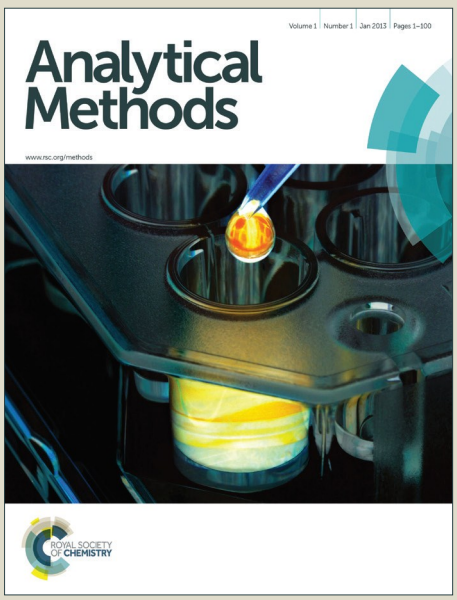
Analytical Methods

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Analytical Methods

RSCPublishing

PAPER

Staining using the lipid dye LD540 in fluorous media: application to sebaceous latent fingermarks

Cite this: DOI: 10.1039/xoxxoooooox

Angelique Qi[a] and Gordon M. Miskelly[a]

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

www.rsc.org/

Abstract Enhancement of the sebaceous components of fingermarks is often performed using an aqueous staining solution. We propose an alternative approach using a bodipy-based lipid-selective dye (LD540) in a fluorous solvent. This fluorous approach can result in excellent retention of the spatial distribution of lipids and can be used on substrates unsuited to treatment with aqueous solutions. We demonstrate the new possibilities enabled by using fluorous solvents by staining fingermarks on an NaCl salt plate and on a block of ice.

The enhancement of fingermarks is often assumed to be a well-developed and routine endeavour. However, there is still a need to understand the processes of fingermark deposition and to determine optimal methods for enhancing fingermarks on different substrates. These investigations are practical, but also provide opportunities for fundamental studies of fingermark composition, fingermark-substrate interactions, solubility and, in the case of sebaceous fingermark enhancement, partitioning of enhancement dyes into lipid-containing material.

Latent fingermarks are most commonly composed of secretions from the sebaceous and/or eccrine glands.[1, 2] The fingertips do not have sebaceous glands, but the lipid-containing material from these glands is transferred to the hands during normal activity.[1, 3] Forensic scientists have targeted sebaceous residues in several scenarios, including after evidence has been immersed in water or after attempted development of eccrine residues.[4-6] While the standard method to enhance sebaceous fingermarks has been with physical developer,[7] because this technique is experimentally challenging several groups have recently reported the successful use of simpler dye partitioning techniques similar to those used for staining lipids in histology.[4-6, 8, 9] Thus, when a sebaceous fingermark is treated with an aqueous solution of a dye such as Nile Red the dye partitions from the aqueous solution into the sebaceous deposit, which is itself insoluble in the aqueous solution. There is active investigation into how the fingermark components enhanced by physical developer differ from those enhanced by aqueous dye partitioning, with some researchers focussing on single solvent properties such as dielectric constant or polarity.[6] The focus on a single parameter to describe solubility in sebaceous fingermark enhancement has led to statements such as "..such (finger)marks are removed by the action of organic solvents."[1]

Several lines of study show that solubility cannot in general be explained with just one parameter. Thus, Curran and co-workers have mapped polarity and fluorophilicity of solvents to rationalise observed behaviour in fluorous separations,[10, 11] while Durkee has applied Hansen's 3-parameter solubility model to rationalise the optimisation of solvents in cleaning applications.[12, 13] Hansen links an overall solubility parameter ($\delta_{overall}$) to parameters associated with the dispersion, polarity and H-bonding properties of a solute or solvent through the equation

$$\boldsymbol{\delta^2_{overall} = \delta^2_{dispersion} + \delta^2_{polar} + \delta^2_{H-bonding}}$$

This model then predicts that solvents are more likely to dissolve a solute when ΔR is small, where

$$\boldsymbol{\Delta R^2 = 4(\delta_{dispersion,\ solvent} - \delta_{dispersion,\ solute})^2 + (\delta_{polar,\ solvent} - \delta_{polar,\ solute})^2 + (\delta_{H-bonding,\ solvent} - \delta_{H-bonding,\ solute})^2}$$

Tables of the Hansen parameters are available, together with operational methods for estimating parameter values for new substances.[13, 14]

The success of partitioning dyes from an aqueous solution into sebaceous secretions can then be predicted from a plot of Hansen parameters (Figure 1). In this Figure, methyl oleate, triglycerides (shown as "lard") and cholesterol are representative of component classes within sebaceous secretions and so have been used to define the region "sebum". Thus, Hansen solubility parameters indicate that an aqueous solution should not dissolve the major sebaceous

components. Nile Red is more soluble in the sebaceous material than in the aqueous solution and so partitions into the fingermark. The focus of this present study, however, is the portion of the graph in Figure 1 at low dispersion and polarity.

The fluorous solvent HFE-7100 (a mixture of methyl nonafluorobutyl ether and methyl nonafluoroisobutyl ether) is a commonly-used carrier solvent in eccrine fingermark enhancement, and we and others have investigated its effect on sebaceous fingermarks. While dyes in HFE-7100 can successfully enhance some sebaceous fingermarks, especially if the fingermarks are charged by first rubbing the fingers on areas of skin rich in sebaceous glands† (vide infra), HFE-7100 can cause the removal of some sebaceous marks, and this is consistent with the comparability of its Hansen solubility parameter values with those of some sebaceous components. Since Nile Red had been successfully used for enhancement of fingermarks we first trialled using fluorous solutions of this dye. Our investigations showed that Nile Red is insoluble in HFE-7100, and when sufficient methanol (0.2% v/v) or isopropanol (4.5% v/v) was added to HFE-7100 to increase the solubility of Nile Red the resulting solution could dissolve sebaceous fingermarks. This is consistent with the use of an HFE-7100: isopropanol azeotrope for specialty cleaning.[12]

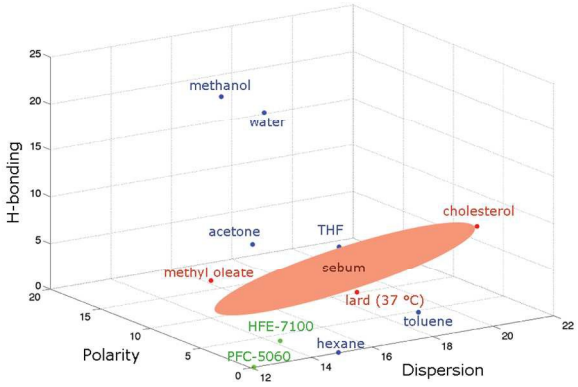
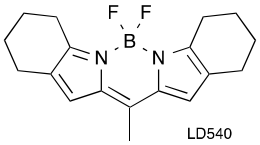


Figure 1. Graph showing Hansen solubility parameters for selected common solvents (blue), two fluorinated solvents (green), and three materials representative of sebaceous sections (red). Units of axes: $MPa^{1/2}$.

We then synthesised the recently-reported lipid-selective bodipy dye LD540,[15, 16] and investigated its solubility in fluorous solvents and partitioning behaviour into fingermarks. LD540 is soluble in neat HFE-7100 and in 1:1 blends of HFE-7100 with perfluoromethyldecalin or perfluorooctane. Blends of HFE-7100 with perfluoroalkanes (e.g. perfluorohexane (PFC-5060), perfluorooctane or perfluoromethydecalin) have Hansen solubility parameters more dissimilar to sebaceous secretions than neat HFE-7100 and so are less likely to dissolve fingermark components. The original reports of LD540 did not provide full chemical characterisation, so we have included that in the Electronic Supplementary Information.



LD540

We have enhanced both charged and uncharged (or "natural") fingermarks on glass substrates to meet the minimum criteria of a Phase 1 trial as proposed by the International Fingerprint Research Group (IFRG).[17] We have then used charged fingermarks to investigate behaviour of the LD540 in fluorous solvents on novel substrates. Exposure of one-day old charged and uncharged fingermarks on glass microscope slides to solutions of LD 540 (0.1 mg mL^{-1}) in HFE-7100: perfluorooctane 1:1 for 5 min resulted in similar enhancement to that observed for a 5 min soak in a basic methanolic aqueous Nile Red solution, although the LD 540 solution resulted in slightly brighter fluorescence and slightly improved retention of fine details in the lipid pattern, Figure 2 and Supplementary Information. LD540 in neat HFE-7100 also developed charged fingermarks and showed some enhancement of non-charged marks but was not as good as the other two solutions, with luminescence intensities 5-10 times lower than for the fluorous solvent blend, Supplementary Information. The brightness reduction may have been due to some dissolution of the fingermark or decreased partitioning of the dye into the fingermark. In all cases, the fingermarks were illuminated with a Rofin Polilight using a filter centred at 505 nm with 40 nm bandwidth, and were imaged with a Canon DSLR camera with 100 mm macro lens and a 550 nm longpass filter, with imaging controlled with DSLR Remote (Breeze Systems) and collecting both jpg and raw (CR2) images. The fluorescence from the charged fingermarks was easily detected, while the uncharged fingermarks gave limited development and required much longer camera exposure times (ca. 10-fold) or wider apertures than the charged fingermarks (Supplementary information).

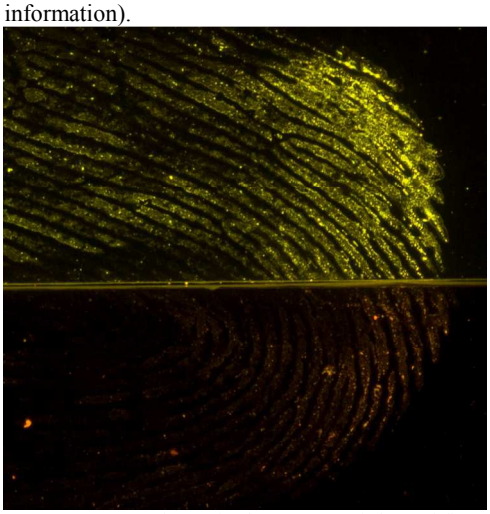


Figure 2. Comparison of enhancement of charged fingermark with LD540 in HFE7100:perfluorooctane 1:1 (upper) and Nile Red in aqueous methanol (lower). Further comparisons of charged and uncharged fingermarks are in the Supplementary Information.

Using fluorous solvents enables the enhancement of lipid deposits on substrates that are not suitable for contact with aqueous solutions. As a first demonstration we deposited charged sebaceous fingermarks on a sodium chloride infrared plate, stored the plate overnight in a desiccator, then developed the fingermarks with LD540 in HFE-7100:perfluoromethyldecalin 1:1 the following day, Figure 3. We also enhanced sebaceous fingermarks on a toffee, as representative of a sugar based food item that was unsuited for water treatment.

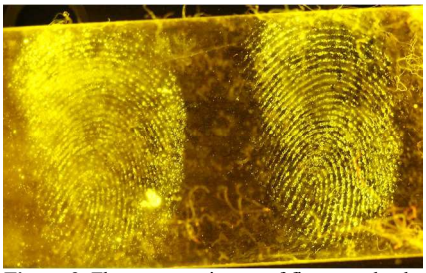


Figure 3. Fluorescence image of fingermarks deposited on NaCl plate and then enhanced with LD540 in HFE-7100:perfluoromethyldecalin 1:1

As a final demonstration, we deposited charged fingermarks on a block of ice at -16 °C and then attempted to enhance fresh fingermarks and fingermarks that were 1 and 2 days old. The enhancement solution was LD540 in either HFE 7100 or HFE-7100: perfluorooctane 1:1 pre-cooled to -16 °C. Some LD540 precipitated during the cooling of the dye solutions, so the solutions were saturated with LD 540 when used. Fluorescent imaging of the fingermarks was performed while the ice was placed in an insulated box cooled with dry ice. Similar quality fingermark images were obtained for both LD540 solutions. This demonstrated a) that sebaceous fingermarks can be deposited on ice b) that these fingermarks can retain their integrity for at least 2 days and c) that the fingermarks can then be enhanced. To our knowledge this is the first report of the enhancement of a fingermark on ice.

Figure 4. Fluorescence image of fingermark deposited on ice at -16 °C, left 2 days, and then enhanced with LD540 in HFE-7100 at -16 °C.

Conclusions

Solutions of LD540 in mixed fluorous solvents can stain lipids and related biological material within sebaceous fingermarks. The fluorous solvent offers advantages in terms of retention of spatial detail of the fingermark minutiae, non-reactivity, and decreased likelihood of dissolution of substrate or materials of interest compared to an aqueous solution. However, as noted by the IFRG further studies of the enhancement of uncharged (natural) fingermarks from a range of donors and with a range of times since deposition are needed before this method can be recommended as a replacement for any current fingermark enhancement technique.[17] Our demonstration that we can enhance sebaceous fingermarks on ice suggests that this fluorous approach might also be useful for staining lipids in frozen sections of tissue or for staining biofilms on ice. These advantages need to be balanced with the substantially greater cost of the fluorous solvents compared to aqueous staining solutions, with the solvents we have blended with HFE-7100 being particularly expensive. In addition, users should be aware that the perfluorocarbons have very high global warming potential, so that the techniques described here should be used sparingly and where possible with capture and recycling of the fluorous solvents.

Acknowledgements

We thank Siyu Peng for performing the NMR characterisation of the LD540. We thank the University of Auckland for financial support of this research.

Notes and references

[a] Forensic Science Programme, School of Chemical Sciences, University of Auckland, Private Bag 92019, Auckland New Zealand 1142.

† For uncharged fingermarks, hands were washed and then normal activities were undertaken for 2 h prior to fingermark deposition. "Charged' fingermarks were prepared by wiping fingers across the forehead immediately prior to fingermark deposition. The fingermark donors consented to their prints being used as part of this study.

Electronic Supplementary Information (ESI) available: [Synthesis and characterisation of LD540, details of enhancement solutions, images of charged and uncharged fingermarks on glass, images of different aged fingermarks on ice]. See DOI: 10.1039/c000000x/

1. C. Champod, C. Lennard, P. Margot and M. Stoilovic, *Fingerprints and Other Ridge Skin Impressions*, CRC Press, Boca Raton, 2004.
2. R. S. Ramotowski, in *Advances in Fingerprint Technology*, eds. H. C. Lee and R. E. Gaensslen, CRC Press, Boca Raton, 2nd edn., 2001, pp. 63-104.
3. R. Gaensslen and H. Lee, *Advances in Fingerprint Technology*, CRC Press, 2001.
4. K. Braasch, M. de la Hunty, J. Deppe, X. Spindler, A. A. Cantu, P. Maynard, C. Lennard and C. Roux, *For. Sci. Int.*, 2013, **230**, 74-80.
5. M. de la Hunty, X. Spindler, S. Chadwick, C. Lennard and C. Roux, *For. Sci. Int.*, 2014, **244**, e48-e55.
6. J. Salama, S. Aumeer-Donovan, C. Lennard and C. Roux, *J. For. Ident.*, 2008, **58**, 203-237.
7. T. Kent, ed., *Manual of Fingerprint Development Techniques*, 2nd edn., Home Office, Sandridge, 1988.
8. A. Becue, S. Moret, C. Champod and P. Margot, *Biotech. Histochem.*, 2010, **86**, 140-160.
9. A. A. Frick, F. Busetti, A. Cross and S. W. Lewis, *ChemComm*, 2014, **50**, 3341-3343.
10. M. Matsugi and D. P. Curran, *Org. Lett.*, 2004, **6**, 2717-2720.
11. W. Zhang and D. P. Curran, *Tetrahedron*, 2006, **62**, 11837-11865.
12. J. B. Durkee, in *Hansen Solubility Parameters: A User's Handbook*, ed. C. M. Hansen, CRC Press, Boca Raton, 2007, pp. 203-229.
13. J. B. Durkee, *Cleaning with Solvents: Science and Technology*, Elsevier, Amsterdam, 2014.
14. C. M. Hansen, *Hansen Solubility Parameters: A User's Handbook*, CRC Press, Boca Raton, 2007.
15. L. R. Morgan and J. H. Boyer, *USA Pat.*, 5,446,157, 1995.

16. J. Spandl, D. J. White, J. Peychl and C. Thiele, *Traffic*, 2009, **10**, 1579-1584.
17. International Fingerprint Research Group (IFRG), *J. Forensic Ident.*, 2014, **64**, 174-200.