A Bufferless Oil Red O Formulation for Developing Fingermarks on Wet Substrates

Chun-Chieh Chen 1,2, M.S.; Sheng-Meng Wang 2, Ph.D.; Te-Chuan Sun 1, B.S.; Chao-Kai Yang 2*, Ph.D.

1 Forensic Science Section, Changhua County Police Bureau, 778 Sec. 2, Jhong-jheng Rd., Changhua City, Changhua 50004, Taiwan ROC
2 Department of Forensic Science, Central Police University, 56 Shu-Jen Road, Kwei-San, Taoyuan City 33304, Taiwan ROC

Received: November 14, 2016; Accepted: November 20, 2016.

Abstract

In crime scenes, relevant documents are sometimes torn or thrown into toilets or ditches to destroy evidence. At the scene of a fire, the environment becomes wet after firefighters extinguish the fire. Under these conditions, small particle reagent (SPR) can be successfully used to develop latent fingermarks on nonporous substrates. However, ninhydrin cannot be used to develop latent fingermarks on porous substrates under wet conditions because amino acids are soluble in water. To overcome this limitation, Oil Red O (ORO), a commonly used stain for detection of lipids in various biological fields, has been used to reveal the presence of residual water-insoluble components. Using bufferless ORO formulations, researchers have examined the effectiveness of sodium hydroxide, methanol, and different buffers. In the present study, we evaluated the effectiveness of a bufferless ORO technique for developing fingermarks on wet and special substrates, such as thermal paper and Bristol boards, in a wet environment in Taiwan. Bufferless ORO was used to develop latent fingermarks on glass, transparent plastic, thermal paper, A4 paper, and Bristol boards in a wet environment. Latent fingermarks successfully developed red ridges with a pink background on thermal paper and discolored the specimen. Bufferless ORO has been approved for use based on its environment-friendly features. Therefore, based on our analysis, the best way to develop latent fingermarks on regular wet paper was to use ninhydrin first, followed by ORO.

Keywords: Forensic Science, Fingermark, Oil Red O, Ninhydrin, Wet substrate, Small particle reagent, Thermal paper

Introduction

The contents of fingermarks come from the secretions of sweat glands and sebaceous glands in the skin. Sweat glands secrete sweat, which contains organic components, such as amino acids, proteins, and uric acid, and inorganic components, such as potassium ions, chloride ions, and calcium ions. Sebaceous glands secrete glycerides, fatty acids, and other components. These two types of glands are extensively distributed throughout the body, whereas the forehead and back are highly enriched with sebaceous glands. Because the hands are in constant contact with the body, particularly the face, fingermarks contain components secreted from both the sweat glands and sebaceous glands [1].

In crime scenes, relevant documents are sometimes...
torn or thrown into toilets or ditches to destroy evidence. At the scene of a fire, the environment becomes wet after firefighters extinguish the fire. Thus, when there is a need to develop latent fingermarks under these conditions, small particle reagent (SPR) is used to develop latent fingermarks on nonporous substrates [2,3]. However, it is difficult to develop latent fingermarks on porous substrates using ninhydrin because amino acids are highly soluble in water, which in turn results in failure of the reagent that interacts with amino acids to develop latent fingermarks. The physical developer allows visualization of latent fingermarks by interacting with water-insoluble components of fingermarks; however, procedures involving physical developer are repetitive and complicated [1].

Since 2004, Oil Red O (ORO), a reagent generally used to stain cells, has been used to develop water-insoluble components, such as lipids and large proteins. Moreover, ORO has been successfully used to develop latent fingermarks on wet paper [4], and the technique is simpler, easier, and more effective than that used for physical developers [5, 6]. ORO was used to develop latent fingermarks in a 21-year-old cold case [7], but was not as effective for fingermarks after more than 4 weeks [8] in other cases. Furthermore, modified ORO formulations have been used to investigate the effectiveness of sodium hydroxide, methanol, and different buffers [8-10].

Therefore, in the present study, we aimed to investigate the effectiveness of a bufferless ORO technique for developing fingermarks on wet and special substrates, such as thermal paper and Bristol boards, in a country with a constant high humidity level.

Materials and Methods

Sample preparation

After washing their hands with detergent, five volunteers (one woman and four men) were restricted from bringing their hands in contact with their bodies. Sweat-rich fingermarks were then deposited after exercising. Sebum-rich fingermarks were obtained after touching the forehead and sides of the nose. The five volunteers deposited their fingermarks vertically onto the different substrates in a single column. Strips of each substrate (except the glass substrate) were cut into two parts, dividing each fingermark into halves for treatment with different developing reagents. The two treated halves were recombined, and a direct visual comparison of the fingermark ridges was made between the two halves. In addition, the fingermarks on the surfaces of specimens were deposited and stored at room temperature for 1 month before developing [11–13]. To simulate “natural marks,” fingermarks were divided into two parts to examine different developing conditions, and the results were compared (Fig. 1). Photographs were acquired using a Canon EOS 20D digital camera with operation conditions set as follows: exposure, automatic white balance, and ISO 400.
Electrophoresis-grade ORO (also called Solvent Red 27) was purchased from Sigma (Germany). Sodium hydroxide (extra-pure grade) was purchased from Shimakyu’s Pure Chemicals (Japan). Methanol (extra-pure grade) was purchased from Nihon Shiyaku Reagent (Japan). To prepare the reagent, 0.77 g ORO was dissolved in 385 mL methanol, and 4.6 g sodium hydroxide was dissolved in 115 mL water. These two solutions were mixed well, the undissolved dye was filtered out, and the solution was stored in an amber glass bottle to prevent light exposure. All specimens were placed in the ORO stain solution for 30 min, removed, and drained. The specimens were immersed for 10 min in a tap water tank that contained no other buffers and were then allowed to surface dry at room temperature. This procedure used less buffer than traditional procedures and was therefore more environmentally friendly. Additionally, because there was no need for buffer preparation, the procedures were less time consuming.

Influence of a wet environment on porous substrates and the effects of staining order

An A4 copy paper was deposited with sweat-rich or sebum-rich fingermarks and cut in half. One half was soaked in water for 24 h and treated with ninhydrin or ORO. The ninhydrin solution was prepared by dissolving 5 g ninhydrin in 45 mL ethanol, 5 mL acetic acid, and 2 mL ethyl acetate, and the volume was then brought up to 1 L with petroleum ether. Thus, we used a total of 25 samples (5 volunteers × 5 strips) in this experiment.

Fingerprint development using different substrates

Sebum-rich fingermarks were deposited on five different substrates, including a porous surface (double A copy paper), semiporous surface (Howen thermal paper and Bristol boards), and nonporous surface (glass [microscope slides, size: 1 × 3 inches] and transparent plastic films [overhead projection film, PET 210 × 297-mm thickness, 0.1 mm]), and cut into half. For preparation of glass-type samples, fingermarks were deposited across the line where the two pieces of glass combined, and each piece of sample was then analysed as follows. One half was soaked in water for 24 h, and the other half was left untreated. Both were simultaneously developed with ORO. There was a total of 125 samples (5 volunteers × 5 substrates × 5 strips) in this experiment.

Differences between ORO and SPR

Fingermarks on five different substrates were developed using white SPR (1.5 g zinc oxide dissolved in 1 L water) and black SPR (from SIRCHIE), and the results were compared. Zinc oxide (extra-pure grade) was obtained from Katayama Chemicals (Japan). There was a total of 125 samples (5 volunteers × 5 substrates × 5 strips) in this experiment.

Results and Discussion

ORO development and the order of staining usage

Ninhydrin staining resulted in purple ridges for sweat-rich and sebum-rich fingermarks when samples were not soaked in water; however, when the fingermarks were soaked in water for 24 h, no ridges developed (Fig. 2). ORO staining did not yield ridges for sweat-rich fingermarks for either condition; however, red ridges were observed for sebum-rich fingermarks for both conditions. Therefore, ORO effectively developed sebum-rich fingermarks (Table 1). Additionally, the red ridges exhibited a pink background that was observable.
in daylight

![Photographs of purple ridges developed on paper using ninhydrin (with or without soaking in water).](image)

**Fig. 2** Photographs of purple ridges developed on paper using ninhydrin (with or without soaking in water).

<table>
<thead>
<tr>
<th></th>
<th>Ninhydrin</th>
<th>ORO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soaked in water</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Not soaked in water</td>
<td>Purple ridges</td>
<td>Red ridges</td>
</tr>
</tbody>
</table>

**Table 1** Development results of Ninhydrin and ORO on A4 copy paper.

*Use of a single reagent and effects of the order of consecutive stains*

For sebum-rich fingermarks, ninhydrin had a lower background, no color interference, and better development in non-water-soaking conditions. However, in water-soaking conditions, ORO, but not ninhydrin reagent, developed red ridges with a pink background.

In practice, without knowing the composition of a latent fingerprint, it is difficult to predict whether the fingerprint may contain more amino acids than lipids or vice versa; thus, application of ninhydrin first for detection of amino acids, followed by ORO for detection of lipids will provide the optimal results during the development of porous surfaces (Fig. 3).
**Fingermark development on different substrates**

Red ridges with light pink backgrounds were detected on five different substrates that were soaked in water for 24 h. Transparent plastic film had a higher background, and this resulted in a lower contrast in color (Fig. 4). Moreover, heating up of the materials increased the contrast of the red ridges.

ORO staining successfully detected red ridges with a pink background on thermal paper; however, the original information on the fax paper gradually faded and disappeared during development (Fig. 5). Although the discoloration in this case was different from that caused by the solvent on thermal paper, according to a previously described formula, ORO may still be useful for fingermark detection on thermal paper, and the vacuum technique may be useful to improve ORO deposition without damaging the thermal paper [14–17].

---

**Fig. 3** Comparison between ninhydrin alone (upper) and ninhydrin first, followed by ORO (lower) during the development of sebum-rich fingermarks under non-water-soaking conditions.
Fig. 4 Ridge development using ORO on different substrates: (a) transparent plastic film and (b) Bristol board.

Fig. 5 Fingermark development on text-printed thermal paper using ORO.
Comparison of the application of ORO and SPR for development of fingermarks on wet specimens

After the five substrates were soaked in water for 24 h, ORO staining yielded red ridges with a pink background. However, SPR failed to detect fingermarks on thermal paper, Bristol boards, and A4 paper because the specimens absorbed the materials. SPR had a better effect on glass and transparent plastic films, with black SPR showing a higher background and white SPR showing a lower background and better ridges (Fig. 6). However, in wet environments, although ORO could be applied to different materials to develop fingermarks, SPR was superior for developing fingermarks on nonporous substrates. For porous substrates and semiporous substrates, only ORO should be used (Table 2).

![Fig. 6](image)

Comparison of fingermark development on transparent plastic film using white SPR and ORO.

<table>
<thead>
<tr>
<th>Porous</th>
<th>Semiporous</th>
<th>Nonporous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copy paper</td>
<td>Thermal paper</td>
<td>Bristol boards</td>
</tr>
<tr>
<td>SPR (ZnO)</td>
<td>Failed</td>
<td>Failed</td>
</tr>
<tr>
<td>ORO</td>
<td>Red ridges</td>
<td>Red ridges but discolored</td>
</tr>
</tbody>
</table>

Table 2 Development results of five different wet substrates.
Conclusions

Latent fingermarks were successfully developed using ORO, which yielded red ridges with a pink background on thermal paper, but discolored the specimen. In the procedure for fingerprint detection, the use of ORO after ninhydrin staining could be helpful for more effective detection of fingerprints. Bufferless ORO has been shown to be environmentally friendly and can therefore be applied without concern for its effects on environmental health. Our results suggested that the optimal method for developing latent fingermarks on wet regular paper was to use ninhydrin first, followed by ORO. The development of red ridges against a light pink background could facilitate the detection and visual analysis of fingermarks.

Acknowledgements

This study was supported by the grants No. 104–0805–05–04–01 from Ministry of Interior, Taiwan (ROC). Special also thanks to Lei-Jang Pang and Yung-Chien Yu, the fingerprint analysts of Criminal Investigation Bureau, for their technical support and professional consulting.

References