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Separation of Latent Fingermark Residue by Thin-Layer Chromatography

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ABSTRACT: A method for the separation of the lipid and nitrogeneous constituents of latent fingermarks using thin-layer chromatography (TLC) is described. The lipid partition is separated into bands corresponding to fatty acids, cholesterol, squalene, and triglyceride controls. Observation of developed plates using ultraviolet fluorescence detection in the region 310–390 nm has determined that the major fluorescent material is lipid in nature and runs with the squalene control band. After running, the TLC plates were treated with the following latent fingermark enhancement procedures: visible fluorescence, ninhydrin, 1,8-diazafluoren-9-one and physical developer. The technique provides an approach to understanding the chemical and photoluminescent processes of latent fingermark enhancement.

KEYWORDS: forensic science, fingerprints, thin-layer chromatography, fluorescence, lipids

The chemical composition of a fingermark is complex. It typically contains a mixture of sweat and lipid material from the skin surface emulsion [*1*]. Additional components may also be introduced through contact of the fingertip with other materials. Furthermore, after deposition, physical and chemical interactions between the fingermark and its environment may alter the initial composition.

Many of the fingermark enhancement procedures used in law enforcement laboratories rely directly on the chemical composition of the latent mark [*2,3*]. Various chemical reagents are used, each reacting with various compounds within the residue. At present, not all the chemical reaction pathways are understood. A method that describes the separation of lipid and nitrogenous material directly from a fingermark laid down on a thin-layer chromatography (TLC) plate is reported here. The procedure enables chemical and photoluminescent enhancement methods to be applied to the separated components. The results provide some insight into the underlying chemistry and photoluminescence enhancement processes. In particular, information has been gained into the nature of the ultraviolet fluorescing compounds in untreated fingermarks.

Experimental Procedure

The initial aim was to separate the fingermark residue into its water insoluble components such as lipids and its water soluble components such as amino acids and proteins. Subsequently, an extraction method was used to separate several major classes of lipid material.

Reagents

All the control chemicals were purchased from Sigma Chemical Company and used without further purification. The triglyceride control was a mixture of 19 triglycerides (kit TRI-19). Analytical reagent grade solvents were used throughout.

TLC Plate Preparation

In all cases the TLC plates were first put through a conditioning procedure. This involved standing the plates in a solvent system until the solvent had run to the top of the plate. The plates were then dried and the fingermarks deposited approximately 1 cm from the bottom of the plate. Sebum-rich fingermarks were obtained from 5 males (aged 20 to 35 years) who wiped the freshly washed fingertip on the forehead and nose before deposition on to a TLC plate. The plates were developed and treated for visualization within one hour of preparation. TLC experiments were carried out in a sealed glass tank with the mobile phase left to equilibrate for one-half hour before introducing the prepared plate.

Lipid Separation

Separation of the lipid material from the deposited fingermark was performed on aluminum-backed non-fluorescent silica gel 60 plates (3 × 7 cm, Merck). Chloroform was used for both the conditioning and separation procedures. Typically the solvent was run for 15 minutes or until the solvent front was approximately three-quarters of the way up the plate.

Subsequently, several classes of lipid material from the deposited fingermark were resolved using glass-backed non-fluorescent silica gel 60 plates (10 × 20 cm, Merck). The plates were conditioned by running them in a mixture of hexane and diethylether (1:1) and then leaving them to stand in overnight. The plates were then baked in an oven at 200°C for one-half hour. A chloroform:acetone:methanol (10/1/1) solvent mixture was used as the mobile phase. The plates were allowed to run for around 18 cm (approx. 2 h). 5 μL of controls of 1% palmitic acid, 0.5% cholesterol, 0.5% squalene, and 0.5% triglyceride mixture in chloroform were used. Earlier experiments had determined the separation order of these controls and hence they could be run in the same lane thereafter. They were spotted onto the TLC plate at a point level with the center of the fingermark.

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Nitrogenous Material Separation

Separation of the nitrogenous material from the deposited fingermark was performed on aluminum-backed cellulose plates (3 × 7 cm, Merck). The conditioning procedure used distilled water. After drying and deposition of the fingermark the plate was run in distilled water for 15 minutes or until the solvent front was approximately three-quarters of the way up the plate.

Plate Treatment

After developing the plates, they were treated for visualization of the partitioned material by the treatments described in this section. Blank plates were also run and developed with the same treatments. No material was visualized with the treatments on these blank controls and they are not discussed further.

Treatment 1: Ultraviolet Fluorescence Imaging—The plates were illuminated by 266 nm laser radiation and the fluorescence imaged through a 60 nm bandpass filter centered at 360 nm [*4*]. Shortwave ultraviolet laser radiation is obtained from the fourth harmonic of a Nd:YAG laser (Spectron Laser Systems SL400). The laser was typically run at 10 Hz with a pulse length of 10 ns, producing a 3 mJ/pulse at 266 nm.

Treatment 2: Visible Fluorescence—The plates were illuminated by a filtered Xenon lamp source (a 'Polilight' Rofin Sinar, Australia) at 530 nm and imaged through a 550 nm long pass filter.

Treatment 3: Ninhydrin—The plates were dipped in ninhydrin solution [2] for 10 seconds and then heated at 75°C at 75% relative humidity for 10 minutes. The plates were then imaged under fluorescent room lighting.

Treatment 4: 1,8-Diazafluoren-9-one (DFO)—The plates were dipped in DFO solution [*5*] for 10 seconds and heated at 75°C and 15% relative humidity for one-half hour. The plates were then imaged under the same conditions as described for visible fluorescence.

Treatment 5: Physical Developer (PD)—The plates were dipped in PD solution and washed in the same manner as routine casework paper samples [2].

Treatment 6: Charring—The plates were dipped into an aqueous solution of 10% copper sulphate/8% phosphoric acid for 10 seconds and then baked in an oven at 200°C for 2 minutes.

Results

Lipid Separation

The use of chloroform as the mobile phase caused the ultraviolet fluorescent component(s) and visibly fluorescent components to run with the solvent front. This is illustrated in Fig. 1 where fingermarks before and after separation can be compared. The efficiency of the separation of the ultraviolet fluorescing material is was typically very good and often complete. The visible fluorescence can be seen at and just below the solvent front. Careful examination reveals two fluorescent bands: a top green band and a lower orange band.

The results of treating chloroform run plates with ninhydrin, DFO and PD are illustrated in Fig. 2. DFO and ninhydrin both react strongly with the fingermark. There was reaction with the material at the solvent front with both these compounds. However, this was particularly weak in the case of ninhydrin and the color change difficult to observe. The greater sensitivity of DFO coupled with fluorescence detection allowed easier observation of the reaction at the solvent front, but this is still weaker than the reaction with the fingermark. This reaction of ninhydrin and DFO with material at the solvent front was not always observed although no correlation with donor was found. PD reacted strongly with the material at the solvent front and also produced ridge detail. The quality of the ridge detail varied, this was probably due the efficiency of the lipid separation process.

After running with chloroform/acetone/methanol four separate bands could be visualized and distinguished by the charring treatment, see Fig. 3. Occasionally the charring process produced some ridge detail at the origin. Careful examination of the treated plates showed that there may be some partitioning of the mobile phase resulting in two solvent fronts. The second front running with the top of the squalene control band.

The four bands correspond to the control bands of triglycerides, squalene, cholesterol, and fatty acid, see Fig. 3. The bands corresponding to squalene and triglyceride control bands run very close and are not always fully separated but could be distinguished by a difference in coloration after charring. Excitation under 266 nm illumination produced ultraviolet fluorescence from the band aligned with the squalene control. Visible fluorescence was observed from the fingermark when the plate was illuminated with 530 nm radiation. Treatment with DFO gave a fluorescing band level with the triglyceride control and a very strong fingermark with good ridge detail. Treatment with ninhydrin enhanced the same regions as DFO although the coloration at the triglyceride position was extremely weak. Again the reaction of ninhydrin and DFO was not always observed. PD treatment enhanced some fingermark ridge detail together with strong visualization of all four bands.

Nitrogenous Material Separation

Figure 4 illustrates the ultraviolet and visible images of the cellulose plates before and after separation procedures. Before running the plates high contrast images of the fingermarks were obtained. After extraction the ultraviolet fluorescing material remained on the fingermark ridges although there was partial extraction of the visible material. The extracted visible material ran with the solvent front.

Developed plates treated with DFO, ninhydrin and PD are illustrated in Fig. 5. Here the strongest reaction for DFO and ninhydrin was with the material at and just behind the solvent front. There was also reaction with material that moved less distance and spread thinly from the fingermark. PD reacted with the fingermark and not with the material at the solvent front.

Discussion

The application of TLC in the analysis of latent fingermark residue is not new but previous work has concentrated on running extractions from either washes or swabs rather than from fingermarks themselves [*6–10*]. Applying a fingermark directly onto a TLC plate is a more realistic method of obtaining residue than either swabbing or washing fingers. This novel method of sample application did not inhibit traditional TLC methods of sample separation.

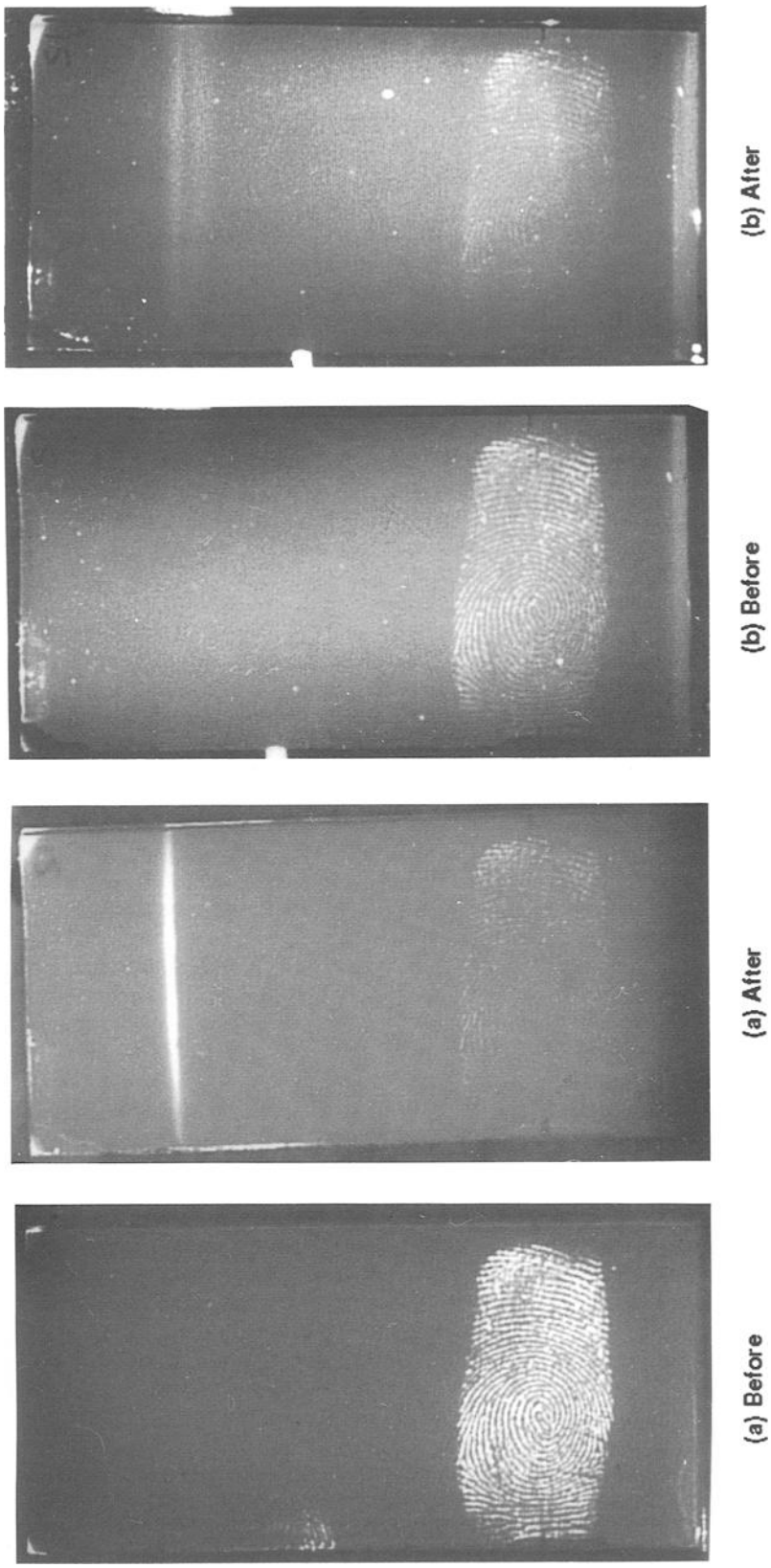

FIG. 1—a) *Ultraviolet and* b) *visible fluorescent images before and after development with chloroform on silica TLC plates.*

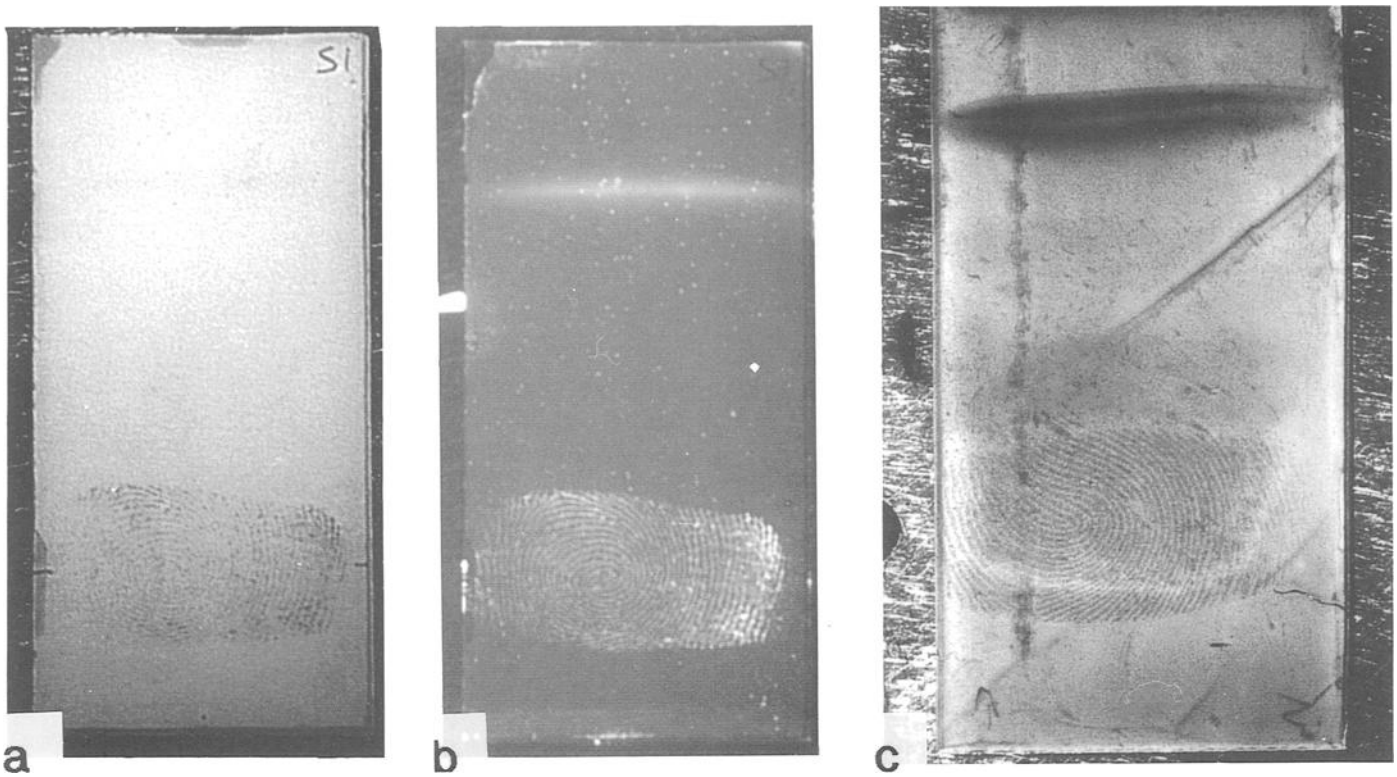

Fig. 2—*Fingermarks on silica TLC plates imaged after development with chloroform and treated with* a) *ninhydrin,* b) *DFO and* c) *physical developer.*

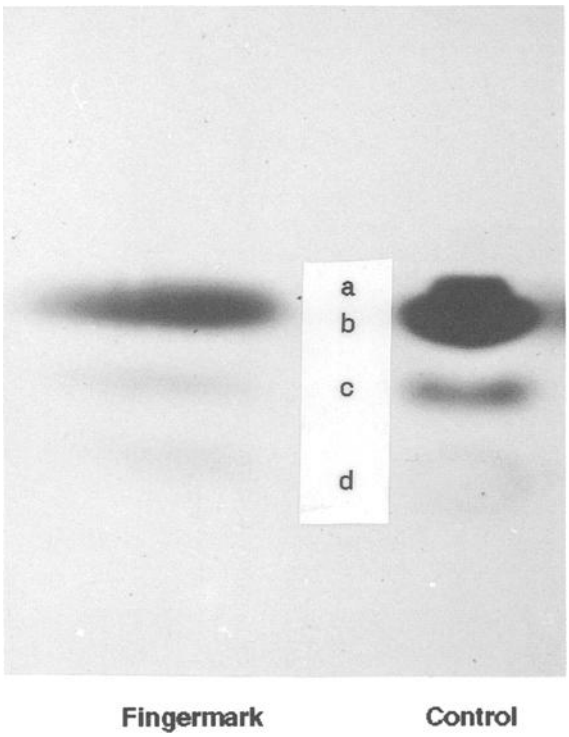

FIG. 3—*Silica TLC plates imaged after development with chloroform:acetone:methanol (10:1:1). Control bands* a) *triglycerides,* b) *squalene,* c) *cholesterol and* d) *fatty acid.*

Chloroform efficiently separated the lipid components from the latent fingermark while leaving the majority of the nitrogenous components unmoved. In contrast water separated the nitrogenous material and left the lipid components relatively untouched. This effect can be seen at its most dramatic by comparing Fig. 4*a* with 5*a*/5*b* where the same fingermark has been enhanced by ultraviolet fluorescence and ninhydrin/DFO after running with water. The former produces a high contrast fingermark with good ridge detail while the latter provides no useful information on the ridge pattern.

On careful examination of the fingermark, after extraction with water, some slight movement of the lipid material has been observed. This causes a slight blurring of the fingermark ridge detail when enhanced with fluorescence or PD. In contrast after running chloroform through the fingermark, clear ridge detail appeared when treated with reagents specific to residue components insoluble in organic solvents, for example, ninhydrin. Such information on the effects of reagent solvent systems upon the fingermark residue could be important in determining future solvent systems and/or treatment sequences.

The use of a three solvent mobile phase separated the lipid material into three main bands and a fourth that was partially resolved. Nitrogenous material observed by reaction with ninhydrin and DFO was with the top lipid band corresponding to the triglyceride control. DFO and ninhydrin are known to react with primary amines [*11*]. Thus, fingermark residue appears to have some neutral lipid materials or other chloroform soluble materials that are relatively non-polar and have amine moieties present. Such chloroform soluble material could include lipoproteins and denatured proteins.

Ultraviolet luminescence from fingermarks has been observed before but together with visible fluorescence the responsible chro-

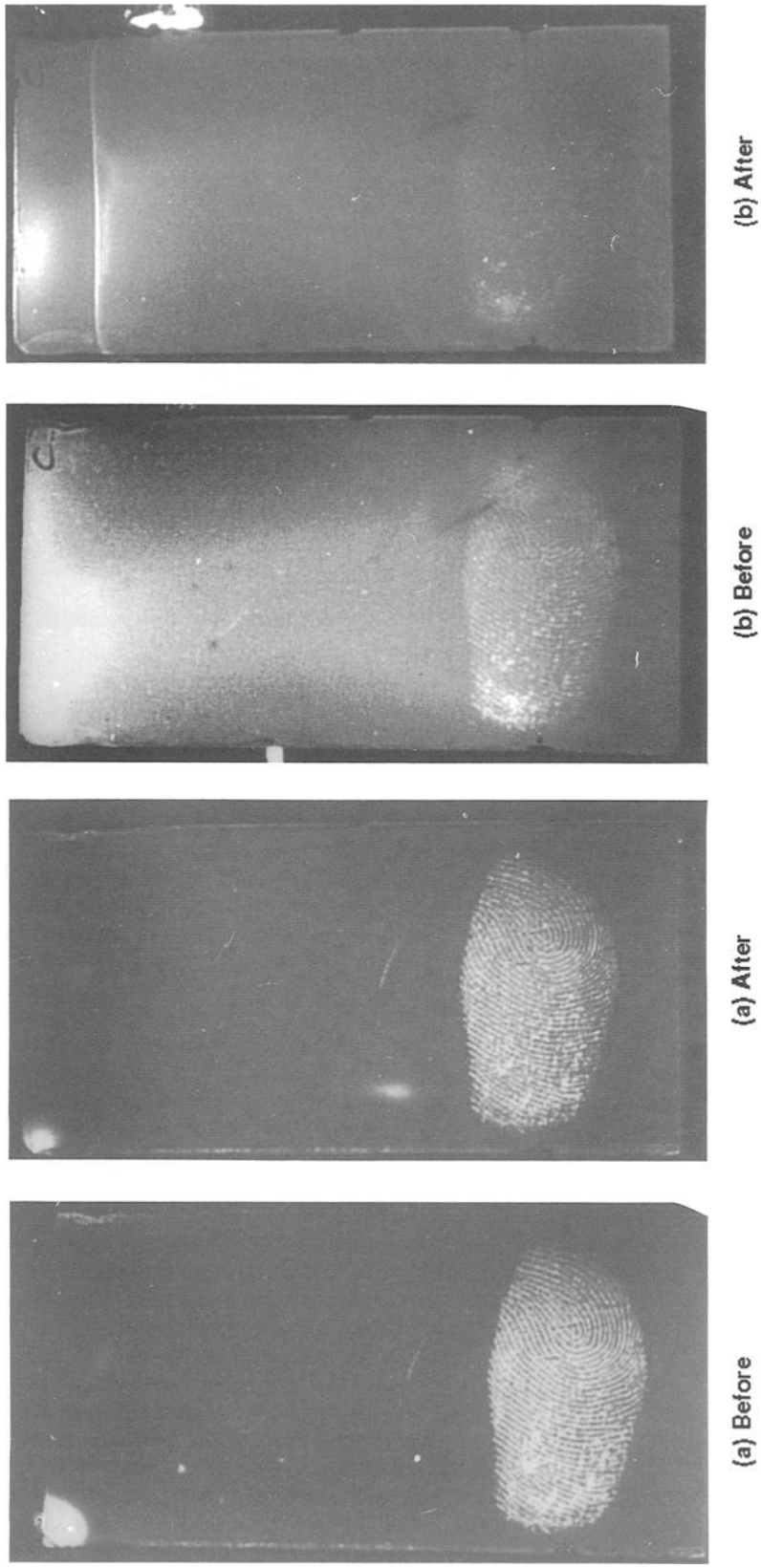

FIG. 4—a) *Ultraviolet and* b) *visible fluorescent images before and after development with water on cellulose TLC plates.*

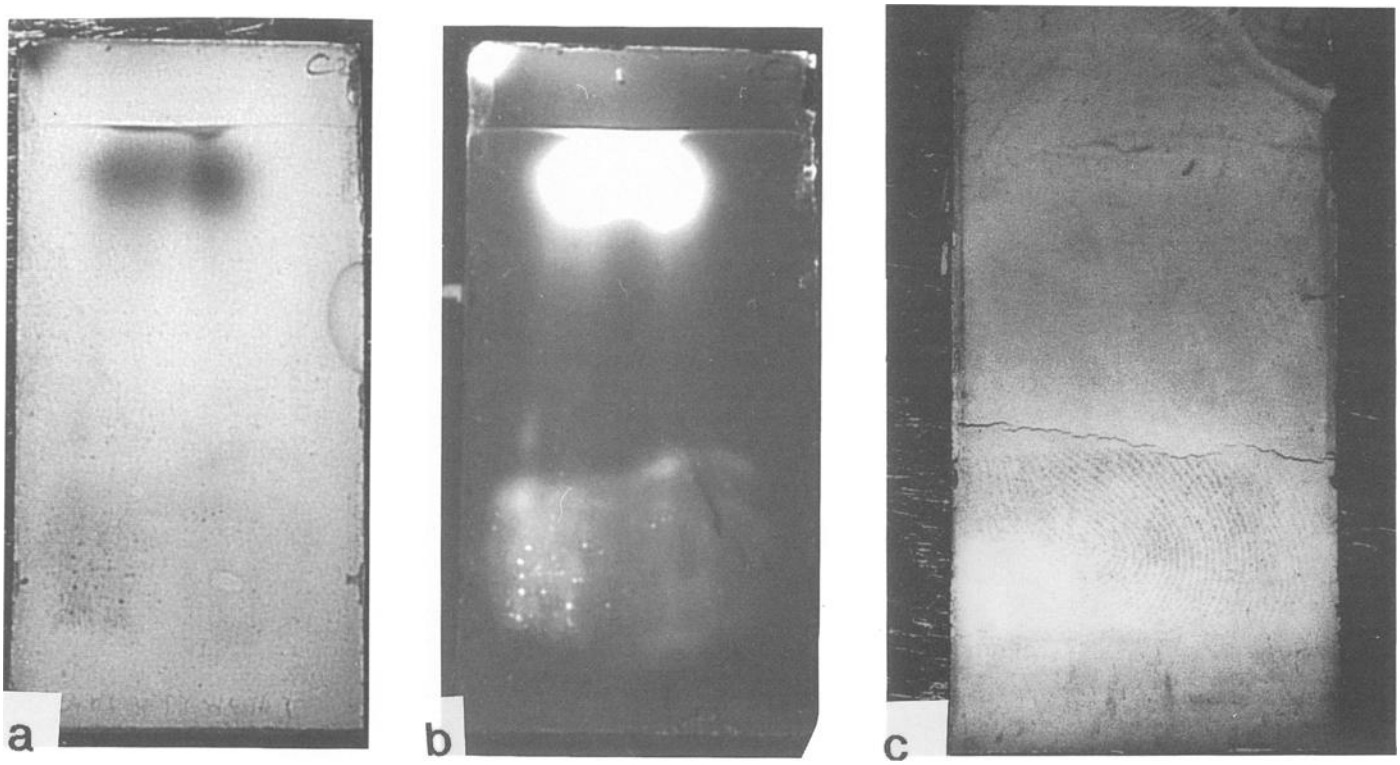

FIG. 5—*Fingermarks on cellulose TLC plates imaged after development with water and treated with* a) *ninhydrin,* b) *DFO and* c) *physical developer.*

mophore(s) is unknown [*4,10,12*]. The experiments reported here have demonstrated that the ultraviolet fluorescing components are removed from fingermark residue using chloroform. Further separation of the lipid material concentrated the fluorescing material in a band corresponding with the squalene control. As the squalene band could possibly be running at a second solvent front there may still be a variety of components within this band. A previous study by Johnson et al. reported that tyrosine and tryptophan accounted for 83% of the ultraviolet fluorescence emission [*12*]. Their HPLC experiments were based on extracts from washings with a mixture of methanol and water (1:1). However, lipids at best are sparingly soluble in methanol and insoluble in water. Therefore, they were only measuring the fluorescence material extracted from the fingers that was soluble in methanol and water. The results reported here suggest that any aromatic amino acids present in fingermark residue only account for a minor proportion of the total ultraviolet fluorescence.

Ohki found ultraviolet fluorescence spectra of fingermark residue to be highly dependent upon the extraction solvent used [*10*]. His samples were obtained from gauze that had been wrapped around clean hands and feet. Extraction was first with ether followed by an equi-volume mixture of ethanol and water. The maximum excitation and fluorescence wavelengths for the ether extract were 300 nm and 350 nm, respectively, and for the ethanol/water extract 315 nm and 400 nm, respectively. Urocanic acid was found to possess similar spectral characteristics to the ether extract but there was no conclusive evidence for it being the source of the observed fluorescence. The difference in fluorescence properties between the two extracts may indicate several different fluorescing components although solvent effects cannot be eliminated.

Previous TLC studies of lipid material from latent fingermark residue are tabulated in Table 1. Direct comparison is difficult because of the different extraction, development and treatments used in each study. This work supports the results of Ohki who found four bands; the lower two corresponding to fatty acid and cholesterol controls. Although the control substances are only a guide to the lipid constituents of fingermarks the results are consistent with the known composition of skin surface lipids [*13*].

TABLE 1—*TLC studies of lipid components in fingermark residue.*

Extraction solvent	Plate type	Mobile phase	Number of bands	Matching controls	Reference
Ether	Silica	Petroleum ether:ether:acetic acid (80:20:1)	4	Cholesterol Fatty acid	10
		Ethanol:water (1:1)	6	Urocanic acid	
Ether	Silica	Ether or acetone	6	none	6
Chloroform	Silica	Chloroform	5	none	8
None	Silica	Chloroform: acetone:methanol (20:1:1)	4	Cholesterol Triglycerides Squalene Fatty acid	This work

Conclusion

Standard TLC procedures have been applied to latent fingermark samples laid down directly onto the plate surface. The major classes of lipid constituents within fingermarks have been found to concentrate and run in discrete bands. This has allowed the running of control samples for comparison.

Nitrogenous and lipid components have been successfully separated directly from latent fingermarks using TLC. The procedure

has demonstrated that chemical reagents used for fingermark enhancement can be reacted with the separated components. In the case of DFO and ninhydrin the majority of the reactive material was shown to be water soluble; however, there is some reaction with a chloroform soluble component. In contrast PD appears to react with the non-aqueous material only.

On resolving four separate lipid components it was shown that the ultraviolet fluorescence observed from fingermarks originates from lipid or lipid soluble components of fingermark residue. Comparison with control standards indicates that such components could include squalene.

The technique also lends itself to observing the effects of different solvent systems on fingermark ridge detail. Such information could be useful when evaluating new fingermark enhancement procedures.

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