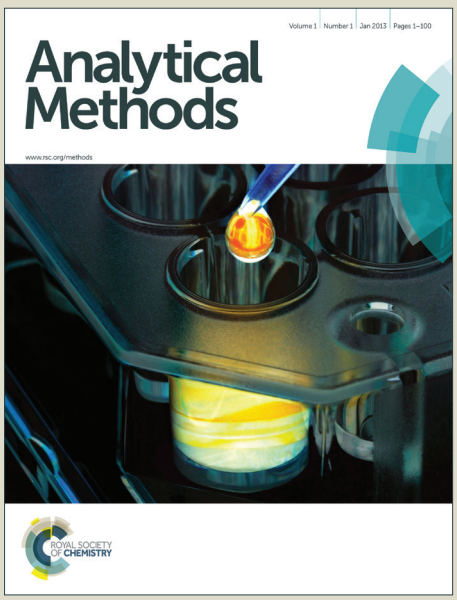
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Journal Name

COMMUNICATION

Fluorescent polyelectrolyte for the visualization of fingermarks†

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Lars van der Mee,[a] Esther S. Y. Chow, [b] Louis C. P. M. de Smet,[a] Marcel de Puit, [c,a] Ernst J. R. Sudhölter,[a] and Wolter F. Jager*[a]

A fluorescent polyelectrolyte, poly(allylamine hydrochloride (PAH) functionalised with the 7-amino-quinolinium chromophore, has been synthesized for the visualization of latent fingerprints. Exposing fingerprints to pH neutral, dilute aqueous solutions of this polymer results in bright green fluorescent images, which are clearly visible by the naked eye.

The surface at the tip of a finger is patterned, which allows one to grip surfaces without slipping. When an individual touches a surface with his hands the pattern on his palms and fingertips is transferred to the surface. The composition of the deposited material is very complex and varies per individual. Most of the deposited material is excreted by glands and sweat pores, another part is foreign material that a person has handled previously. Fingermarks found at the crime scene have been used for over 100 years to lead investigators to the suspect.[1] This form of individualisation is still popular as two individuals with identical ridge details on a finger are yet to be found. In addition to ridge details, mapping of sweat pores at the fingertips can be used for identification purposes as well.[2] Identification by fingerprint pattern can be augmented by chemical analysis of the various constituents present in the fingerprint deposition that confirm the identity of the donor, for example DNA.[3] Foreign material in the fingerprint residue that is relevant for forensic investigations, such as gun powder residues, explosives, or drug metabolites can be detected as well.[4]

Various methods are presently used to colour fingerprints[5] and to make them visible to the human eye. Colour development may be the result of a chemical reaction on the fingerprint that produces a coloured species,[6] or attachment of a dye, either covalently or as a result of physical adherence. In most cases dyes adhere to the deposited material on the fingerprint , thus generating a positive image.[7] Direct colorimetric detection of specific components in the fingerprint, such as amino acids,[8] enzymes,[9] explosives, or drug metabolites[10] has been reported as well.

Fingerprint visualisation by fluorescence, as opposed to optical absorption, is highly attractive because the detection limit of fluorescent dyes is very low, in principle down to the single molecule. [11] Even for detection by the human eye minute amounts of a staining material are required. Various methods for attaching fluorophores have been described, such as the deposition of luminescent (nano)particles,[10.12] either from an aerosol or from solution. Other methods for fluorescence staining are the use of lipophilic dyes, which are generally deposited from solutions in organic solvents,[13] and attaching fluorescent labels to cyanoacrylates.[14]

Here we report the development and application of a water-soluble fluorescent polymer for fingerprint visualisation. The fluorescent polymer adheres to the fingerprint by non-covalent interactions[15] and can be applied by exposing a fingerprint to a pH neutral, dilute aqueous solution. To the best of our knowledge, this is the first time a water-soluble fluorescent polymer has been used for fingerprint visualization.

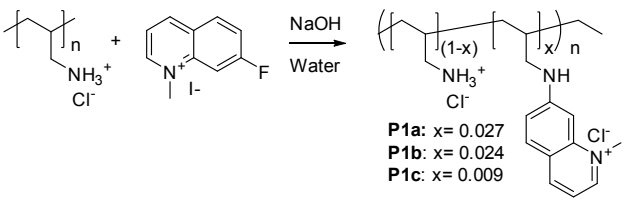


Figure 1. Synthesis of the fluorescent polymers **P1a**-**P1c**.

Polymer **P1** is synthesised by a polymer analogous reaction of poly(allylamine hydrochloride (PAH) with 7-fluoro-1-methyl quinolinium iodide,[16] in water (Figure 1).§§§ We have synthesised

[a] Department of Chemical Engineering
Delft University of Technology
Julianalaan 136, 2628BL Delft, The Netherlands.
[b] Department of Chemistry
University of Leicester
University Road, Leicester, LE1 7RH, United Kingdom.
[c] The Netherlands Forensic Institute
Laan van Ypenburg 6, 2497 GB, Den Haag, The Netherlands.

† Electronic Supplementary Information (ESI) available: Experimental details and supporting Figures. See DOI: 10.1039/x0xx00000x

the polymer with feed ratios of 5, 2.5 and 1.25 %, resulting in polymers with degrees of functionalization of 2.7, 2.4 and 0.9 %, respectively, according to their UV-Vis spectra.[17] In the ^{1}H NMR spectrum the resonance signals of the 7-amino-1-methylquinolinium chromophore are clearly visible, but the degree of substitution cannot be determined from the NMR spectrum by peak integration (Figure S1).

Table 1. Optical properties of polymer **P1a** in water as a function of the pH

	pH = 3	pH = 7	pH = 9
λ_{abs} (nm)	411	417	418
λ_{em} (nm)	502	506	510
Φ_F	0.50	0.36	0.10

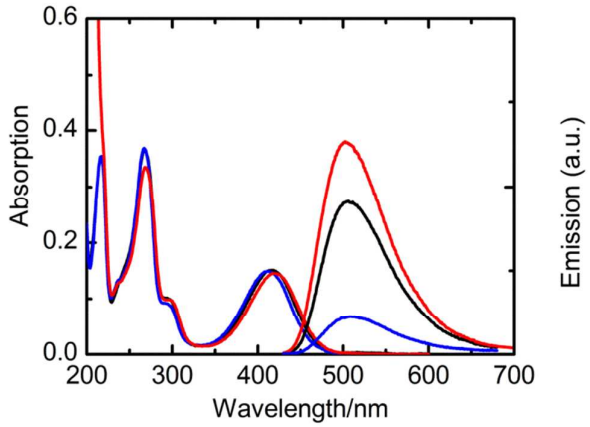


Figure 2. Emission of **P1a** as a function of the pH. Emission and absorption of **P1a** at pH= 3 (red), pH= 7, (black) and pH= 9 (blue).

The spectroscopic properties of **P1a**-**P1c** in water have been investigated, and are summarised in Tables 1 and S1 and Figure 2. The absorption and emission wavelengths of **P1**, 417 and 506 nm, respectively, are similar to those of the parent chromophore **1** (Figure 2).[17] The fluorescence quantum yields (Φ_F) and lifetimes (τ_F) of **P1a**-**P1c** in solution are lower than the values of 0.63 and 11.5 ns, respectively that have been reported for the chromophore **1** (Figure S2).[16a] A decrease of the quantum yield Φ_F with increasing degrees of functionalization is clearly visible in Table S1. At pH = 3 Φ_F of **P1c** is 0.61, a value close to the 0.63 measured for 7-(methylamino)-1-methyl-quinolinium iodide **1** (Scheme S1). By increasing the degree of functionalization, however, Φ_F decreases to 0.50 for **P1a**. This may be due to concentration quenching.[18,19] A decrease in Φ_F upon increasing the pH is also evident from Tables 1 and S1. Upon increasing the pH, the fluorescence quantum yields of **P1a** decreases by a factor 5. This fluorescence quenching is caused by the electron donating amino groups on the PAH chain, which quench the quinolinium fluorophore by photo induced electron transfer (PET).[21] In summary, efficient fluorescence of polymer **P1** in aqueous solution has been observed. The fluorescence intensity is highly sensitive to pH, exhibiting strong fluorescence at low pH values, and is moderately sensitive to concentration quenching.

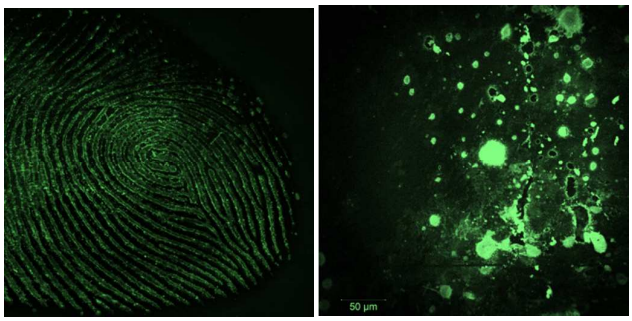


Figure 3. a: Fluorescence image of fingerprints stained by a 0.2 % mg/ml solution of **P1a** at pH 7 (left). b: Confocal images of the fluorescence of a fingerprint stained with **P1a**, taken at a height of 4.5 µm above the glass substrate (right). The fingerprint residue is deposited on the right side of Figure 3b. Samples were excited at 458 nm.

When latent fingerprints on glass were dipped in a 0.2 mg/ml solution of **P1a** in water for times as short as 1 second, brightly fluorescent green fingerprints were obtained. §§ The images were positively stained, implying that the deposited material of the fingerprint was highly fluorescent against a dark background (Figure 3a). Fresh prints give excellent images and older prints still provide good quality images, with sufficient detail for identification (Figure S3).

In order to explore the scope and limitation as well as the mechanism of the staining process, we have varied a number of parameters during the development procedure, such as exposure time, polyelectrolyte concentration and pH. We also examined compound **1**, the fluorophore present in **P1**, as a fluorescent dye for fingerprint development (Scheme S1).

Measurements at different concentrations of polyelectrolyte **P1a**, showed that the best visualisation was obtained at a concentration of 0.2 mg/ml (~5.7×10^{-5} mol/l), with typical exposure times of 5 minutes (Figure S4). At lower concentrations, (0.02 mg/ml), prints are hardly visible. At higher concentrations, (2 mg/ml), prints are visible, but the contrast is inferior. In all cases, the brightness of the fingerprint has changed, while the background remains virtually non-fluorescent. The decrease of contrast upon increasing the concentration of **P1a**, was somewhat unexpected, and may have been caused by a diminished deposition of **P1a**, or by a decreased fluorescence quantum yield of the deposited polymer. A decreased deposition at higher polymer concentrations due to an increased ionic strength, is unlikely because an increase in the ionic strength (induced by adding NaCl) did not influence the fingerprint staining using a "standard" 0.2 mg/ml solution of **P1a**.

Changing the pH, as shown in Figure S5, has a very pronounced influence on the quality of the developed fingerprints. At pH = 3, when the polymer is fully protonated and highly fluorescent ($pK_A \approx$ 8.8 for PAH),[21] a very weakly fluorescing image is obtained. At pH =

7, when the majority of amino groups on the PAH is protonated, the best images, with the highest contrast between background and fingerprint are obtained. At pH = 9, when most of the amino groups are non-protonated, a weakly fluorescent image is obtained. In all cases the background remains virtually non-fluorescent, while the brightness of the fingerprint contact area varies. The decreased fingerprint brightness at pH = 9, is fully in line with the observation that the fluorescence of **P1** in solution decreased upon increasing the pH, due to photo induced electron transfer from adjacent amino groups at the PAH backbone. The low fingerprint emission at pH = 3 is probably due to poor binding of **P1** to the fingerprint material. This may be caused by positive charging of the fingerprint surface by protonation of amino functionalities present in the fingerprint deposition.

We have tested the visualisation of fingerprints by exposure of a dilute pH neutral aqueous solution of **P1a** on different substrates. Staining fingerprints on different background materials gave positive images in all cases. Staining of the background is limited in all cases, which indicates limited affinity for binding at the surface or efficient fluorescence quenching of surface-bound fluorophores. Images of developed fingerprints on phenol resin based high-pressure laminate (Trespa interior panel), plastic and metal substrates are depicted in Figure S6. From Figure S6 it is clear that the best quality prints have been obtained on glass.

The information provided so far indicates that **P1** is an excellent staining agent for the visualisation of latent fingerprints, in terms of the rate, simplicity, safety of the entire procedure, and the quality of the images. It is, however, not clear why **P1** specifically binds to the deposited fingerprint material. In terms of its chemical composition the main question is whether the chromophore itself, which composes 0.9-2.7% of the material[22] plays a crucial role, or whether the polycationic nature of the PAH backbone is essential. Obviously, the fact that the backbone is a polyelectrolyte, facilitates the water-solubility of the fluorophore-modified polymer to a large extend. It should be explicitly mentioned that the use of water as the solvent for fingerprint development is a significant advantage with respect to safety, substrate compatibility and, as far as the lipophilic components are concerned, retaining the composition and integrity of the fingerprint.[23]

When a 0.015 mg/ml solution of the water-soluble 7-methylamino-1-methyl-quinolinium iodide **1**, which contains a similar concentration of the quinolinium chromophore (~5.0×10^{-5} mol/l), was used for staining, a positive image was developed (Figure S7). However, the quality of this image was inferior in comparison to those obtained with **P1a**. In particular significant background fluorescence was observed, which severely lowers the contrast. Staining experiments with other water-soluble fluorescent dyes, like the cationic Nile Blue and the anionic fluoresceine gave results that were similar (cationic dye) or even worse images (anionic dye) compared to those obtained with compound **1**. These results suggest that individual chromophores do not exhibit preferential binding to the fingerprint material and that cationic chromophores perform slightly better than anionic chromophores. These results also suggest that chromophore attachment to a cationic polymer is a prerequisite for effective fingerprint staining. Apparently, the exact nature of the fluorescent chromophore is of less important, which suggests that PAH loaded with any other fluorophore may be suitable for staining fingerprints.

The observation that **P1a** does not stain glass, despite the fact that polycations are known to bind to negatively charged surfaces like glass and silica,[24] deserves extra attention. From X-ray photoelectron spectroscopy (XPS) experiments on clean glass plates, deposition of C and N onto the glass surface after development with **P1a** is clearly visible (Figure S8). This result implies that **P1a** is deposited on a clean glass slide, although the amount of deposited material may be very small. Close examination of glass plates that have been exposed to aqueous solutions of **P1a**, reveals weak fluorescence from the exposed surface, thus confirming this observation, see Figure S9.

Finally, the question remains why **P1** prefers to reside on the fingerprints, which consist to a large extent of sebaceous material. Based on the notion that fresh fingerprint material is composed mainly of water, containing small amounts of bio(macro)molecules and fatty components, fresh fingerprints can be regarded, at least partly, as emulsions. For such emulsions, part of the water-lipid interface will contain amphiphilic compounds. It is expected that **P1** will bind to such interfaces, which based on the isoelectric points of the commonly encountered human sweat proteins are negatively charged at pH 7.[25] In Figure 3b confocal images of the fluorescence of a fingerprint is depicted. The images in Figure 3b clearly show that fluorescent material is unevenly deposited on the fingerprint residue, which is present on the right part of the image in Figure 3b. Polymer **P1a** is accumulated in large aggregates with typical diameters ranging from 2 to 20 μm. A stack of confocal images revealing the depth profile of the fluorescence, depicted in Figure S10, indicates that the fluorescent material is deposited over several micrometres, roughly at a distance of 3.6-9.8 μm from the glass substrate. Thus fluorescent particles with μm dimensions are formed which are attached to the fingerprint residue. This explains why the emission intensity from the print largely exceeds that of the flat glass surface that is coated with **P1** as well.

In conclusion, we have developed the fluorescent water-soluble polymer **P1** for the visualisation of fingerprints. To the best of our knowledge **P1** is the first water-soluble[26] polymer used for fingerprint development. Polymer **P1** enables excellent visualisation of fingerprints by a very fast, safe, simple and robust procedure; just dipping the print in a dilute pH-neutral solution of **P1** in water. Molecules like **P1** can also be used to visualize fingerprints on large substrates that are less compatible with the dipping approach, because the deposition of polyelectrolytes can also be realized by spray-coating.[27] Fingerprints are stained by the deposition of μm-sized polymer aggregates on the fingerprint residue. Further research on structure property relations of fluorescent polyelectrolytes for fingerprint staining, the mechanism of polymer deposition on fingerprints and the development of probes with

differently coloured chromophores with increased emission intensity, are currently underway.

Experimental Details

§: Synthesis of **P1a**: Poly(allylamine hydrochloride) PAH (1.00 g, 10.68 mmol), 7-fluoro-1-methylquinolinium iodide (0.16 g, 0.554 mmol, 5.0 mol%) and NaOH (0.47 g, 11.9 mmol) were dissolved in H_2O (20 ml), heated to 50 °C and stirred for three hours. The solution was transferred to dialysis tubing (Sigma Aldrich D0530, 32mm, 12400 MWCO) and immersed in demineralized water for seven days, water being refreshed twice daily. Subsequently, the product was freeze-dried to remove water and isolated as a bright yellow powder. Yield 0.7 g 60%. ^{1}H NMR (400 MHz, D_2O, 25°C, 3-(trimetylsilyl)propionic-2,2,3,3-d4 acid, sodium salt): δ=8.61 (b, 2H; CH), 7.96 (b, 1H; CH), 7.42 (b, 2H; CH), 6.76 (b, H; CH), 4.31 (b, 3H; CH3), 2.88 (b, PAH; CH2 backbone), 1.87 (b, PAH; CH), 1.41 (b, PAH; CH2). Polymers **P1b** and **P1c** were synthesised by an identical procedure, using 80 and 40 mg 7-fluoro-1-methylquinolinium iodide, respectively.

§§: Glass slides (75 × 25 mm) were washed with a glass cleaner, rinsed with deionised H_2O and acetone and dried with air. A fingerprint was made on the plates and left to dry for 24 hours. Stock solutions of **P1a** were made by dissolving 100 mg, 1 mmol **P1a** in 50 ml water and adjusting the pH to 7 using 1N HCl. The plates with fingerprints were immersed in solution for five minutes, rinsed with H_2O and dried with air. The results described in the manuscript are from the prints of a single individual. Tests on a collection of latent fingerprint from a dozen individuals obtained from the Netherlands Forensic Institute (NFI) confirmed that our staining method works in all cases.

Acknowledgements

Daniela Ullien, Sumit Sachdeva and Alexandre Olive (Delft University of Technology) are acknowledged for their help with the XPS measurements and fluorescence microscopy, respectively.

Keywords: fluorescent polymers • fingermarks • forensic methods • fluorescence spectroscopy • polyelectrolytes

Notes and references

1 R. Ramotowski, *Advances in Fingerprint Technology*, **2012**, CRC Press.
2 a) M. Pyo, J. Lee, W. Baek, C. W. Lee, B. J. Park, J-M. Kim. *Chem. Commun*, **2015**, *51*, 3177-3180; b) J. Lee, M. Pyo, S-h. Lee, J. Kim, M. Ra, W-Y. Kim, B. J. Park, C. W. Lee, J-M. Kim *Nature Commun*. **2014**, *5*, Article Number: 3736.
3 R. A. van Oorschot, M. K. Jones, *Nature*, **1997**, *387*, 767.
4 a) M. J. Bailey, R. Bradshaw, S. Francese, T. L. Salter, C. Costa, M. Ismail, R. Webb, I. Bosman, K. Wolff, M. de Puit, *Analyst*, **2015**, **DOI:** 10.1039/C5AN00112A. b) G. Groeneveld, M. de Puit, S. Bleay, R. Bradshaw, S. Francese, *Nature Sci. Rep*, **2015**, *manuscript accepted for publication*.
5 B. Friesen, *J. Chem. Educ*. **2015**, *92*, 497-504.
6 W. Troll, R. K. Cannan, *J. Biol. Chem*. **1953**, *200*, 803.
7 Negative staining has also been reported, see: N. Jaber, A. Lesniewski, H. Gabizon, S. Shenawi, D. Mandler, J. Almog. *Angew. Chem. Int. Ed*. **2012**, *51*, 12224-12227.
8 X. Spindler, O. Hofstetter, A. M. McDonagh, C. Roux, C. Lennard. *Chem. Commun*, **2011**, *47*, 5602-5604.
9 M. Wood, P. Maynard, X. Spindler, C. Lennard, C. Roux. *Angew. Chem. Int. Ed*. **2012**, *51*, 12272-12274.
10 O. S. Wolfbeis, *Angew. Chem. Int. Ed*. **2009**, *48*, 2268-2269.
11 R. A. L. Vallee, N. Tomczak, L. Kuipers, G. J. Vansco, N. F. van Hulst, Phys. Rev. Lett, **2003**, 91, 038031.
12 a) S. Moret, A. Bécue, C. Champod, *Nanoscience*, **2014**, *25*, Article Number: 425502; b) J. Dilag, H. Kobus, Y. Yu. C. T. Gibson, A. V. Ellis, *Polym. Int*. **2015**, DOI: 10.1002/pi.4861.
13 A. Beaudoin. *J. Forensic Identif*. **2004**, 54, 413-421. b) A. Qi, Miskelly, G. M. *Anal. Methods*, **2015**, *7*, 1265-1268.
14 a) A. Bentolila, J. Totre, I. Zozulia, M. L. Elad, A. J. Domb. *Macromolecules*, **2013**, *46*, 4822-4828; b) C. Prete, L. Galmiche, F-G. Quenum-Possy-Berry, C. Allain, N. Thiburce, T. Colard, *Forensic Sci. Int.* **2013**, *223*, 104-112; c) G. Groeneveld, S. Kuijer, M. de Puit, *Science and Justice*, **2014**, *54*, 42-48.
15 Non covalent binding of polyelectrolytes to surfaces has been exploited for layer by layer deposition (LbL), using polyelectrolytes of opposed charges, see: G. Decher, *Science*, **1997**, 277, 1232-1237.
16 a) O. van den Berg, W. F. Jager, S. J. Picken, *J. Org. Chem*. **2006**, *71*, 2666-2676; b) W. F. Jager, T. S. Hammink, O. van den Berg, F. C. Grozema, *J. Org. Chem*. **2010**, *75*, 2169-2178.
17 For **P1a**-**c** both the absorption and the emission wavelength are slightly pH dependent, which may reflect the fact that protonation of the amines in the polymer reduces the electron donating capacity of the amino donor at the chromophore.
18 a) Lakowicz, J. R. Principles of Fluorescence Spectroscopy; Kluwer Academic and Plenum Publishers: New York, **1999**; b) Valeur, B. Molecular Fluorescence; Wiley-VCH, Weinheim, **2001**.
19 The fluorescence lifetimes of **P1a**-**c** decrease with the chromophore loading as well, and scale with the fluorescence quantum yields. In the polymers **P1a**-**c** the lifetimes are multi exponential. For compound **1** a single lifetime is recorded
20 J. F. Callan, A. P. de Silva, D. C. Magri, *Tetrahedron* **2005**, *61*, 8551-8588.
21 J. Choi, M. F. Rubner, *Macromolecules*, **2005**, *38*, 116-124.
22 Fingerprint visualization works well for **P1a**, **P1b** and **P1c.**
23 We have soaked latent fingerprints in various solvents, methanol, ethanol, acetone and toluene, for 30 minutes and tested the staining with **P1a** (standard condition) afterwards. Only after exposure to water or toluene stained fingerprints could be observed.
24 The pK_A for the deprotonation of the SiOH group is around 6, which implies that the degree of dissociation is 10^{-3}, 0.9 and 0.999 at pH = 3, 7, and 9, respectively.
25 D. Baechle *et all*, *J. Biol. Chem*. **2006**, *281*, 5406-5415.
26 Insoluble polymers, notably (nano)powders have been used frequently, see reference 12b.
27 J. B. Schlenoff, S. T. Dubas, T. Farhat. *Langmuir*, **2000**, *16*, 9968–9969.

A fluorescent polyelectrolyte, poly(allylamine hydrochloride (PAH) functionalised with the 7-amino-quinolinium chromophore, has been synthesized and employed for the visualization of latent fingerprints.
319x277mm (72 x 72 DPI)