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Replacing Synperonic® N in the Physical Developer Fingermark Visualisation Process: Pseudo-Operational Trial and Parameter Studies

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Abstract

A reformulated physical developer (PD) solution has been devised to replace the use of Synperonic® N for environmental reasons. The performance of the replacement solution has proved promising in laboratory trials using planted fingermarks [1] (Thomas-Wilson et al, 2020) however; this may not always represent how a reagent works on real world samples. This paper therefore explores the effectiveness of the decaethylene glycol monododecyl ether (DGME)-based PD formulation through a pseudo-operational trial. A range of naturally handled, porous substrates were processed, which totalled over 600 samples that had been previously treated with amino acid reagents (1,2-indandione (IND) or 1,8-diazafluoren-9-one (DFO) and ninhydrin). The trial was representative of the operational use of PD at the end of a processing sequence for porous exhibits. The results from the trial establish that DGME is an effective replacement detergent for Synperonic® N in PD solutions and demonstrated the added benefit of using PD as a sequential treatment.

Planted mark studies to assess the parameters of the DGME-based PD formulation are also included in this paper. These studies explored the preparation, processing and storage temperature required for the solution as well as the shelf life. The effectiveness of DGME-based PD on items that have been previously wetted was also investigated. These studies show the formulation is suitable for use in an operational laboratory and is therefore an effective replacement formulation for the Synperonic® N-based PD.

Key Words

Physical developer (PD); Fingermark; Synperonic® N; Decaethylene glycol monododecyl ether (DGME), Pseudo-Operational Trial, Wetted surfaces.

Introduction

Physical developer (PD) is primarily used to develop fingermarks on porous exhibits recovered from crime scenes. In order to maximise fingermark recovery, it is well recognised to treat porous substrates sequentially with amino acid reagents followed by PD [2].This is due to the aqueous solutions used in PD dissolving amino acids, rendering 1,2-indandione (IND), 1,8-diazafluoren-9-one (DFO) and ninhydrin ineffective if used subsequently. Research suggests that certain fingermark constituents need to be present for development by PD [3-5]. It has been proposed that PD targets eccrine material but only when other constituents are present, for example insoluble components of a fingermark may trap eccrine material thus allowing the development of additional marks when used sequentially [3-5]. This theory demonstrates why PD has the unique ability to visualise fingermarks on porous items that have previously been wetted [6-8].

The chemistry of PD relies upon a redox reaction between the silver, ferrous and ferric ions in the solution. The equation below illustrates the reaction whereby the iron (II) ions reduce the silver ions to elemental silver [9; 10].

$$Ag^{+}(aq) + Fe^{2+}(aq) \leftrightarrow Ag(s) + Fe^{3+}(aq)$$

The reduction reaction is favoured due to the presence of the citric acid in the solution which forms a complex with the iron (III), thus removing it from the equilibrium system. The use of surfactants are therefore required to stabilise the silver and prevent precipitation by the formation of micelles around the silver particles. Once the exhibit is immersed in the solution, the fingermark constituents cause the destabilisation of the micelles formed allowing the silver particles to deposit and grow on the mark ridges, resulting in the visualisation of the fingermark [1; 4; 10; 11].

The surfactants previously recommended in the United Kingdom (UK) for PD consisted of a cationic surfactant; n-dodecylamine acetate (nDDAA) and a non-ionic surfactant; Synperonic® N [2]. Issues have arisen with the availability and manufacture of both these surfactants. With regard to n-dodecylamine acetate, issues with batch to batch variation have been reported leading to inconsistencies in the purity of the reagent [2; 12]. Although this is an ongoing concern, researchers are currently favouring a single supplier due to the bespoke manufacture required [13; 14]. Additionally, Synperonic® N was covered by the EU directive 82/242/EEC [15], which sought to monitor and phase out environmentally harmful chemicals, leading to its subsequent ban from industrial use (EU directive 2003/53/EC [16]). Therefore, Synperonic® N is no longer commercially available and the remaining stocks held in the UK for making fingerprint reagents are dated and depleting in quantity [1; 9; 17].

A replacement surfactant for Synperonic® N has been addressed by other researchers by the implementation of Tween® 20 [3; 5; 9; 17-19]. However, little agreement has been reached on the quantities of Tween® 20 used in the stock detergent, with quantities including 1.5 mL/L, 2.8 g/L, 3 mL/L, and 4 g/L being used around the world (all with equal amounts of nDDAA present) [5; 17; 19-23].

Research previously conducted in the UK discovered issues with the stability of Tween® 20 in PD solutions [1]. These trials found that Tween® 20 produced cloudy solutions causing

unpredictable efficacy during use. There were also additional concerns that the solution requires aging to obtain optimal performance [17; 18; 21; 22] and this timeframe was deemed to be at least two weeks in UK laboratory conditions [1; 24]. Based on these difficulties, Tween® 20 was determined not to be a reliable and effective replacement for Synperonic® N within the UK and alternative surfactants were subsequently explored using planted mark studies [1; 24].

Observations and results from this work, including exploring detergent ratios, have led to an optimised PD formulation. The reformulated stock detergent solution incorporated decaethylene glycol monododecyl ether (DGME) as a replacement for Synperonic® N and comprised 1.25 g of DGME and 1.5 g of nDDAA in 1 litre of reverse osmosis, deionised water. The PD working solution contained 50 mL of the DGME-based stock detergent solution and was found to be comparable in effectiveness to the current Synperonic® N-based formulation at developing fingermarks in planted mark studies and on aged cheques [1; 25]. Therefore, the purpose of this work was to conduct a larger and more realistic comparison study using pseudo-operational items, with the aim to establish the potential impact on police casework of implementing this new PD formulation.

The use of PD is known to require care and specific parameters, such as the temperature and shelf life of the solutions have been established to aid effective performance. Previous research by this group[1] has shown issues with the stability of the silver when preparing solutions at lower temperatures. It was therefore recommended that the Synperonic® N-based PD working solution should always maintain a temperature greater than 17ºC [1; 2; 26]. Furthermore, the shelf life of Synperonic® N-based PD working solutions ranged in published work from 5 days, as recommended by the Fingermark Visualisation Manual (FVM) [2], to 10-15 days as recommended by USA researchers [17]. Subsequent research has shown that working solutions that incorporate Tween® 20 have a greater shelf life ranging from 2 to 3+ months [17; 18; 21; 22]. However, these results have not been able to be reproduced with Tween® 20 formulations in UK laboratory conditions [1]. Therefore, the purpose of this work was to challenge the limitations of the new PD formulation incorporating DGME and to establish the parameters required for the operational use of this reformulated solution.

Further to this, the effectiveness of PD on wetted items required assessing, as PD is the recommended process on porous items that have been wetted. Current PD formulations employed in the UK and in literature have demonstrated the ability to develop marks on wetted items [2; 7; 8]. The work described here involved two stages:

Stage 1: Pseudo-operational trial using realistically handled items following on from the encouraging results from previous planted mark studies [1].

Stage 2: Establishing the parameters of the reformulated PD solution including preparation, processing, and storage temperatures, shelf life and effectiveness on wetted items.

[1] Group refers to the fingermark research team at Dstl (which integrated across from the Home Office Centre for Applied Science and Technology) and works in collaboration with universities.

Material and Methods

The chemical treatment process utilised for this study began with ensuring all glassware was thoroughly washed with reverse osmosis, deionised water and scratch free to minimise premature silver deposition. For larger items plastic trays were also used and these were lined with plastic sheeting. Solution preparation followed guidance outlined in the FVM [2], with the reformulated solutions being the only deviation. All items were first submerged in a tray of maleic acid for approximately 10-15 minutes, or until bubbles had ceased, in order to remove alkali fillers from the substrates which would otherwise induce premature silver deposition. Each item was then submerged in a tray containing the appropriate PD working solution, rocked occasionally, and observed for fingermark development. The items were monitored in the working solution until a sufficient contrast between the mark and background was achieved, or, if no marks were observed, until the background became sufficiently darkened. Processing times in the working solution ranged from approximately 10-25 minutes with factors such as the solution formulation, donor variability, substrate and the amount of items processed influencing the length of time required for optimal development. After removal from the working solution, the substrates were washed in a series of baths containing reverse osmosis, deionised water, followed by a print-washer using running tap water to remove any last residues of the working solution. The substrates were then dried at room temperature before examination [2].

Tables 1 and 2 contain details of the chemicals and the solution formulations utilised. Two brands of nDDAA were used due to availability issues, the original supply used (ICN Pharmaceuticals) is no longer available. Analysis and effectiveness of the replacement chemical was assessed when determining it was suitable [1; 12].

Table 1- Details of the chemicals and quantities for both DGME and Synperonic® N-based PD formulations

Chemical	CAS Number	Chemical grade	Quantity	Supplier
		Maleic acid solution		
Maleic acid	100-16-7	ReagentPlus© ≥99% (HPLC)	25 g	Sigma Aldrich* (Gillingham, UK)
Reverse osmosis, deionised water	N/A	Grade 2 (as defined in ISO 3696)	1 L	In-house Sartorius water purification system
		Redox solution		
Ammonium iron (II) sulphate hexahydrate	7783-85-9	ACS reagent 99% BioUltra ≥99%	80 g	Sigma Aldrich* (Gillingham, UK) Honeywell Fluka (Bucharest, Romania)
Iron (III) nitrate nonahydrate	7782-61-8	ACS reagent BioReagent	30 g	Sigma Aldrich* (Gillingham, UK) Honeywell Fluka (Bucharest, Romania)
Citric acid anhydrous	77-92-9	Redi-Dri ACS reagent	20 g	Sigma Aldrich* (Gillingham, UK)
Reverse osmosis,	N/A	Grade 2 (as	900 mL	In-house Sartorius

deionised water		defined in ISO 3696)		water purification system
Synperonic® N-based stock detergent solution				
n-Dodecylamine acetate (nDDAA)	2016-56-0	As supplied	2.8 g	ICN Pharmaceuticals (Plainview, NY, USA)
Synperonic® N	9016-45-9	As supplied	2.8 g	BDH Chemicals (Now Merck*)
Reverse osmosis, deionised water	N/A	Grade 2 (as defined in ISO 3696)	1 L	In-house Sartorius water purification system
DGME-based stock detergent solution				
Decaethylene glycol monododecyl ether (DGME)	9002-92-0	As supplied	1.25 g	Sigma Aldrich* (Gillingham, UK)
n-Dodecylamine acetate (nDDAA)	2016-56-0	As supplied	1.5 g	Pfaltz & Bauer (Waterbury, CT, USA)
Reverse osmosis, deionised water	N/A	Grade 2 (as defined in ISO 3696)	1 L	In-house Sartorius water purification system
Silver nitrate solution				
Silver nitrate	7761-88-8	Puriss. p.a., ACS reagent, reag. ISO, reag. Ph. Eur., ≥99.8% Puriss. p.a., ≥99.5% (AT)	10 g	Sigma Aldrich* (Gillingham, UK) Honeywell Fluka (Bucharest, Romania)
Reverse osmosis, deionised water	N/A	Grade 2 (as defined in ISO 3696)	50 mL	In-house Sartorius water purification system

*Sigma Aldrich and Merck Chemicals were consolidated into Merck Life Science UK Ltd in July 2020.

Table 2 - Volume of each component used within the two PD working solution formulations

Reformulated DGME-based PD working solution	**Current Synperonic® N-based PD working solution [2]**
900 mL of redox solution	900 mL of redox solution
50 mL of DGME-based stock detergent solution	40 mL of Synperonic® N-based stock detergent solution
50 mL of silver nitrate solution	50 mL of silver nitrate solution

Stage 1: Pseudo-operational trial

All items processed in the pseudo-operational trial were accumulated from a prior study by this group exploring the effectiveness of IND compared to DFO [27]. These items were chosen as they had been previously treated in 2015 (approximately three years prior to PD

treatment) with amino acid reagents (either IND or DFO, followed by ninhydrin) as per the UK Home Office recommended sequential processing charts [2; 14]. By using PD on these items, it was possible to compare the reformulated DGME-based PD to the current Synperonic® N-based PD formulations as well as verify the added benefit of using PD after amino acid reagents.

662 porous items were included in the trial and these had been previously sourced from waste bins, goods packaging and donations from staff such as receipts, envelopes and greetings cards. The range of items processed is shown in figure 1 and was tailored to be representative of those submitted to an operational laboratory [28]. The descriptions used for most of the paper types are self-explanatory; however, 'general paper' mainly consisted of printer paper, with most items in this category having some kind of printing on them. It also consisted of a mix of low quality and high quality paper types. Thermal paper consisted of paper with a coating that changes colour when exposed to heat, such as receipts. It was not possible to formally include 'semi-porous' items in this study, although items such as magazines and leaflets were included in the general paper category and these items may have locally affected areas of lower porosity due to heavily printed regions. Additionally, the envelopes sourced were separated into two categories: brown envelopes and envelopes, (which comprised all other colours excluding brown). This was due to brown envelopes being of poorer paper quality causing variations in effectiveness.

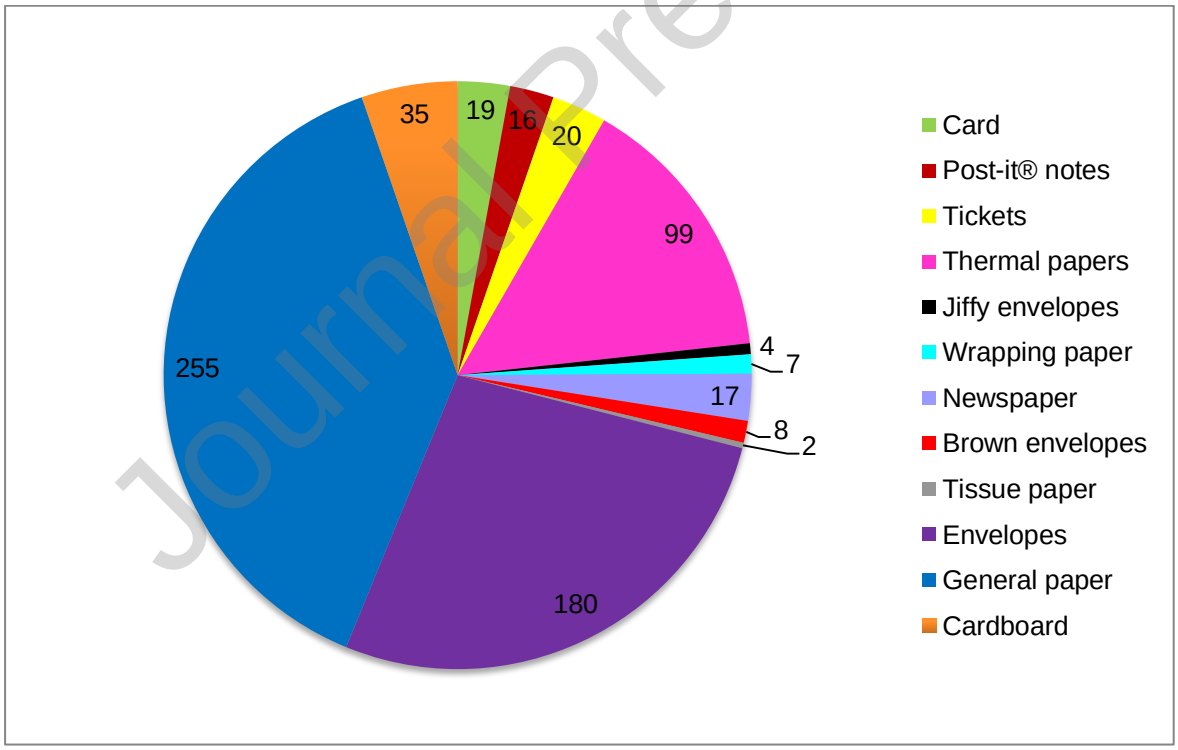

Figure 1: Number of items and types of substrates included in the PD pseudo-operational trial

Items were divided evenly into two batches as far as practically possible; they were split based on substrate type, size and the amount of marks previously developed with amino acid reagents. Each batch was selected blindly for treatment with either the reformulated DGME-based PD or the Synperonic® N-based PD formulation. Prior to treatment, some of

the larger items (typically cardboard) required cutting into smaller pieces for practicality reasons and envelopes were cut open along the seams to ensure an even chemical exposure was achieved over the surface area when processing.

After chemical treatment, the items were searched using a magnifying glass under white light. Items with dark or heavily patterned backgrounds were also examined using reflected infrared lighting conditions to aid fingermark visualisation by reducing the interference of background inks. Visualised marks were counted using an area criterion such that any area of clear continuous ridge detail was counted as one mark if the ridge detail was equal or greater than an area of 64 mm^2; examples of marks counted are shown in figure 2 [27; 29]. This area criterion is commonly used in pseudo-operational trials, and was first introduced by this group. This is because it was found that almost all areas over 64 mm^2 contained at least eight 'second level detail' points, which most UK police forces consider sufficient for a database search [29]. All items were re-searched and marks counted by a second individual in a blind verification.

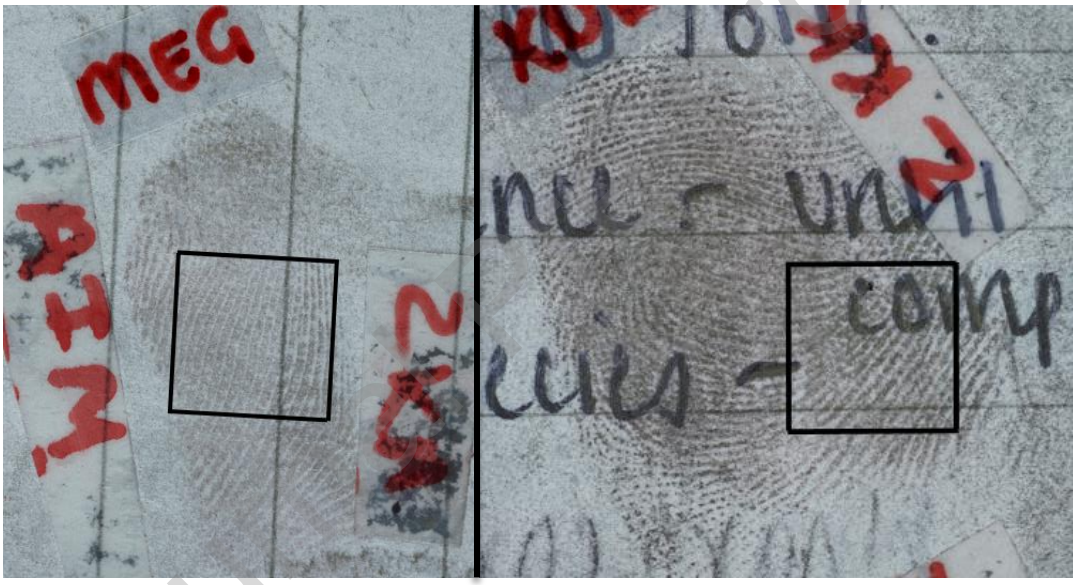

Figure 2: An example of the approximate criterion used to count fingermarks on two marks; visualised by DGME-based physical developer (left) and Synperonic® N-based physical developer (right). In these particular examples, both areas of ridge detail exceed 64mm^2 and would be considered as a countable mark

Stage 2: Planted mark studies – establishing parameters

Laboratory trials were conducted to establish the parameters of the new DGME-based PD formulation, the trials established:

- Minimum preparation and processing temperature of the PD working solution and storage temperature of the stock detergent solution.
- Shelf life of the stock detergent solution and PD working solution.
- Effectiveness of the PD working solution at developing fingermarks on wetted items.

All experiments were conducted with natural planted fingermarks (i.e. not controlled or deliberately groomed) and donors were asked not to wash their hands or apply hand lotion for at least 30 minutes before depositing marks. Table 3 lists the different substrates used in the planted mark studies. Not all of the substrates were used throughout; the ID number of the substrates used for each experiment is referenced in the respective section.

Table 3 – Thirteen porous substrates used in trials to establish parameters of the DGME-based stock detergent and PD solutions

ID Number	Manufacturer	Paper Type	Details
1	Xerox	Plain White paper	A4 performer copier paper, 80 gsm
2	Wilkinson's	Plain White paper	A4 paper, 80 gsm
3	Banner	Plain White paper	100% recycled A4 copier paper, 80 gsm
4	Pukka Pad	Lined White paper	Jotta writing paper, 80 gsm
5	3M	Yellow Post-it® note	Super sticky big notes yellow Post-it® note pad
6	Blake	White Envelopes	Purely everyday recycled envelopes (324 x 229 mm)
7	Not known	Plain Brown card	A4 card sheets
8	Not known	Brown Envelopes	A4 Manilla envelopes, cut along the seams
9	Not known	White Envelopes	A4 white envelopes, cut along the seams
10	Not known	Brown Cardboard	Corrugated cardboard boxes (light brown), cut into A4 size
11	Boofle	White Wrapping paper	Printed wrapping paper (one side white, one side printed beige)
12	NCR	Thermal Paper	Thermal POS Printer Rolls
13	Jiffy AirKraft	White Jiffy Envelope	A4 Jiffy envelopes, cut along the seams and the bubble wrap removed

Throughout the experiments outlined, once the marks were processed they were examined using white light and a magnifying glass, and graded 0-4 depending on the area of ridge detail developed [29; 30]. The grading scheme used is outlined in Table 4.

Table 4 - Grading scheme used for assessment of developed marks.

Grade	Description of level of detail present
0	No evidence of fingermark
1	Evidence of contact but no ridge detail observed
2	Less than ⅓ clear ridge detail present across original contact area
3	⅓ to ⅔ clear ridge detail present across original contact area
4	Over ⅔ clear ridge detail present across original contact area

A second grading scheme was used to interrogate the marks further in experiment 2.4 assessing the shelf life of the DGME-based PD working solution. The grading scheme assessed the quality of each mark comparatively against its corresponding half/quartered mark. A score of zero was assigned if two halves/quarters were of equal quality, a score of -1 was assigned if the half/quarter mark was of lesser quality and a score of +1 was assigned if the half/quarter mark was better in quality.

When assessing the marks post processing, a minimal number of half marks were discarded from some of the experiments due to obscured marks as a result of inadequate maleic acid exposure and donor placement. When this occurred, the corresponding half mark was also eliminated from the study.

2.1, 2.2 and 2.3 Preparation and processing temperatures of DGME-based PD working solution and storage temperature of DGME-based stock detergent solution

The same experimental design was used to assess the preparation and processing temperatures of the PD working solution and storage temperature of the stock detergent solution. The storage of the working solution was not assessed as it was deemed unlikely that the working solution would be stored for a length of time based on the results of the shelf life experiment.

Twelve donors were asked to deposit a depletion series of six natural marks down a column with the same finger (shown in figure 3). Three different substrates were used (types 1-3 as summarised in Table 3), and two sets of marks were collected, resulting in six sets of marks for each experiment. The age of the fingermarks varied depending on the experiment and ranged from one to three weeks. The marks were cut in half to form split depletions for processing to allow two temperatures to be compared at once. These were 15ºC and 20ºC for the preparation and processing temperatures of the working solution and 10ºC and 15ºC for the storage temperatures of the stock detergent solution (referenced as A and B in figure 3). Note that A and B were swapped throughout the experiment to account for changes in pressure distribution of the fingermark residue when donating.

Only one parameter was changed in each respective experiment, for example, when assessing the preparation temperature of solutions at 15ºC and 20ºC, the storage and processing temperatures remained at room temperature. These temperatures of 15ºC and 20ºC were chosen to explore whether 17ºC would cause cloudy solutions, as previously experienced with the Synperonic® N-based PD solution [26]. Lower temperatures of 10ºC and 15ºC were chosen to assess the storage temperature due to environmental monitoring indicating these lower temperatures were reached in the laboratory outside of standard operating hours and during winter. The solutions were placed in a calibrated incubator (Labcold™ RLCG01502 Incubators). The temperature in the incubator was monitored by a

datalogger (D 753-651, TC Direct Ltd.) to ensure uniform stability, and the temperature of the solutions was monitored using a calibrated thermometer probe (Lollipop thermometer, Control Company).

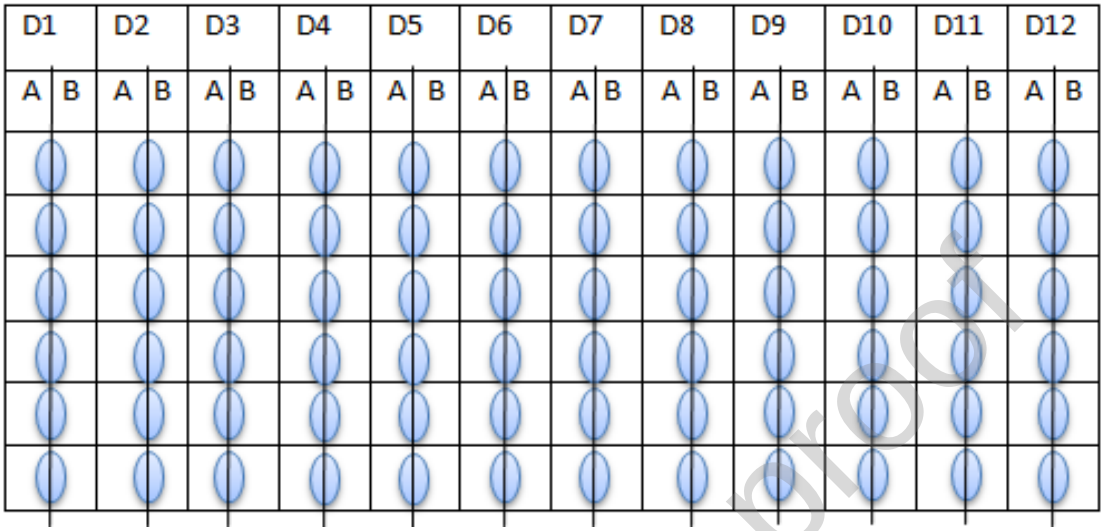

Figure 3: Schematic showing the method of donation to obtain a split depletion series

2.4 Shelf life of the DGME-based stock detergent solution

The shelf life of the DGME-based stock detergent solution was assessed by comparing the quality of half and quartered marks prepared as shown in figure 4. Donors were asked to place a mark of their thumb in the middle box followed by four fingermarks in the remaining four boxes. 24 donors were used, the fingermarks were aged for one week and the experiment was repeated to create a larger data set. Four substrates were used (types 1-4 summarised in table 3) and the samples were cut into quarters, each quarter being subsequently processed with a fresh working solution containing an aged DGME-based stock detergent solution. The ages of stock detergent solution tested in this experiment were 0 days, 1 month and two batches of 18 months old stock detergent, which had been stored in the dark at room temperature before use. 0 days refers to fresh working solution that was prepared on the same day processing occurred.

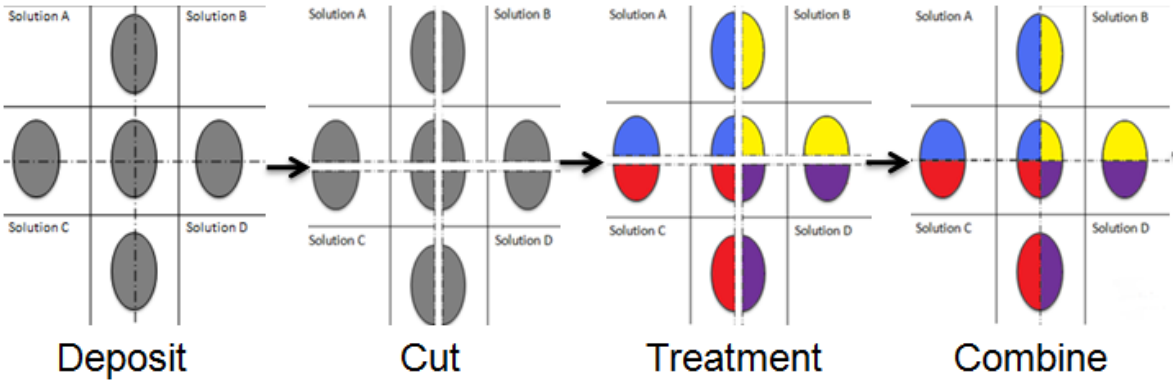

Figure 4: Schematic showing the method of donation and treatment in the experiment to establish shelf life of detergent solution

2.5 Shelf life of DGME-based PD working solution

The shelf life of the working solution was assessed by observing working solutions kept for up to five days at room temperature in dark conditions, due to five days being the recommended shelf life in the UK for Synperonic® N-based PD [2]. The working solutions were observed daily with regular agitation of the solutions to assess silver precipitation. An experiment was also carried out to determine the effectiveness of the working solution over time by assessing the quality of split half marks using three ages of working solution (0 days, 2 days and 4 days) in a three-way comparison (0 v 2, 2 v 4, and 0 v 4). As shown in figure 3 a six mark depletion series was obtained using six donors, three substrates (1, 5 and 6, as referenced in table 3), and the fingermarks were aged for three weeks. This experiment was then repeated, therefore 648 fingermarks were obtained overall, resulting in 432 half marks being processed with each aged solution respectively.

2.6 Effectiveness on wetted items

The effectiveness of fingermark development of the reformulated DGME-based PD working solution on items that had been previously wetted was assessed by a comparative study. Two sample sets of ten substrates were used (types 2, 4, 5, 7 – 13 detailed in table 3) and 30 donors were asked to place a single mark in one box on each substrate, as shown in figure 5. A single mark donation from a broad range of donors was selected rather than a depletion from a smaller donor set, in order to incorporate a wide range of chemical compositions in the marks. This allowed the comparison of the environmental conditions the substrates and fingermarks had been exposed to; the sensitivity of the process was outside the remit. The donation sequence was repeated to create two sets of aged marks: one week and one month. Therefore, overall 1,200 fingermarks were collected for this experiment. Half of the substrates/samples were submerged in rainwater for approximately 8 hours then allowed to dry at room temperature prior to processing. The remaining half of the samples were processed without any prior wetting. This wide range of substrates was selected in order to assess surfaces similar to those encountered in operational work [28].

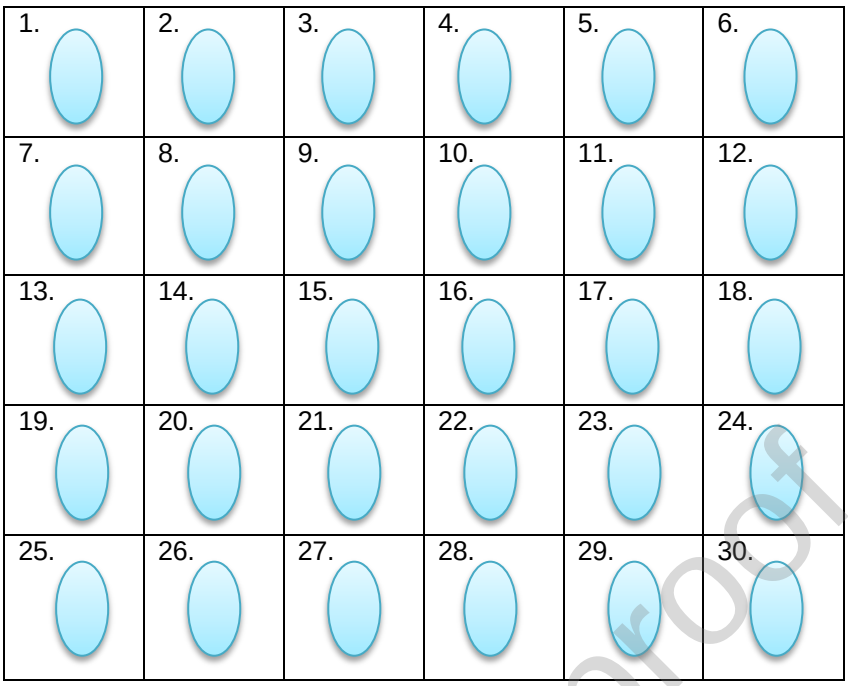

Figure 5: Schematic of mark donation

Results and Discussion

Stage 1: Pseudo-operational trial

Overall 662 items were processed in the pseudo-operational trial, which consisted of 330 items treated with the DGME-based PD formulation and 332 items treated with the Synperonic® N-based PD formulation (noted here that although two extra items were processed with Synperonic® N-based PD formulation it does not affect the outcome).

The results across the trial are summarised in figure 6, and show that the reformulated PD solution incorporating DGME proved to be equivalent in effectiveness, visualising 217 marks, compared to the Synperonic® N-based PD formulation, which visualised 200 marks in total. Furthermore, observations when processing showed similar apperances of both solutions, as well as similar processing times (10-25 minutes), and the developed marks had comparable contrast and background development (see figure 2). The use of reflected infrared as a additional viewing processes on dark and pattern backgrounds did not apply to many exhibits and therefore is not discussed in this section.

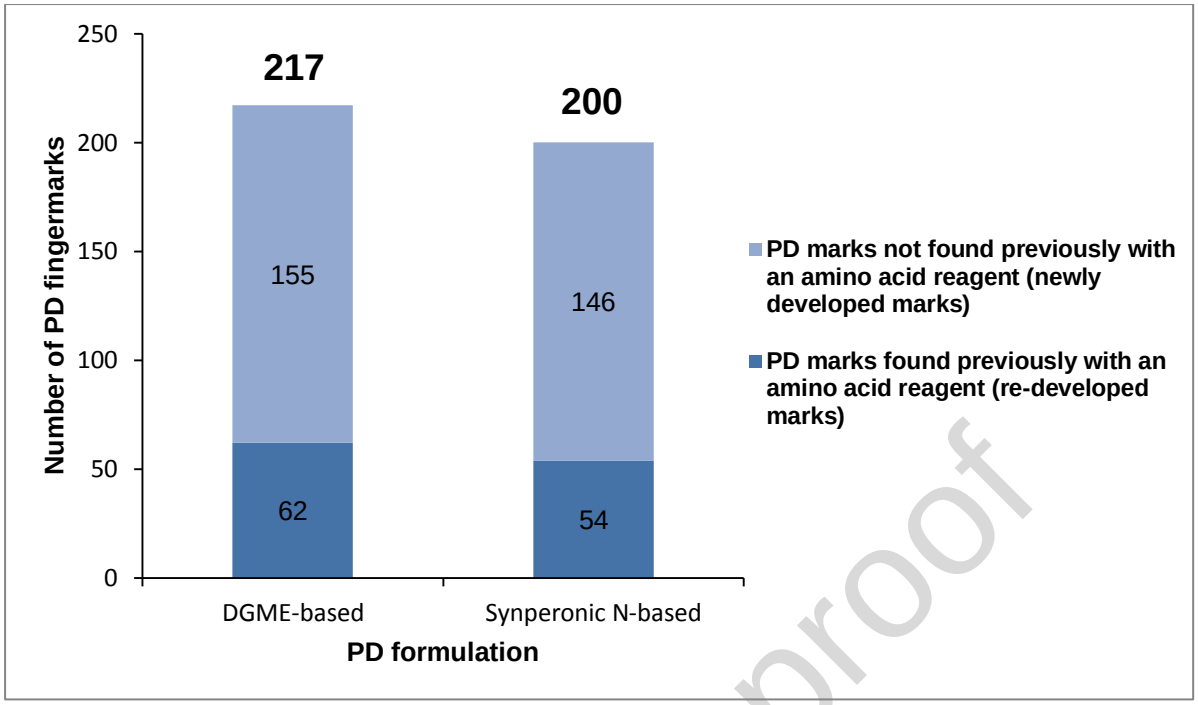

Figure 6: The breakdown of fingermarks visualised (≥64 mm^2) based on the PD formulation used

The graph also shows that approximately three-quarters of all marks developed by PD were unique to the PD chemical treatment process, independent of the PD formulation utilised, which reinforces the performance similarity between the two formulations. These marks were not previously found at the amino acid reagent stage as all marks found were compared with previously labelled marks, photos and the data from the processing of amino acid reagents. The development of new marks is most likely due to the PD chemical process acting on different constituents present in the fingermark compared to amino acid reagents.

An extra 301 marks (155 DGME marks and 146 Synperonic® N marks) were developed following amino acid reagents with PD. This highlights the added benefit of using PD in sequential processing as an extra 30% of marks were developed that were not previously found at the IND/DFO or ninhydrin stage (shown in Figure 7). This could be considered a high percentage of new marks, as other studies employed have shown smaller percentage increase in the number of marks developed by PD [31; 32]. However, the study by Bleay et al on old cheques showed an approximate increase of 30% extra marks developed solely by PD which is concurrent with the findings observed [25].

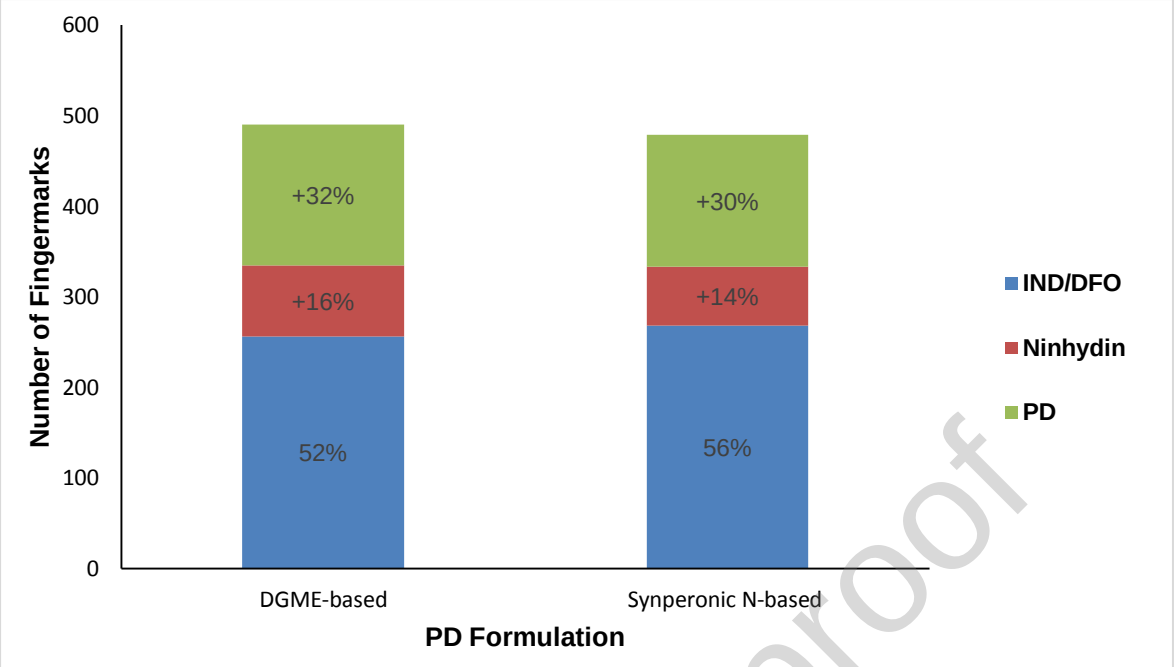

Figure 7: Percentage of marks and extra marks developed by sequential processing (DFO/IND and ninhydrin data was taken from the DFO/IND study by Luscombe et al [27]).

Further to this, the data was analysed based on the average number of marks per item, to ensure the different sequences employed (IND-ninhydrin-PD and DFO-ninhydrin-PD) did not negatively affect the data. The results are tabulated below (table 5) and show that the average number of marks developed differ rather significantly between IND and DFO, supporting that IND is a superior amino acid reagent [27]. However, the results for ninhydrin and PD are comparable across all four data sets. Therefore, it can be concluded that in this study the ninhydrin and PD mark recovery are not affected by the initial amino acid reagent utilised, which is concurrent with previous studies [25].

Table 5: Average number of fingermarks per item separated based on the processing sequence

DGME-based PD working solution				**Synperonic® N-based PD working solution**			
IND	1.2	**DFO**	0.5	**IND**	1.3	**DFO**	0.6
Ninhydrin	0.2	**Ninhydrin**	0.3	**Ninhydrin**	0.2	**Ninhydrin**	0.2
PD	0.4	**PD**	0.5	**PD**	0.4	**PD**	0.5

A limitation of the pseudo-operational trial is that only older marks were incorporated which is not representative of all types of police casework. However, the preliminary PD reformulation studies [1] and planted mark studies to establish parameters included marks that were fresher, for example aged for 1-2 weeks, and the new DGME-based PD formulation was found to be effective at developing these marks.

Stage 2: Planted mark studies – establishing parameters

2.1, 2.2 & 2.3 Preparation and processing temperature of the DGME-based working solution, and storage temperature of the DGME-based stock detergent solution.

Table 6 shows the data obtained from the 1,645 fingermarks graded overall in the experiments regarding the preparation, processing and storage temperatures, with 864, 360 and 421 marks treated per study respectively. 72 marks were excluded due to maleic acid damage and 11 marks were discarded due to incorrect donor placement. The data (shown in table 6) highlights the limited number of marks graded three and four throughout the experiments and this may be attributable to the donor dependency associated with PD leading to poor mark recovery and not a result of processing conditions. For each assessment, two temperatures were compared: 15ºC and 20ºC for the preparation and processing temperature of the DGME-based working solution and 10ºC and 15ºC for the storage temperature of the DGME-based stock detergent solution.

Table 6 - Results table for experiments on preparation temperature of the DGME-based working solution (2.1), processing temperature of the DGME-based working solution (2.2), and storage temperature of the DGME-based detergent solution (2.3)

	2.1 Preparation temperature		**2.2 Processing temperature**		**2.3 Storage temperature**	
	15ºC	**20ºC**	**15ºC**	**20ºC**	**10ºC**	**15ºC**
Percentage of half marks graded 3 & 4 (%)	16.1 (139 half marks)	17.7 (153 half marks)	9.7 (35 half marks)	6.4 (23 half marks)	12.6 (53 half marks)	10.5 (44 half marks)
Percentage difference between temperatures (%)	1.6		3.3		2.1	

The results from the experiments show a similar number of marks were graded equivalently, independent of the temperature. No changes were observed in the working solutions (other than the anomaly highlighted below), with all working solutions remaining clear with no observations of cloudiness.

As a note, an anomaly was recognised during the preparation of one batch of working solution, where a slight precipitation of a silver colour was observed. The preparation experiment was repeated and the solution prepared a second time to explore this further. However, second solution showed no precipitate and the same trends as the original data set were seen therefore, the results were combined and not considered void.

Overall, the results demonstrate that preparation and processing of the working solution can occur at lower temperatures than previously observed with Synperonic® N-based PD solution, without adversely affecting the solution or processing times. Therefore, standard

laboratory temperature can be recommended for the DGME-based PD working solution, as it is more tolerant of lower temperatures. In regards to the DGME-based stock detergent solution, processing can occur when the detergent solution has been stored at 10ºC, and therefore the storage temperature for the detergent solution is not anticipated to be a future problem.

2.4 Shelf life of DGME-based stock detergent solution

Overall, 2,264 marks were graded in this experiment, consisting of half marks and quartered marks. Three ages of stock detergent solutions were compared: 0 days, 1 month and 18 months and the number of marks graded three and four are shown in figure 8. Two different batches of the 18 month old stock detergent solution were included in the comparison study to explore the potential effect of batch to batch variation. The results show little difference between the effectiveness of the three ages of solutions at developing marks graded three and four (a maximum variation of 25 marks was seen across the dataset). The 18 month old stock detergent solutions have both performed equivalently, thus supporting the reliability of the dataset.

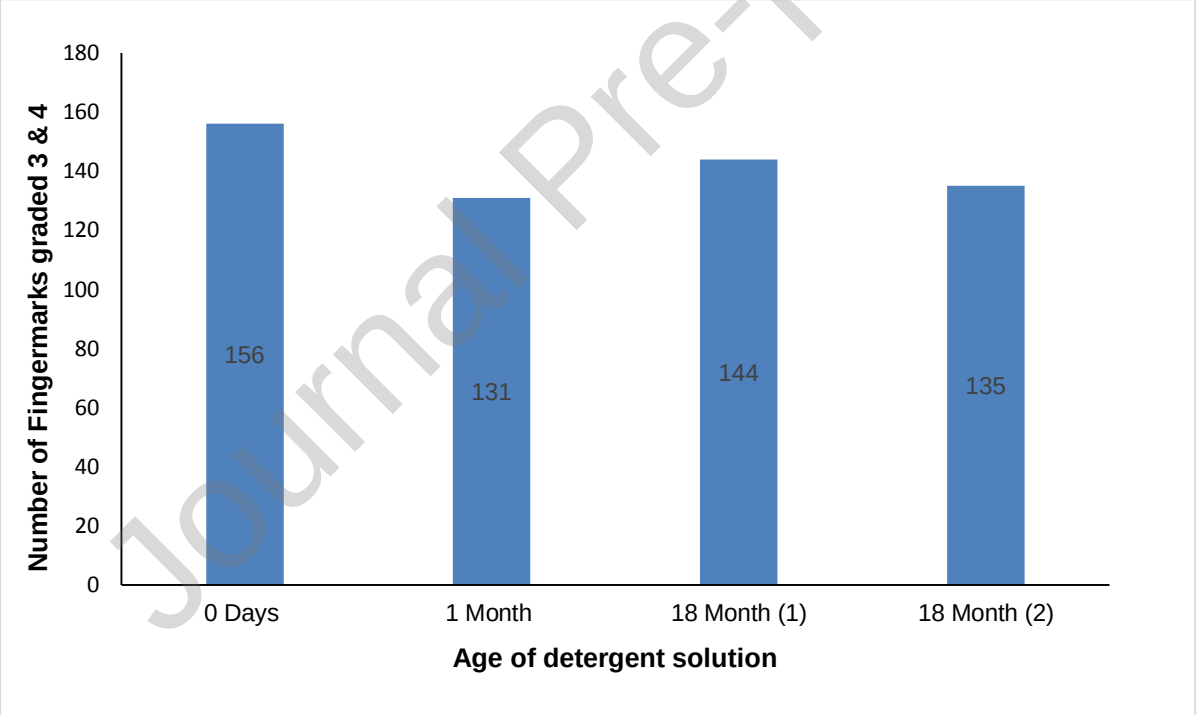

Figure 8: Number of marks graded 3 & 4 based on detergent age

The same fingermarks were interrogated further using an alternative grading scale assessing the quality of the marks by comparatively assigning scores of 0, +1 and -1. It should be noted that this comparative grading scale does not account for overall quality of the dataset as a grade three mark can be compared to a grade four mark and would be assigned a score of -1. Figure 9 shows a trend in the results of the comparison indicating that the older solutions were slightly less effective in comparison to their corresponding half/quarter mark. The fresh (0 days) stock detergent solution gave a higher percentage of

better marks (30% achieved +1 score); with 18 months old (1) & (2) solutions achieving only 22% and 17% of better marks. However, the difference in better marks between 0 days and 18 months (2) equates to 98 marks (13%), which when compared to the number of marks graded overall (2,264) is only a small variation in results.

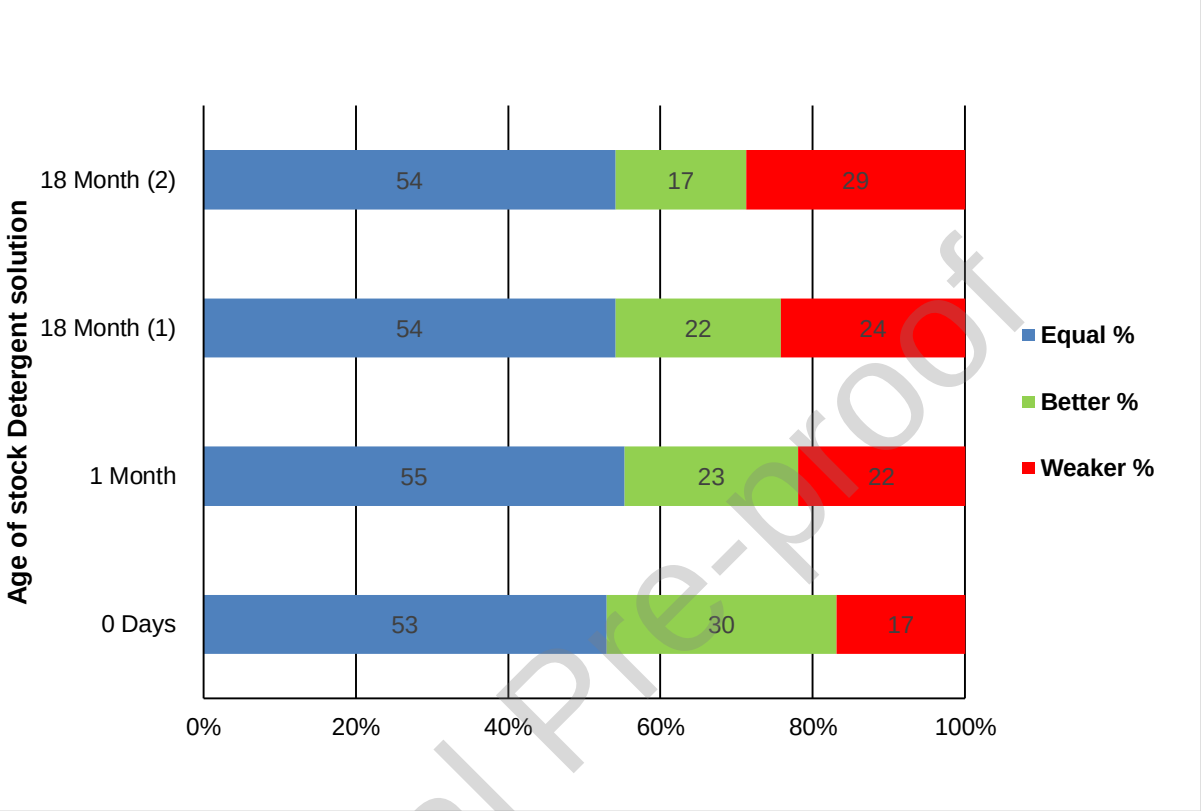

Figure 9: Percentage effectiveness of DGME detergent solution based on comparative scoring

The two different grading schemes used to analyse the data show slight variations in the trends observed. These variations in the analysis highlight the benefits from comparing two grading scales. It also demonstrates that the slight decrease observed with solution age accounts for minor differences in the data sets, indicating that 18 month old solution is still effective.

However, it would be practical to assume that a shelf life of 12 months would produce satisfactory results as the level of decline from 0 days to 18 months was minimal and it is not anticipated the chemistry of the stock detergent would alter over this timeframe. Due the timing of experiments, it was not possible to include a 12 month old stock detergent solution in this study, however a one year shelf life conforms with standard warrantees stated by manufacturers [33]. It is also not anticipated that laboratories would store a chemical beyond this age unless stated otherwise by the manufacturer.

2.5 Shelf life of DGME-based PD working solution

PD working solutions of varying ages (0, 2 and 4 days old) were compared in a small-scale experiment using 635 fingermarks and the grade three and four results are shown in Figure 10. The results reveal the decreasing effectiveness of the working solution over time and, over a four-day period, grade three and four fingermark recovery decreased by 39%.

Working solutions that were stored in the dark at room temperature were observed in order to document their visual stability over a five-day period. Small quantities of silver coloured precipitate was observed at day 2 (48 hours after preparation) in the working solution as shown in figure 11, and the amount of precipitate present in the working solution continued to increase with solution age. It should be noted that the solutions were regularly agitated in order to observe the silver precipitation more clearly. As this study indicates that the working solution shows signs of degradation approximately 24-48 hours after preparation, it is therefore considered most beneficial to use the working solution on the day of preparation in order to achieve optimal fingermark development.

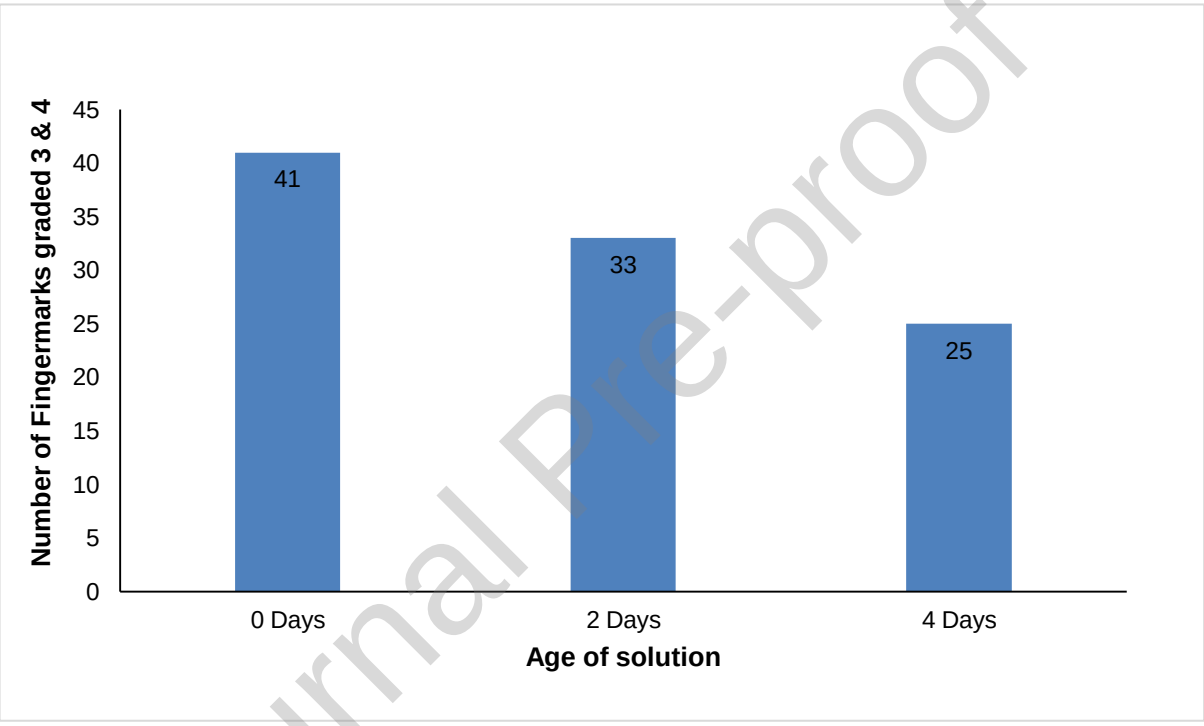

Figure 10: Number of fingermarks graded 3 & 4 for varying ages of the DGME-based working solution

Figure 11: Photograph of the underneath of a bottle of a DGME-based PD working solution - on the day of preparation showing clear solution (left), aged for 48 hours exhibiting some silver deposition (right).

2.6 Effectiveness of DGME-based PD on wetted items

In this experiment, 1,200 fingermarks were graded and the results show minimal difference between the marks that were previously submerged in water and those that remained dry (figure 12). The effectiveness of the Synperonic® N-based PD on wetted marks is already known and the solutions used in the chemical process are water based, including the maleic acid pre-wash [6; 7]. However, as wetted items were not included in previous planted mark studies or the pseudo-operational trial conducted by this group, this experiment satisfies the criteria that the DGME-based PD formulation is capable of developing marks on this item type. The results suggest that submersion of the marks in water before chemical treatment does not affect the recovery rate to any extent and supports the theory that PD targets insoluble constituents and residue trapped by insoluble constituents in the fingermark [4; 5].

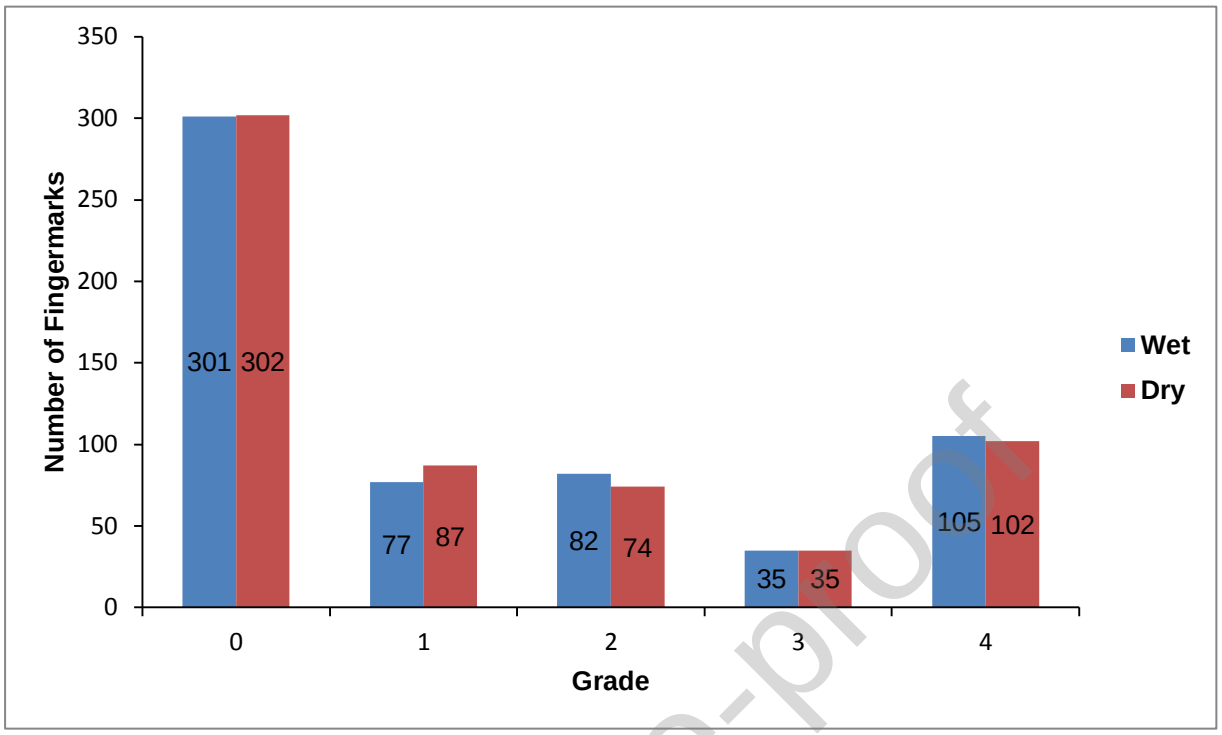

Figure 12: Number of marks based on their grade and pre-treatment conditions

Conclusions

Stage 1: Pseudo-operational trial

This study is unique because items were retained from a previous pseudo-operational trial exploring the effectiveness of amino acid reagents, and then utilised for the comparison of PD formulations. This has allowed older marks on a wide range of naturally handled substrates to be exposed to PD following treatment with IND/DFO and ninhydrin. The trial has demonstrated that the fingermark recovery of the new DGME-based PD formulation is as effective as the Synperonic® N-based PD formulation currently in use in the UK operational fingerprint laboratories. Furthermore, this study has provided supplementary evidence to emphasise the added benefit of using PD as a sequential treatment [25]. An additional 30% of marks were developed with PD that had not been visualised after amino acid treatment, thus demonstrating the success of pseudo-operational material in comparison to planted marks studies which showed poor mark recovery.

It should be noted that the marks used in this part of the study were aged for at least three years prior to PD processing, due to utilising the items collected through the previous trial. Due to the processing time required for PD, it was not possible to cover all variables in the pseudo-operational trial. However, this journal paper and preliminary studies have included fresher marks, wetted items and substrates not previously processed with a chemical treatment and the reformulated DGME-based PD solution has proven to be effective at developing these fingermarks [1].

It would still be highly advisable for fingerprint laboratories to perform an operational trial if they are implementing this new PD formulation on police casework. This would allow the variabilities that could occur with real criminal case exhibits, such as the fingermark residue left by a suspect committing a crime, to be taken into account. The added reassurance of an operational trial would provide confidence in the performance of DGME-based PD in these circumstances.

Stage 2: Planted mark studies – Establishing parameters

The parameter studies sought to challenge the boundaries of the reformulated DGME-based PD formulation in order to provide evidence as to its suitability as a replacement for the Synperonic® N-based PD. Operational fingerprint laboratories require a process that is useable as well as effective and this was found to be challenging with a Tween® 20 PD formulation [1]. From the results of the parameter studies using the DGME-based PD solution, the following conclusions were drawn:

- The preparation and processing temperatures of 15ºC or 20ºC for the PD working solutions were explored and there was no significant difference in the effectiveness of the solutions at these two temperatures. Therefore, the minimum 17ºC required by the Synperonic® N-based PD working solution [2; 26] no longer applies to this new PD working solution and the processing times observed at the temperatures tested still fall in the expected range of 10-25 minutes.
- There was little difference in the results observed when comparing the storage temperature at 10ºC and 15ºC. Therefore, it is not anticipated that the storage temperature of the DGME-based PD solutions should be a concern.
- The shelf life of the stock detergent was evaluated by comparing the effectiveness of three aged solutions; 0 days, 1 month and 18 months. The results from this experiment showed a small variation in the data depending on the grading scale used, however even the older solutions were still producing good quality marks therefore a guideline expiry period of 12 months can be recommended for the DGME-based stock detergent.
- The shelf life of the working solution was evaluated over a five-day period and it was determined that the solution should be used within 24 hours of preparation to achieve optimal performance.
- As PD is unique in its ability to develop marks on wetted items, this was explored with the new formulation and it was found that the DGME-based PD is effective on substrates that have been wetted and then dried before processing.

Overall, from the results of the pseudo-operational trial, the DGME-based PD formulation has shown to be effective and comparable to the Synperonic® N-based PD formulation. The planted mark studies also showed that the DGME-based PD formulation had practical usability and stability. Thus it can be concluded that the DGME-based PD formulation is a suitable replacement for Synperonic® N-based PD formulation in operational laboratories.

Tween® and Synperonic® are registered trademarks of Croda International [34].

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CRediT authorship contribution statement

E. M. Cartledge: Formal analysis, Investigation, Visualisation, Writing - original draft; **Z-Y. Guo:** Formal analysis, Investigation, Visualisation; **S. M. Bleay:** Investigation, Writing - review & editing; **V. G. Sears:** Conceptualisation, Methodology, Writing - review & editing, Supervision, Project administration; **L. J. Hussey:** Conceptualisation, Methodology, Visualisation, Writing - review & editing, Supervision, Project administration.

Highlights

- DGME-based PD is compared to Synperonic N-based PD in a pseudo-operational trial.
- DGME-based PD performed equivalently to Synperonic N-based PD when used in sequence.
- Reinforces benefit of using PD in sequence as additional 30% of marks recovered.
- DGME is recommended as a suitable replacement for Synperonic N