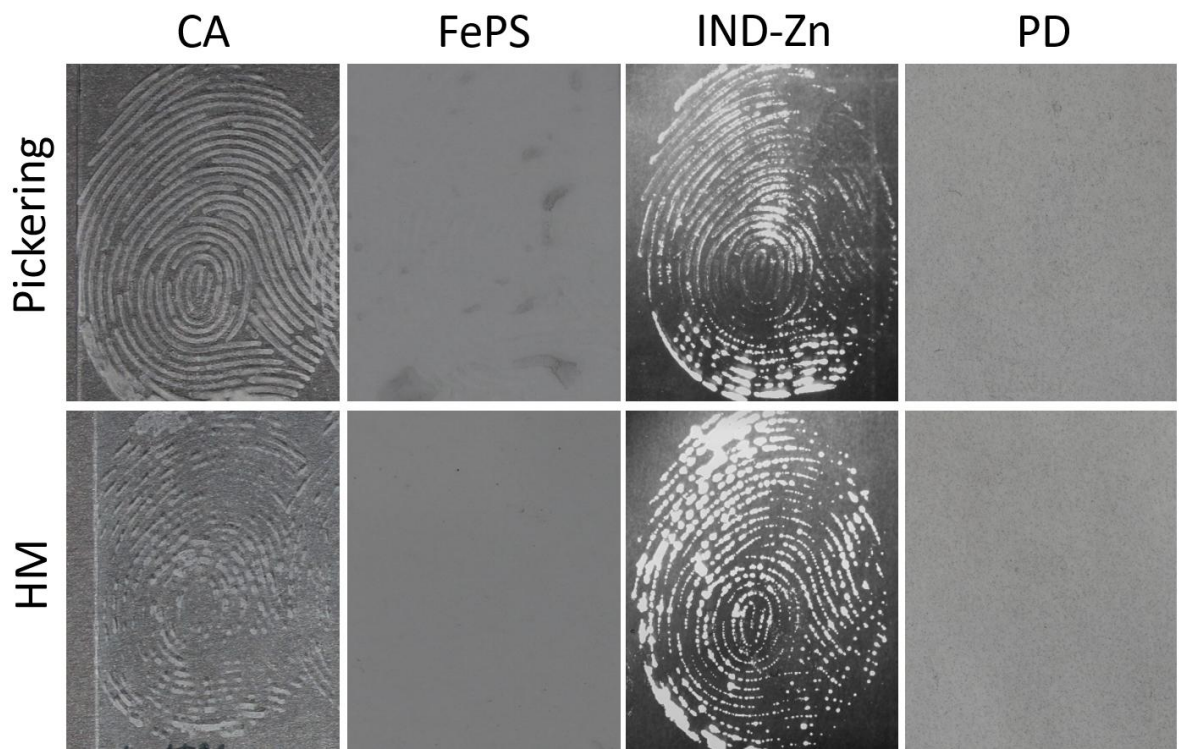


Figure 4.7 Development of HM and Pickering emulsions applied with large acrylic stamp on non-porous (CA and FePS) and porous (IND-Zn and PD) surfaces

4.3.4 Results - Comparison of simulants

Before the deposition and development of any fingermarks, clear physical differences between the artificial simulants and natural marks were observed. Both emulsions were cloudy white and the HM emulsion had a watery consistency whereas the Pickering was more foamy and oily. When the simulant stamped marks that were deposited on glass were observed under an optical microscope (Figure 4.8), it was found that the consistencies of the emulsions affected their ability to maintain the boundaries of the stamp ridges. The HM emulsion dried in inconsistent watery spots along the ridges whereas natural fingermark ridges and Pickering emulsion stamps maintained clear ridge boundaries.

From the images in Figure 4.8, it was also clear that the physical appearance of the artificial residues does not mimic natural donor fingermark emulsions. The Pickering simulant contained larger aggregations within the residue, however the distribution of these components was not homogenous along the ridges. In the HM emulsion, smaller aggregates were observed and were concentrated mainly around the edges of the dried spots. Figure 4.8 illustrates that neither simulant is deposited with compounds homogenously distributed in the same manner as natural fingermark residue. The voids present on the natural ridges are created by the presence of pores along the ridge skin, which were not mimicked on the stamps. This is important to note, as the drying process may be affected by the depth and volume of residue present, which is reduced in natural marks due to the presence of pores.

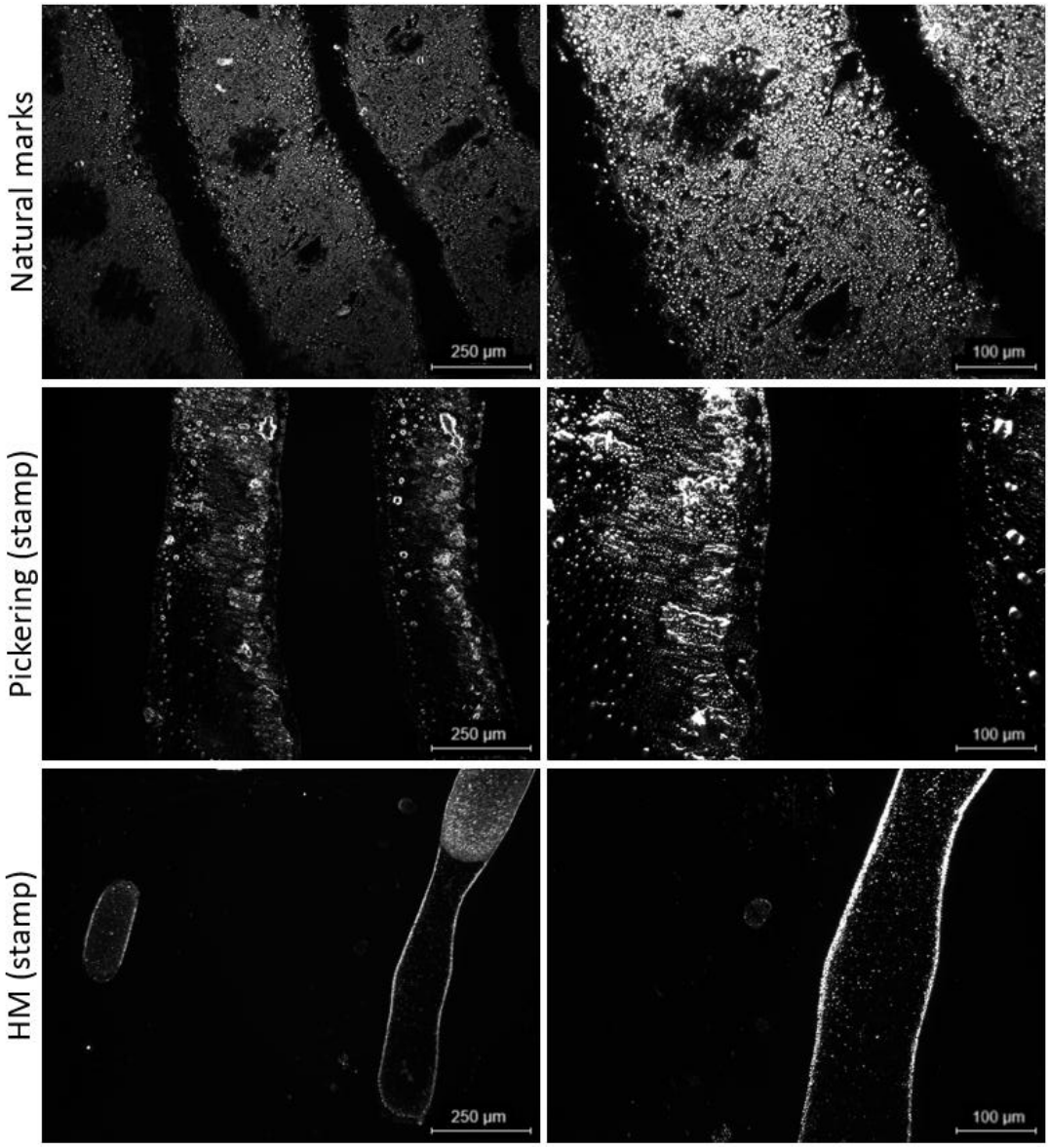


Figure 4.8 Images of undeveloped natural fingermarks and artificial simulant ridges deposited using a rubber stamp on glass at 10x (left) and 20x (right) magnification

4.3.4.1 HM emulsion

The HM emulsion marks could be developed to some degree by all techniques except for PD, shown in Figure 4.9 and Figure 4.10. On glass, FePS produced the highest quality development on HM emulsion with all marks graded 2 or 3, while CA development only produced scores of 1 or 0, and enhanced CA marks appeared quite spotty (Figure 4.10). On paper, PD did not develop any fingermarks while IND-Zn mostly produced marks scored 1 or 2 but had some scores of 0 for no detection. The developed spot tests shown in Figure 4.10 demonstrate weak development for CA, no reactivity with PD and development with FePS and IND-Zn only occurred on the outside edges of the spots.

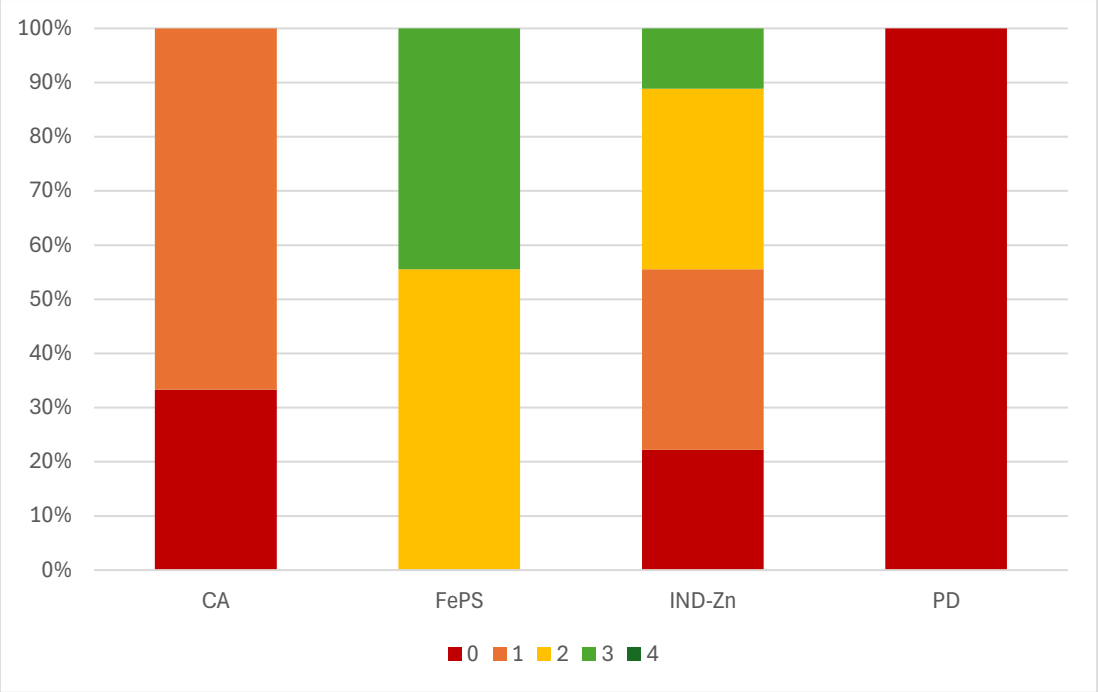


Figure 4.9 CAST scores produced by development of each technique on HM emulsion deposited with rubber stamp

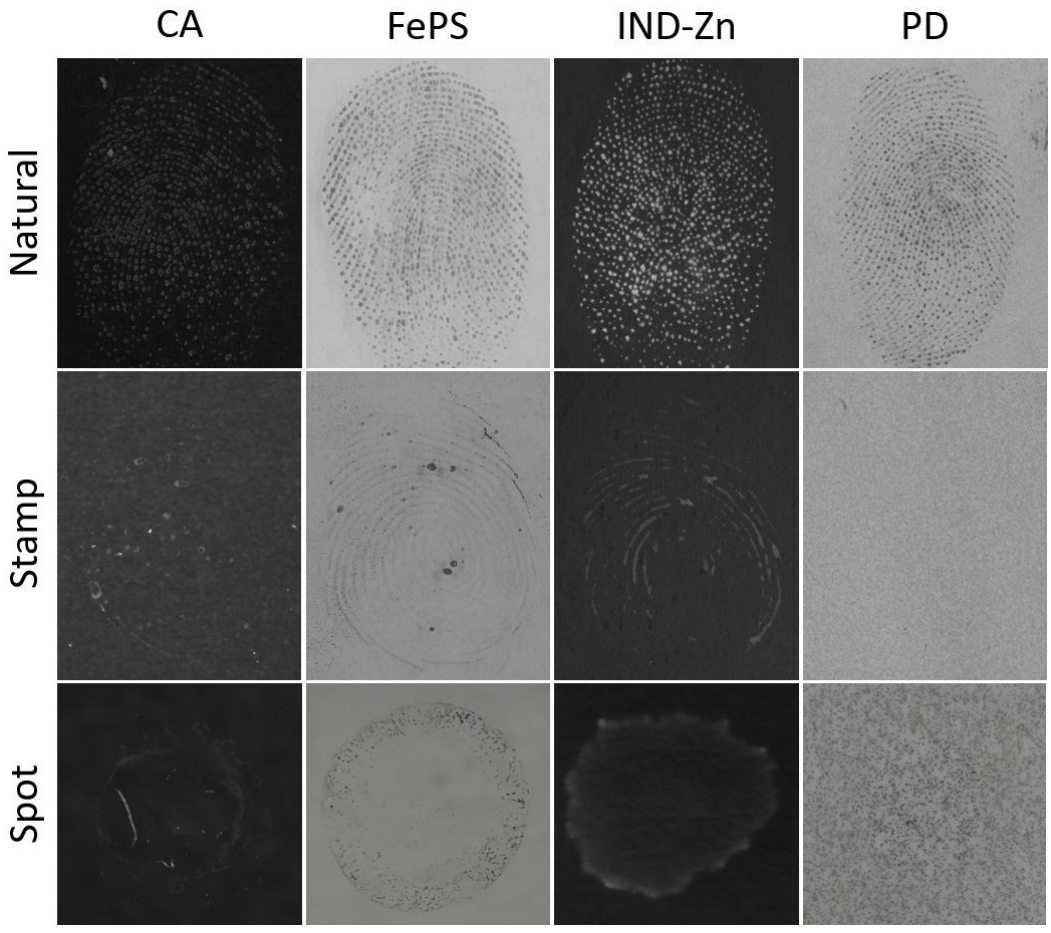


Figure 4.10 Examples of development produced on HM emulsion stamps and spots with natural fingermarks from donor 1 with all techniques on non-porous (CA and FePS) and porous (IND-Zn and PD) surfaces

Various formulations of the HM emulsion have been developed and tested by de la Hunty *et al.* (2017) and Steiner *et al.* (2022) [82, 97]. The formulation used in this study was investigated by de la Hunty, while Steiner modified the ratios used to increase the amount of sebum in the formulation due to better quality development produced. The spotty development of stamped HM emulsion with CA observed in this study was also reported by Steiner when the HM emulsion was applied to non-porous substrates (acetate sheets) using a modified inkjet printer [82]. The authors concluded that despite the emulsion not creating stabilised ridges like natural marks, the CA was polymerised by the simulant and therefore was still able to catalyse a development reaction. In 2023, the authors also reported successful IND-Zn and no development with PD [96].

The efficacy of CA and IND-Zn demonstrate the presence of their target compounds in the artificial simulants. IND-Zn is reactive to amino acids and it is thought that CA requires the presence of water-soluble components found in eccrine sweat to initiate polymerisation of the cyanoacrylate monomer, as the CA technique is less effective on marks exposed to water [18, 33, 51]. Studies have suggested that possible initiate compounds of this technique are lactic acid, ammonia, acetic acid, amines, alcohols, amino acids, alkanes, and proteins [11, 147, 148]. The target compounds of FePS and PD are not currently well understood, however both techniques can be used on fingermarks which have been wetted and it is theorised that they both require more complex mixtures of fingermark residue compounds to initiate development. The clear difference in development quality using the HM emulsion suggests that the target compounds for these techniques are either different, or the emulsions are interacting differently with the two substrate types, altering the residue composition and therefore affecting development. These hypotheses are also not mutually exclusive

4.3.4.2 Pickering emulsion

The Pickering emulsion was able to be developed by all techniques, however as shown in Figure 4.11, only two marks were developed using FePS and PD and these were scored very low. High quality marks (all scored 3 or 4) were produced using CA and IND-Zn. The Pickering spot tests could not be developed with FePS and PD, however development with CA and IND-Zn in Figure 4.12 show that the simulant emulsion separated into two phases on both substrates. CA development shows lighter development around the edges of the spot test and IND-Zn enhanced ‘rings’ within the jagged boundaries of the spot. It is possible that the oils within the Pickering emulsion were diffused into the paper fibres, or that a capillary-type separation is occurring within the paper. Again, IND-Zn development was strongest on the outside edges of the spot, however the technique also reacted with some compounds left in the centre.

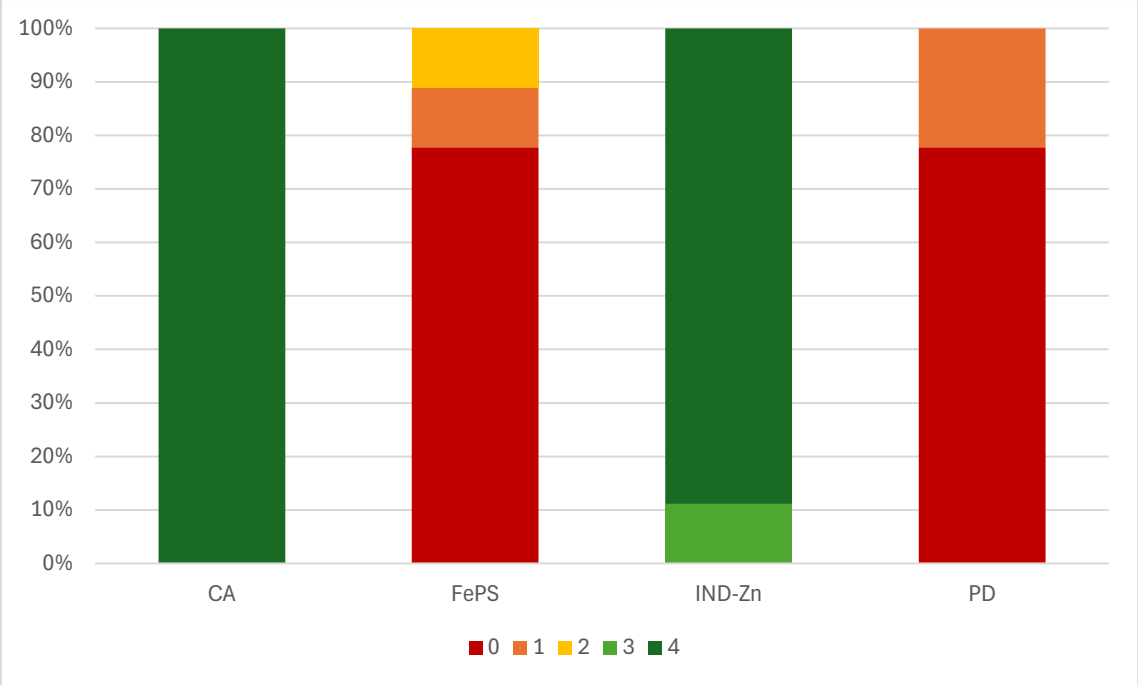


Figure 4.11 CAST scores produced by development of each technique on Pickering emulsion deposited with rubber stamp

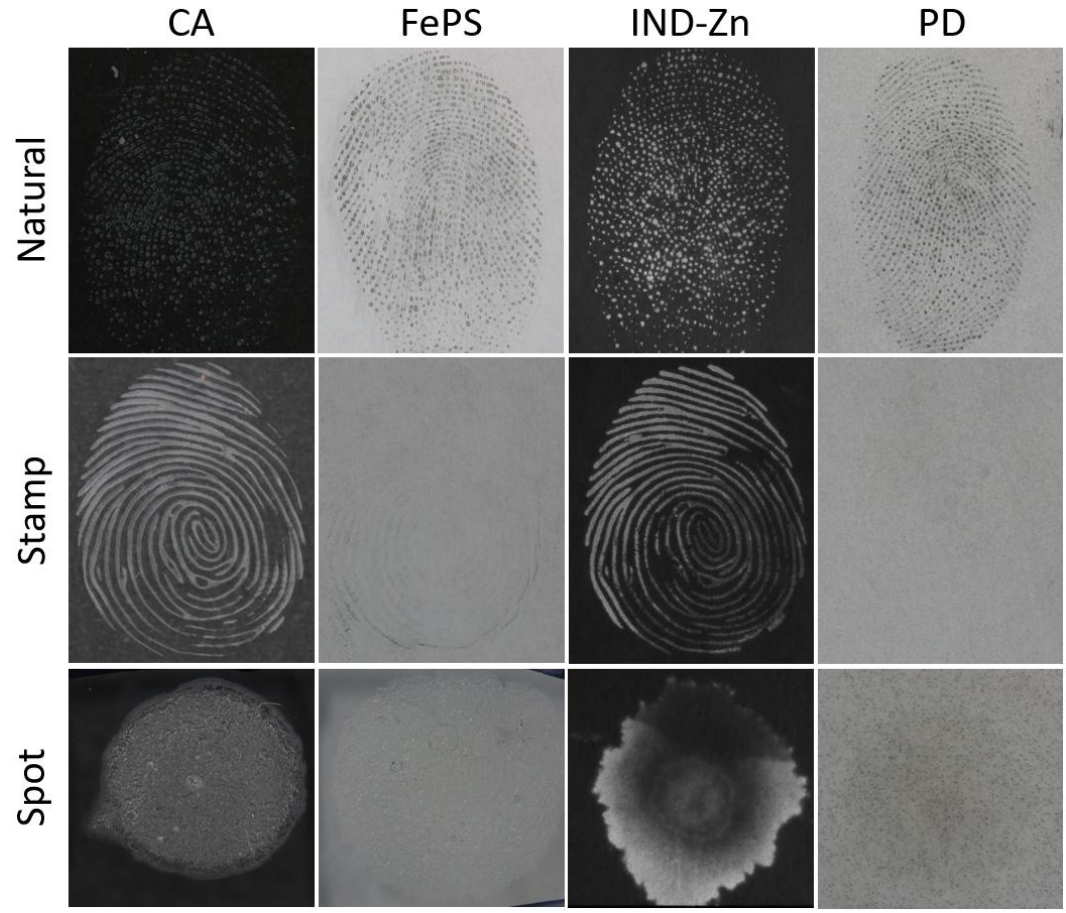


Figure 4.12 Examples of development produced on Pickering emulsion stamps and spots with natural fingermarks from donor 1 on non-porous (CA and FePS) and porous (IND-Zn and PD) surfaces

The Pickering emulsion has not been previously investigated for fingermark development, however the company's artificial perspiration (artificial eccrine sweat) has been tested for use as an amino acid test control by the United States Army Criminal Investigation Laboratory. In a 2015 presentation at the International Association for Identification, they suggested it to be a suitable replacement for commercially available amino acid test pads [149]. The emulsion is manufactured for use in cosmetic testing, such as testing if sweat may stain a new type of clothing fibre or dye [145]. It has been used by LeSassier *et al.* (2019) as an artificial fingermark representative in a study comparing recovery methods of DNA and proteins from fingermarks [138]. In this study, the simulant was mixed with DNA material and additional eccrine perspiration then spotted onto a substrate and was not used for assessment of fingermark development techniques.

4.3.4.3 Similarity to natural development

The CAST scores produced in this experiment were used to compare the similarity of artificial mark development to that of natural donor marks. This was done to suggest which simulant was best able to represent natural fingermark residue and if this varied by donor. To compare between the natural fingermark and the emulsion marks, a percentage similarity assessment was performed. To do this, natural marks were compared to the different simulant marks at the same depletion number and a percentage similarity was then calculated for each time equivalent scores were awarded across the categories, shown in Figure 4.13. For example, Figure 4.13 suggests that all fingermarks from donors 2-4 obtained the same quality grade ('poor' (score 0), 'medium' (scores 1-2) and 'good' (scores 3-4)) as stamped Pickering marks when developed with CA. This data shows that the Pickering emulsion was better able to represent natural development with CA and IND-Zn, while HM emulsion performed more like donor marks when developed with FePS. Neither emulsion could consistently produce similar grades for PD. A lack of consistent similarity in development indicates that the emulsions are interacting with the development techniques very differently compared to natural residue, despite theoretically containing similar chemical compounds. This may be due to differences in the stability or state of emulsions compared to fingermark residue. The similarity scores were fairly consistent between donors, with a notable exception of donor 1 producing the only marks similar in quality to HM emulsion (33%) when developed with CA. It must be noted that the use of combined grades (eg. natural mark score of 3 being "similar" to either a 3 or 4 artificial score to indicate 'good' development) in producing this similarity assessment provides a higher degree of similarity than considering only grades which are the same. This was done upon consideration of the use of a subjective scoring system and use of only one assessor, however because of this the results should be interpreted with caution.

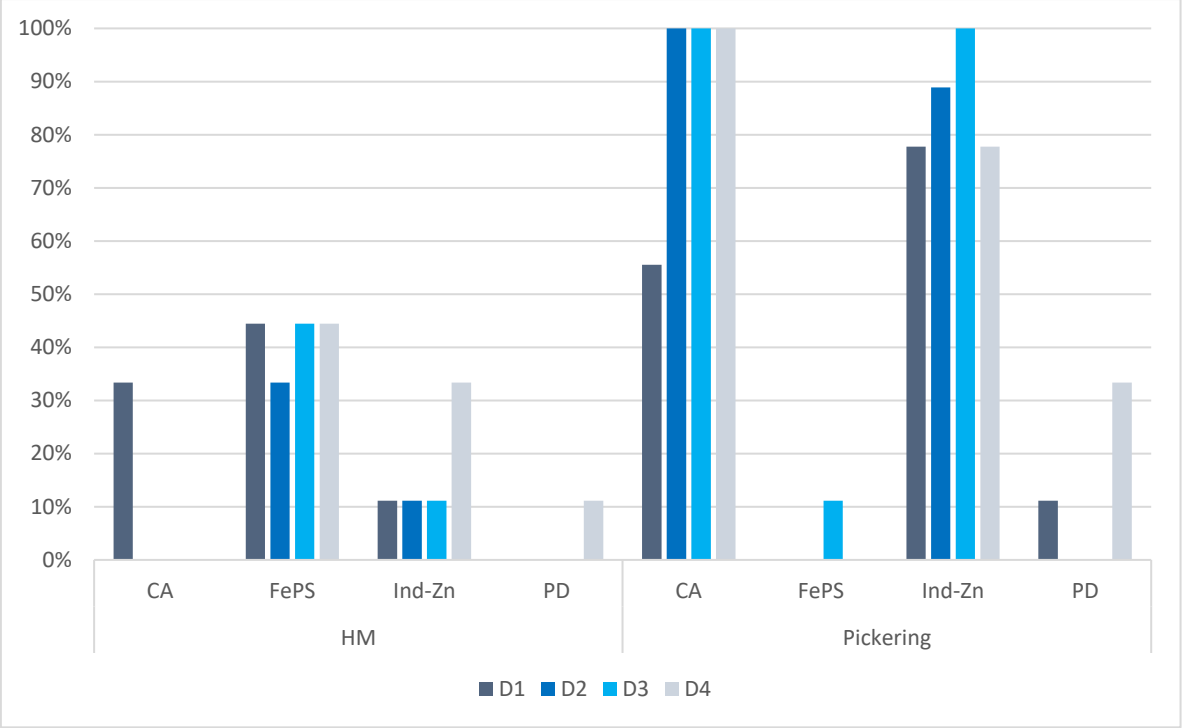


Figure 4.13 Percentage of similarity between scores given to each donor and the two artificial emulsions developed by all techniques

The main aim of this investigation was not to produce a fingermark simulant that can replace natural residue in research, but to evaluate the viability of using these emulsions in future research to investigate the possible composition and subsequent targets of FePS formulations contained in donor marks. The comparison between natural marks of four donors and artificial mark development showed that neither emulsion was able to accurately and reliably mimic the same development as natural residue for all techniques. This shows that either the assessed simulants are not accurately representing compounds present in natural residue or there is a difference in the structure or emulsion state of deposited residue (possibly due to unknown interactions of fingermark residue occurring within the friction ridge skin) which is impacting development.

The results of this investigation illustrated the highly complex nature of fingermark residue and development. The HM emulsion has been investigated in previous research in an attempt to represent a standardised fingermark. Most of the work investigating artificial fingermark simulants has focussed on porous substrates and subsequent amino acid-targeting techniques, which this investigation demonstrated to be effective with development using IND-Zn [98, 141]. Successful IND-Zn development confirms that both simulants contain amino acids that are effective targets for these techniques. The differences between each donor's similarity assessment were expected as it is well documented that donor variability results in different amounts of endogenous and exogenous compounds [150-152]. Previous investigations involving artificial simulants on porous substrates have

compared results to natural mark development from a range of donors [98, 143]. The only other published work involving artificial emulsions on non-porous substrates is from Steiner *et al.* (2023) and natural marks from only one donor was used for comparison as it was a proof-of-concept study [143]. Because of this gap in research, there is little data to compare the results of this study to and limited conclusions can be drawn. This highlights a need for comparison of artificial simulants with a much wider range of donors on non-porous substrates to better understand the differences in deposited residues and their interactions with non-porous substrates.

Overall, the results of this study showed that artificial simulants can be effectively developed by some techniques, however neither of the emulsions tested could wholly, reproducibly, and accurately mimic natural fingermark residue with the assessed development techniques. Successful development using IND-Zn, FePS, and CA on stamped marks confirm the presence of important target compounds, however each technique and substrate type affected development quality. The utilisation of artificial fingermark residues is therefore a useful tool in the exploration of foundational mechanisms of lesser understood techniques and may also provide insight into the complexities of natural residue interactions with friction ridge skin, substrates and detection techniques.

4.4 Part 2: Impact of surfactants on development of artificial residues with FePS

The artificial residues investigated in section 4.3 were developed using FePS formulations made with different surfactants to investigate if the type of surfactant influenced what residue fractions could be developed. Simulants were deposited using the rubber stamp on non-porous substrates before being developed with the optimised FePS formulations for each surfactant. This development was also compared to natural fingermark development.

4.4.1 Fingermark deposition

Both artificial simulants and natural fingermarks were deposited in this study. Artificial simulants made in section 4.2 were used to compare development of each simulant to natural marks and deposited using a rubber stamp to prevent any contamination of natural residue. Four types of artificial residue were used; individual fractions of eccrine sweat and sebaceous residue made in sections 4.2.2.1 and 4.2.2.2 respectively, as well as the HM emulsion outlined in section 4.2.2.3 and the commercial emulsion from Pickering Laboratories. A different rubber stamp was used to deposit each simulant on clean substrates also used in section 2.2.2; glass slides, white glossy ceramic tiles and resealable PE plastic bags (Table 2.2). Natural marks were deposited from one donor, and all marks were deposited in series of five depletions to also assess the impact of reduced residue volume.

The deposition of marks is illustrated in Figure 4.14. Deposited marks were aged in laboratory conditions for 24 hours before development.

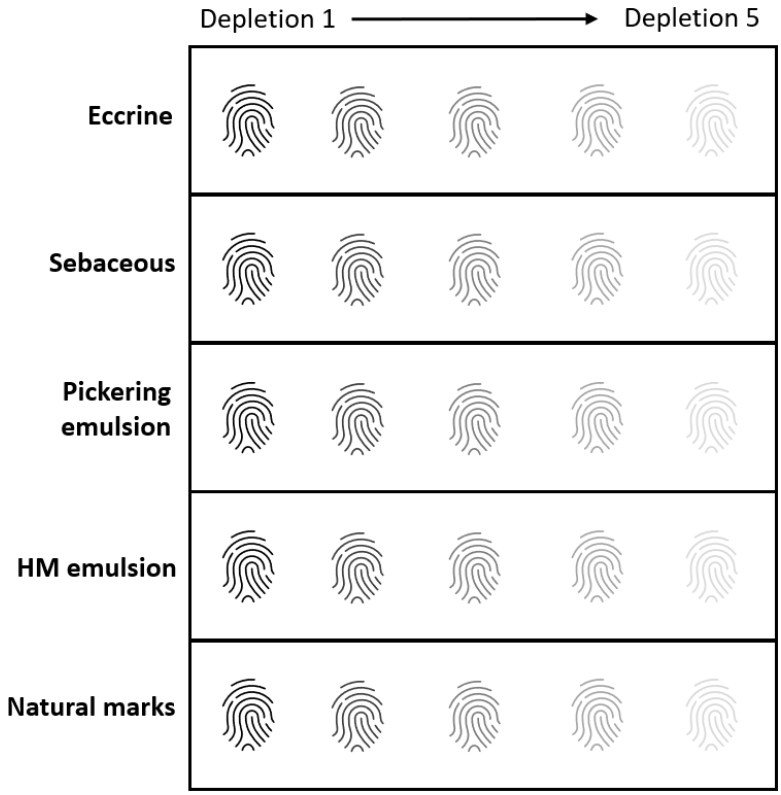


Figure 4.14 Illustration of fingermark deposition method on all substrates

4.4.2 Fingermark development and analysis

The optimal formulations of each surfactant, as determined in section 2.3.3, were used to develop fingermarks. The formulations are outlined in Table 2.7. FePS formulations were made and applied as described in section 2.2.3. One formulation made with only water was used as a control. All developed marks were photographed using a Canon EOS 800D DSLR camera with a Canon EF-S 60mm macro lens and visualised under standard laboratory ambient lighting. Developed marks were graded by one assessor for the quality of their ridge detail, contrast, and background development using the same scales as in section 2.2.4 (Tables 2.3 to 2.5). A total of 825 artificial and natural fingermarks were assessed in this study.

4.4.3 Results

4.4.3.1 Reactivity of simulants

All stamped simulants were able to be developed by FePS formulations, however the different surfactants used did influence the quality of development for natural fingermarks and artificial residues. Scores of background development were not included in these analyses as the focus was on

areas of simulant deposition rather than the surrounding area, and scores of background development for all formulations on glass were previously assessed in section 2.3. Figure 4.15 illustrates a comparison between only ridge detail scores given to each simulant, to demonstrate the general level of quality produced. From these results it can be seen that the sebaceous simulant and Pickering emulsion developed a similar rate of reactivity across all surfactants. This may suggest that the Pickering emulsion is a water-in-oil (w/o) emulsion. The eccrine simulant produced the highest number of 0 scores while natural marks developed the lowest. This shows that overall, the sebaceous residue and Pickering emulsion were the most reactive artificial simulants, while eccrine residue and HM emulsion were the least reactive with the tested FePS formulations. This may suggest that the HM emulsion exists as an oil-in-water (o/w) emulsion.

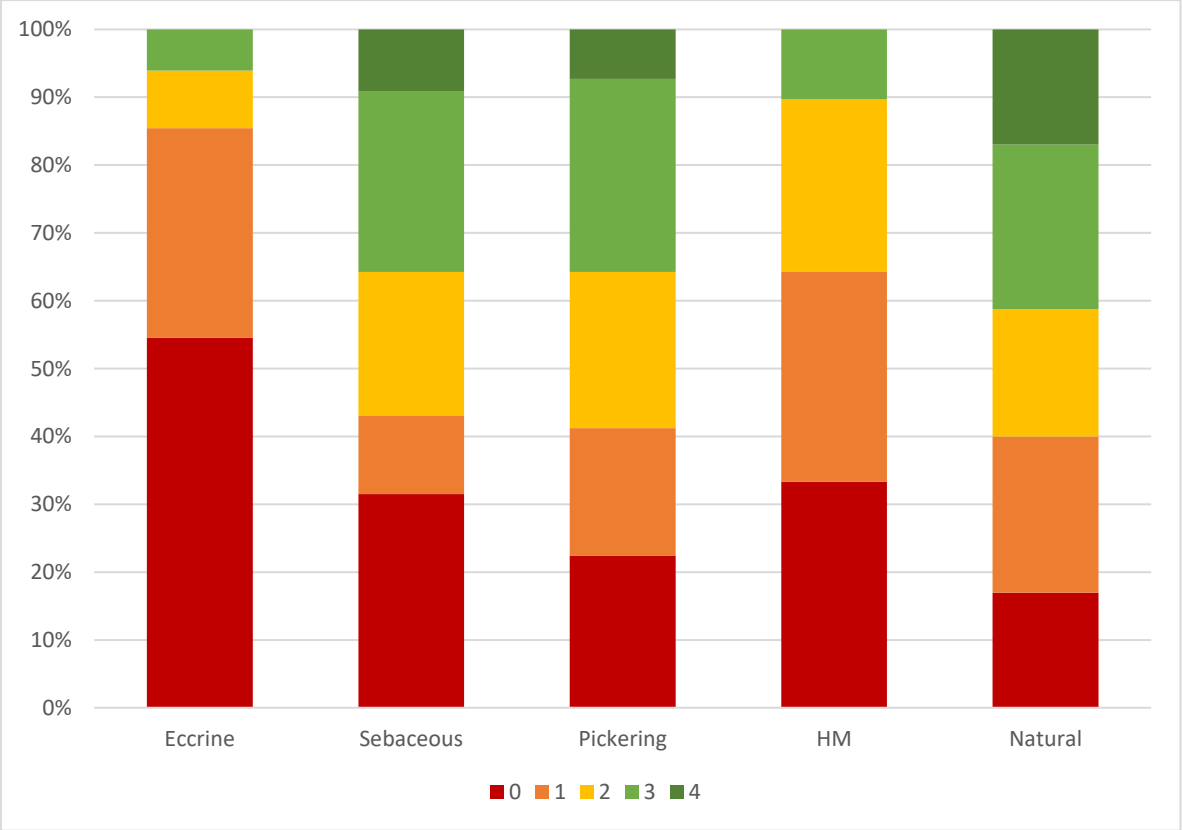


Figure 4.15 Combined ridge detail scores of all surfactants assessed in FePS formulations for each simulant and natural fingermark residue

The ability of FePS formulations to develop artificial simulants differed for each surfactant used. This suggests that the addition of different surfactants affects the target compounds of FePS formulations. The ability of each surfactant to develop different residue types was therefore assessed further. Table 4.3 indicates the rank of each surfactant in its ability to develop ridge detail and contrast for both natural and artificial fingermarks. LN did not produce any development and therefore scored the

lowest for all residues. For this reason, LN was removed from any future comparison of residue development. Further investigation into the cause of this is explored in chapter 5.

The two surfactants which produced the highest scores of ridge detail and contrast for the artificial simulants were n-DDAA and DOSS, as seen in Table 4.3. Formulations made with only water were also effective at developing stamped simulants. Surfactants which are known to be highly effective at developing natural fingermarks, such as KP, TX100 and T20 as determined in section 2.3, were generally not as effective in enhancing the artificial residues. Examples of the variable effectiveness of each surfactant in developing both artificial and natural residues is further illustrated in Figure 4.16. A table representing simulant development from all surfactants can be found in Appendix E. This shows the high level of ridge detail produced by water formulations and those made with n-DDAA, especially on sebaceous and Pickering residues. This is consistent with the assumption that the Pickering emulsion is a w/o emulsion, as the continuous oil phase would not be removed by a water wash. These formulations however did not develop high quality natural marks due to the heavy background development obscuring any ridge detail. Interestingly, development of eccrine marks with n-DDAA formulations produced reverse development on ceramic and glass substrates, shown in Figure 4.16, which scored the highest grades for ridge detail for this simulant.

Table 4.3 Rank of combined ridge detail and contrast scores for which surfactant best developed each type of residue using FePS made with different surfactants

Surfactant	Eccrine	Sebaceous	Pickering	HM	Natural
TX100	9	10	10	6	4
T20	8	6	6	5	2
T80	8	7	7	7	10
SP80/T80	5	5	4	3	8
KP	3	9	8	8	1
DOSS	2	2	1	2	7
SDS	7	8	9	10	9
LN	9	11	11	11	11
CTAB	6	3	5	9	3
n-DDAA	1	1	3	1	5
Water	4	4	2	4	6

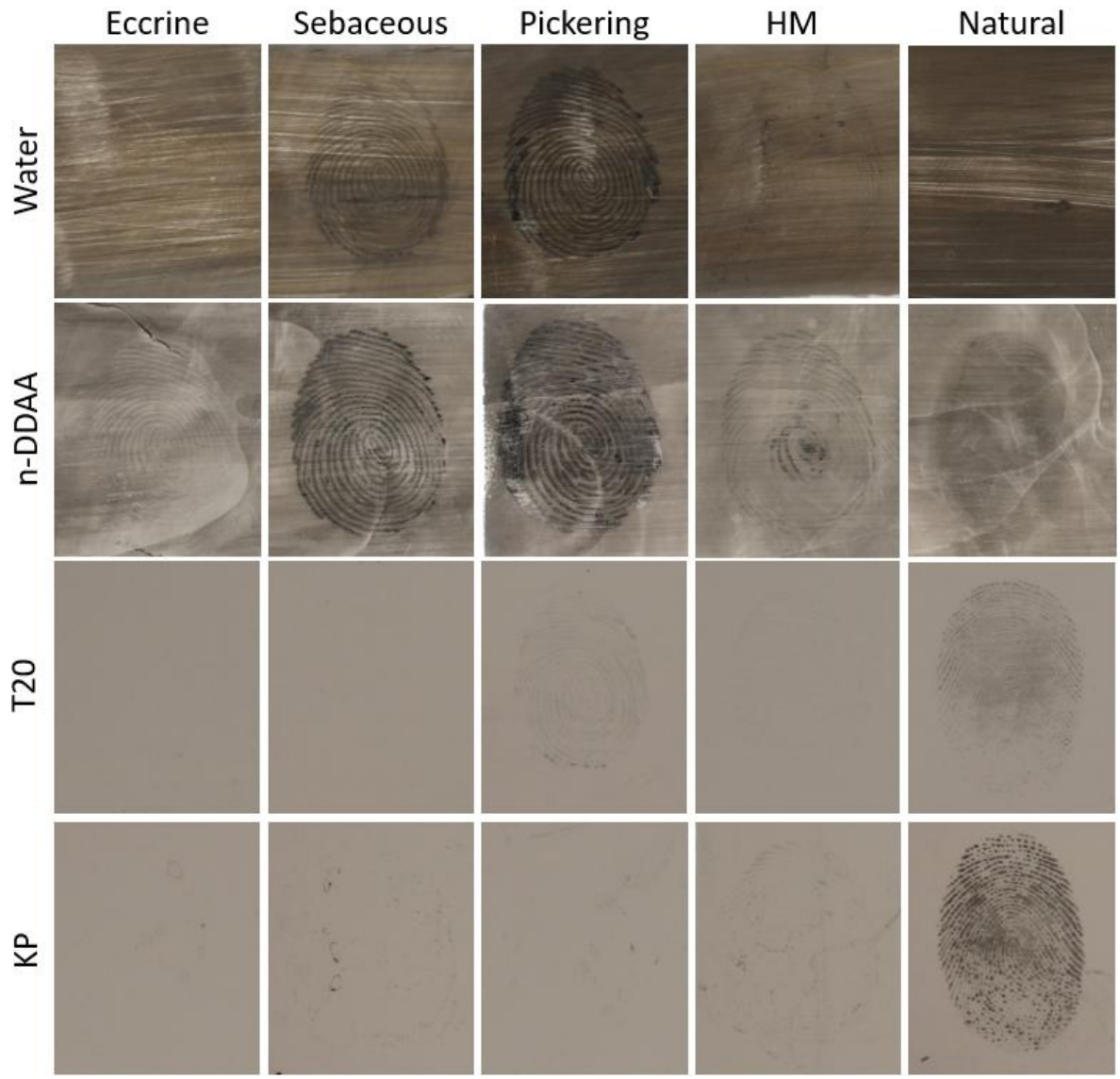


Figure 4.16 Comparison of the variable efficacy of four formulations in developing natural (donor 1) and artificial fingermarks on ceramic

Overall, these results demonstrate that the addition of different surfactants influence what components (or emulsion state in terms of continuous phase) of fingermark residue is being targeted during development with FePS. It is known that surfactants have varying abilities to suspend and disperse insoluble materials in order to carry out the function for which they are designed [57, 70]. Their application within techniques used for fingermark residue are therefore likely impacted by this ability, either removing certain components or being repelled by them. In terms of the FePS mechanism, the most common hypothesis is that the technique relies on the presence of eccrine compounds which are preserved by sebaceous material, allowing the water-soluble compounds in eccrine sweat to remain after application of water both before and during development. This hypothesis is based on previous success with development of wetted and aged marks and investigations performed by Stubbs-Hayes into the target compounds of FePS [26, 144]. The lack of

eccrine development indicated in the results of this study agree with the suggestion that it is affected by the aqueous nature of the technique, and the highest ridge detail scores for eccrine residue came from reverse development with n-DDAA. This surfactant also produced heavy background development, indicating the surfactant either easily deposits powder on a substrate or the micelles are not suspending the particles in the same way as other surfactants, as indicated by similar development occurring with formulations made with only water. The reverse development around eccrine residue however shows that this surfactant may be repelled by some part of eccrine residue, or the application of eccrine simulant interacted with something on the glass and ceramic substrates to repel subsequent powder deposition.

4.4.3.2 Comparison to natural development

Formulations previously determined to be highly effective and recommended for developing natural marks (such as KP, T20 and TX100) generally were not able to produce high quality artificial mark development. This is shown in Figure 4.17. The T20 formulation was able to produce some high-quality development on the sebaceous and Pickering simulants, however for all formulations ridge detail was greatly improved on natural fingermarks. Interestingly, the development of artificial emulsions using T20 differed slightly from the results produced in section 4.3.4 where the FePS formulation involving the Sigma nanopowder and 10% T20 solution was unable to develop the Pickering emulsion, but some HM development was achieved. This is likely due to either the difference in powder type or possibly the difference in T20 concentration, as the surfactant used in this part of the experiment was diluted to 25% of the FVM recommendation.

Figure 4.17 Ridge detail scores of FePS formulations made with KP, T20 and TX100 surfactants to develop natural (donor 1) and artificial fingermarks on all substrates

Poor artificial simulant development from these surfactants despite their previous success on natural fingermarks suggests that there are either specific components not adequately represented in any of the simulants that these formulations require for powder deposition, or there is a difference in the physical properties of the simulant preventing development (such as emulsion state in terms of continuous phase) or a combination of both. Theoretically, an emulsion such as the Pickering and HM simulants contains both eccrine and sebaceous components, and if all FePS formulations targeted the eccrine compounds, provided they are protected from water solubilisation by sebaceous material, it would be expected to see high quality development with the emulsions.

As this study did not produce these expected results, it can be assumed that either FePS is not solely targeting the eccrine components, or the simulant emulsions are structurally different in a way that is not facilitating fingermark development in the same way natural residue does. It is possible that the structure of the emulsion present in natural residue is playing a larger role in the successful development of fingermarks than the presence of specific individual components as is currently thought. The presence of different surfactants may be emulsifying or removing parts of the residue or altering the emulsion state (such as inverting the emulsion from one continuous phase to the other)

which explains the varying ability of surfactants to develop different fractions. As this technique is well known to be effective on wetted and aged marks, there is likely a part of these degradation processes that allows the residue to be better preserved and developed with the FePS technique [31, 41, 48]. Bleay *et al.* suggested that drying out of the residue leads to decreased proximity between surfactant micelles and encapsulated soluble residue (eccrine) components, allowing for more effective deposition of powder [26]. If this proposed mechanism is correct and natural residue is present on a substrate with eccrine compounds protected by a 'barrier' of sebaceous material, it is possible that the artificial emulsions are structured as a more homogenous mixture, however further ageing and drying tests using the simulants may improve this understanding. Similarly, the physical deposition mechanism with friction ridge skin may have some impact on the presence and structure of the compounds and how they form into an emulsion and how they are maintained as a stable emulsion. Overall, this study has again demonstrated the highly complex nature of interactions between FePS and fingermark residue, and suggested the presence of specific compounds is likely not the sole catalyst for preferential powder deposition.

4.4.3.3 Effect of substrate

The substrate type used to deposit natural and artificial marks influenced the quality scores of developed marks. Substrate type most greatly influenced the contrast scores of developed marks in this study, which are shown in Figure 4.18. These results show minimal differences between ceramic, glass and plastic substrates for natural fingermarks, and much wider differences for the artificial simulants. This graph shows the combined contrast scores for all surfactants and therefore the general effect of each substrate type. Interestingly, the plastic substrate produced the highest contrast scores for all artificial simulants while glass produced the lowest. Sebaceous residue and Pickering emulsion produced the most variation between substrates, an example of which is illustrated in Figure 4.19.

Developed sebaceous residue produced a 'bubbling' pattern around the edges of the stamped ridges. This was observed in development with all surfactants and some examples are illustrated in Figure 4.20. This is likely due to build up of residue around the edges of the stamp, however it was only present on the plastic substrate which suggests that substrate type is playing a role in the physical spreading of this simulant when applied with a rubber stamp.

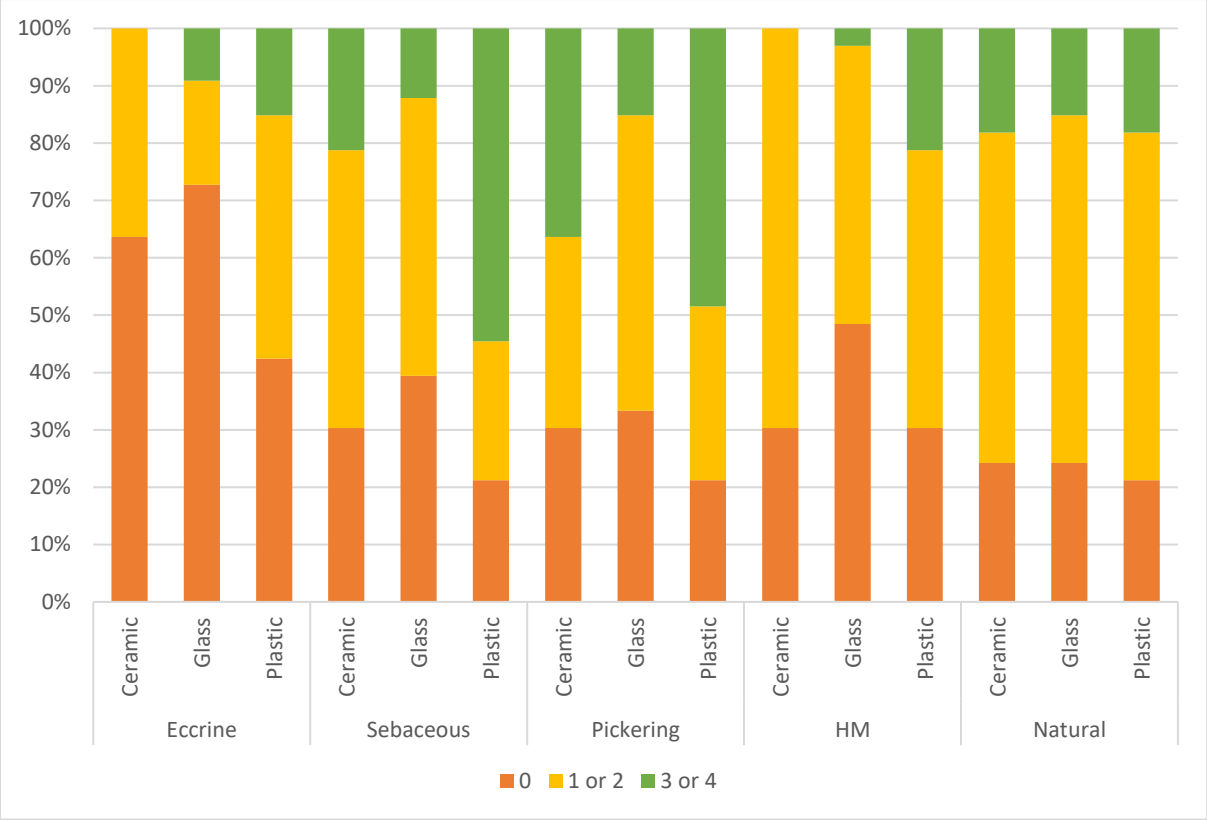


Figure 4.18 Combined contrast scores of all FePS formulations to develop natural (donor 1) and artificial fingermarks on each substrate

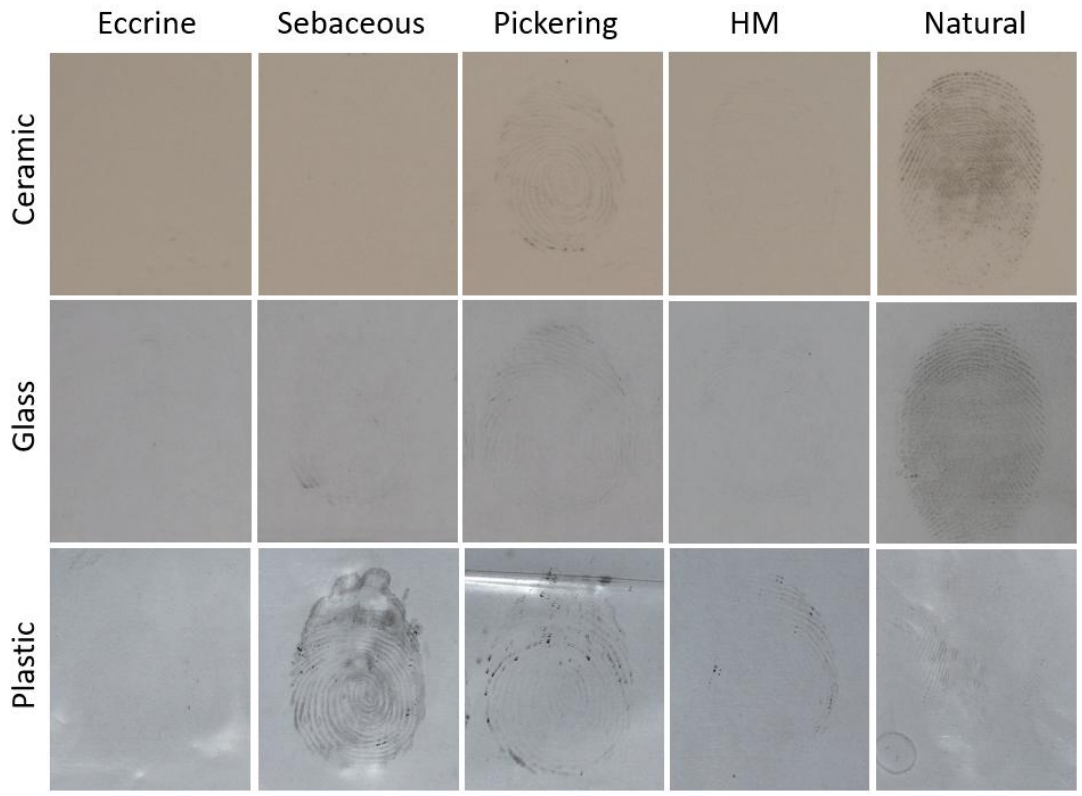


Figure 4.19 Examples of development of natural (donor 1) and artificial fingermarks developed using FePS made with T20 on each substrate

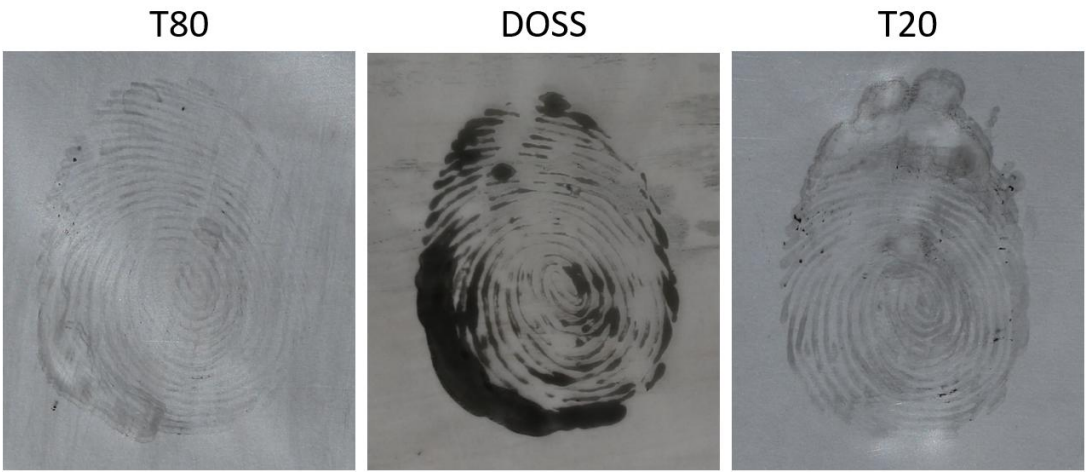


Figure 4.20 Examples of 'bubbling' produced by stamped sebaceous artificial residues on plastic substrate, developed using FePS made with T80, DOSS and T20

4.4.3.4 Effect of depletion

Artificial simulants and natural marks were deposited in a set of five sequential depletions in this study so the effect of reduced residue volume could be assessed. Figure 4.21 shows the combined ridge detail and contrast scores given to each depletion for the two assessed artificial emulsions and natural marks. Generally, little variation was observable between developed depletions. Natural residue demonstrated a very slight decrease in quality between depletions 1 and 5, while the emulsions slightly decreased in quality between depletions 1 and 3 before improving again from depletions 3 to 5. An example of this observation is shown in Figure 4.22. These images show that improvement in ridge detail in later depletions was partly due to improved consistency in the ridges, which may occur by physical interaction with the substrate during deposition better homogenising the emulsion on the stamp. The separated eccrine and sebaceous resides followed a similar pattern as the emulsions.

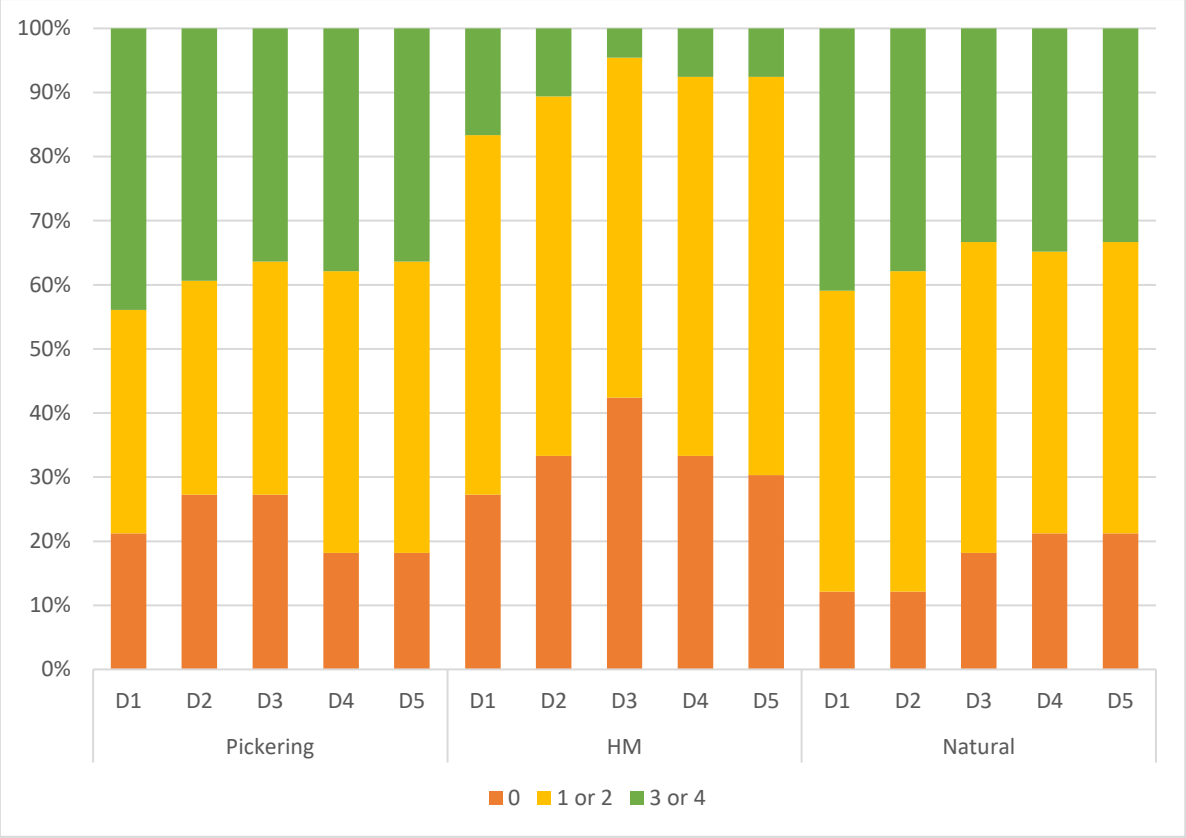


Figure 4.21 Combined ridge detail and contrast scores of all FePS formulations to develop natural (donor 1) and artificial emulsion fingermarks (Pickering and HM) on each depletion

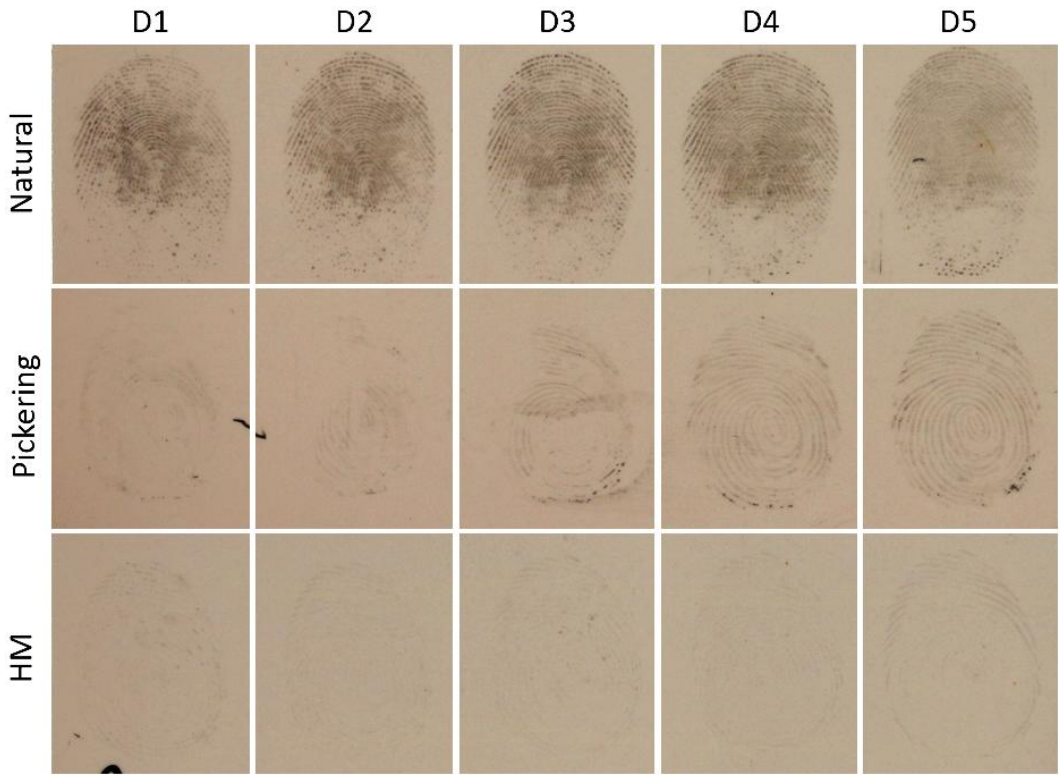


Figure 4.22 Example of effect of depletion on development of artificial emulsions (Pickering and HM) and natural (donor 1) fingermarks using FePS made with T20

This study has shown a high level of reactivity by FePS formulations with stamped sebaceous and Pickering residues. These simulants had a thicker consistency than the other residues, and it can be assumed that some part of the increased powder deposition along the ridges of these simulants was due to powder being 'trapped' within the residue during development. Although natural residue is known to vary wildly in the volume and composition of sebaceous residue, the stamping method used in this study is likely not able to replicate the very small amount present in natural marks [7, 150, 151]. However, improved development with later depletions of sebaceous and Pickering residues suggests that more simulant does not lead to more powder deposition, subsequently indicating that there is a more complex interaction with the powder depositing on these residues that is not wholly reliant on a physical 'trapping'. Surfactants, which were more effective at producing ridge detail and contrast on artificial marks were also prone to heavy background development, such as water formulations and those made with n-DDAA, DOSS and SP80/T80. This may be due to the surfactant allowing indiscriminate powder deposition which is better able to physically adhere to the larger volume of simulant residue.

Overall, this research has highlighted the importance of further research in the area of artificial fingermark simulants for research purposes. They have been shown to be useful in investigating the impact of variables in fingermark development techniques on residue targets and interactions, however the conclusions made from this work are limited by gaps in our understanding of residue composition and emulsions. The current capabilities of artificial emulsions have shown that the interactions between FePS formulations and compounds found in fingermark residue are likely not dependent on the presence of a specific compound, but rather a complex mixture which cannot currently be replicated artificially. Future work focussing on the composition of fingermark residue and development of more complex artificial emulsions will aid in better understanding of these residue targets.

4.5 Conclusion

These investigations were conducted to explore how specific fractions of fingermark residues are targeted by surfactants during FePS development using artificial fingermark simulants. The results of both studies agree that none of the tested simulants are able to adequately represent natural fingermark residue. It has highlighted the extremely complex nature of fingermark residue fractions and development techniques, and how little these relationships are currently understood. In the first part of the study, the donor differences present in marks loaded with simulants suggests that the physical properties of friction ridge skin play a larger role than previously suggested in influencing variability between natural donor fingermarks and their subsequent ability to be enhanced by

detection techniques. This may be the physical contact of deposition changing the components of present residues, such as the skin absorbing compounds, or depositing the emulsions in such a way that cannot yet be replicated by other techniques such as spot tests and stamping. No doubt several intrinsic factors influence this interaction, such as pH, temperature, and pressure of the donor, however it seems likely that the physical mechanism of deposition is impacting fingermark residue emulsions. These parameters are difficult to investigate specifically due to issues controlling and consistently reproducing them.

There seems to be an assumption that FePS formulations are targeting specific residue fractions, based on understanding of other technique mechanisms, and is most likely interacting with eccrine compounds protected by sebaceous residue. However, the poor development of artificial emulsions which contain both these products by formulations effective in developing natural marks in the second part of this study suggest it is not only the presence of specific compounds allowing preferential powder deposition. It can be suggested that the structure of the emulsion is also playing an important role, and that the addition of different surfactants influence what part of the residue is targeted in this technique, or what form an emulsion must be in (w/o or o/w) to be effectively developed. The results of both parts of the study are further indication that the physical act of deposition is affecting the residue emulsion in a way that is not replicated by artificial simulants but is vital to the successful and consistent development of fingermarks.

Overall, this study has further shown how complex the nature of latent fingermark residue is and indicated that successful enhancement using FePS requires a more intricate structure of residues than other techniques. It is clear that the type of surfactant used in this technique indeed influences what fraction of the fingermark residue is being targeted and this plays an important role in the successful development of fingermarks.

Chapter 5: An investigation of surfactant interaction with fingermark residue

5. *An investigation of surfactant interaction with fingermark residue*

5.1 Introduction

It is well documented that different development techniques interact with different components found in fingermark residue. For example, IND-Zn reacts with amino acids, while black powder sticks to moisture within the residue [22, 25, 27, 28]. Because of this, the amount and type of residue present in a fingermark greatly influences the ridge detail developed, and therefore the success of a development technique. However, when assessing fingermark development quality, it can be difficult to determine if poor development is due to issues with the technique or if the fingermark residue is lacking the relevant compounds for visualisation. The variability of natural residue between donors is similarly well documented, and it has been shown that the composition of fingermark residue changes through post-deposition processes such as ageing, wetting or heat exposure of the latent mark. Often these processes result in the removal or degradation of important compounds, typically to the detriment of fingermark enhancement methods [10, 16, 80, 130, 153, 154].

Despite this, several techniques may still be used on fingermarks that have degraded. Examples are PD and IND-Zn effectively developing aged fingermarks on porous substrates, while PS and VMD are effective on wetted and aged marks on non-porous substrates [7, 8, 20, 31, 41, 44, 81]. Understanding the effect of environmental influences and degradation processes on fingermark residue composition helps to determine suitable development techniques. Some techniques also require the use of water baths to rinse the item or heat, however there is limited research into how this may alter fingermark residue during development. Much of the research looking at natural residue composition has focussed on the process of ageing, as all latent fingermarks found at a crime scene will be aged to some degree. If the success of a development technique is measured by the amount and quality of fingermark ridges enhanced, scenarios where residue compounds may be altered or removed (through environmental processes or technique application) require further investigation to accurately evaluate technique performance and improve fingermark recovery rates. Surfactants are widely used for cleaning to remove fats and oils, and as such, may be impacting the amount of fingermark residue present for development.

5.1.1 Fingermark ageing

There is a general assumption that the processes of wetting and ageing fingermarks lead to a reduction in water content and water-soluble compounds, either through evaporation or dissolution [16, 155, 156]. A 2016 review by Kent into the water content of fingermarks within the context of handling recommendations for museum policy, stated that there was a widely held belief that fingerprint residue was 98-99% water, which implicates only a very small fraction of residue to contain organic or inorganic compounds that may potentially be damaging to delicate surfaces [13]. The authors note a lack of distinction between the amount of residue produced by the eccrine glands, what may actually be present on the fingertip and what is deposited into a fingermark. The paper suggests that soon after deposition, a fingermark is likely to contain an average water content of 20% or less due to evaporation both on the fingertip and after contact with a substrate [13]. In 2022, Boseley *et al*. wrote about the water content of fingermarks being evaporated, finding that the amount of water decreased over time and that there was also notable variation between donors [155].

All studies into the lipid composition of fingermark residue note the extreme difficulty of detecting trends in ageing studies due to donor variability, both between different donors and the same one on different days [14, 16, 137, 150, 151, 157]. Because of this, several studies have introduced fingermark 'charging' methods to improve consistency. Charging fingermarks to 'load' secretions with relevant compounds is used in some fingermark research, especially in early phase or proof-of-concept studies. For example, sebaceous-charged marks can be produced by touching the face and hairline where high concentrations of sebaceous glands are located. Eccrine-charged marks can be made by washing hands to remove contamination and either placing hands in clean PE bags to initiate sweating or not touching any surfaces before deposition to allow the eccrine glands to produce sweat. This allows for slightly more control over the composition of fingermark residue which can be useful when investigating the residue targets of a specific technique, or in scenarios where it is required to reduce donor variability and improve consistency of fingermark residue [14, 81, 129, 135, 144, 158, 159]. They are sometimes used as 'positive controls' to ensure certain compounds are present to react with a development technique. However, there are issues with using this method as charged marks are not usually representative of marks found in casework, causing either less sensitive techniques to appear more effective or hinder development using more sensitive techniques [80, 130, 153].

The changes in lipid concentrations of fingermark residue in aged fingermarks have been previously investigated. Mong *et al*. investigated the effect of fingermark ageing using gas chromatography/ mass spectrometry (GC-MS) analysis of the residue in 1999, and noted the inherent variability between donors affecting the author's ability to make generalised conclusions [157]. This investigation utilised

a large donor pool, however for the main study participants were encouraged to engage in "typical grooming motions" such as touching the face or forehead prior to development to ensure sebaceous residue was present. In an initial study, marks that were not charged and were deposited after handwashing, found that the only identifiable compounds present were water soluble salts. In the charged marks, despite donor variability, the main compounds present were squalene, cholesterol, oleic acid and palmitic acid. The identified compounds all demonstrated degradation in the 60-day timeframe, the amount decreasing over time and possibly degrading into smaller molecules. The authors suggest fingermark residue develops a waxy coating due to evaporation of water and transformation of unsaturated compounds to saturated analogues, which in turn may hinder the interaction of important residue components with development techniques [157].

These results were supported by Archer *et al*. in 2005, in an investigation which also used GC-MS analysis on sebaceous-charged fingermarks aged up to 33 days and reported rapid squalene loss after a few days [16]. For saturated fatty acids such as palmitic and stearic acid however, the authors noted a trend towards increasing levels up to about 20 days before falling back to or below original levels. The authors postulate that this was due to wax esters breaking down and/ or triglycerides degrading into fatty acids. This study also noted some differences in samples that were stored in dark or light conditions, with light generally leading to more pronounced degradation [16]. O'Neil *et al.* (2018) investigated the diffusion of fatty acids and triglycerides in ageing fingermark residue on various substrates, with the aim of creating a diffusion model to estimate the age of a deposited fingermark [160]. The authors found that diffusion of compounds assessed was greatly altered by the substrate's surface characteristics and temperature and note that the composition and behaviour of aged fingermark residue was more complex and variable than expected.

5.1.2 Fingermark wetting

Despite extensive research into the effect of ageing on fingermark residue composition, very little research has been performed into the effects of wetting fingermarks. Research has focussed on the success of traditional techniques on various substrates after wetting, however these investigations do not focus on the effects of wetting on the fingermark residue composition. Madkour *et al*. compared the success of CA, SPR and black powder on sebaceous-charged fingermarks on non-porous substrates that had been submerged in fresh or salt water [161]. The results found CA to be the most effective technique on wetted items, with SPR and black powder having variable results depending on substrate type. Salt water was more damaging to latent fingermarks than fresh water, and generally mark quality decreased with longer submersion times (up to 60 days). PS was not used in this study. Soltyszewski *et al*. (2007) investigated the enhancement of natural and charged fingermarks (using black magnetic

powder, aluminium powder and CA fuming) and recovery of DNA on glass slides submerged in water up to 6 weeks [162]. The results presented were brief but suggested that water submersion made fingermarks more difficult to develop, especially using aluminium powder, whilst no DNA recovery was achieved from submerged fingermarks. Several studies have demonstrated the effectiveness of PS formulations on non-charged aged and wetted fingermarks on a range of non-porous and adhesive substrates [44, 48, 55, 163]. Published literature only indicates that the effect of water submersion on fingermarks is likely to be removing the water-soluble components of residue, suggesting that effective techniques do not target these components.

Several development techniques, including PD, PS, SMD and SPR, are aqueous in nature and require a liquid surfactant solution to facilitate the successful enhancement of fingermarks [26]. These techniques are often effective on previously wetted fingermarks, suggesting that their development targets are either water-insoluble, or are protected by water-insoluble compounds during development. Surfactants are successful in their role as cleaning detergents by removing impurities such as oils, dust and dirt from a substrate. This is achieved through the formation of micelles, whereby oils and lipids are attracted to the hydrophobic part of a surfactant (the 'tail' of the molecule) and encased within the micelle and therefore removed from a substrate (further discussed in section 1.4) [56, 57, 70]. It can therefore be hypothesised that surfactant solutions, especially at higher concentrations, may be removing parts of the fingermark residue during the application of aqueous development techniques containing them. There is no current published research investigating how fingermark residue is affected by either water or surfactant solutions in the context of fingermark development. Better understanding these interactions will improve our capabilities of identifying residue targets of aqueous techniques, and in turn may allow the improvement of technique specificity and effectiveness.

The results of previous work presented in this thesis has demonstrated examples of no ridge development following development using some FePS formulations (such as lack of development using DOSS at 50x CMC in section 3.3.3). However, it has been unclear whether this lack of development is due to inadequate powder deposition (i.e. surfactant type or concentration is preventing powder deposition) or if the application of the surfactant solution is removing components of the fingermark residue. It is possible that surfactant solutions, especially at higher concentrations, are removing water-insoluble components and 'cleaning' the fingermarks off the substrate. It is also possible that surfactants, or other chemicals contained within fingermark developers, are altering the emulsion state of the residue. Due to a lack of previous research involving interactions between surfactant solutions and fingermark residues, this serves to limit the analysis of such results. An

investigation into the possible removal or alteration of fingermark residue using surfactant solutions will facilitate better understanding of the FePS development mechanism.

5.2 Part 1: Effect of surfactant wash on residue volume

5.2.1 Materials and method

To investigate the interaction between fingermark residue and surfactant solutions without powders, fingermark residues were microscopically observed before and after rinsing with surfactant solutions to visually determine if residues are being removed or modified. Rinsed marks were then developed with FePS to determine how much residue is required to be present for successful ridge development.

5.2.1.1 Deposition of fingermarks

Natural and charged marks from two donors were used in this study, as well as the four artificial simulants (Pickering and HM emulsions, eccrine and sebaceous simulants) used in section 4.4. All fingermarks were deposited on clear glass tiles which were cleaned with acetone and Kimtech™ wipes prior to deposition. Donors were asked to charge both eccrine and sebaceous-charged fingermarks as recommended by Moret (2015) and Sears (2012) [130, 159]. Eccrine-charged marks were produced by washing hands before waiting 15 minutes without touching anything to allow the eccrine perspiration to replenish on the fingertips. No current published research is available regarding replenishment times, however anecdotal evidence indicates 15 mins is sufficient for fingers to become eccrine-rich after handwashing. Sebaceous-charged marks were created by donors washing their hands before rubbing their fingertips around the nose for 10 seconds where sebum is produced. To deposit natural, uncharged marks, donors were asked to wash their hands before going about normal daily activities for an hour. For all types of donor marks, the fingertips of both hands were rubbed together before deposition to evenly spread the residue constituents. For each sample, one finger was used to deposit marks in a set of three depletions. Ethics approval for collection of donor fingermarks was completed through the University of Technology Sydney (ETH18–2521, Appendix A) and participants were required to consent to the collection of their fingermarks prior to deposition.

All artificial simulants were deposited by pipetting 5 µL of the simulant onto a glass tile before rubbing a rubber fingerprint stamp tested in section 4.3.2 into the residue to coat the surface. The stamps were then pressed onto the sample substrate in a set of three depletions with an approximate deposition pressure of 400-500 g. Eleven samples with donor and artificial fingermarks, as illustrated in Figure 5.1, were created to be washed with different surfactant solutions. A total of 330 fingermarks (donor and artificial) were deposited and all marks were aged in laboratory conditions for 24 hrs before microscopic visualisation and washing with surfactant.

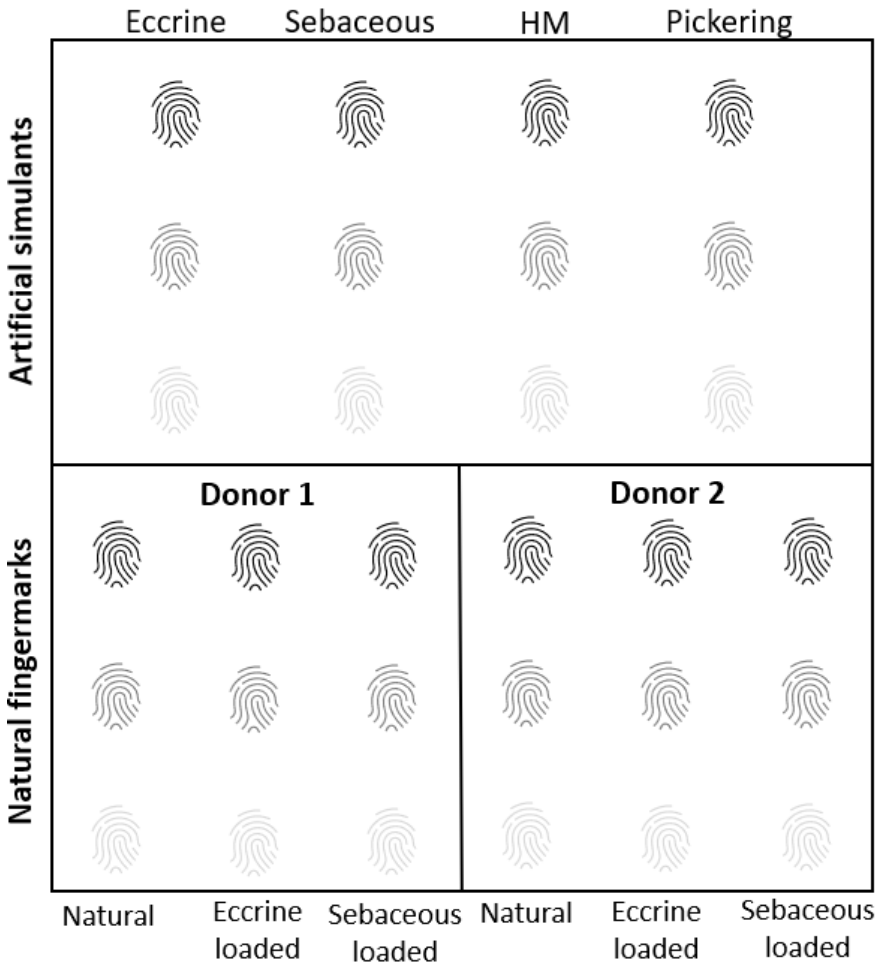


Figure 5.1 Deposition layout of artificial fingermark residue simulants using a rubber stamp, and donor fingermarks on one glass tile for use in surfactant wash experiments

5.2.1.2 Surfactant wash

Eleven samples were prepared with the marks described above in section 5.2.1.1 and each sample washed with a different surfactant solution. Ten surfactant solutions, as used in the optimised formulations (omitting the iron oxide powder) determined in section 2.3.3, were assessed as well as one with only water used as a control. The concentrations of surfactant washes used are outlined in Table 5.1. The surfactants were applied using a clean squirrel hair wet powder brush in the same manner as FePS has been applied for the duration of this study, by soaking the brush and gently brushing across the substrate. Surfactants were then gently rinsed off the substrate with running water.

Table 5.1 Surfactant solutions used to wash fingermarks, based on optimised formulations determined in section 2.3.3

Surfactant	Surfactant code	Weight of surfactant in 100 mL water (g)
Triton X-100	TX100	13.4
Kodak Photo-Flo	KP	25
Tween 20	T20	2.75
Tween 80	T80	6.6
Span 80/ Tween 80	SP80/T80	10
Dioctyl sulfosuccinate	DOSS	0.75
Sodium dodecyl sulfate	SDS	2.9
Liqui-Nox	LN	25
Hexadecyl trimethyl ammonium bromide	CTAB	0.728
n-dodecylamine acetate	n-DDAA	0.490

5.2.1.3 Visualisation and quantification of residue

All fingermarks were visualised under a microscope before and after the surfactant wash to allow for comparison of residue coverage. Residue was visualised as recommended by Moret (2015) using phase contrast microscopy on the Leica DMLS microscope under 4x magnification equipped with Leica DFC295 camera and controlled with Leica Application Suite software, version 4.1.0 [159]. Before initial visualisation, small boxes were drawn on the approximate centre of each fingermark to use as a reference so that precisely the same area could be visualised before and after washing. After fingermarks were washed with surfactant solutions, samples were left to dry on a laboratory bench for 24 hrs before visualisation and image capture.

Once each fingermark had been imaged before and after washing, the images were scaled and processed. First, image processing software GIMP (version 2.10.24) was used to crop sections of each image to 1000x1000 pixels to ensure that the washed and unwashed image sections were visualising the same area. These images were then used to determine how much of the visualised area was covered by fingermark residue with Image J (version 2.9.0/1.54d) image processing software. In Image J, all images were first converted to 8-bit so they were presented in greyscale. A colour threshold was then run so the software highlighted residue present within the image, as shown in red in Figure 5.2, and calculated the percentage of the image covered. These values were then exported to a Microsoft Excel sheet for further analysis. With this method, the amount of residue present on the same area of

a fingermark was able to be quantified (as a percentage of area covered) before and after surfactant washing.

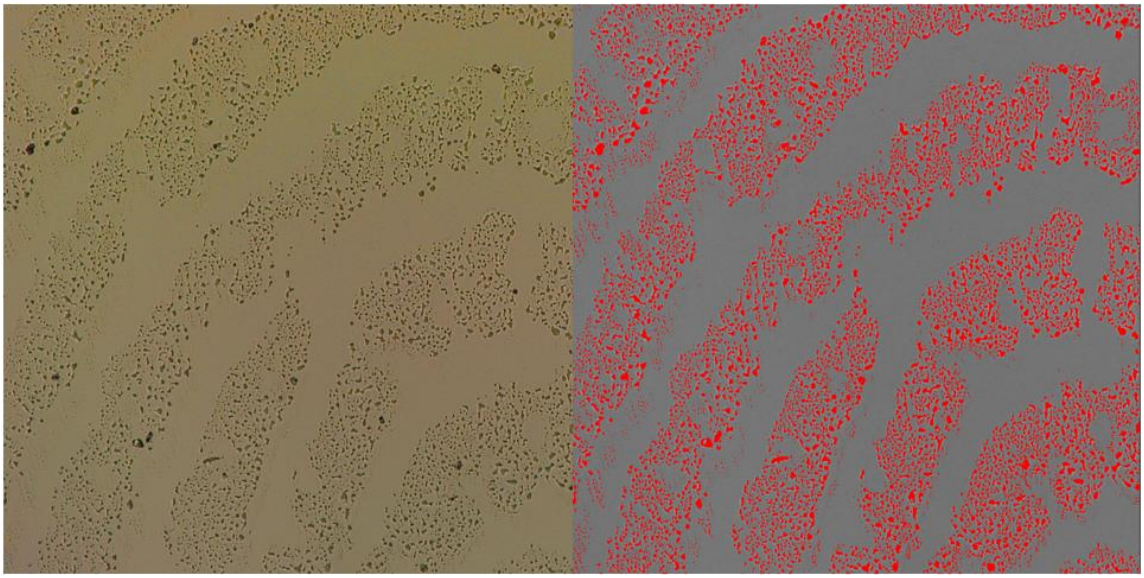


Figure 5.2 Example of Image J threshold residue quantification process

5.2.1.4 Analysis of results

The amount of residue present on the substrate of interest was calculated by determining what percentage of the imaged area contained visual fingermark residue components, both pre and post surfactant wash. Two subsequent calculations were used to assess the difference between surfactants in their ability to remove fingermark residue. The first was calculating the percentage difference, simply a deduction of the percentage of residue present post-wash from the value calculated pre-wash. This was used to infer the relative amount of residue present on the substrate, as it was shown that the amount of pre-wash residue present varied greatly due to donor variability.

Because of this donor variability, reliance on only percentage difference would result in highly skewed data. This is because if the initial percentage of residue present is very low, there is less to be removed by a surfactant regardless of how effective it was at removing residue. Therefore, the result of each surfactant wash was heavily dependent on the initial fingermark volume present. As current fingermark research capabilities do not allow for control of fingermark residue, the percentage difference was not the only value to be considered. To minimise the impact of donor variation, percentage change was also calculated. This was done to represent the value differences as a percentage of the starting residue volume, and the pre-wash percentage was considered 100% of the residue available after the wash was conducted. To do this, the percentage change previously calculated was represented as a portion of the initial area. For example, if a fingermark initially covered 2.5% of the imaged area this would be considered the total volume possible to be recovered

after surfactant washing. As such, if the post-wash residue covered 0.05% of the area, this was a reduction of 2.45% (percentage difference) but could also be considered a removal of 98% of starting residue (percentage change). Both values were considered during the analysis of the results.

5.2.1.5 Development of washed marks

After the fingermarks had been washed and visualised under the microscope, FePS was applied to washed samples to determine if marks could still be enhanced with this technique. An optimised formulation using DOSS in the same concentration as the surfactant wash combined with Fisher Chemicals magnetic iron oxide powder in a 1:2 w/v (powder (g): surfactant (mL)) ratio was used. This formulation was chosen due to previous success in developing both donor and artificial fingermarks in sections 2.3 and 4.4.3 respectively. The DOSS formulation had been shown to produce heavy background development in previous work, however the use of a FePS that usually produced background development allowed for additional observation of whether the surfactant wash step influenced the surface-wide deposition of powder or not. The developed fingermarks were photographed using a Canon EOS 800D DSLR camera with a Canon EF-S 60mm macro lens and graded using the CAST scale as shown in Table 5.2 by one assessor.

Table 5.2 Modified CAST scale used to assess fingermarks [80]

Grade	Detail visualised
0	No evidence of a fingermark
1	Some evidence of a fingermark
2	Less than 1/3 clear ridge detail
3	Between 1/3 and 2/3 clear ridge detail
4	Over 2/3 clear ridge detail

5.2.2 Results

5.2.2.1 Visualisation of residues

Before quantification of the amount of residue present was conducted, clear differences were observed between different types of residues (natural, charged and artificial) as well as between donors. All fingermarks were collected on the same day to minimise intra-donor variability, however notable differences were still observed.

Examples of how each type of residue was visualised are illustrated in Figure 5.3. This shows that for both donors, clearer ridge boundaries could be seen with the sebaceous-charged marks compared to

natural and eccrine-charged. The charged fingermarks of each donor also showed clear differences, demonstrating that despite the same method being followed by each donor, variation in the amount of eccrine and sebaceous residue was reflected in the percentage of area covered by residue. None of the artificially stamped marks were visually similar to donor marks, with the sebaceous and Pickering residues generally containing larger aggregate areas and the eccrine and HM emulsion appearing spotty, without clear ridges. These observations are consistent with results presented in chapter 4.

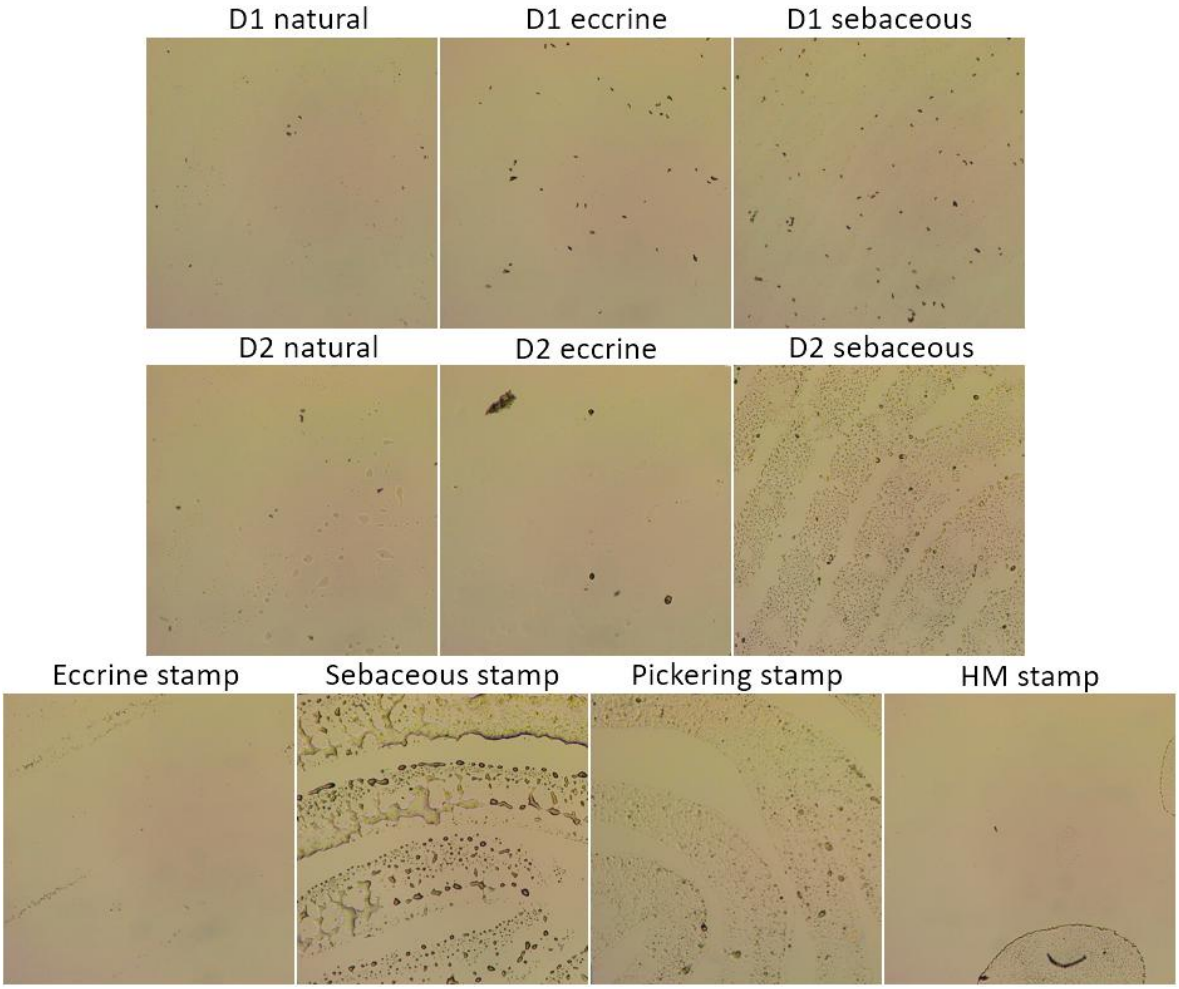


Figure 5.3 Examples of each residue type deposited on glass visualised with phase contrast microscopy at 4x magnification

The highest amount of residue present after deposition (indicated by a higher percentage of the imaged area being covered) was produced by artificial sebaceous and Pickering residues, shown in Figure 5.4. There was also a wide variation in the spread of the area coverage of these simulants, showing that the stamp application methodology did not lead to uniform or consistent deposition volume. The eccrine and HM simulants covered much less of the imaged area, due largely to the spotty deposition resulting in very inconsistent ridge visualisation. The aggregate areas present in these residues also appeared smaller than the other artificial simulants, as seen in Figure 5.3. Variations

were also seen in the percentage coverage produced by natural donor marks, shown in Figure 5.5. The average percentage coverage, as well as the spread between scores (difference between highest and lowest percentage), was much less than the artificial simulants, showing that volume of natural donor residue deposited is generally more consistent than that of the rubber-stamped artificial marks. The results show that donor 2 marks charged with sebaceous residue had the highest percentage of area covered by residue that could be visualised, as well as greater variation in scores. For both donors, eccrine-charged residue resulted in the least amount of visualised residue.

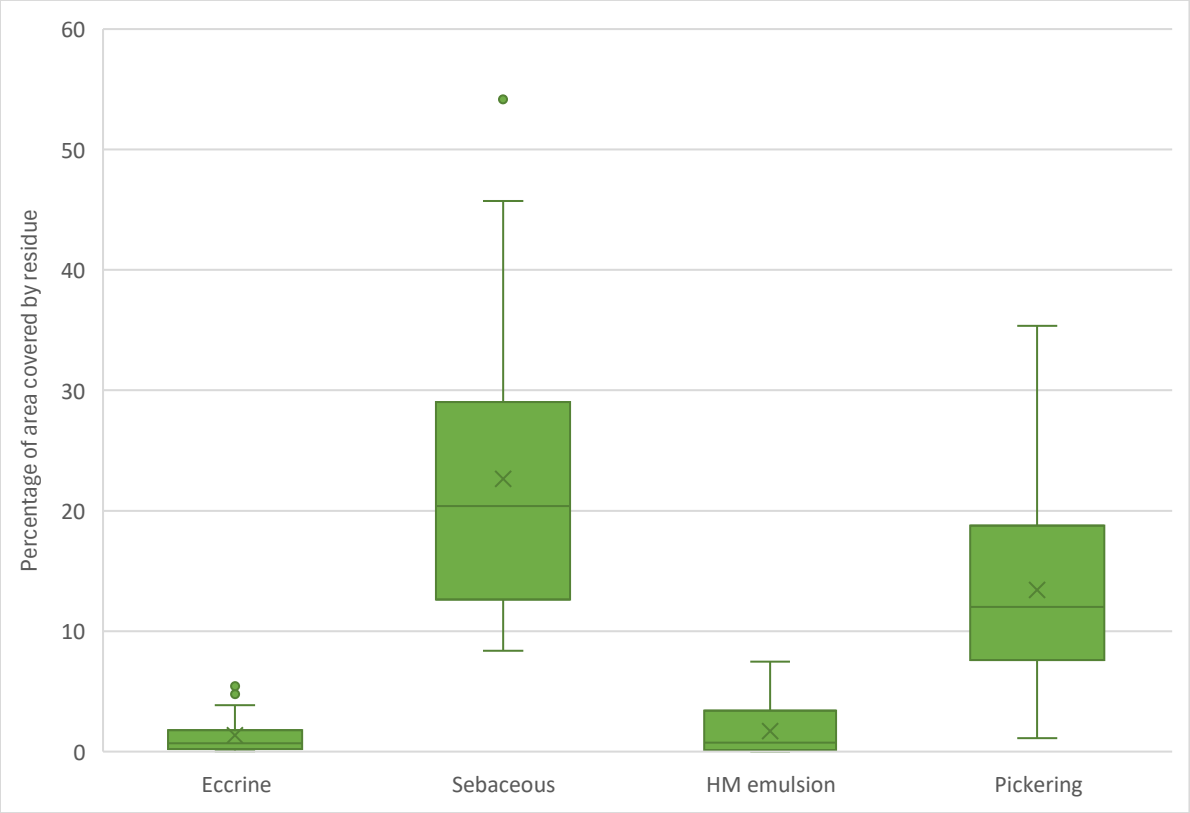


Figure 5.4 Percentage coverage spread of pre-wash artificial residues present in imaged area of all artificial fingermarks assessed

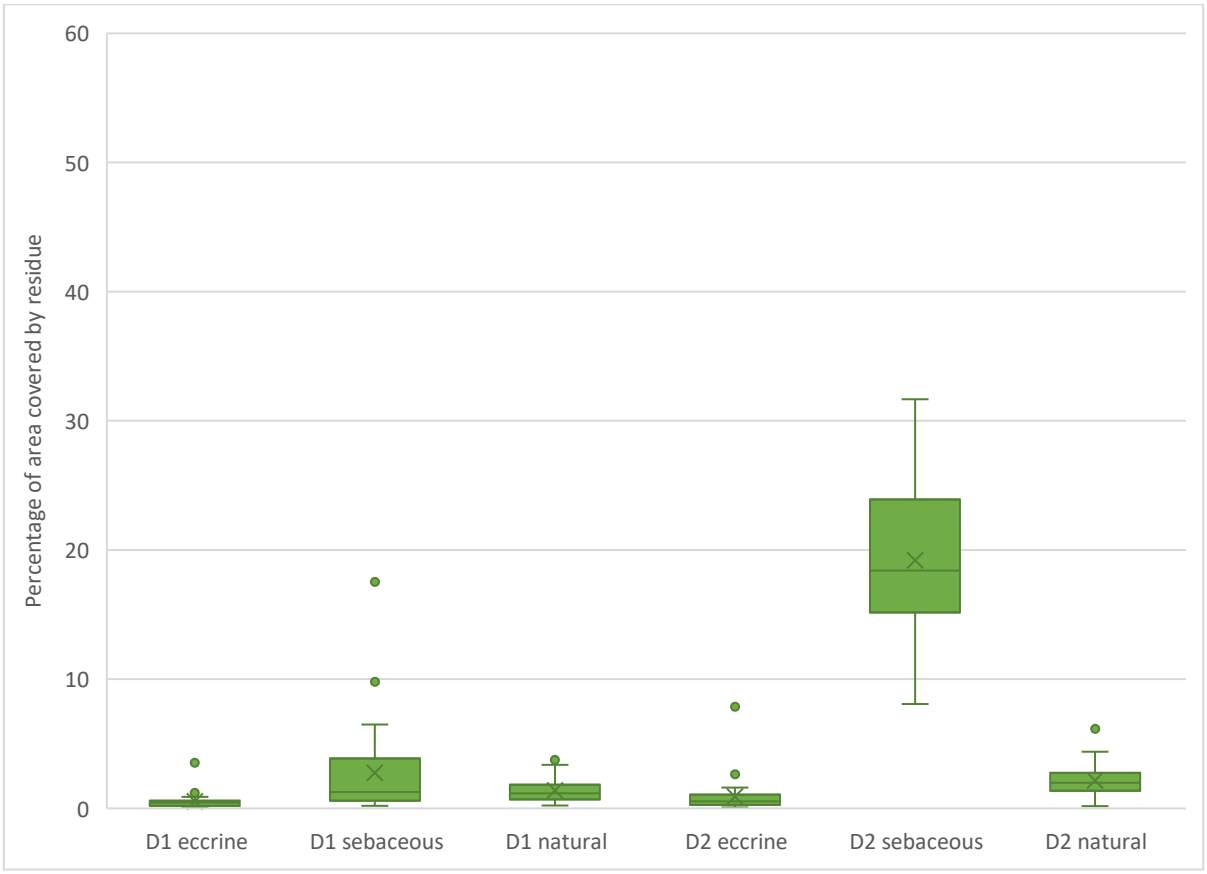


Figure 5.5 Percentage coverage spread of pre-wash donor residues present in imaged area of all donor fingermarks assessed

These results show that donor variability was an important factor to consider when using this method. Due to the high degree of variation between stamped artificial residues, the results for these marks were not included in subsequent analysis, and only donor fingermarks were used for later surfactant comparison. The method used to quantify the amount of residue present on the substrate also had limitations, as it was not able to consider the height of residue present. Because of this, the actual volume of residue on a substrate, especially for sebaceous residues (artificial and natural) containing larger compounds, could not be determined. This impacted the artificial residues most of all, further suggesting that the use of stamped artificial residues was not appropriate for comparing amount of residue removed in this way. Results from previous investigations using the artificial simulants demonstrated they are not currently capable of mimicking fingermark residue composition, and application using the stamp provides difficulty in controlling the amount of residue present. Artificial residues were initially included to provide a more consistent chemical equivalent for comparison, however the outlined issues in using this method demonstrated they were unsuitable for further analysis. Further investigations using a deposition method that can better control the volume of residue deposited, such as use of a modified inkjet printer investigated in previous literature, may be conducted to minimise this issue [96, 98, 141, 164, 165].

Clear ridges were much more visible using phase microscopy in fingermark residue containing sebaceous material, such as the sebaceous-charged donor fingermarks and the two artificial emulsions. This may suggest that the lipids present in sebaceous material are more easily visualised with this method. Moret *et al*. (2015) showed that the use of phase contrast microscopy to visualise natural and charged fingermarks on glass was a sensitive and non-destructive method of visualisation [159]. The authors found that 'weaker' eccrine-charged marks could be visualised, however sebaceous-charged marks were the most clearly defined. This research also noted that using microscopy, different droplet sizes and shapes were observed within the residues.

The appearance of heterogenous fingermark residue is supported by Dorakumbura *et al*. (2018), who demonstrated that fingermark residue (charged and natural) is not deposited uniformly within the ridges [137]. The authors investigated the spatial distribution of compounds within fingermark residue and note that because of this heterogeneity, studies investigating ageing on fingermark residues should ensure to visualise exactly the same area on a mark. This research also found that eccrine-charged donor fingermarks contained more lipids than expected. As such, it cannot be assumed that the eccrine-charged fingermarks used in this study do not contain any lipids despite difficulty in visualising these residues.

5.2.2.2 Surfactant comparison

The variation between the percentage change of natural residues from both donors was calculated and shown in Figure 5.6. The sample size for these results was small, with a total of 6 fingermarks assessed for each surfactant. It is clear from Figure 5.6 that there is huge variation in the percentage change scores. It should be noted however that the majority of removal scores after the water wash were lower than for any surfactant, indicating that the addition of surfactant to the wash was generally removing more residue that just water. These observations, however, were not consistent for each type of residue (eccrine or sebaceous charged) tested so these results cannot be used to indicate that one surfactant was better able to remove residue than others. This is likely due to the small sample size used and the variety of pre-wash residue coverage, however it is also possible that each surfactant is interacting with each residue type (or components of each residue) in a different way. Further work investigating the chemical composition of residues before and after processes such as wetting would aid in furthering this understanding. Techniques that may be utilised for such future investigations include matrix assisted laser desorption/ ionization mass spectrometry (MALDI MS) and GC-MS analysis to better understand what chemical species are present in fingermark residue before and after water or surfactant washes.

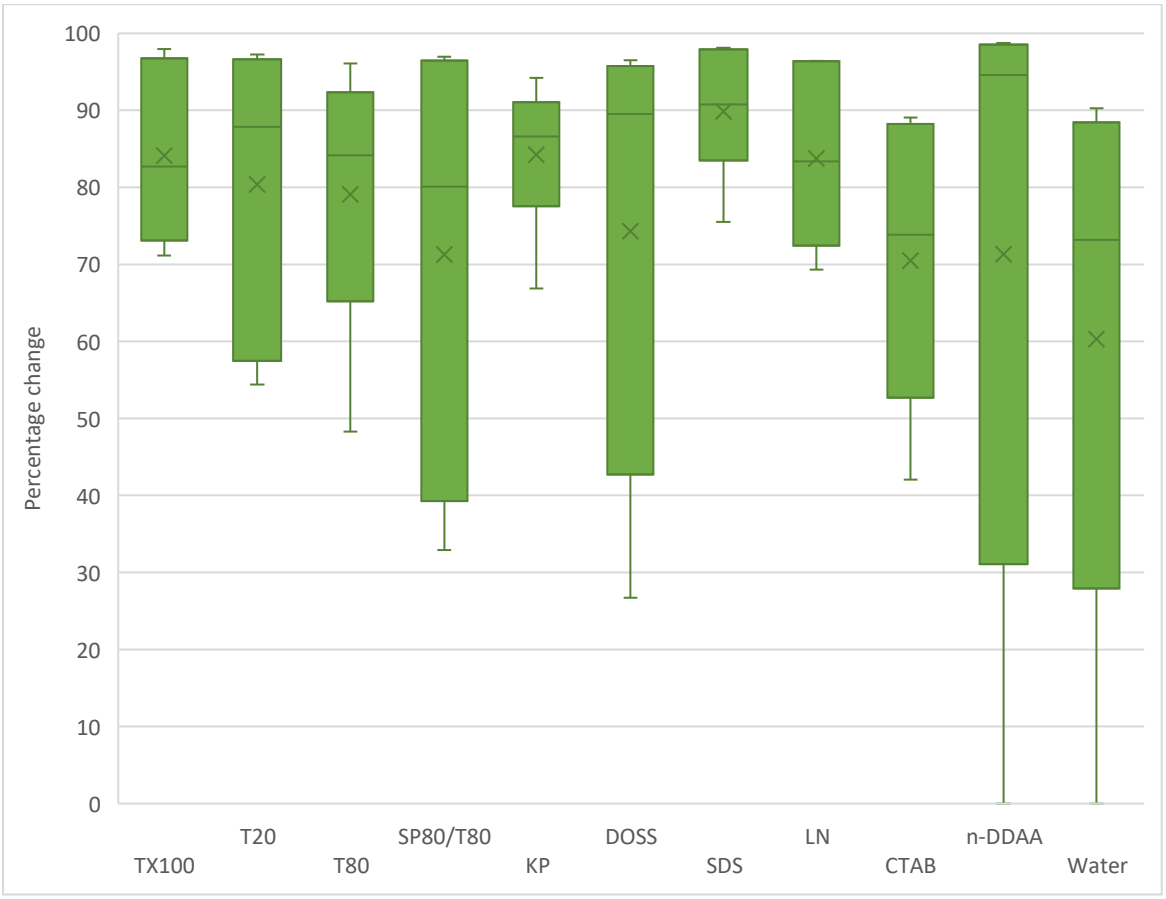


Figure 5.6 Spread of percentage change scores (how much of initial residue was removed) of all natural fingermarks assessed for each surfactant

There is currently no published literature investigating the removal of fingermark residue during the application of aqueous fingermark development techniques. In the context of stain removal from fabrics, Rosik states that "it is well known that surfactants remove sebum", and that sebum with higher concentrations of squalene are more difficult to remove [166]. A 1985 shampoo evaluation study by Thompson *et al*. reported that polar sebum components are more readily removed than non-polar components, the success of which depends on the surfactant used [166, 167]. Due to donor variability, the ability of surfactants to remove some fingermark residue components and not others may depend on the amount of squalene present in each donor's fingermarks.

The removal of any oily or fatty residues by a surfactant is logically reliant on the concentration of surfactant present, as well as the physical application method. Anionic surfactants are often used in laundry and detergents due to their ability to effectively remove grease and oils from a range of substrates [57, 60]. The manufacturer of Liqui-Nox detergent, Alconox, state on their website that this surfactant can "effectively remove fingerprint residue" and is effective for scrubbing, soaking or sonicating [168]. None of the tested surfactants completely removed the presence of visible fingermark residue, which may be attributed in part to the gentler application of the surfactants with

a squirrel hair brush compared to other common cleaning methods, showing the physical interaction is playing a larger role than just the presence of micelles in a solution. The lack of removal may also be due to the use of lower concentrations than recommended for washing (LN concentration was 25% of the manufactured product) as well as the application being relatively gentle. In FePS formulations, it is likely that surfactant micelles are largely present in solution encapsulating powder particles. This study has shown that in the absence of powder particles, the surfactant micelles present are still not interacting with fingermark residue to completely remove the components. This suggests that with the application technique used, free surfactant micelles are not responsible for fingermark residue removal. Only one concentration of each surfactant was investigated in this study, and future work may consider testing higher and lower concentrations as well as a wider range of surfactants and application methods.

5.2.2.3 Development of washed marks

After the washed fingermark residue was visualised using microscopy, the substrates were developed with FePS to determine if the washed marks could still be detected using this technique. A formulation made with DOSS was used despite previous results showing background development with this formulation, to assess the impact of surfactant washes on background development. The CAST scores given to developed fingermarks (natural and charged, total of 60 marks per surfactant) are shown in Figure 5.7. The scores given to developed fingermarks washed with only water are represented in the background colour of the graph. These results show that addition of a surfactant wash does not have a large impact on ridge detail development with DOSS FePS. The least ridge detail was produced by CTAB, n-DDAA and LN surfactants, suggesting that these surfactants may be removing some parts of fingermark residue. However, fingermarks up to grade 3 were able to be developed with FePS after washing with all assessed surfactants, showing that none of the surfactants (at the concentrations tested) removed fingermark residue enough with the application method used to notably impact development.

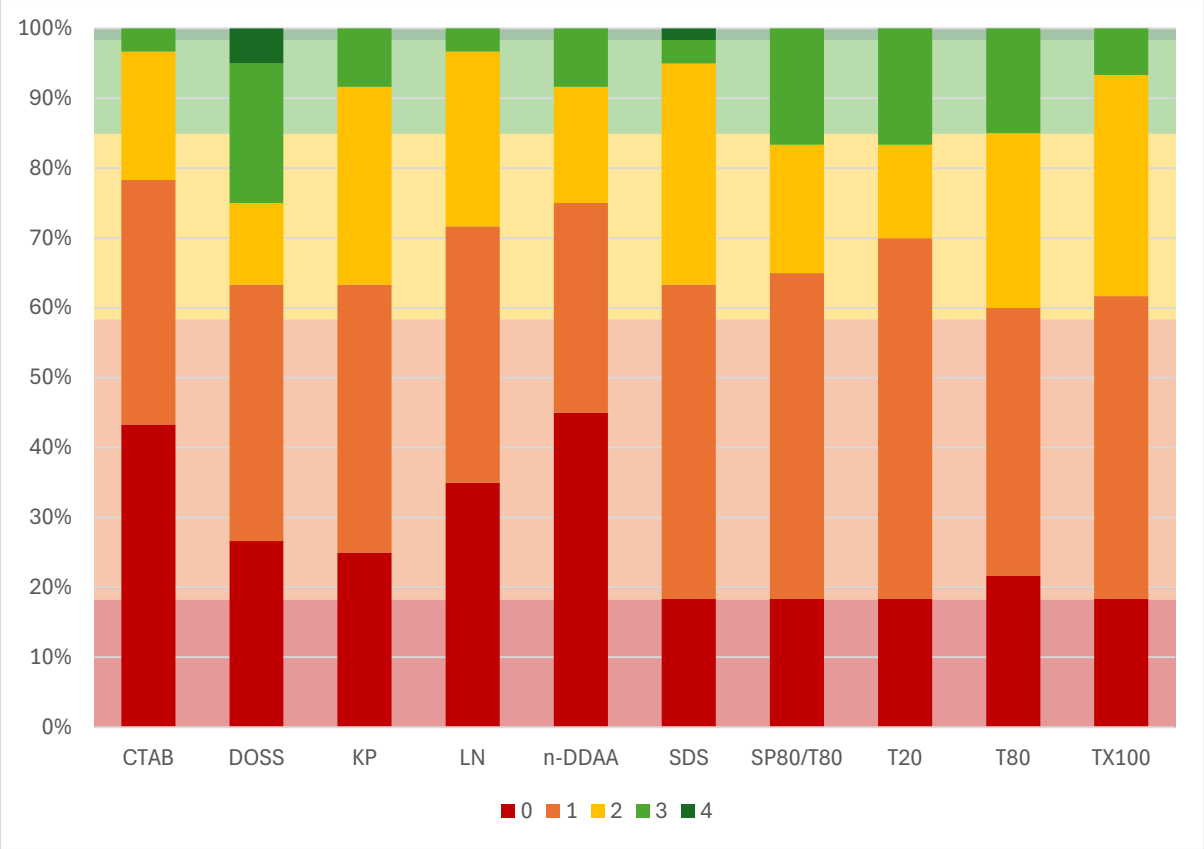


Figure 5.7 CAST scores of donor fingermarks developed with DOSS FePS formulation after being washed with each surfactant. Scores of development after washing with water-only shown in background

Previous results showed that FePS formulations made with DOSS were prone to background development (section 2.3.3), however it was noted in this investigation that depending on the surfactant wash applied before development, this background development could be reduced. Examples of these variations are shown in Figure 5.8. Background development was reduced by TX100, T20, LN and KP surfactant washes. These are all surfactants that previously produced little to no background development when used in FePS formulations and it is possible that some residual surfactant was present on the substrate after the washing step, despite being rinsed with water. Therefore, it may be suggested that the small amount of residual surfactant left on the substrate was enough to prevent powder deposition, either by repelling powders or forming micelles and creating a more stable suspension than the one made with DOSS. The reduction of background development was not consistent with the ridge detail scores, showing that less background development does not lead to higher ridge detail scores.

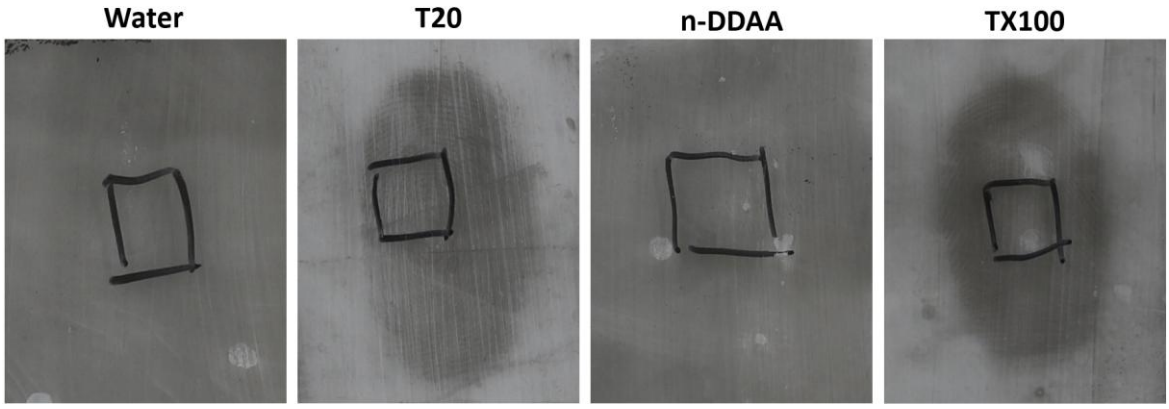


Figure 5.8 Examples of background development produced by DOSS FePS development of donor 2 natural fingermarks after water, T20, n-DDAA and TX100 surfactant washes. Squares drawn indicate area visualised during microscopy

The scores of developed fingermarks were then compared with the amount of residue visualised after washing to determine if a certain amount of residue must be present to develop high quality fingermarks. The spread of residue percentage post-wash for each CAST quality grade of developed marks is shown in Figure 5.9. Many fingermarks contained only a small amount of residue that was visualised using phase contrast microscopy both before and after washing, especially eccrine-charged marks (Figure 5.5). Despite the lack of visual residue, ridge detail was still able to be developed with many of these fingermarks. Notably, artificial eccrine simulant and HM emulsion stamped marks did not show any visible ridges under the microscope (Figure 5.4), appearing rather as concentrated spots of residue. However, when the FePS formulation was applied, ridge detail was visualised that was not apparent using microscopy. This indicated that for these residues, some compounds were present that aid in preferential powder deposition with FePS but cannot be visualised with phase contrast microscopy. The spread of visualised residue present for each CAST grade shown in Figure 5.9 does suggest, however, that fingermarks with higher residue coverage mostly produce greater ridge detail grades. Despite this, natural donor fingermarks with very small amounts of visual residue did not prevent the development of high-quality ridge detail scores (3 or 4), indicating again that the interaction between FePS and fingermark residue is highly complex and not dependent on the presence of one specific residue fraction.

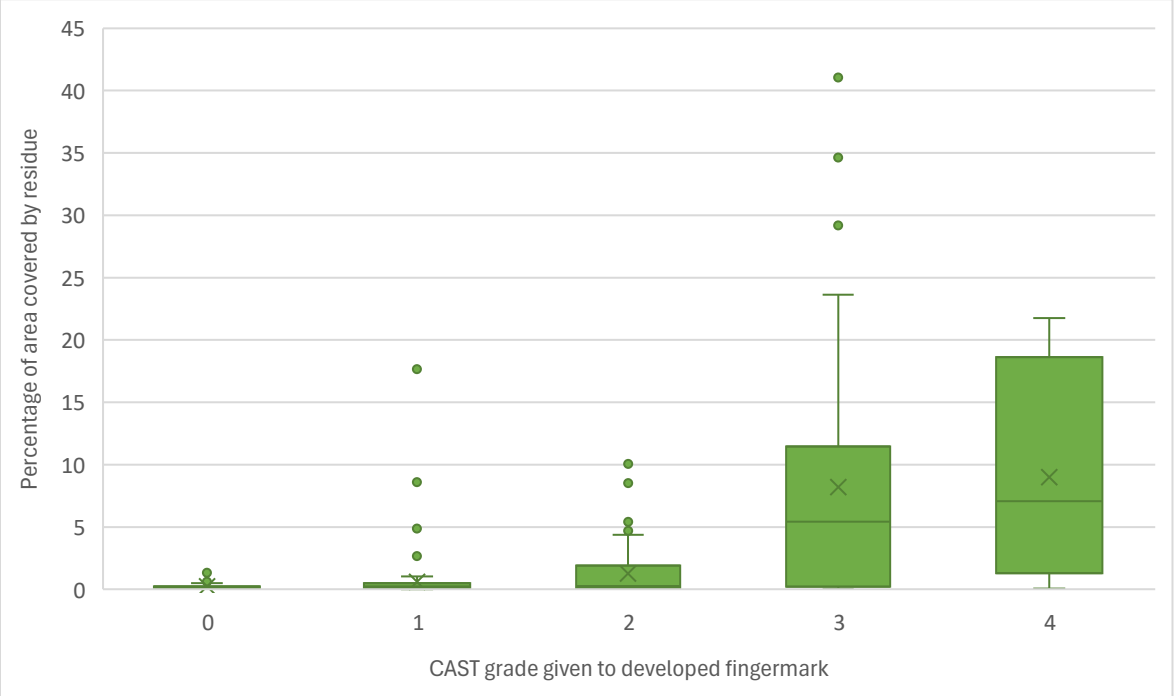


Figure 5.9 Percentage of area covered by donor fingermark residue post-wash for fingermarks developed with each CAST grade. ('0' n= 66, '1' n= 145, '2' n= 78, '3' n= 37, '4' n= 4)

5.3 Part 2: Effect of surfactant concentration on residue removal

5.3.1 Materials and method

To better observe the effect of surfactant wash concentration on fingermark development, various concentrations of T20 were used to wash natural fingermarks before developing with a T20 FePS formulation. T20 was chosen as it is the currently recommended surfactant for use in FePS and is therefore more likely to have practical applications [41].

5.3.1.1 Deposition of fingermarks

Natural fingermarks were collected from four donors on glass tiles for this study. The glass tiles were prepared in the same way as in section 2.2.2. Donors were asked to not wash their hands or obviously contaminate them with residues, such as food oils and cosmetics, but otherwise go about normal activities for an hour before depositing. Fingermarks from the three middle fingers of either hand were deposited as split marks with the middle finger across the seam of two samples, in a set of three depletions (as in section 3.2.3, Figure 3.3). Fingermarks were left to age inside a drawer in controlled laboratory conditions for 24 hrs before being washed and developed with FePS. A total of 900 fingermarks were tested in this stage.

5.3.1.2 Surfactant wash and fingermark development

Five T20 wash solutions were made to encompass a range of concentrations previously used throughout this thesis. Concentrations at 0.5x and 50x CMC were made (as in section 3.2.2), as well as the standard (10%) and quarter (2.5%) concentrations used in section 2.2.1, as shown in Table 5.3. A control wash of only water was also used (T1).

Table 5.3 Concentrations of T20 surfactant wash solutions applied to fingermarks before development with FePS

Surfactant code	Mass (g) Tween 20 in 1 L water	How many x CMC	Previous use
T1	0 (water)	n/a	n/a
T2	0.0429	0.5	Chapter 3
T3	4.29	50	Chapter 3
T4	27.5 (quarter)	320	Chapter 2 and 4 (2.5%)
T5	110 (standard)	1280	Chapter 2 (10%)

Once fingermarks were deposited, half of the split sample was washed using one of the wash solutions (T1-5), as shown in Figure 5.10. This was done to better compare the effect of washing fingermarks with various concentrations of surfactant on subsequent development. The surfactant washes were applied by saturating a wet squirrel-hair brush used for FePS development and gently brushing it along the substrate. The washed samples were then rinsed under tap water to remove any surfactant residue and left to air dry on a bench in controlled laboratory conditions.

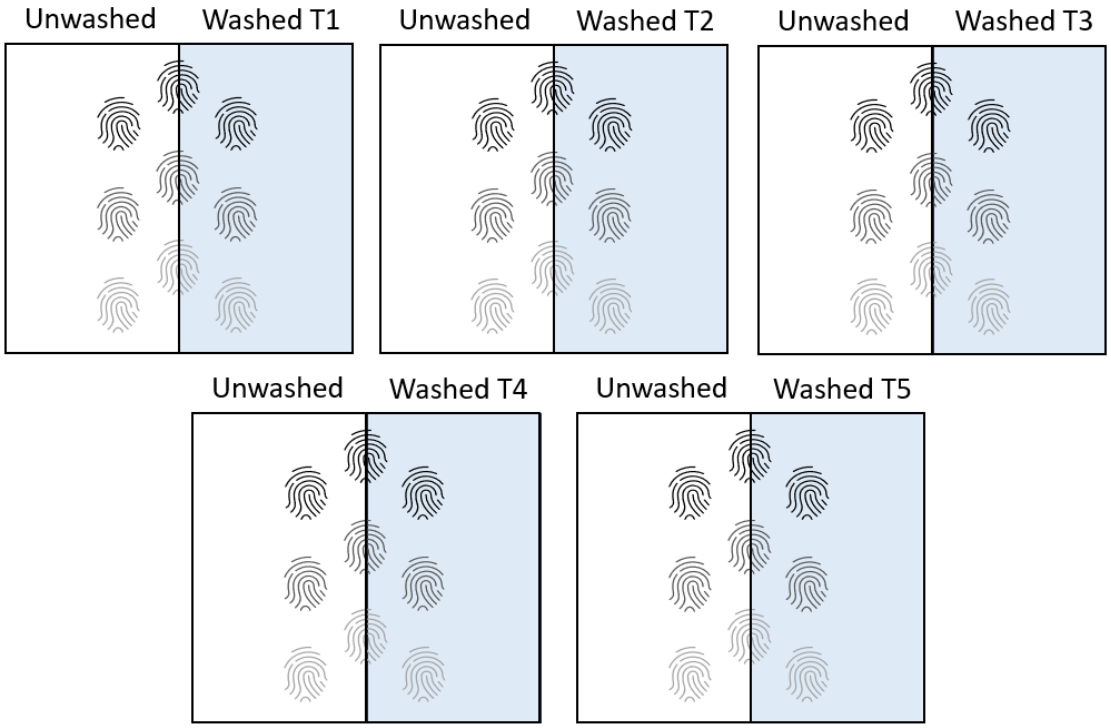


Figure 5.10 Deposition of one 'set' of split fingermarks on glass substrates. Marks on the washed side (right) rinsed with wash solution (T1-5) before development. Repeated for all four donors

Once the washed samples were dry, all marks were developed with FePS. Each 'set' of samples (illustrated in Figure 5.10) were developed with a FePS formulation made with the same concentrations of surfactant solutions (T1-5) using the method described in section 2.2.3. The FePS formulations and their relationship to the wash concentrations are outlined in Table 5.4. Five sets of fingermarks were processed in this way, to ensure all combinations of washing (T1-5) and development (FePS1-5) were assessed. Fingermarks were left to dry on a bench in controlled laboratory conditions overnight and then photographed using a Canon EOS 800D DSLR camera with a Canon EF-S 60mm macro lens and visualised under standard laboratory ambient lighting.

Table 5.4 FePS formulations made to develop washed and unwashed fingermarks at a range of T20 concentrations

FePS Code	Surfactant solution used (10 mL mixed with 5 g iron oxide powder)
FePS1	T1 (water)
FePS2	T2
FePS3	T3
FePS4	T4
FePS5	T5

5.3.1.3 Analysis of fingermarks

All fingermarks were graded based on the quality of their ridge detail, contrast, and background development using the scales outlined in section 2.2.4. A modified University of Canberra (UC) scale was also used to directly compare the benefits or detriment of surfactant washes on technique performance. This is outlined in Table 5.5. All fingermarks were graded by one assessor.

Table 5.5 Modified UC scale used to compare washed and unwashed fingermark development [92]

Grade	Definition
+2	Development of washed marks is significantly more effective compared to unwashed marks
+1	Development of washed marks is slightly more effective compared to unwashed marks
0	Both methods are indistinguishable in quality
-1	Development of unwashed marks is slightly more effective compared to washed marks
-2	Development of unwashed marks is significantly more effective compared to washed marks
00	No detection of the fingermark using either method

5.3.2 Results

The UC scale results were used to determine if the process of washing fingermarks may be improving the quality of development. The scores of development for all suspensions are shown in Figure 5.11. In this graph, the number of split marks in which fingermark quality was improved through washing (regardless of wash concentration) is shown in blue, while the number of marks for which quality was reduced by washing are shown in red. These results indicate that when developed with FePS formulations below the CMC (FePS1 or FePS2), the quality of fingermarks is improved when they are washed before development. Above the CMC (FePS3 to FePS5), the number of marks with either no change or negatively impacted by the wash step increases with surfactant concentration.

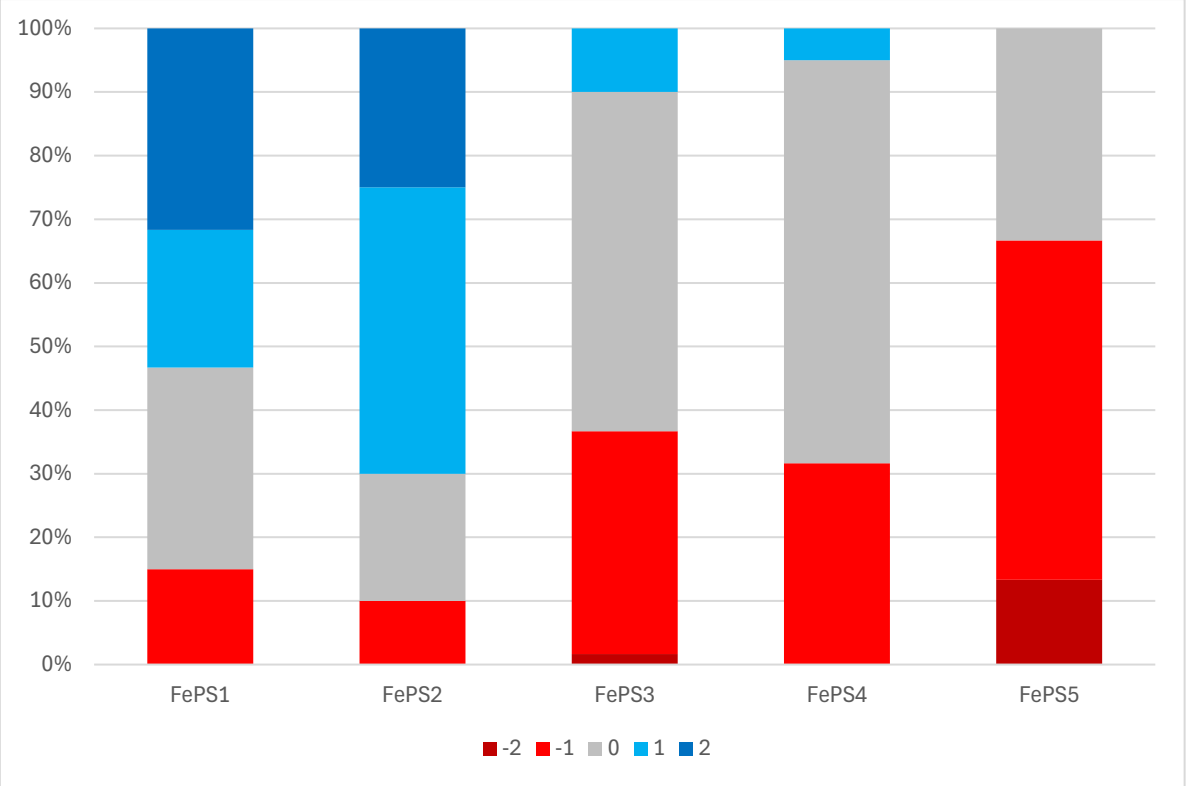


Figure 5.11 UC scores given to development with each FePS formulation (FePS 1-5), positive (blue) scores indicating improvement with washing step and negative (red) indicating detriment with washing step

5.3.2.1 Background development

FePS formulations with concentrations below the CMC (FePS 1 and 2) produced heavy background development, which was expected and consistent with results from chapters 2 and 3 which indicate that at surfactant concentrations below the CMC (or without any surfactant), FePS formulations produce indiscriminate powder deposition. The positive UC scores given to FePS1 and FePS2 (improvement of quality from surfactant wash) was due to the addition of a higher concentration surfactant wash preventing this background development. An example of this is shown in Figure 5.12. The reduction of background development after surfactant wash above the CMC (T3 to T5) is consistent with observations made in section 5.2.2.3, where background development produced by DOSS FePS was reduced after washes with T20, TX100, KP and LN. This shows that the surface-wide powder deposition caused either by insufficient surfactant concentration or presence of a surfactant which is not stable enough in suspension for selective powder deposition (such as DOSS) can be reduced by higher concentration surfactant washes without damaging fingermark residue. After the application of the surfactant wash, the substrate was rinsed well to remove the surfactant, however these results indicate that there is sufficient residual surfactant to impact fingermark development. As the results of section 3.3.4 show that below the CMC surfactants did not reduce background development when used in FePS, it may be assumed that the amount of surfactant left on the

substrate is either above the CMC or is somehow providing a coating on the substrate which is repelling powder. The images shown in Figure 5.12 also show differences in the intensity of powder deposited on the fingermark ridges, however this is due to intra-donor variability as the change in contrast was not consistent between donors or concentrations. All fingermarks presented in this image were deposited by the same donor on the same day, however at different timepoints and therefore it can be assumed that these are expected differences in the residue composition.

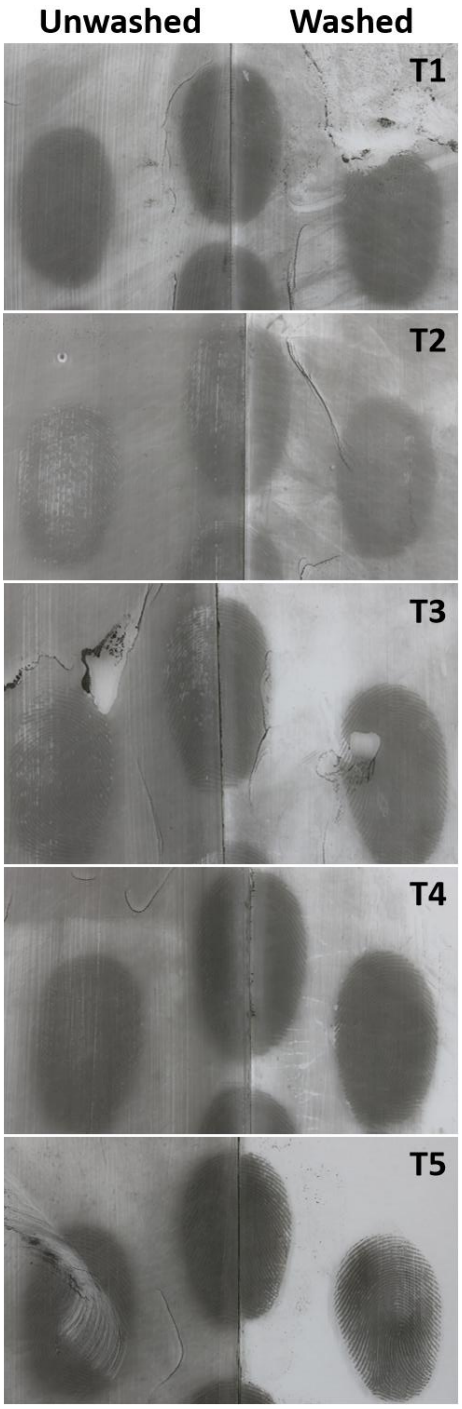


Figure 5.12 Donor 1 fingermarks developed with FePS2, showing reduction in background development with higher concentration T20 wash (right side of split marks)

For development with FePS1 and FePS2, the addition of a higher concentration surfactant wash only removed the powder deposition around the outside of the mark, however background staining still occurred between fingermark ridges. An example is shown in Figure 5.13. This occurred for all donors washed with T3 to T5 (above CMC) developed with FePS2, and half the donors washed with the same concentrations but developed with FePS1. None of the fingermarks developed with above CMC suspensions (FePS3 to FePS5) produced this inter-ridge staining on washed or unwashed marks.

As this result was not observed with all marks developed with FePS1, it cannot be stated that the reason for this inter-ridge development is strictly due to a combination of below CMC FePS development and above CMC washes. It appears that for some of these developments however, that where the addition of a surfactant wash is providing enough residual surfactant to prevent surface-wide background deposition, the wash is perhaps not able to penetrate within the fingermark ridges. This may be due to a higher volume of sebaceous material somehow repelling the surfactant, so it is only able to settle around the fingermark boundaries. The inconsistency of this observation between donors with FePS1 development supports the hypothesis that individual composition is influencing how the surfactant wash can interact with the substrate. When higher surfactant concentration is used in development however (FePS3 to FePS5), the lack of inter-ridge development (washed and unwashed) shows that the technique is relying on the presence of fingermark residue for powder deposition, making the suspension more stable.

Figure 5.13 Comparison of development produced by FePS1 and FePS4 showing difference in inter-ridge background development

5.3.2.2 Contrast of developed marks

The UC scores of fingermark development achieved with FePS3 to FePS5 (above CMC) showed that often the unwashed side of the developed marks produced the best quality, as illustrated by negative scores in Figure 5.11. Especially with development using the most concentrated surfactant (FePS5,

2022 FVM recommended concentration), where all fingermarks developed were either of equal or higher quality when not washed. Generally, this was due to some streaking which appeared on washed fingermarks, leading to a slight reduction in contrast as illustrated in Figure 5.14. These images show however that less contrast in developed washed marks did not increase with surfactant concentration and was fairly consistent regardless of which wash had been applied (T1 to T5). This possibly indicates that the physical action of washing the marks is removing the residue rather than the presence and concentration of surfactant micelles being solely responsible. This supports the conclusion that T20 surfactant up to 10% concentration (T5) is not removing fingermark residue.

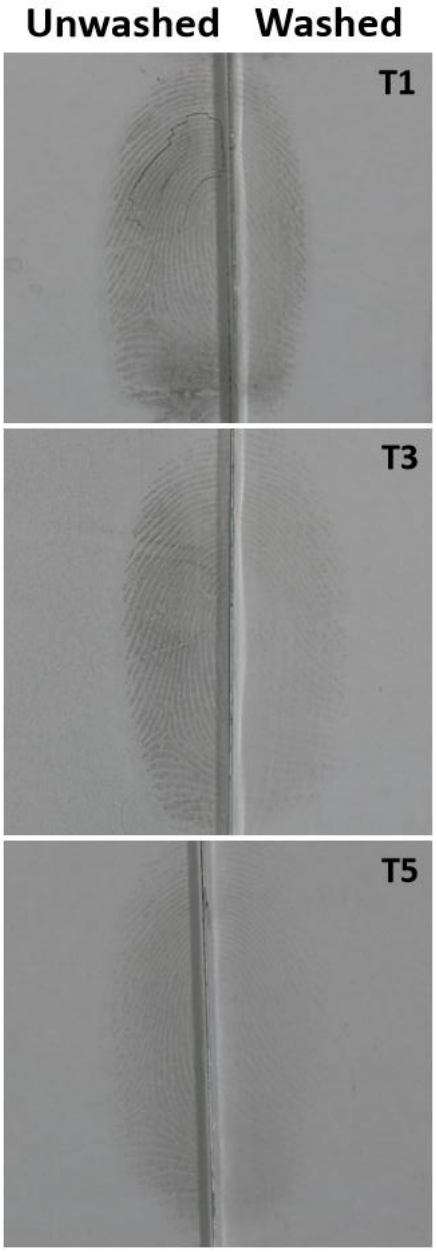


Figure 5.14 Comparison of FePS5 developed marks (donor 1) washed with T1 to T5, showing slight reduction in contrast of washed marks for all washes however not increasing with surfactant wash concentration

The concentration of the wash applied before development did not have a notable impact on the appearance of marks developed with the same FePS formulation. The contrast of developed fingermarks depended mostly on the concentration of T20 used in FePS, as shown in Figure 5.15. For FePS formulations below CMC (FePS1 and FePS2), contrast was greatly improved by any concentration of pre-development wash due to the reduction of background development. When FePS formulations made with T20 concentrations above the CMC were applied (FePS3 to FePS5), contrast was reduced by all wash concentrations (T1 to T5). Overall, this indicates that at the highest surfactant concentration tested (T5, 10%) and below, T20 is not removing fingermark residue and the reduction in powder deposition is due to increased suspension stability. As such, surfactant concentration may be controlled to reduce background deposition without the concern that fingermark residue is being removed in this process.

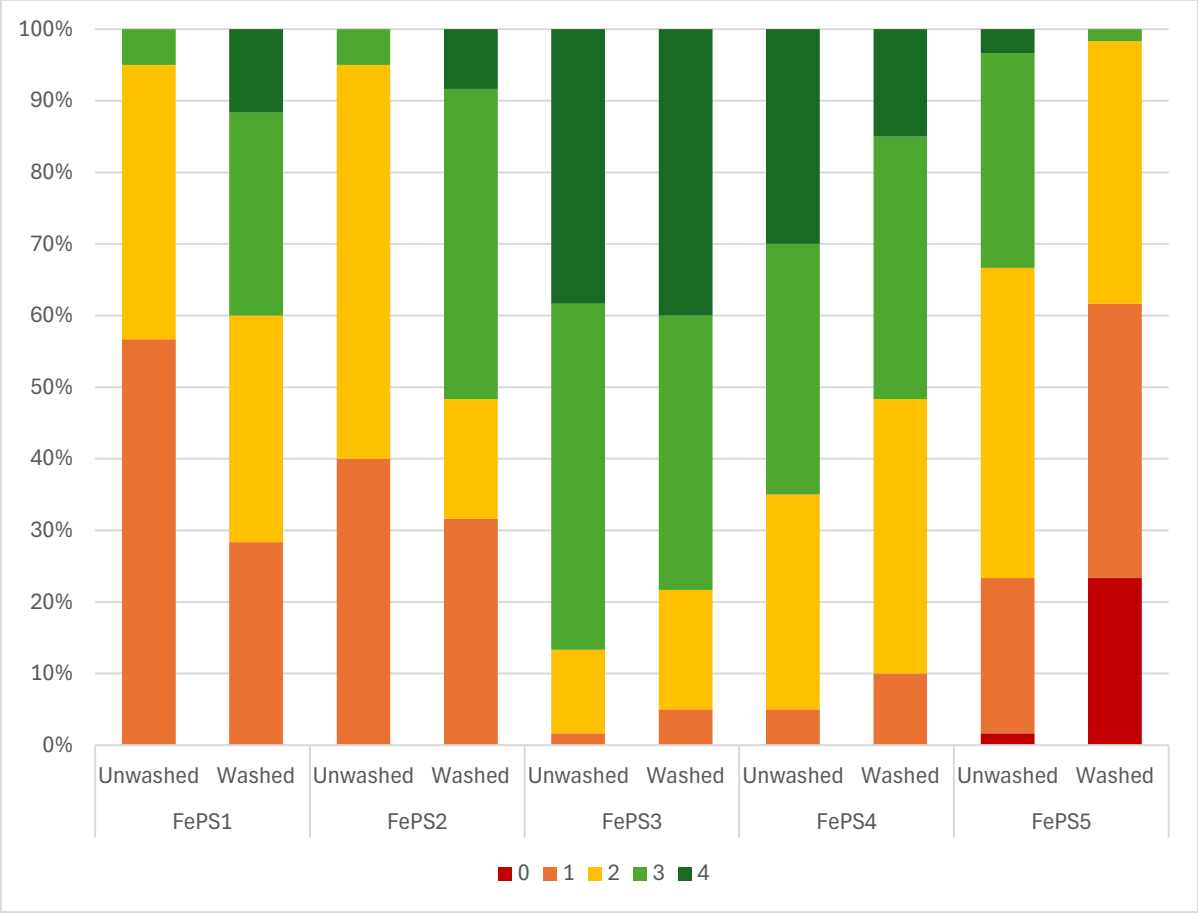


Figure 5.15 Contrast scores of washed and unwashed fingermarks developed with each FePS formulation

The contrast scores presented in Figure 5.15 show that the best contrast of both washed and unwashed marks was produced using FePS4 (2.5% T20), and increasing this concentration up to FePS5 (10% T20) makes the suspension more stable which results in weaker ridge contrast. These observations, coupled with results presented in section 2.3, indicate that the recommended 10% T20

FePS formulation (FePS5) could be optimised by reducing the T20 concentration to 2.5% (FePS4) to allow for heavier powder deposition on fingermark ridges without additional background development.

5.4 Conclusion

This study was comprised of two parts, the first of which investigated the percentage removal of donor and artificial fingermark residues exposure to different surfactant washes to determine if the surfactants may be removing residue, an important consideration when using surfactants in FePS formulations. The second part investigated different combinations of varied T20 concentrations (T1 to T5) to wash and develop fingermarks in an FePS formulation (FePS1 to FePS5) to better understand the impact of surfactant concentration on both residue removal/alteration and quality of development. The investigation found that at optimal concentrations for use in FePS, the surfactants assessed are not completely removing fingermark residue. Because of this, cases of no development with optimised FePS formulations are likely due to important residue fractions not being present in the first place to catalyse powder deposition from the suspension system. Difficulties were faced with the method employed to visualise and quantify residues before and after washing, indicating that the use of phase contrast microscopy may not be appropriate for this kind of comparative investigation. Some fingermark residues, especially those eccrine in nature, could be developed with FePS formulations despite lack of visible residue with this technique.

The results of the second part of this study show that T20 does not remove fingermark residue up to 10% concentration. Because of this, lower contrast of developed marks observed with higher concentration FePS development was not due to a lack of residue present, but rather an increased stability of suspension that prevented powder deposition. This shows that the ability of a surfactant to develop fingermark in FePS is closely linked with the stability of suspension formed by the presence of surfactant micelles. High quality fingermarks could be detected after exposure to water showing that the likely degradation of residues (both eccrine and sebaceous fractions) do not hinder the effectiveness of FePS. Due to difficulties presented in previous literature about identifying and quantifying the chemical composition of natural fingermark residue, these results cannot be used to identify the constituent (or constituents) responsible for successful FePS development. This further outlines the complexity of fingermark residue interactions, however as FePS is not hindered by these processes, it is valuable to understand that surfactant concentration can be controlled with a focus on reducing background development without impacting fingermark residue.

Chapter 6: Future work and conclusions

6. *Future work and conclusions*

6.1 Future work

Continuous optimisation of current and emerging fingermark development techniques will improve the rate and quality of fingermark recovery for casework. FePS is a fingermark development technique which is used operationally around the world and is the subject of global research, demonstrating its importance in fingermark development efforts. To date, such research has focussed on the "how" of this technique; the effect of variables such as different powders, surfactants, fingermark donors, and substrates. This has been effective in creating optimised FePS formulations and exploring the boundaries of specific formulations. However, research efforts have provided limited understanding of the mechanism involved in this technique. As such, any variation to chemical recommendations of relevant components results in a knowledge reset, and new proposed formulations must be tested again against all relevant variables to be validated for use in casework. This leads to more time and resources being spent during the reformulation process, and understanding of results produced can be limited.

Recently, there has been growing interest into improving the foundational understanding of fingermark development techniques to aid in more efficient and effective optimisation efforts. By focussing research on the "why" questions of technique interactions with fingermark residue components and substrates, we improve the base knowledge from which operational research can be conducted. Gaps in knowledge surrounding the mechanisms involved in fingermark development techniques limit our understanding of results produced during optimisation studies, and therefore filling these gaps will improve the sustainability of future fingermark research practices. The success of the research presented in this thesis to improve understanding of the role surfactants play in fingermark development with FePS demonstrates the potential of further fundamental research to be performed on other development techniques. Extensive research investigating the changing of specific properties in development techniques will improve our understanding as a whole of the mechanisms involved in development methods.

Overall, this work has highlighted important gaps in knowledge relating to the composition of fingermark residue and technique interactions. However, it is difficult to improve this knowledge when faced with the issue of inherent fingermark residue variability, which is widely noted as a key roadblock in current research efforts. The use of artificial fingermark simulants is one option of mitigating this issue and has been proposed to develop a repeatable emulsion of a known chemical

composition for use in fundamental interaction research. This research, as well as published literature, has identified the limitations of current simulants in that they are currently not able to accurately mimic fingermark development [129, 143]. However, further research into altering current emulsions, testing additional deposition methods and assessing impacts such as residue drying time will aid in better developing artificial simulants. This kind of research will expand the capabilities and uses of artificial simulants, so they may one day be used more broadly to investigate residue interactions in fundamental research. Parallel to artificial fingermark simulant research, further work involving residue compounds present in natural fingermarks should be undertaken. A number of studies have focussed on this in recent years, however the scale of required research limits progress [14, 15, 137, 154, 155]. A particular focus should be on the effect environmental processes such as wetting, heating and drying may have on fingermark residue. This will aid in better understanding residue targets of development techniques which are effective fingermarks exposed to such processes.

For FePS formulations, further fundamental research into the powder component should be conducted. Current research into the capabilities of various iron oxide powders has established that the powder component plays an important role in successful development [45, 48, 78]. However, future work should focus on the impact of specific physical features such what size and shape of powder particles being are deposited on and off ridge during development, to assess if selectivity is controlled by this or if the surfactant component plays a more primary role. Some recent work has explored this possibility with specific powders, however expanding this work to multiple powder types will provide a more foundational understanding of the powder component [29, 46]. This will complement the research presented in this thesis and provide a robust evaluation of the factors involved in fingermark development with FePS.

The currently recommended surfactant T20 was shown to be highly effective in FePS formulations in this work, when combined with the precipitated magnetic iron oxide from Thermo Fisher Scientific. This research found that a more dilute T20 formulation improves the contrast of developed marks, and further work into testing the more dilute T20 on a wider range of powders may aid in further optimising the current FePS formulation. This work would also help to determine if the results of surfactant efficacy presented in this study can be more broadly applied to FePS formulations, or if relevance is contained to a formulation made with the powder from Fisher scientific. Notably, widening the amount and types of substrates tested with the more dilute T20 formulation (such as including adhesive substrates) will expand the possible applications of the formulation. This will also provide interesting insight into possible chemical interactions occurring between surfactants, powders and adhesives. As previous literature has identified issues with background development using FePS

on adhesive substrates, further work could be conducted into using a surfactant pre-wash step when developing these substrates [31, 41, 48]. The results presented in chapter 5 showed reduced background development with formulations prone to heavy background staining when a pre-wash with a more concentrated surfactant was applied on non-porous substrates. It is possible similar observations could be made on adhesive substrates to minimise the negative impact of FePS formulations on some adhesives. Further investigations into the effect of different substrate types will similarly aid in improving fundamental understanding of the interactions involved in this technique.

6.2 Conclusions

The main goal of this thesis was to aid research efforts by improving fundamental understanding of the FePS mechanism by investigating the role of the surfactant in fingermark development. To fulfil this objective, the research was divided into four main experimental parts. The first investigated a wide range of surfactants of different ionic natures to gain an overview of how different surfactant types and concentrations influence fingermark development quality. The second part focussed on the importance of micelle presence in successful fingermark development using FePS and aimed to identify if any physical or structural surfactant features could be used to predict surfactant efficacy in this technique. The third sought to identify possible residue targets responsible for initiating powder deposition on fingermark ridges. To achieve this, commercial and house-made artificial fingermark residue simulant emulsions were first evaluated for use by assessing their reactivity with other established development techniques. Finally, the interaction between surfactant micelles and fingermark residue in the absence of powder particles was investigated to better understand their role in ineffective formulations and evaluate the interaction between fingermark residue and surfactant micelles.

6.2.1 Effect of surfactant type and concentration

Throughout all experimental work detailed in this thesis, a range of surfactant types, concentrations and powder to surfactant ratios were assessed with respect to their effect on fingermark development in FePS. This resulted in a robust evaluation of surfactant efficacy in this technique and represents the most thorough investigation of the impact different surfactants have in FePS to date. The quality of developed fingermarks was assessed on their ridge detail, contrast, and background development.

The ridge detail and contrast of a developed fingermark is heavily influenced by surfactant type and concentration. All assessed surfactants produced varying quality of developed fingermark ridges, however overall this research showed that the most effective surfactants play a larger role in controlling the volume and/or size of powder particles deposited on-ridge than previously thought

[45, 48]. This is influenced heavily by the stability of the suspension, as surfactants used at too high a concentration produced light coloured fingermark ridges or did not allow any powder deposition to occur. These higher concentrations contain more surfactant micelles and deposit less powder on the ridges, leading to lighter coloured developed fingermarks with poorer contrast. Even within wide concentration ranges however, some surfactants (such as SP08/T80, T80 and LN) were much less effective in producing selective powder deposition than others. This demonstrates clearly that the type of surfactant used greatly impacts the interaction between FePS formulations and fingermark residue.

This research has also demonstrated that the addition of surfactants in FePS plays a vital role in the prevention of background powder deposition, that subsequently allows the technique to develop preferentially on fingermark residue. Formulations made only with water and powder were shown to consistently produce heavy background development that obscured ridge detail on all non-porous substrates assessed. This indicates that the presence of optimal surfactants (such as T20, KP and TX100) in FePS formulations are producing a suspension stable enough to prevent powder particles from depositing indiscriminately on a substrate. The concentration of surfactant required for appropriate stability varies with each surfactant, and some surfactants have a much wider optimal range than others and are subsequently resistant to minor formulation variations that can occur during solution preparation. Despite these variations between surfactants, this research demonstrated that all surfactants must have a concentration above their CMC for optimal development. This proves the importance of micelle presence in the creation of a suitably stable suspension to allow for optimal fingermark development.

The efficacy of fingermark development techniques is often assessed based only on their ability to enhance ridge detail. The results of this study, however, show the importance of evaluating development against multiple quality parameters such as ridge detail, contrast and background development to better discriminate between different formulations and identify what specific variables are impacted. As current research efforts into fingermark development techniques focus more on mechanistic understanding than ever before, this investigation has shown the importance of utilising a range of quality parameters in this type of research in the future.

6.2.2 Residue compounds responsible for powder deposition

Previous attempts to identify the compounds required in fingermark residue for development techniques like FePS to be effective have been hindered by the inherent variable nature of donor fingermark residue [14-16, 81, 151]. This research therefore investigated the use of artificial

fingermark simulants to assess possible fingermark residue targets required for the initiation of powder deposition, and the effect different surfactants may have on these targets. An artificial fingermark emulsion tested in previous literature was made and compared to a commercially available simulant by developing them with benchmark development techniques on porous and non-porous substrates. A range of deposition methods for the emulsions were assessed, and it was determined that the use of a rubber fingermark stamp was the most effective way to replicate the size and complexity of a real fingermark. This research found that neither of the tested emulsions could accurately mimic the development of natural fingermark residues when developed using CA, FePS, IND-Zn and PD. However, the development that did occur for some of the techniques demonstrated the presence of some important initiate compounds.

The development of four different artificial fingermark simulants with FePS formulations made with a range of surfactants demonstrated variation in the compounds targeted with this technique depending on type of surfactant used. The results showed that surfactants which promote the selective deposition of powder on natural fingermarks, such as T20, KP and TX100, were not very effective in developing artificial simulants, while formulations prone to heavy background development were more likely to develop ridge detail on the artificial residues. This suggests that optimal surfactants are stable enough to prevent indiscriminate powder deposition (due to the presence of micelles), however are destabilised by the complex mixture of compounds within natural residue. Either these relevant compounds are not present in the artificial emulsions tested, or they are not present in a form that allows the FePS formulations to interact with them. This mechanism has been theorised in previous literature, and this research provides further support while also demonstrating that the use of different surfactant changes this interaction, highlighting the complex relationship between surfactant micelles and fingermark residue components [31]. It is therefore only possible to make conclusions about residue targets of specific formulations tested without better understanding of natural fingermark residue components. Overall, this research furthered understanding of artificial simulants especially for use on non-porous substrates and demonstrated great potential for future applications in fundamental fingermark research.

The results of this project have consistently highlighted the importance of suspension concentration and stability in FePS interactions. It has been shown that higher surfactant concentrations may lead to no development of ridge detail, and this research suggests that the lack of development is more likely due to increased suspension stability rather than removal of fingermark residue. Experiments conducted using T20 washes and development in FePS formulations has shown that as the concentration of this surfactant is increased, more stable suspensions are created which increasingly

prevents powder deposition. At lower concentrations this is ideal, as surface-wide background development is prevented, however at higher concentrations this results in poorer ridge contrast. Where previous results of this thesis suggested that higher concentration surfactants may be removing fingermark residue and therefore resulting in poorer contrast or lack of ridge development, this experiment demonstrated that the surfactant is more likely controlling ridge development through preventing powder deposition rather than residue removal. This further supports the great importance of micelle formation and concentration in the control of suspension stability influencing fingermark development with FePS.

6.2.3 Formulation considerations

The goal of this thesis was to improve foundational understanding of surfactants in FePS to aid in future optimisation efforts. To that goal, several conclusions can be made which are directly relevant to practical research applications. The main conclusion of this research is that all surfactants interact differently with fingermark residue, powders in suspension and substrates, and therefore cannot be simply substituted for one another in FePS formulations. Any future reformulations must provide a robust evaluation of the newly chosen surfactant including testing over a wide concentration range. It has been established, however, that the presence of micelles in a solution is critical to preventing background deposition in FePS formulations and therefore producing optimal development. Because of this, surfactant concentrations must be used above their CMC for fingermark development on non-porous substrates.

This research investigated any structural properties of surfactant monomers or micelles that may correlate with an increase or decrease in developed fingermark quality. From the results obtained, none of the parameters assessed could aid in predicting FePS efficacy, such as ionic nature, micelle shape and size, or surfactant monomer length and complexity. At the same point above their CMC, surfactants with varying complexity and carbon chain tail lengths of between 8 and 18 carbons produced similar fingermark development quality. This further highlights the complexity of the interaction involved between surfactants, powders in suspension and fingermark residue in this technique. However, through all experimental work conducted, T20 was a highly effective surfactant over a range of concentrations, substrates and fingermark donors. As such, this research supports the current recommendation for T20 surfactant in FePS formulations used on non-porous substrates. This work also shows that the iron oxide powder recommended in the 2014 FVM (Thermo Fisher Scientific precipitated magnetic iron oxide) is effective for use with this surfactant [25]. The 2022 FVM suggests that this powder may still be used as a cheaper alternative to the currently recommended nanopowder, and this research adds further support to this claim [41]. With the Fisher powder,

optimal development was produced in all conditions with a T20 formulation diluted to a quarter of the currently recommended 10% concentration and therefore may be tested further for possible future operational use. This surfactant fits operationally relevant suggestions that an ideal surfactant would be non-toxic, inexpensive, have a long shelf-life and remain effective over a wide range of concentrations to account for human error during solution preparation.

Overall, the work presented in this thesis has highlighted a need to focus future research on the fundamental understanding of fingermark detection technique interaction. By improving this base understanding, future technique development and optimisation efforts will be more efficient and effective, ultimately improving the rate and quality of fingermark detection and aiding forensic investigative procedures.

Appendices

Appendix A: Ethics

UTS UNIVERSITY OF TECHNOLOGY SYDNEY

PARTICIPANT INFORMATION SHEET
COLLECTION OF BIOLOGICAL SAMPLES FOR FORENSIC ANALYSIS
UTS HREC APPROVAL NUMBER ETH18-2521

WHO IS DOING THE RESEARCH?
My name is *Lumikki Clover Ree* and I am a student at UTS. My supervisor is Dr Scott Chadwick (scott.chadwick@uts.edu.au)

WHAT IS THIS RESEARCH ABOUT?
The aim of this research is to improve our understanding of powder suspensions, a commonly applied fingermark development method. Fingermarks are used in criminal investigations to identify people of interest, and the enhancement of fingermarks is an important process in this process. This project aims to investigate the mechanism by which powder suspensions are able to enhance fingermarks, and in turn aid in improving the technique and quality of marks detected. More specifically, this project aims to understand the role of surfactants in this technique,

WHY HAVE I BEEN ASKED?
You have been invited to participate in this study because you have fingerprints which are one of the most common trace types in forensic science. In order to get a good representation and understanding of fingermarks it is important to get a range of people with different backgrounds, diets, lifestyles etc. You have not been selected for any other reason. You must be over the age of 18 to participate.

IF I SAY YES, WHAT WILL IT INVOLVE?
If you decide to participate, I will invite you to do the following:
- provide approximately 1000 fingerprints over the course of the study in a laboratory environment at UTS

You are free to provide any or none of these.

ARE THERE ANY RISKS/INCONVENIENCE?
Yes, there are some risks/inconvenience:
- You will have to spend about 30 minutes of your time in repeated deposition sessions
- Failure to maintain social distancing may result in transmission of SARS-CoV-2

Your samples (fingermarks) will have all identifiers (e.g. name and personal details) removed and replaced with a code (unique participant ID).

DO I HAVE TO SAY YES?
Participation in this study is voluntary. It is completely up to you whether or not you decide to take part.

WHAT WILL HAPPEN IF I SAY NO?
If you decide not to participate, it will not affect your relationship with the researchers or the University of Technology Sydney. If you wish to withdraw from the study once it has started, you can do so at any time without having to give a reason, by contacting Lumikki Clover Ree (Lumikki.ree@student.uts.edu.au) and quoting your unique participant ID.

If you withdraw from the study, your fingerprints corresponding with your unique participant ID will be destroyed. Any associated data that has not already been incorporated into a larger study or published will also be destroyed.

CONFIDENTIALITY
By signing the consent form you consent to the research team collecting and using personal information about you for the research project. All this information will be treated confidentially. Your questionnaire responses, fingerprints, tissue sample and any sub-samples will only have your unique participant ID associated with them. No-one will be able to associate you personally with any of these. Fingerprints that have been deposited will be stored in a secure laboratory facility with swipe card access prior to

Participant information – v1, 2019 03 06 *Page 1 of 2*

enhancement and imaging Only authorised persons involved with forensic research will have access to any of these. These may include UTS researchers and students.

We would like to store your fingerprints for future use in research projects that are an extension of this research project We may store them for a period longer than five years. Your samples will not be released for any use without your prior consent, unless required by law. In all instances, they will be treated confidentially.

We plan to publish the results in international, peer-reviewed journals, conference presentations and research theses. In any publication, information will be provided in such a way that you cannot be identified.

WHAT IF I HAVE CONCERNS OR A COMPLAINT?

If you have concerns about the research that you think Ior my supervisor can help you with, please feel free to contact us on *Lumikki.ree@student.uts.edu.au or Scott.chadwick@uts.edu.au*

You will be given a copy of this form to keep.

NOTE:
This study has been approved in line with the University of Technology Sydney Human Research Ethics Committee [UTS HREC] guidelines. If you have any concerns or complaints about any aspect of the conduct of this research, please contact the Ethics Secretariat on ph.: +61 2 9514 2478 or email: Research.Ethics@uts.edu.au], and quote the UTS HREC reference number. Any matter raised will be treated confidentially, investigated and you will be informed of the outcome.

YOUR UNIQUE PARTICIPANT ID

Below is your unique participant ID. This is the only identifying information associated with your questionnaire responses, fingerprints and tissue samples. If at any time you wish to withdraw them, please quote this ID.

Appendix B: CMC determination graphs

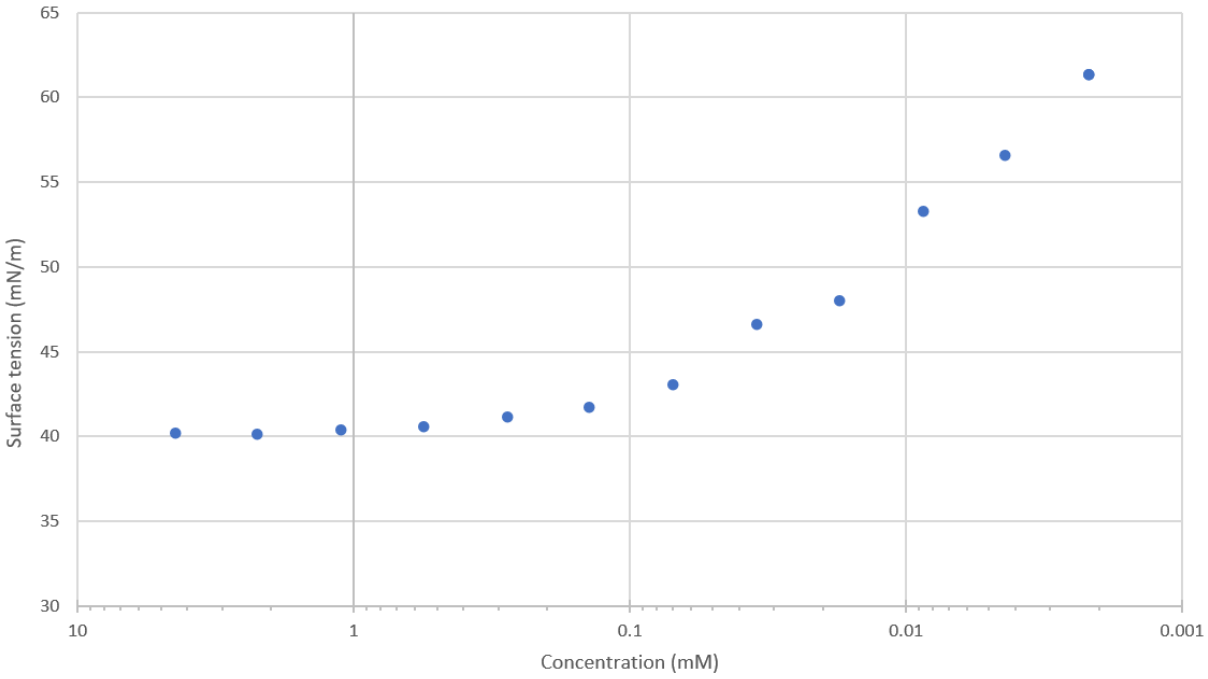


Figure B 1 Plot of ST measurements used to determine CMC of T20

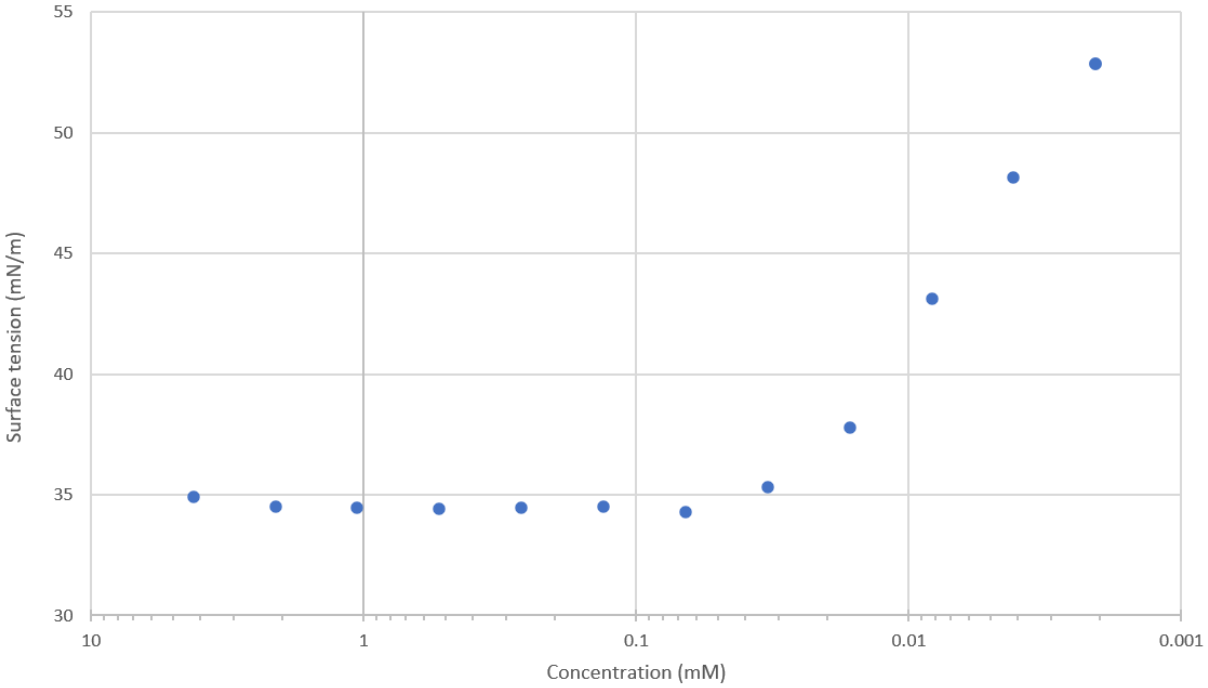


Figure B 2 Plot of ST measurements used to determine CMC of TX100

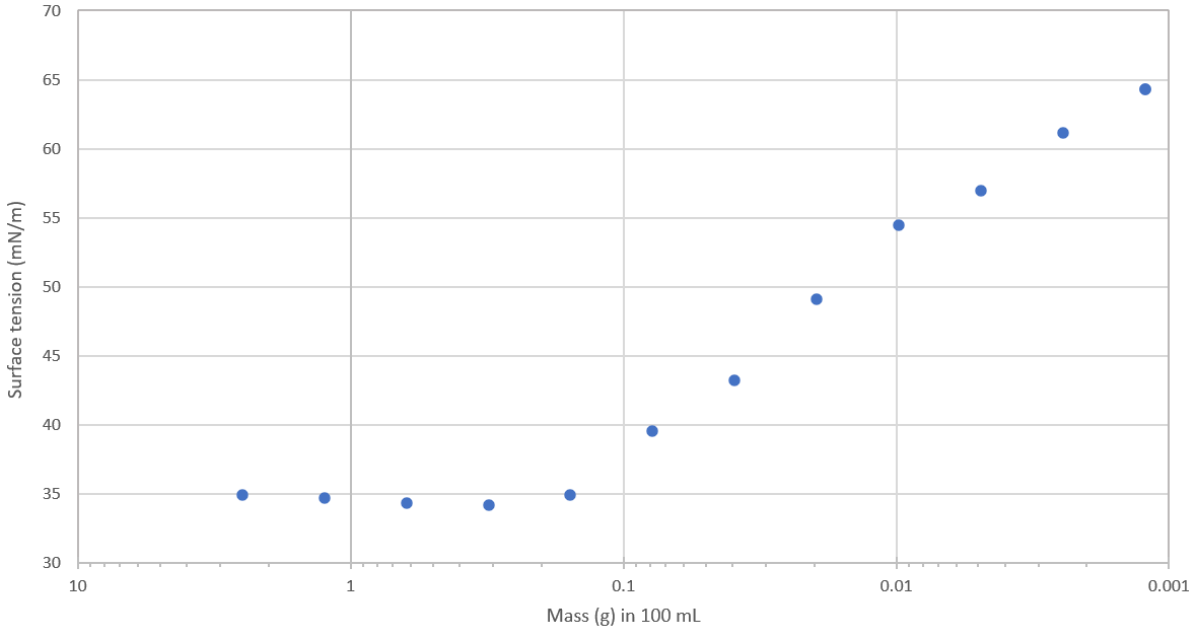


Figure B 3 Plot of ST measurements used to determine CMC of KP

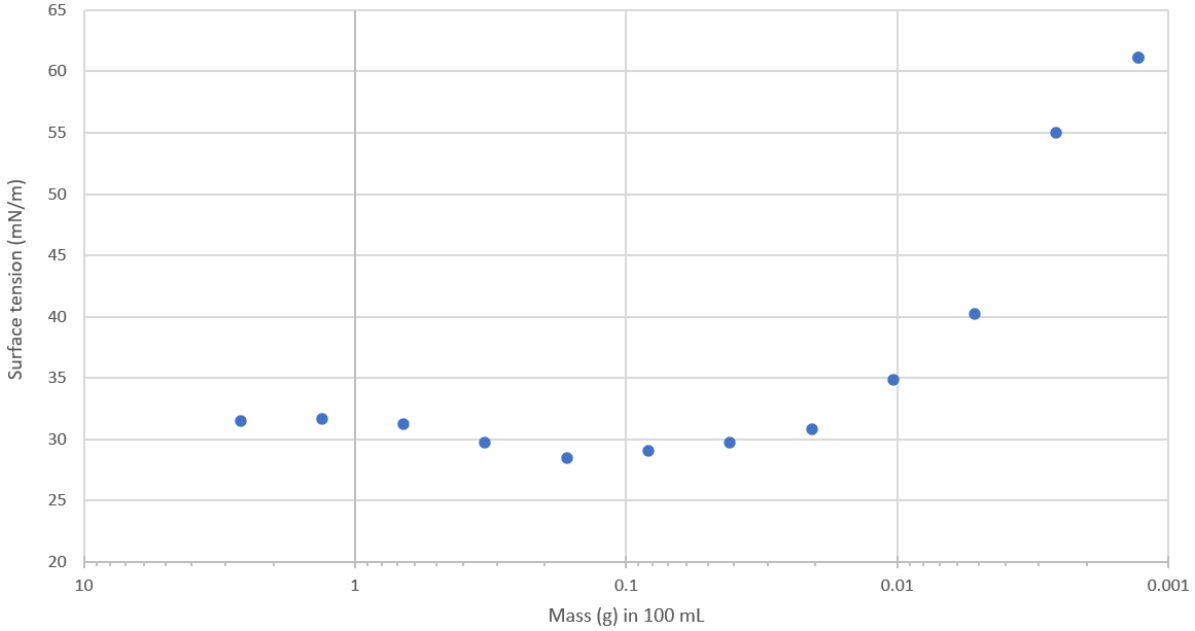


Figure B 4 Plot of ST measurements used to determine CMC of LN

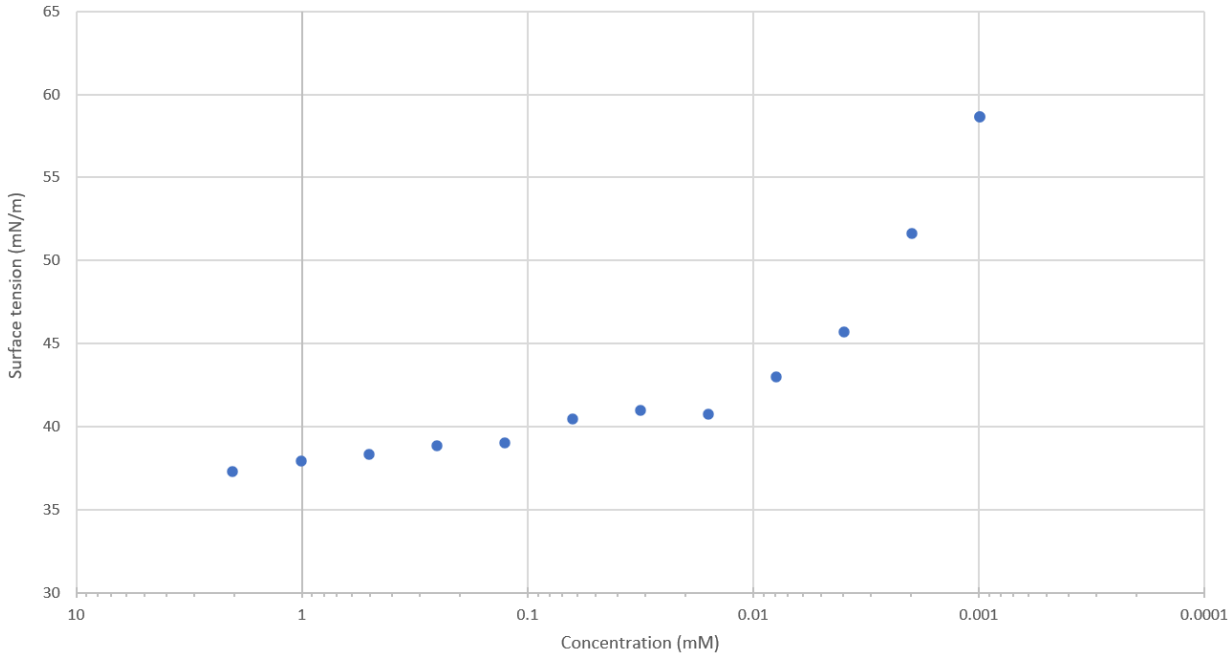


Figure B 5 Plot of ST measurements used to determine CMC of T80

Appendix C: Micelle size results

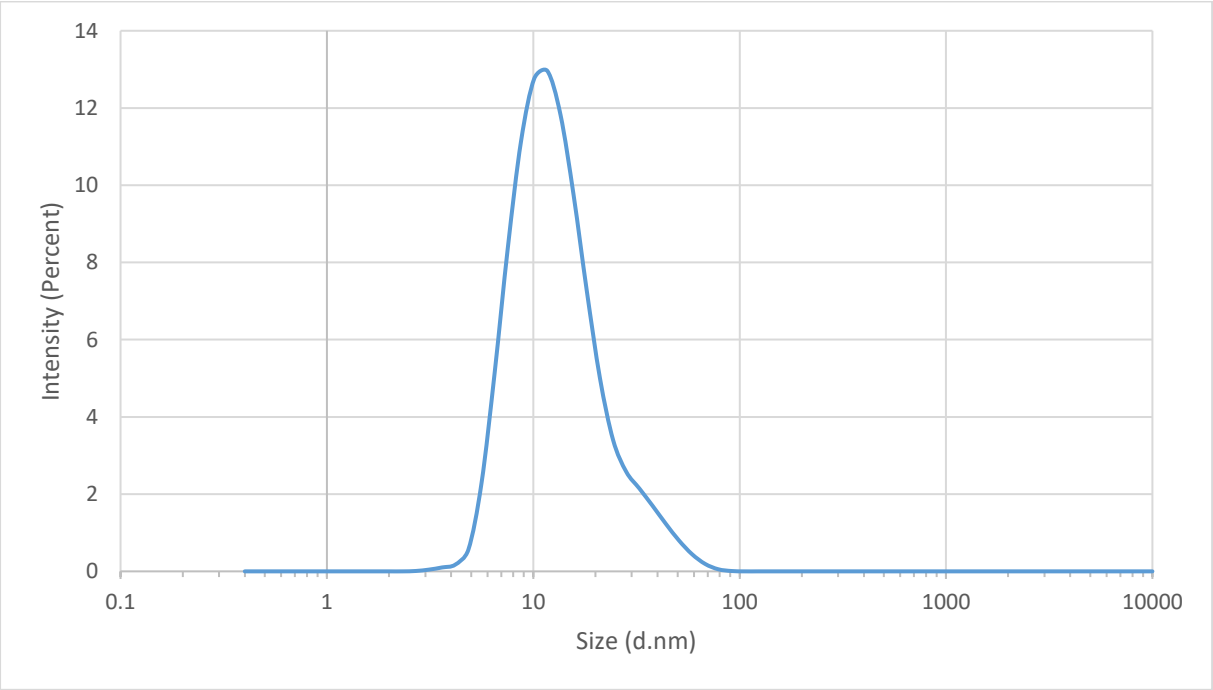


Figure C 1 TX100 micelle diameter results from DLS measurements

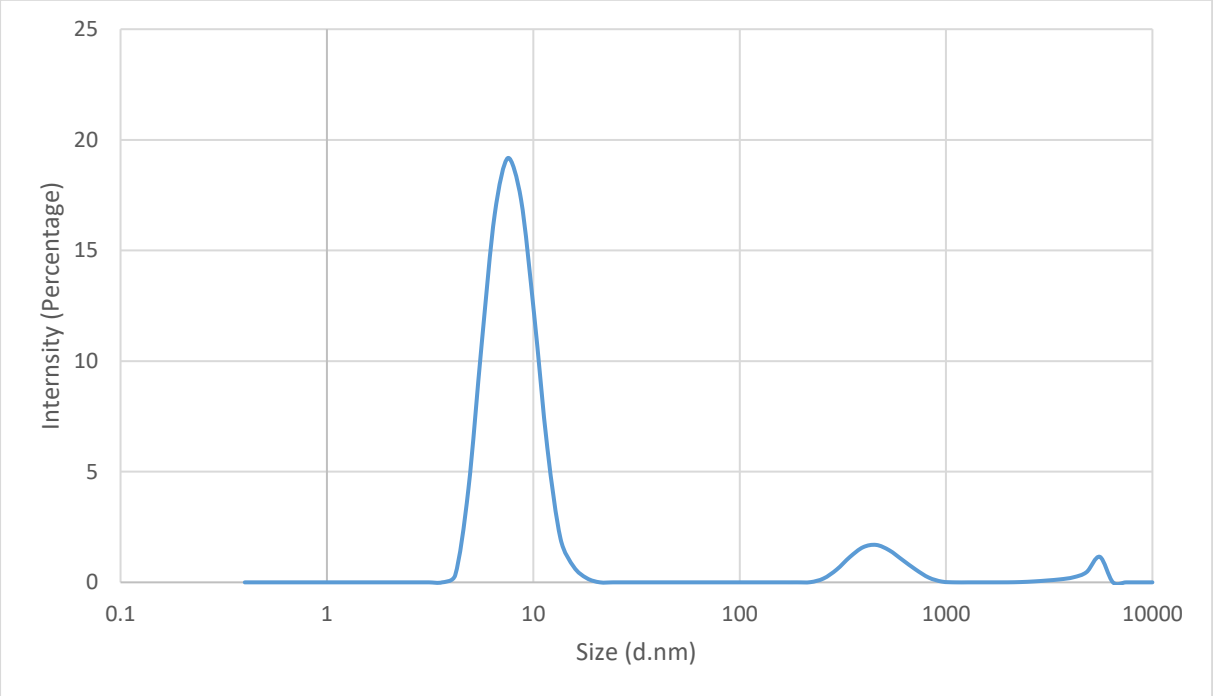


Figure C 2 T20 micelle diameter results from DLS measurements

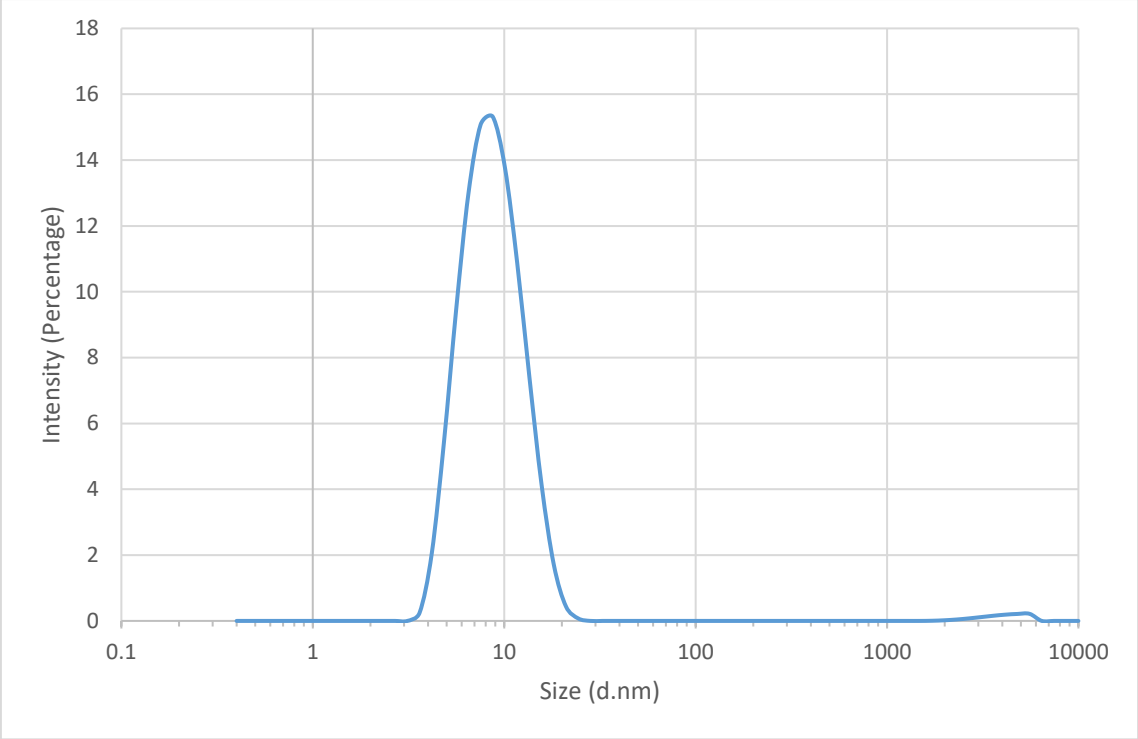


Figure C 3 KP micelle diameter results from DLS measurements

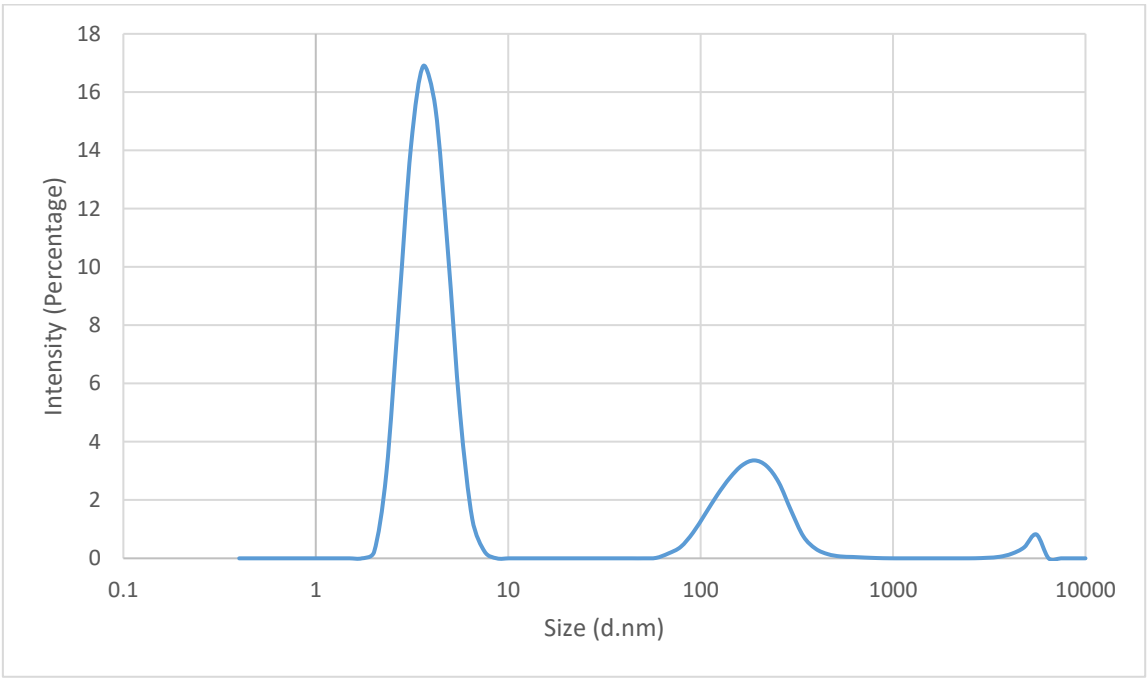


Figure C 4 LN micelle diameter results from DLS measurements

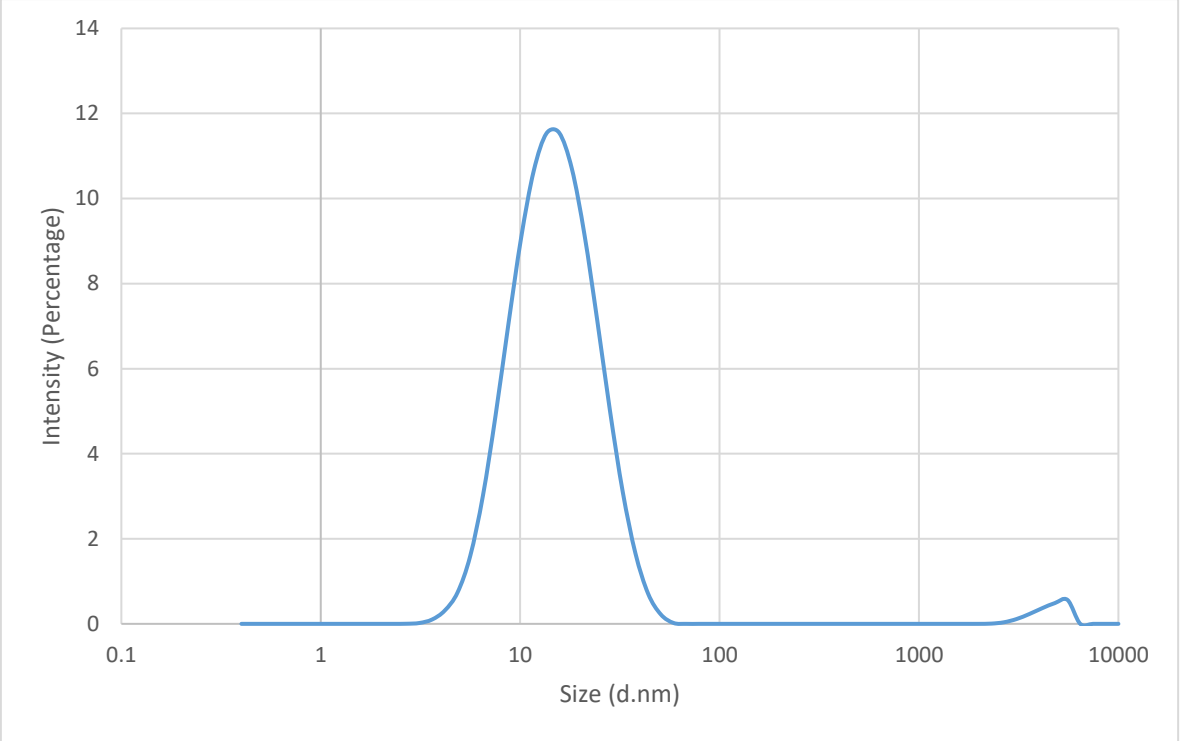


Figure C 5 T80 micelle diameter results from DLS measurements

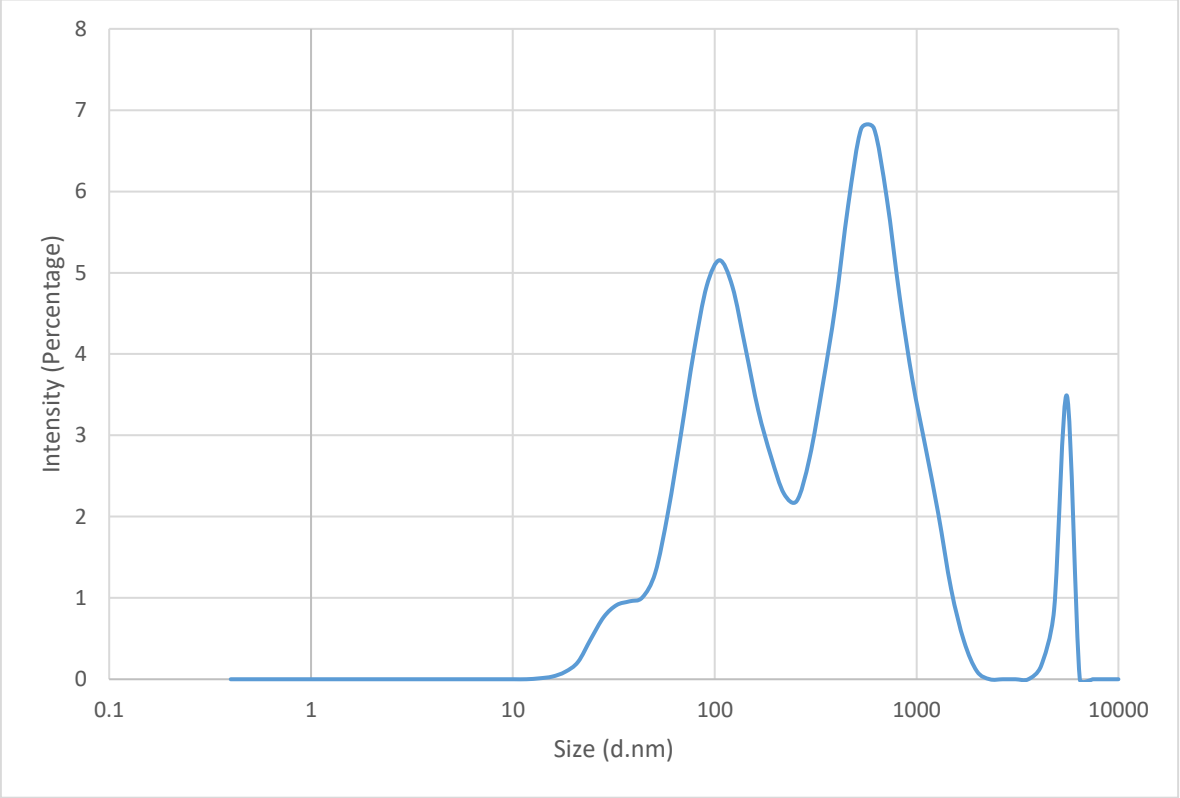


Figure C 6 SP80/T80 micelle diameter results from DLS measurements

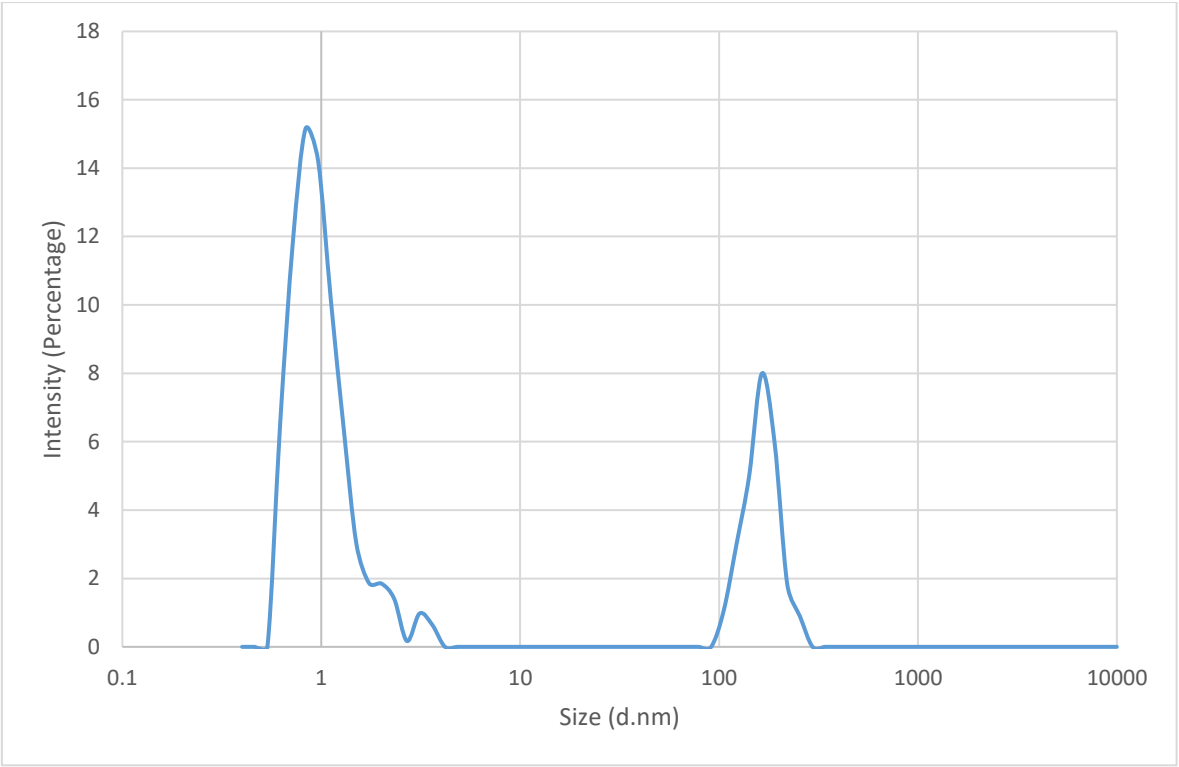


Figure C 7 SDS micelle diameter results from DLS measurements

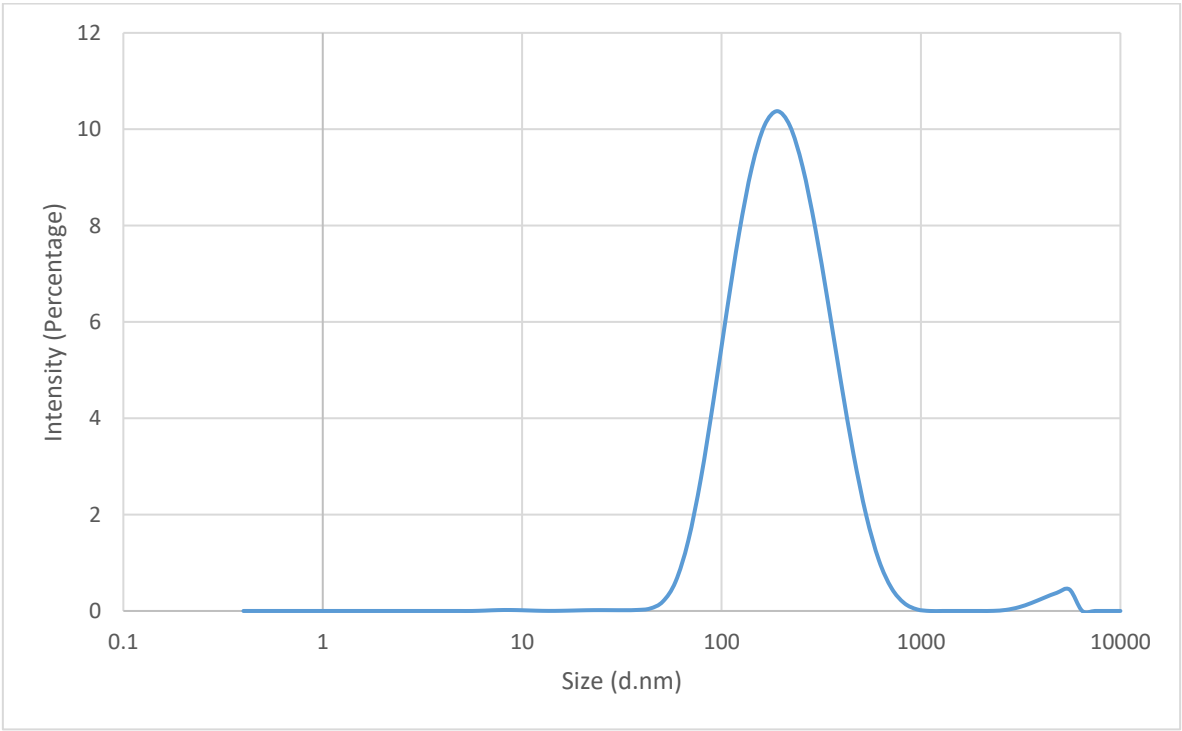


Figure C 8 DOSS micelle diameter results from DLS measurements

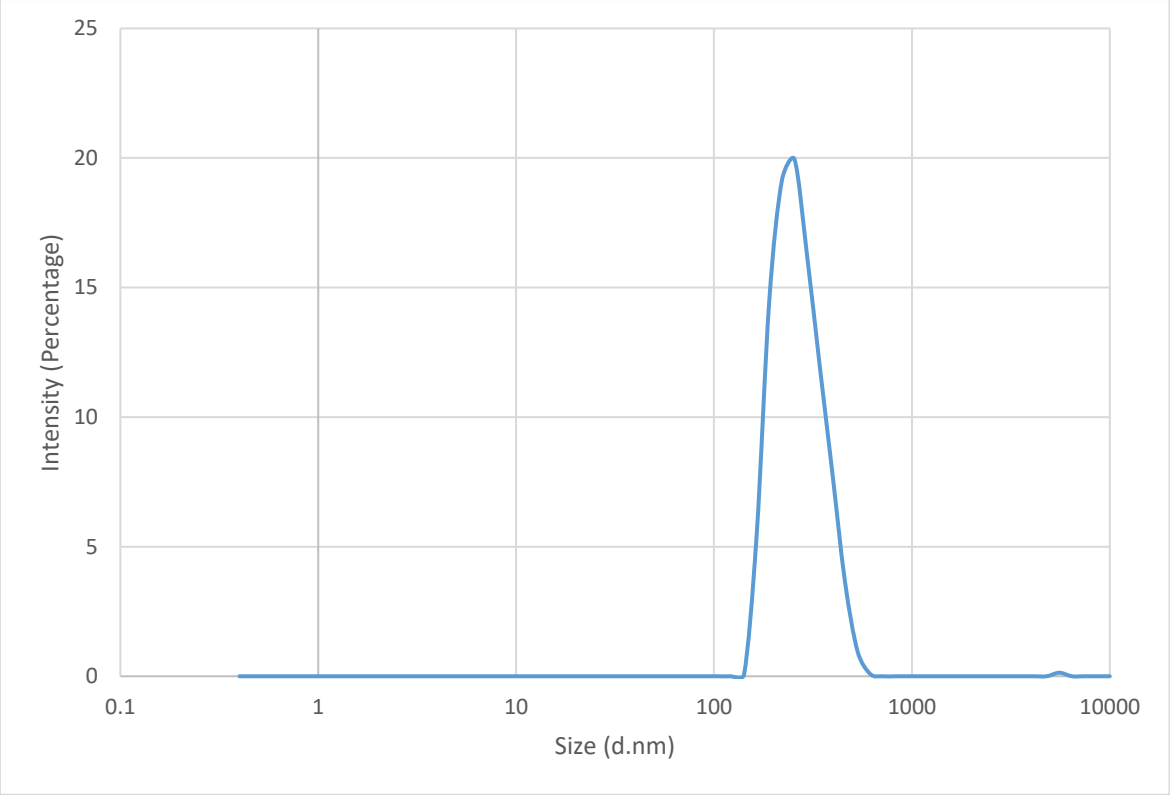


Figure C 9 n-DDAA micelle diameter results from DLS measurements

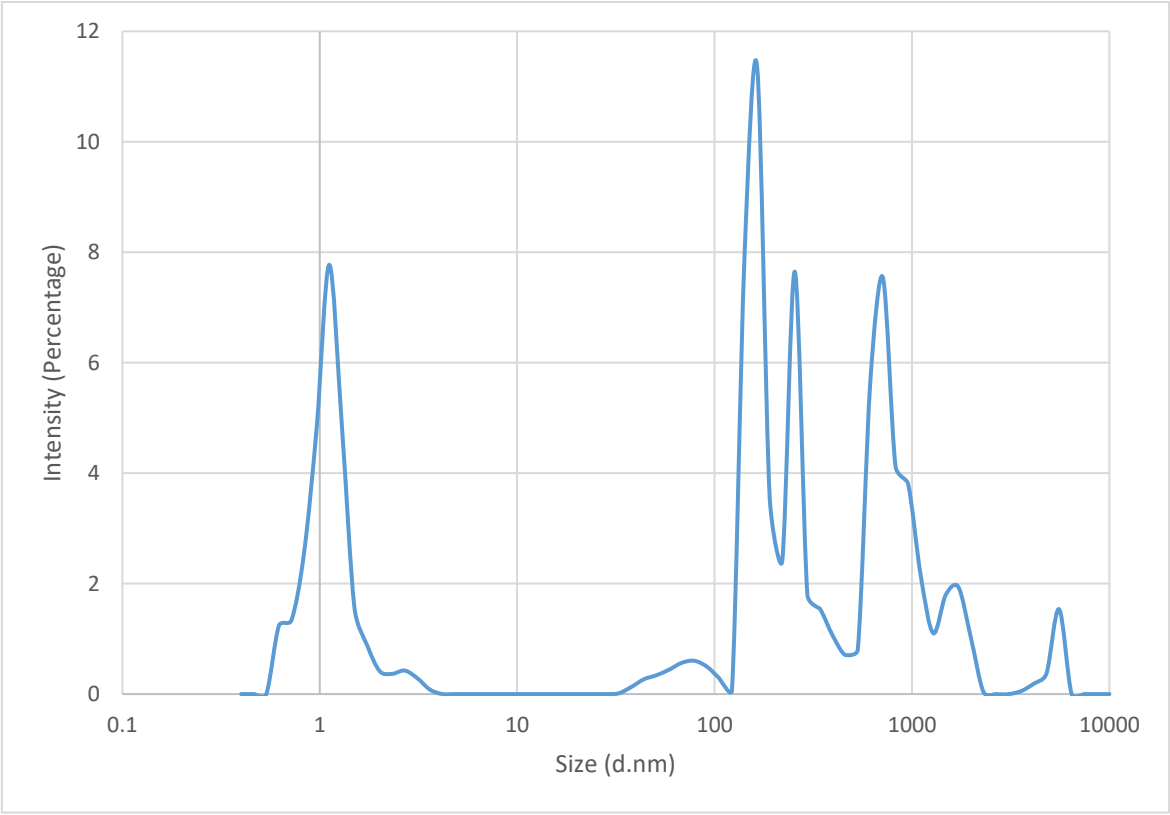


Figure C 10 CTAB micelle diameter results from DLS measurements

Appendix D: Fingermark development above and below CMC

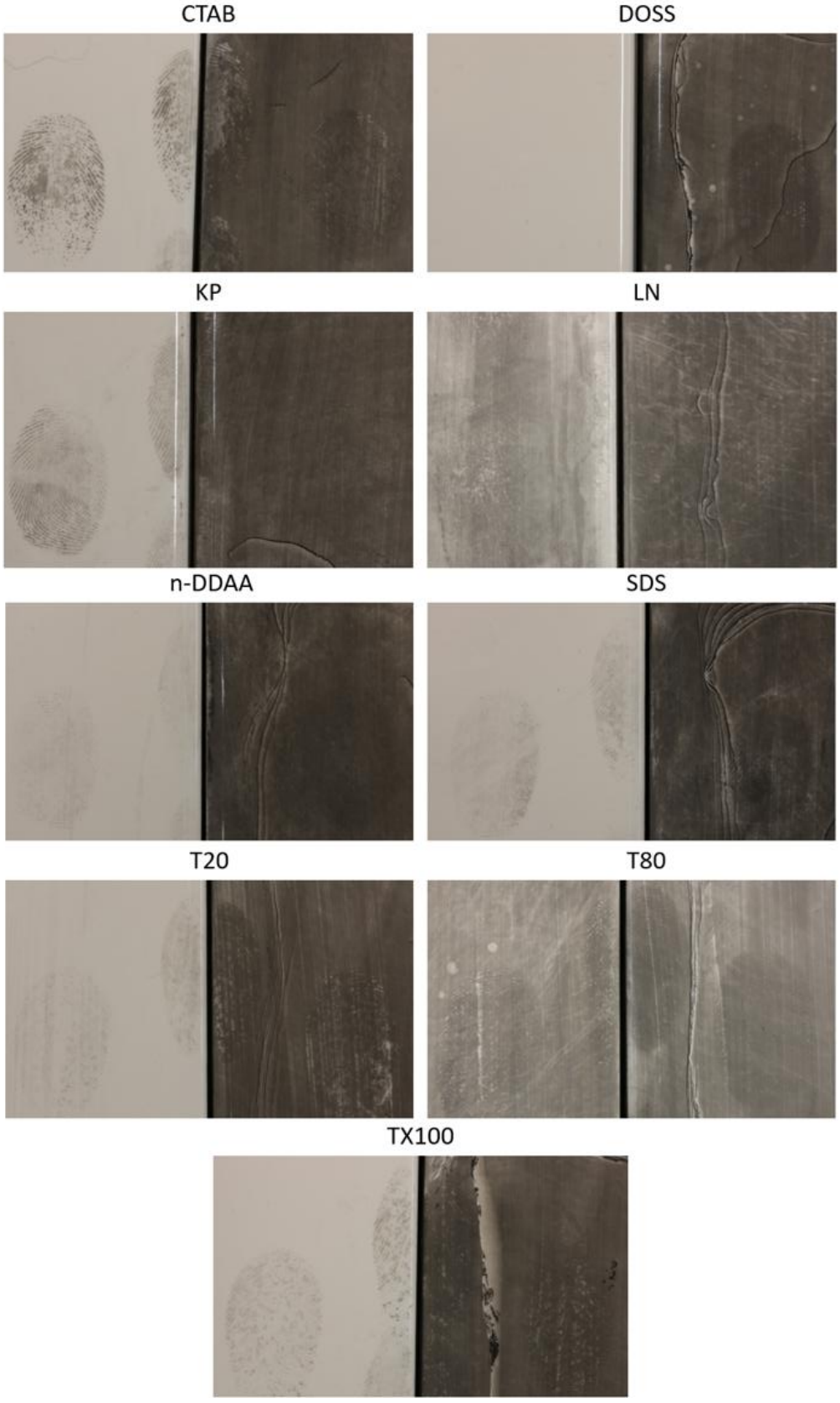


Figure D 1 Examples of development produced by all surfactants at 50x (left) and 0.5x (right) CMC on fingermarks deposited by one donor on ceramic

Appendix E: Artificial fingermark residue development

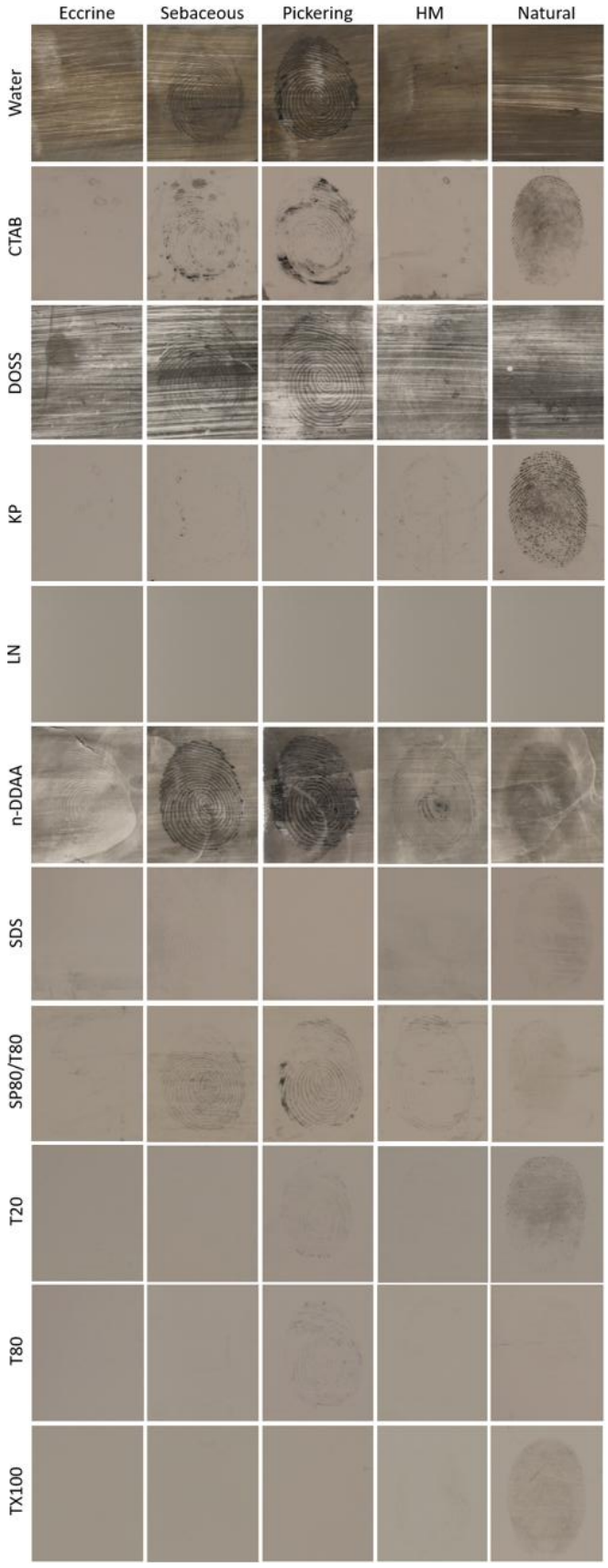


Figure E 1 Development of artificial residue fractions and natural marks by all surfactants on ceramic

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