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Rapid Communication

Synthesis and application of an aqueous nile red microemulsion for the development of fingermarks on porous surfaces

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ABSTRACT

An oil-in-water microemulsion containing a luminescent dye, nile red, has been synthesised using a solvent-diffusion method. This has been demonstrated to be effective in developing fresh latent fingermarks on porous surfaces. The working solution is made using a binary surfactant solution to create a lactescent dual organic–aqueous phase intermediate, which subsequently results in a transparent microemulsion after the organic solvent has evaporated. The solution is non-toxic and performs comparatively with a previously published methanolic formulation but at a much lower cost and with an extended shelf life. The microemulsion outperforms a previously reported aqueous nile blue formulation for the development of both charged and natural fresh fingermarks, and requires lower exposure times for image recording.

1. Introduction

Latent fingermarks occur as a result of the residues that are deposited onto a surface when the friction skin of the hand comes into contact with that surface. There are three major glands in the human body that are responsible for the secretion of bodily fluids through the skin medium: the eccrine glands, the sebaceous glands and the apocrine glands [1]. The secretions from the eccrine and sebaceous glands are most commonly found in fingermark residue; eccrine constituents are endogenous to friction ridge skin, whilst sebaceous constituents are present through contact of friction ridge skin with the face and other regions of the body that have hair follicles. Fingermark residue also contains lipids that originate in the epidermis and are contained within a hydrolipid film (also known as the acid mantle) that is present on the surface of the skin [2–4].

The detection of lipids in latent fingermark investigations is crucial for items that have been wet, as the apocrine and eccrine components are water soluble; however, the water insoluble lipid component of sebum from the sebaceous glands and from the hydrolipid film on the surface of the epidermis, will remain after water exposure and can be targeted for development. The method of choice to target these lipids is physical developer (PD) [5]. PD works by selectively reducing silver ions to metallic silver on reactive nucleating sites of the water insoluble fraction of fingermark residue [6]. PD is the only technique in routine use for the development of fingermarks on porous surfaces that have been wet. It can also be sequentially used after treatment with amino-acid sensitive techniques if the sample has not been wet, in which case it can develop additional detail or additional fingermarks. Much research has been undertaken to optimise the PD method as the working solution has a short shelf life due to its inherent instability, although this is difficult when the exact mechanism by which the technique operates is still largely unknown; the technique is also time consuming and laborious to apply. PD can interfere with the forensic examination of handwriting, ink, paper, indented impressions and body fluids [7], so planned sequential analyses are important. Due to the inherent difficulties encountered when using PD, the implementation of alternative or complementary techniques is of significant interest.

Oil Red O (ORO) is a lysochrome commonly used for biological purposes to colour lipoproteins that have been separated by electrophoresis on cellulose acetate [8]. In recent years it has been shown to be a viable reagent for the development of latent fingermarks on porous surfaces that have been wet, resulting in red ridges on a pink background with no further visualisation techniques needed [4,8–11]. While these studies have collectively shown that ORO presents some practical advantages over PD, and

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that it can be used in sequence prior to PD treatment, it is less effective on marks older than 4 weeks.

Nile red has been used to develop fingermarks on porous surfaces that have been wet, with promising results [12]. Nile red is a luminescent benzophenoxazine dye that has been used as a post-cyanoacrylate (CA) stain for the luminescence enhancement of CA developed marks on non-porous surfaces [13,14]. More recently, in a pseudo-operational study on five year old exam booklets, nile red in a methanolic-aqueous working solution has shown great promise as a lipid sensitive fingermark development reagent that develops more marks when used in sequence with PD than can be developed by PD alone [12,15], demonstrating the importance of sequencing nile red with PD. The nile red solution did not appear to enhance previously developed PD marks, but targeted undeveloped fingermarks, indicating that the techniques are complementary. This is possibly due to the two techniques having discrete lipid targets that vary across aged fingermarks from different donors.

Despite the demonstrated abilities of the published nile red formulation, improvements were needed to avoid the deposition of nile red particles onto the substrate, which is caused by the hydrophobic nature of nile red in the methanolic-aqueous working solution resulting in precipitation during the treatment of exhibits. This phenomenon also resulted in a very short shelf-life for the working solution. Research has been undertaken to synthesise nile red derivatives with increased water solubility; however, organic solvents are still needed for the complete solubilisation of these compounds in the working solutions [15]. The removal of potentially hazardous organic solvents from the working solution is also of interest from an occupational health and safety perspective.

Recently, a new formulation of the nile red working solution has been developed by our research group. This formulation results in a working solution that solubilises the nile red by incorporating the dye into an aqueous microemulsion. Microemulsions can be formed by mixing a primary surfactant with water and an oil (in this case, a hydrophobic compound dissolved in an organic solvent) to create a lactescent emulsion that is then titrated with a secondary surfactant until the mixture becomes clear. The volatile organic solvent is then evaporated from the solution. For this study, the method has been adapted by initially adding the oil (nile red in dichloromethane) to stirring water, and then adding an aqueous solution containing both primary and secondary surfactants. The formulation has been created with a view to ease of operational implementation as the surfactant solution employed is the same as the surfactant solution used in the PD working solution (Tween 20 formulation [16]), with Tween 20 and *n*-dodecylamine acetate acting as the primary and secondary surfactants, respectively.

It is known that nile red targets the lipids found in sebaceous gland secretions, and it has been demonstrated to develop five year old uncharged marks that were previously undeveloped by PD (with PD possibly targeting the epidermal originating lipids) [12,15]. To be effective operationally, the nile red microemulsion needs to be sensitive enough to develop sebaceous lipids that have not been placed there in high concentrations immediately prior to development (which is the case when marks are "charged"), but persist in lower concentrations from normal hand to face contact (referred to as "natural" marks).

The recently published guidelines by The International Fingerprint Research Group (IFRG) [17] for the evaluation of fingermark development techniques has provided a structural framework for the effective and comprehensive development of fingermark enhancement techniques. It stipulates directives for undertaking an unbiased initial assessment of novel development techniques. The IFRG guidelines [17] encourage the use of both natural and charged fingermarks so that the significance of preliminary results is not overstated. It has therefore been deemed important to use a combination of both appropriately charged marks (which provide a positive control) and natural marks (which are more realistic) when assessing and optimising the microemulsion or when comparing it with other development solutions.

Frick and co-workers [18] recently published an aqueous nile blue formulation that is effective as a fingermark development method, with this being attributed to the presence of nile red in the solution. The nile red is present as an impurity in nile blue, and may also be formed as a hydrolysis product of nile blue [19]. The formulation was shown to develop fresh, charged fingermarks on paper, and is claimed to outperform the previously published methanolic nile red solution. However, no results were mentioned for the development of natural marks.

In this research, a nile red microemulsion has been compared with (1) the methanolic nile red working solution [12] and (2) the nile blue solution [18] to evaluate and compare the effectiveness of each solution on both charged and natural fingermarks. This research is ongoing to satisfy the Phase 1 and Phase 2 directives specified by the IFRG guidelines. At this stage, one fingermark donor, known to produce good natural and charged fingermarks, and one substrate were chosen for the preliminary comparison of the three fingermark development solutions. All experiments were repeated three times and the fingermark collection parameters were meticulously controlled.

2. Materials and method

All solvents (AR grade), were obtained from BDH-Prolabo Chemicals (VWR International Pty. Ltd., Australia). Nile red (BioReagent, Sigma–Aldrich, USA), sodium hydroxide (Merck Chemicals, Australia), nile blue (Revector Microscopical Stain, Hopkin & Williams Ltd.), polyoxyethylenesorbitan monolaurate (Tween 20) (Sigma–Aldrich, Australia) and *n*-dodecylamine acetate (MP Biochemicals Inc., Germany) were used as supplied.

2.1. Surfactant solution (Solution A)

1.5 mL Tween 20 and 1.5 g *n*-dodecylamine acetate were added to 500 mL deionised water with stirring until dissolved.

2.2. Nile red stock solution (Solution B)

10 mg nile red was added to 1000 mL dichloromethane with stirring until dissolved.

2.3. Nile red microemulsion (Solution 1)

100 mL deionised water was placed in a beaker containing a magnetic stirrer bar. 12 mL of Solution A was added, followed by the slow addition of 8 mL Solution B. The solution was vigorously stirred until the dichloromethane had evaporated; this process was observed by the transformation of a two-phase solution (clear aqueous and bright pink organic phase), to a clear, single-phase, light purple microemulsion. The solution was then stirred for a further 10 min to ensure complete dichloromethane evaporation.

2.4. Nile red methanolic working solution (Solution 2) [12]

170 mL 0.1 mg/mL sodium hydroxide solution was slowly added to 230 mL 0.1 mg/mL nile red in methanol with constant stirring and was used immediately.