Article

Assessing Phosphomolybdic Acid as a Fingermark Enhancement Reagent

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Abstract: The efficacy of an ethanolic solution of phosphomolybdic acid (PMA) was investigated as a latent fingermark visualization reagent, primarily on porous substrates. After treating samples and exposing them to ultraviolet radiation, the PMA solution was shown to develop fingermarks of high quality. Unlike the common amino acid reagents that are used for the development of fingermarks on porous substrates (e.g., ninhydrin and 1,8 diazafluoren-9-one), PMA stains a range of other compounds that are found in fingermark deposits, including lipids. The lysochrome diazo dye Oil Red O (ORO) was used for comparative purposes because of its application in staining some of the same components of fingermark residues for which PMA would be proposed. Initial results indicate that PMA is comparable to ORO at developing fingermarks on porous surfaces and may also have applications on nonporous surfaces.

Introduction

Fingermarks are generally regarded as the most reliable method of personal identification and are therefore viewed as some of the most important contact evidence recoverable from a crime scene [1]. For the successful retrieval of fingermarks from a scene, they first have to be detected. To achieve this, a range of techniques have been developed to visualize such marks. Several chemical and physical methods are currently employed to

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develop latent fingermarks that are invisible to the naked eye [2]; however, these visualization techniques are constantly evolving. Recent years have seen researchers exploring novel approaches to fingermark development, both instrumental and chemical in nature, and in some instances, even repurposing techniques that are not normally utilized for fingerprint work [3–21].

It was with the aforementioned repurposing in mind that phosphomolybdic acid (PMA) was considered as a possible fingermark development reagent. Phosphomolybdic acid (H₃[PMo₁₂O₄₀]) [22] is a heteropolyacid that is commonly used in histology as a component of Masson's trichrome stain and as an indicator in thin-layer chromatography, where it is used to visualize a great many compounds including steroids [23], sterols [24], lipids [25], fatty acids [26], and triglycerides [27]. It was PMA's versatility in identifying these compounds that led to its 1973 investigation as a possible fingerprint visualization reagent [28], although this has not been pursued further in the following decades.

PMA has a 1:12 tetrahedral structure [22] (Figure 1), which is structurally identical to its species counterparts with the formula [XM₁₂O₄₀]ⁿ-, where X is the heteroatom (P^V, As^V, Si^V or Ge^{IV}, among others) and M is the addenda atom (usually Mo^{VI} or W^{VI}). Phosphomolybdic acid is reduced to molybdenum blue in the presence of conjugated, unsaturated compounds. Burstein found that the blue color becomes more intense with an increase in the number of double bonds in the molecule being stained [29]. This suggests that the primary use of PMA as a fingermark development reagent would be to detect the water-insoluble, sebaceous constituents of fingermarks, as originally suggested by Vincent [28].

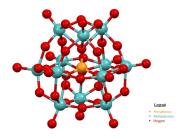


Figure 1
Phosphomolybdate anion.

Vincent [28] originally considered PMA as a spray reagent for use on porous surfaces, including fabrics and paper, and concluded in a report that of 25 reagents tested for the purpose of fingerprint development, PMA outperformed all except ninhydrin. Recent feasibility studies [30, 31] strived to optimize the technique by using different carriers as well as by varying the development process and confirmed this development reagent merited further investigation for the purposes proposed by Vincent. There are several fingermark visualization reagents that have been researched or recommended for staining fatty acids, lipoproteins, and triglycerides on porous surfaces: Nile red, Europium Chelate, and Oil Red O (ORO) [32]. ORO (Figure 2) was chosen as a comparison reagent for this study because it is more widely used operationally, is less expensive than Nile red and Europium Chelate to formulate, and does not require fluorescent illumination conditions to view developed marks. ORO is a lysochrome, or lipid stain, similar to Sudan black (solvent black 3). Initially, ORO was investigated as an azo-dye for the staining of lip prints deposited on tissue paper [33], then as a replacement for physical developer for the development of latent fingermarks on substrates that had been wetted [34]. Beaudoin [35] reported a formulation of ORO that was capable of developing fingermarks, not just on porous surfaces, but also on porous surfaces that had been wetted. It was also found to be effective on semiporous and soiled paper. The process is completed in three stages: (1) an ORO dip bath for up to 90 minutes, (2) immersion in a buffer solution, and (3) submersion in a water bath [35].

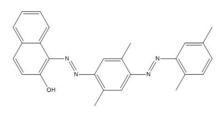


Figure 2
ORO structure.

However, sebaceous fingermarks are not encountered only on porous surfaces, and the application of PMA to nonporous surfaces has not yet been explored. On nonporous surfaces, the development reagent solvent black 3 [36, 37] is most widely recommended for the development of sebaceous and grease-contaminated marks. On dark surfaces, natural yellow 3 has recently been suggested as an alternative [38]. Both reagents are prone to causing excess staining of the background for long exposure times, and alternative reagents are desirable. Although originally proposed for the development of sebaceous-rich marks on porous surfaces, small particle reagent works well on nonporous substrates [39]. On adhesive surfaces, as well as on nonporous, basic violet 3 (gentian violet) is recommended [40]. Iodine solution has been found to be effective on both porous and nonporous substrates [41].

There has also been continued interest in developing fingermarks on metal surfaces (e.g., brass and stainless steel). Recent focus has been on processes that are tailored towards different classes of metal surface, for example electrochromic deposition (stainless steel [14]), and longer established processes may also be used to target different metal types (gun blueing on brass and steel, aluminum black on aluminum).

The purpose of this initial study was to investigate the breadth of applications for PMA on nonporous substrates and to conduct an assessment of its performance in comparison to an existing lipid visualization reagent (ORO) on porous surfaces.

Materials and Method

PMA Study

The substrates that were used in this study were paper, acetate, aluminum, and stainless steel. These are substrates that are found in common, everyday items. The substrates were prepared for fingermark deposition by cutting 12 cm by 3 cm sized samples. These were labelled using photographic twin check labels with the twinned label being logged with details of sample type, fingermark deposition method, donor number, and development day. The 13 donors who were used in this study were a mix of males and females ranging in age from 22 to 40 whose potential to leave fingermark deposits was unknown. Donors had not washed their hands for at least 30 minutes prior to depositing their fingermarks; no extra sebaceous deposits were loaded on the hands, therefore providing more natural deposits

from the donors' hands. Fingermark deposition was carried out by having the donor deposit a mark from each finger of one hand; each donor deposited all of his or her marks at the same time. After the fingermarks had been deposited, the samples were stored in cardboard boxes, in the dark, at room temperature for 1, 2, 4, 8, 14, 21, or 28 days before being processed. In total, 182 samples of each substrate (13 donors, 7 different ages, 2 deposition methods) were used (728 samples in total).

PMA versus ORO Comparison Study

The substrate that was used in this study was paper, which was an 80 gsm copier paper made by Polaroid. The substrates were prepared for fingermark deposition by cutting 12 cm by 5 cm sized samples to allow ample space for the fingermark to be deposited and split down the center. This allowed for the direct comparison between the chosen development reagents. Three donors were used in this study. They were a mix of males and females ranging in age from 22 to 40 whose potential to leave fingermark deposits was unknown. Donors had not washed their hands for at least 30 minutes prior to depositing their fingermarks. Fingermark deposition was carried out by having the donor deposit a single mark from each finger along the center of the substrate (five marks per sample). Each donor deposited all of his or her marks at the same time.

PMA and Wetted Samples

Paper samples had a mixture of natural and sebaceous marks deposited on their surface. These were subsequently placed into a water bath to soak for up to an hour. Samples were allowed to air-dry, or they were placed in an oven at approximately 50 °C to dry out. Once dry, the samples were treated in the same manner as the previous samples.

Reagents

The prepared samples were treated with a 10% w/v PMA solution, prepared from phosphomolybdic acid hydrate (Sigma Aldrich–221856) in absolute ethanol. The samples were sprayed with the PMA solution using an ECOSPRAY (Labo Chimie France) and were developed for 15 minutes under a 15 W longwave UV lamp [42].

Treating fingermarks with ORO is a three-part process: (1) the ORO stain bath, (2) a buffer solution, and (3) a water bath. The ORO stain was prepared by adding 0.77 g ORO (Sigma Aldrich-O0625) to 385 mL methanol, then separately adding

4.6 g sodium hydroxide to 115 mL of deionized water. These two solutions were then mixed together and filtered before being stored in a brown bottle away from light. The buffer solution was prepared by adding 26.5 g of sodium carbonate to 2 liters of deionized water while stirring until dissolve. To this solution, 18.3 mL of concentrated nitric acid was added. Finally, the total volume was made up to 2.5 liters by adding more deionized water.

Samples treated with ORO required up to 90 minutes in the stain bath, after which they were placed in the buffer solution for up to 5 minutes before being rinsed in the water bath and being left to dry.

Treated samples were then photographed using a Nikon D5200 [43] digital camera with an AF-S DX Nikkor 18–55 mm F/3.5–5.6G VR [44] lens. The visualized marks were then graded using one of the CAST grading scales (Table 1) [45, 46].

Grade	Comment
0	No development.
1	Signs of contact, but <1/3 of the mark has continuous ridges.
2	1/3-2/3 of the mark contains continuous ridges. Of sufficient quality to potentially be identified.
3	>2/3 of mark has continuous ridges, but not quite a perfect mark.
4	Full development. Whole mark continuous ridges.

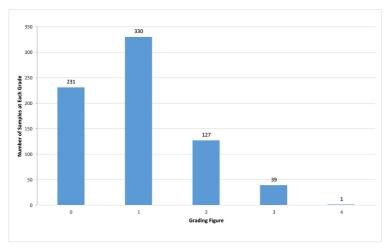
Table 1 Fingermark grading scale.

Results and Discussion

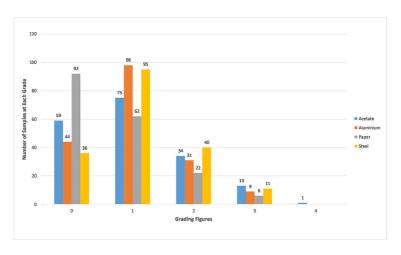
PMA Study

There were 728 sample substrates that were treated, with 4 to 5 fingermarks on each. After treatment, the best developed fingermarks from each sample were graded from 0 to 4 after a visual examination. Of the 728 samples, 45% were graded as 1, 17% were graded 2, and 6% were graded 3 and above (Graph 1).

Paper provided the most 0 graded marks (those containing no development), double the amount of some of the other substrates. Consequently, the number of paper samples within each individual grading value above 0 was markedly less than those of the other substrates (Graph 2). The metal samples (aluminum and stainless steel) both performed very similarly despite having slightly different finishes (i.e., aluminum had a slightly brushed



Graph 1
Distribution of grading values.



Graph 2
Distribution per substrate type.

finish; stainless steel had a clean smooth finish). Performing slightly behind the metals were the acetate samples, which exhibited problems because of high instances of background staining. The paper's poorer performance was expected because of the fingermark residues being absorbed into the paper's porous surface. This is also consistent with the recommended use of amino acid visualization reagents on this type of surface because of the higher proportion of eccrine constituents that are present in natural sweat deposits. Conversely, all constituents of the fingermark residue sit on the surface of the nonporous metal and acetate substrates and are available to interact with the PMA.

The differences in the finishes of the metals made a difference in the visualization of the fingermarks that were present on the surface. The slight brushed finish on the aluminum caused some marks to be visible only at an oblique angle, especially faint marks. There were some instances where the PMA caused high background staining on the substrate, leaving the surface awash with blue staining, although some did show signs of ridge detail, which was broken and spotty in places (Figure 3). Many of the samples that presented usable prints were observed to have little in the way of background staining, and the ridge detail appeared to be lighter than the background (Figure 4). This suggests that for this metal surface, the primary interaction occurred between the PMA and the aluminum metal substrate.

The stainless steel had the greatest number of grade 2 and grade 3 marks; however, it also suffered from the occasional background staining issues that the aluminum had (Figure 5). The fingermarks on the stainless steel substrates developed differently from the aluminum, with ridges presenting as a dark blue or black (Figure 6) and, in some cases, with some yellow staining between the ridges. This suggests that the principal interaction on stainless steel was between the PMA and the fingermarks, which represents a difference in development mode between the two metals that were used.



Figure 3
Aluminum-stained background.

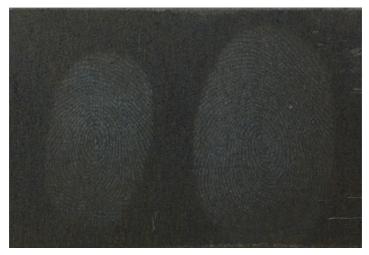


Figure 4
Aluminum with ridge detail.



Figure 5
Stainless steel-stained background.



Figure 6
Stainless steel with ridge detail.

The acetate substrates performed better in terms of fingermark development than the aluminum samples, but to a lesser degree than the stainless steel samples. When applying the PMA to the acetate surface, the PMA would sit in pools around the mark and stain the acetate a color similar to the yellow-green of the original solution. This produced a "halo", probably because the solution was repelled by the oils in the fingerprint residues (Figure 7). The marks or ridges within the voids, however, were visible as the characteristic dark blue or black of the molybdenum blue. The background staining that was observed was similar to that which occurs when using cyanoacrylate fuming and basic yellow 40 dye on certain substrates (e.g., tin foil) [2]. The visualized marks on the acetate surface were very fragile and easily destroyed by light touches (Figure 8). Despite this fragility and the instances of background staining, the acetate did produce instances of grade 2 to 4 prints (Figure 9).

As mentioned previously, the application of PMA to the paper substrates did not produce the same level of fingermark visualization as did the other samples. Over 50% of the paper samples returned no development whatsoever, with only 15% giving marks of grade 2 and above. However, as stated above, this is not inconsistent with operational observations that amino acid development reagents will be more effective on this substrate, and ideally, PMA should be compared to another process targeting noneccrine constituents to get a more representative measure of its effectiveness. Background staining was also noted in the paper samples, albeit to a lesser degree than the other substrates that were tested. The staining was observed to be of a variety of colors ranging from the aforementioned yellow-green to a pale blue. Grade 2 and 3 marks that were present were often faint; however, some very good ridge detail was observed (Figure 10).

No clear pattern was established in the results of the finger-mark aging series. This was due to natural fingermarks being used for the study. Because PMA primarily reacts with constituents of sebaceous secretions (which are present in varying and uncontrolled concentrations) and not all experiments commenced on the same day, the intra- and inter-donor variability made clear trends difficult to establish. To confirm the specificity of PMA to sebaceous constituents, two additional, shorter studies were conducted: (1) sebaceous material was artificially added to the finger tips, and (2) eccrine-only fingermark deposits (sweat from the hands only) were used as a control. The number of donors was dropped from 13 to 4, and the age of the fingermark deposits was lowered from 28 days to 8 (1, 2, 4, and 8 days). The number of sample substrates remained the same at four.

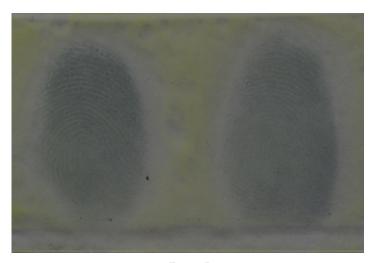


Figure 7
Acetate background staining.

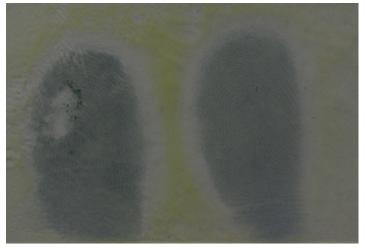


Figure 8

Damaged print on acetate.

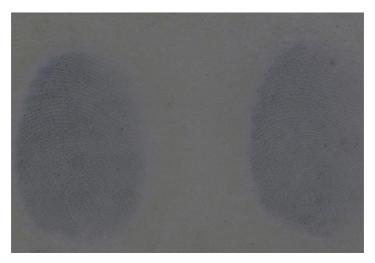


Figure 9
Ridge detail on acetate.



Figure 10
Ridge detail on paper.

For the eccrine study, the donors washed their hands then donned nitrile gloves for up to 30 minutes to allow the hands sufficient time to sweat. Marks were then deposited on the sample substrates. When conducting the sebaceous study, donors were asked to rub their fingertips around their hairline and nose areas where sebaceous sweat glands are abundant. Marks were then deposited upon the sample substrates.

Eccrine Study

The eccrine study failed to yield any positive mark visualization (Table 2), as expected. This occurred across all of the substrate materials. Some showed signs of background staining that was due to the PMA, but no signs of any ridge detail staining.

	Acetate	Aluminum	Paper	Steel
Grade 0	16	16	16	16
Grade 1	0	0	0	0
Grade 2	0	0	0	0
Grade 3	0	0	0	0
Grade 4	0	0	0	0
Total	16	16	16	16
% of Grade 2+ Marks	0	0	0	0

Table 2
Eccrine study results.

Sebaceous Study

The sebaceous study provided many positive marks and instances of fingermark development that could be used in an operational capacity to identify the depositer of the marks (Table 3). The acetate substrate showed the most grade 2 and above marks, although they still suffered from the delicacy mentioned before. The paper samples also showed a noticeable improvement in the amount of grade 2 and above marks that were developed (Figure 11). The age of the fingermark did not appear to influence the results that were gained over the time frame that was studied.

	Acetate	Aluminum	Paper	Steel
Grade 0	2	3	2	3
Grade 1	2	4	3	3
Grade 2	5	3	5	4
Grade 3	5	5	4	4
Grade 4	2	1	2	2
Total	16	16	16	16
% of Grade 2+ Marks	75%	56.25%	68.75%	62.5%

Table 3
Sebaceous study results.



Figure 11
Sebaceous study (paper).

This discovery not only sheds some light on the high number of no-detail results that were gained in the primary trial, because these contained marks that had not been artificially charged with sebaceous deposits, but these success rates are also comparable to other visualization reagents that target sebaceous constituents, such as ORO [47]. Despite this technique's limitation in only developing fingermarks with sebaceous material present, there may well be merit in using PMA in sequence with DFO \rightarrow indandione \rightarrow ninhydrin, much in the same way ORO is proposed for use at present [48].

PMA versus ORO Comparison Study

Fresh samples that were treated within a day of the fingermarks being deposited showed developed marks that were comparable between the two processes. Once the individual halves were treated and recombined, it was relatively easy to follow the ridge flow of the fingermark from one half to the other. The only noticeable issue was that the halves of the fingermarks were slightly misaligned; this was due to shrinkage of the paper that was treated with ORO (Figure 12). The ORO-treated halves were also prone to warping.

Samples that had the fingermarks deposited and were then left for over 4 weeks before being developed looked markedly different from those that were developed the day after the fingermarks were deposited. The half treated with ORO barely showed any marks from the fingermark residues, whereas the PMA-treated halves showed development, albeit slightly fainter than previously achieved (Figure 13). This suggests that the constituents of fingermarks targeted by PMA are more persistent within the deposited mark than those targeted by ORO (Table 4).

	1 Day	2 Weeks	4 Weeks
PMA (% of Grade 2+ Marks)	100%	80%	80%
ORO (% of Grade 2+ Marks)	80%	20%	0%

Table 4
Direct comparison percentage grade 2+ marks.

While experimenting with the two reagents sequentially, it was discovered that by using PMA in the first instance, ORO could be used additionally after 4 weeks. The developed fingermarks presented darker with a superior definition than when the ORO was used alone after the same period (Figure 14). On these split comparisons, the ORO developed 20% grade 2 marks and 80% at grade 1, whereas the PMA followed by ORO provided developed marks of 40% at grade 2 and 60% at grade 3.

Using PMA after ORO did nothing to enhance deposited fingermarks beyond what the ORO had already achieved.

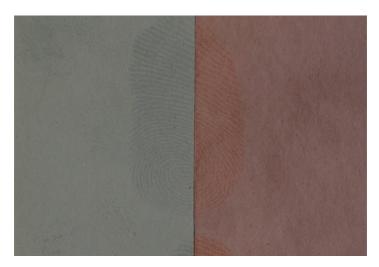


Figure 12
ORO vs phosphomolybdic acid.



Figure 13
ORO vs PMA (4+ weeks).



Figure 14
PMA + ORO used in sequence vs ORO.

PMA and Wetted Samples

Once the paper had been placed in the water bath and had completely soaked, fingermarks could be observed on the paper's surface (Figure 15). When they were dry, the samples were sprayed with PMA solution in the same manner as the previously processed dry samples were (i.e., sprayed and a 15 minute UV exposure). After treatment, however, the marks were not developed to the same standard as dry samples. Although faint fingermarks were visible once the development process had been completed, these marks lacked any usable ridge detail (Figure 16).

The fact that PMA is capable of developing fingermarks on both porous and nonporous substrates makes it a potentially more versatile visualization reagent than ORO. Although ORO formulations have been explored for the visualization of fingermarks on nonporous surfaces [2], it was found to be inferior to other dyes, such as basic violet 3 and solvent black 3, which are used for this purpose. Although this pilot study has demonstrated the ability of PMA to develop marks on nonporous substrates, it is recognized that future phases of the work would also need to include comparisons with solvent black 3 and basic violet 3 formulations to establish whether PMA offers any benefit over these existing processes on nonporous surfaces.

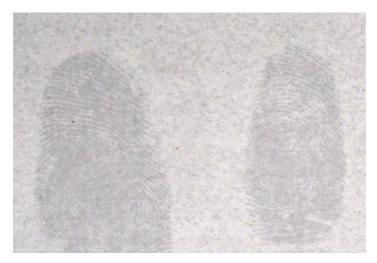


Figure 15
Fingermarks visible on wet paper.

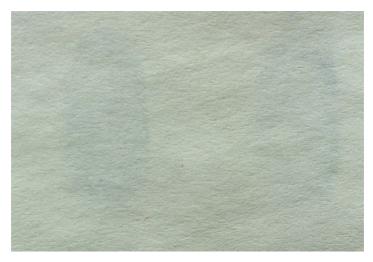


Figure 16
Wetted paper treated with PMA.

Conclusion

Of the substrates tested, it appears that the nonporous substrates provided more positive results than did the porous substrates. On stainless steel, the PMA technique produced positive mark development 80% of the time, 28% of which were grade 2 and above. This was closely followed by the aluminum, with positive mark development 76% of the time, 22% of which were grade 2 and above. The acetate substrate produced positive mark development 68% of the time, 26% of which were grades 2 and above. The smooth, nonporous surface of the acetate meant, however, that the fingermark developed was very fragile and easily wiped away. The paper substrate performed the worst, achieving positive mark development only 50% of the time, only 15% of which were grade 2 and above. Fingermarks aged up to 28 days were still enhanced to grade 2 and greater on all substrate surfaces except the paper.

An addendum trial, using a single donor, found that prints containing only eccrine sweat deposits returned no positive mark development whatsoever, whereas marks with charged sebaceous sweat deposits produced up to 75% positive mark development with greater ridge detail present.

When comparing the efficacy of PMA against ORO on porous substrates, the two seemed to be comparable on newer marks (those less than a week old). However, PMA easily outperformed ORO on older marks (those older than 1 week), possibly because it targets a wider range of constituents, some of which may be more persistent than the constituents targeted by ORO. The degradation observed with PMA after 4 weeks was less than that observed for ORO, so it would be expected that PMA would continue to develop marks for a little longer than the 4-week limit

PMA struggled to develop marks on paper substrates that had been wetted. Developed marks were visible, however, these lacked any ridge detail. This was an instance where ORO outperformed the PMA. Future studies would further investigate whether this could be resolved or whether it is an instance where ORO would be the preferential treatment.

PMA could be considered as a less expensive (~\$175 U.S. per liter vs ~\$250 U.S. per liter for ORO), faster alternative to ORO for the same type of development. By using the PMA as a precursor to ORO, fingermarks were able to be developed long past the point where ORO normally fails as a development reagent.

PMA has a potentially broad application across porous and nonporous surfaces; it is quicker and potentially more effective than ORO, and possibly physical developer, on porous surfaces (particularly for marks older than a month). However, it is unlikely to outperform an amino acid development reagent, and there are some health and safety issues in its flammability and corrosive nature, which would need to be addressed. Overall, PMA has shown significant promising trends in performance that merit further research, particularly with a larger study group to see whether these observed trends are maintained. Other areas that warrant further investigation are the ability of PMA to work in concert with amino acid visualization reagents and its performance comparative to other lipid development reagents on nonporous surfaces.

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