Abstract: Amido black (acid black 1) is the dye currently recommended by the UK police service for the enhancement of blood-contaminated fingerprints. Acid black 1 is a general protein stain and can be used for enhancing fingerprints in blood in either a methanol or water-based formulation to produce blue-black fingerprints. As both the water and methanol-based formulations have problems associated with their use, a program of research has been carried out to examine alternative formulations of the dye to find the most effective method for the enhancement of fingerprints in blood. After carrying out systematic evaluation on a range of typical surfaces, an alternative solvent system for amido black has been developed.

Introduction

Many reagents and formulations exist for the enhancement of blood-contaminated fingerprints, but there are few reports in literature describing their relative effectiveness on differing surfaces.

The Police Scientific Development Branch (PSDB) has undertaken to do a comprehensive review of all the most commonly used techniques for enhancement of blood-contaminated fingerprints. Also in-
cluded this study were related techniques that could be applied to this end.

The necessarily broad scope of the project meant the work had to be divided into three manageable sections:

1. optimization of amido black;
2. other protein stains;
3. reactive techniques.

PSDB currently recommends amido black for the enhancement of fingerprints in blood in one of two formulations based on methanol [1, 2] or water [2, 3]. The water-based formula is recommended for general use at scenes of crime and on all surfaces likely to be damaged by methanol treatment. The methanol formula is recommended for use when treating suitable articles in the laboratory, i.e., surfaces that are not effected by methanol.

Amido black, which is also known as acid black 1 (C.I. 20470), is a diazo dye which stains proteins blue-black. It does not react with the normal constituents of sweat in uncontaminated latent fingerprints so should be used in sequence with other techniques when blood-contaminated surfaces are examined [4].

Experimental protocol

Initial experimental work was carried out on nine surfaces typically found at scenes of crime:

porous surfaces - photocoppying paper, paper wallpaper and vinyl matt paint;
semi-porous surfaces - lacquer-coated wallpaper and vinyl satin paint;
non-porous surfaces - vinyl wallpaper, (either blown polythene wallpaper or pvc tablecloth), vinyl silk paint and glass.

For more detailed comparisons the following additional surfaces were used:
porous surfaces - cardboard, newspaper and untreated wood;
non-porous surfaces - aluminum metal, floppy disc (matt black), melamine, polythene bag, steel and varnished wood.

**Preparation of experimental samples**

Ideally, the blood used for depositing fingerprints would have been fresh so that the test sample would be as realistic as possible, i.e., what a scene of crime officer would usually be dealing with in practice. As this was impractical, experiments were undertaken using blood obtained approximately every six months. Blood was stored in a blood transfusion bag containing anti-coagulant, at 4°C.

A finger was covered in blood and gently touched on the surface to give a sequence of six depleted bloody fingerprints with the heaviest at the top. Fingerprints were left to dry for approximately 12 hours, after which time the strips could be used for experimental work. The fingerprints were guillotined through the center so that one half of a set of depleted bloody fingerprints could be treated with one technique and

![Figure 1](image)

*Figure 1*

*Diagram to show format for laying and splitting of bloody fingerprints*
the other half with another, enabling a comparison of techniques to be made. Each set of fingerprints on hard surfaces was laid over the join of two separate strips butted up to one another to avoid the need for cutting.

Using a series of depleted fingerprints, the relative performances of various techniques could be evaluated with respect to the quantity of blood present in the fingerprint. However it was found that sometimes fingerprints were laid with differing pressures on each side. To overcome this problem, for in-depth assessments, two strips of depletions were used. Each treatment was carried out on opposite halves of each fingerprint set.

Each technique was evaluated depending on the amount of background staining produced, the color intensity and the detail of the stained fingerprints. Techniques were also evaluated as to whether any adverse surface reaction took place, e.g., damage to varnishes, paints, etc.

It was found that sometimes when fingerprints were laid they were slightly smudged as the finger was lifted, giving the impression of diffuse ridges or leaching of the blood. This led to a more accurate method being introduced to assess ridge diffusion. The ink reservoir of a draftsman’s pen with a nib size of 0.25 mm was filled with blood, enabling lines of the set width to be drawn. An additional assessment was carried out to test the effectiveness of fixing solutions. This involved using drops of blood (0.5 mL to 0.75 mL) to see the amount of the leaching that occurs when heavier areas of blood are treated.

**Experimental**

PSDB currently recommends two formulations of amido black for the enhancement of fingerprints in blood. The methanol-based formulation [1] has been used since the 1970s with the water-based formulation [3] only relatively recently introduced. However, there appears to have been nothing published on how these formulations were arrived at, and little on the effects of any of the components except one fixing agent, 5-sulfosalicylic acid [5]. Therefore it was considered essential that knowledge of how the amido black process worked be gained before considering other techniques.
To this end experiments were carried out to find the best possible enhancement procedure for fingerprints in blood using amido black. Each component of each step (fixing, staining and destaining) of the two amido black formulations was examined, as well as possible alternatives published in literature, to determine the part it plays in the enhancement process. It was hoped that this work might lead to a single amido black formulation being produced.

Articles are usually fixed for five minutes and then stained for three minutes, although heavy deposits of blood need more time. Washing times depend on the nature of the surface being treated and cannot be standardized; porous surfaces require longer washing times and more changes of solution to destain non-porous surfaces. Articles are washed until excess dye has been removed from the background and greatest contrast is achieved between fingerprints and background. Several changes of solution may be required to achieve this.

Experiments were also conducted on aged bloody fingerprints. Fingerprints were used which had been laid six months, two months and one day before they were used.

**Current PSDB-recommended formulations for amido black**

Enhancement of the bloody fingerprints using amido black takes place in three principle stages: fixing the blood residue, staining the fingerprints and destaining the surface. The current recommended formulations are shown in table 1.

**Advantages of current formulations**

The methanol-based formula is very effective, inexpensive and an easy method to use for enhancing fingerprints in blood. It gives good ridge definition, little background staining and produces dark blue-black fingerprints.

The water-based formula does not use flammable or toxic solvents and can therefore be used safely at the scene of a crime as well as in a laboratory. It is also an easy process to use and inexpensive to carry out.
Table 1
Current PSDB Recommended Amido Black Formulations

<table>
<thead>
<tr>
<th></th>
<th>Methanol-based method</th>
<th>Water-based method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixing solution</td>
<td>Methanol (99%+)</td>
<td>20 g 5-sulfosalicylic acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000 ml distilled water</td>
</tr>
<tr>
<td>Staining solution</td>
<td>2 g acid black 1</td>
<td>2 g acid black 1</td>
</tr>
<tr>
<td></td>
<td>900 ml methanol</td>
<td>20 g citric acid</td>
</tr>
<tr>
<td></td>
<td>100 ml acetic acid</td>
<td>1000 ml distilled water</td>
</tr>
<tr>
<td>Destaining solution 1</td>
<td>900 ml methanol</td>
<td>Distilled water</td>
</tr>
<tr>
<td></td>
<td>100 ml acetic acid</td>
<td></td>
</tr>
<tr>
<td>Destaining solution 2</td>
<td>950 ml distilled water</td>
<td>Distilled water</td>
</tr>
<tr>
<td></td>
<td>50 ml acetic acid</td>
<td></td>
</tr>
</tbody>
</table>

Disadvantages of Current Formulations

The methanol-based solutions are toxic by ingestion and skin absorption. Methanol is also a highly flammable solvent. Although this formulation can be used safely in a laboratory, its use at scenes of crime is not recommended due to potential ignition or the possibility of absorption of methanol through the skin. Leaching of blood from heavy deposits also occurs with this formulation unless long fixing times are used. The methanol-based formulation may also soften or destroy some surfaces including paints, varnishes and some plastics, damaging or obliterating ridge detail.

The water-based formula does not always produce optimum results as it may give diffuse fingerprint ridges, especially on porous surfaces. On porous surfaces, the contrast between fingerprint and background can sometimes be poorer than that achieved when using the methanol-
based formulation, because of relatively high background staining and the intensity of the ridges may be less.

Results and discussion

Fixing

5-Sulfosalicylic acid proved to be an extremely effective fixing agent before the water-based stain, but did not show the same capability before methanol-based amido black. Concentration experiments were carried out and the optimum amount of 5-sulfosalicylic acid dihydrate was confirmed to be 20 g [5].

Methanol was very effective at fixing blood before the methanol-based amido black, but proved ineffective before the water-based amido-black formulation without extremely protracted fixing times.

Other published blood/protein fixing agents were assessed as to their effectiveness at preventing the diffusion of blood during fingerprint enhancement procedures [6, 7, 8]. Methods that involved materials that are toxic, carcinogenic or cause other health related problems, such as formaldehyde, picric acid and osmium tetroxide were eliminated from the study without testing. Water-based solutions of varying concentrations of acetic acid proved completely ineffective at fixing blood and absolute ethanol performed exceptionally poorly, taking in excess of one hour to fix average amounts of blood.

Preprocessing articles with superglue to develop latent fingerprints does not fix the blood. Therefore the amido black process must be carried out in full after treatment with superglue to develop latent fingerprints [9]. Also it was found that superglue treatment inhibited the dying process. Staining of fingerprints was patchy and less dark. The prior use of aluminum powder does not appear have any detrimental effect on the amido black results.

It was found that fixing average deposits of blood for five minutes gave the best results before both formulations. However, if heavy coatings of blood are to be treated, it is advisable to fix for longer, fifteen minutes or more, otherwise leaching of the blood will often occur.
Staining

Solutions that fixed and stained in a single step [10] were not stable over a period of a few days and proved to be not as effective as separate fixing and staining solutions.

Various experiments were carried out changing citric acid concentrations in the working solution for the water-based amido black. They showed that entirely removing the citric acid from the working solution gave stained fingerprints a greenish tinge, which greatly reduced the contrast with the background. Using higher concentrations of citric acid did not improve results. It was also found that the citric acid might be replaced by another organic acid, such as acetic, without detriment to the process.

Various concentrations of acid black 1 were examined and it was found that when used in concentrations of less than 0.1% w/v the stained fingerprints were less dark. The experiments also showed that using higher concentrations of dye did not improve the results.

The use of ethanol as an alternative to methanol in the amido black formulation was attempted as this would reduce toxicity. This had limited success as the staining solution produced good results but only after fixing for very long times.

Propan-2-ol in a distilled water and acetic acid mixture [8] was examined as a new solvent system. This produced good results after using 5-sulfosalicylic acid as the fixing agent. This led to a series of experiments involving ethanol, distilled water and acetic acid as the working and subsequent wash solutions. Ethanol was chosen in place of propan-2-ol to reduce the toxicity of the solutions.

Timed treatments were carried out for staining solutions in the methanol-based and water-based formulations. It was found that articles should be left in the staining solution for three minutes as this produces optimum results.

Destaining

Acid black 1 is very soluble in water, but while this is very effective for destaining after the water-based treatment on non-porous surfaces, it is less efficient on porous surfaces where it leaves higher backgrounds and can cause ridge diffusion.
Citric or acetic acids added to the wash solution produce higher background staining, but help retain dense blue-black colored fingerprints.

Methanol is an efficient destainer, which helps to prevent diffusion, but can remove coloration from the fingerprint as well as the background. Ethanol is as effective as methanol at destaining porous surfaces.

It was observed that amido black faded when left in the light over periods of a few days. There is a possibility that this property might be used to increase contrast between stained fingerprints and background on porous surfaces.

**Ethanol/water-based formulation of amido black**

Using the information learned from examining each stage of the amido black process, a new formulation was developed. This formulation uses the 5-sulfosalicylic acid fixing solution with staining and destaining solutions based on water, ethanol and acetic acid. This formulation is inexpensive and easy-to-use and is non-toxic and non-flammable.

This formulation proved more effective than the water-based amido black, giving more intense staining and no ridge diffusion, even on porous surfaces. Also it proved as effective as the methanol-based amido black formulation on porous surfaces. Although the background staining was noticeably higher, closer investigation showed the fingerprints were correspondingly darker. However, on some non-porous surfaces the staining of fingerprints was less intense.

When tested on aged bloody fingerprints it was found that the performance of the new formulation remained the same irrespective of age.

The ethanol/water-based formulation should be prepared as follows.

Fixing solution - 20 g of 5-sulfosalicylic acid is placed in a clean, dry beaker to which 1 liter of distilled water is added. The solution should be stirred with a plastic stirring rod or magnetic stirrer until all the sulfosalicylic acid has dissolved.
Staining solution – 1 g acid black 1 (CI 20470) is placed in a beaker and to this is added 250 ml ethanol, 50 ml acetic acid and 700 ml distilled water. The solution should be stirred using a magnetic stirrer for 5 minutes.

Destaining solution – 250 ml ethanol, 50 ml acetic acid and 700 ml distilled water are poured into a beaker and stirred.

Conclusions

Small-scale experiments have shown that the new ethanol/water-based formulation is at least as effective as the water-based formulation. It is also non-flammable, making it suitable for use on all surfaces at scenes of crime. Also it may be used in the laboratory on all porous surfaces and those non-porous surfaces that react with methanol.

The methanol-based formulation should be used only in the laboratory on nonporous surfaces that give no adverse reaction.

No firm recommendation about this or any other formulation will be made by PSDB until the research program is completed and extensive trials have been carried out.

Research into alternative protein stains and reactive techniques continues.

For further information, please contact:

Vaughn G. Sears
Fingerprint Research Group
Police Scientific Development Branch
Home Office
Sandridge
St. Albans
Hertfordshire AL4 9HQ
Great Britain
+44 (0) 1727 816 216

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