Electronic Supplementary Material (ESI) for Analytical Methods.

Incorporating hydrogels into fingermark development: indicative viscosities for preserving ridge detail on paper surfaces

Supporting Information

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1. *Chemicals*

All chemicals were purchased from Sigma Aldrich, including iodine ≥99.99%, soluble starch (from potato), xanthan gum, glycerol ≥99.5%.

2. *Synthesis of starch-containing hydrogels*

Method 1
This method of hydrogel synthesis was adapted from the fingerprint literature wherein it was used to create a starch spray for iodine fixation on paper.[1]
A 0.5%, 1.0%, or 2.0% (w/w) starch solution was made by first creating a slurry by stirring either 2.5 g, 5 g, or 10 g of starch in 80 mL of water heated to 70 ^{0}C. Once the slurry was homogenized into a paste, a further 420 mL of 70 ^{0}C water was added with 5 minutes of additional stirring. The resulting hydrogel was stood for 60 minutes until cooling to a temperature of 25 °C prior to analysis / use.

Method 2
This method was adapted from method 1 to produce more viscous gels, and better approximates other research into starch gel behaviour.[2]
A 2.0% or 4.0% (w/w) starch gel was made by heating 80 mL of water to 100 ^{0}C and creating a paste using either 10 g or 20 g of soluble starch. This was then added to 420 mL of boiling water with stirring. Each solution was then stirred at 100 ^{0}C for a further 7 minutes. Finally, the gels were stood for 60 minutes, or until they had cooled to 25 °C, prior to analysis / use.

3. *Synthesis of xanthan gum hydrogels*

Two non-starch hydrogels were prepared to fix iodine fumed fingermarks: a 1% and a 2% xanthan gum in 10% aqueous glycerol. Depending on the concentration, 5 g or 10 g of xanthan gum was stirred in 50 mL of glycerol, and the homogenous solution was then diluted with 500 mL of water. The resulting gels were stood for 5 minutes prior to analysis / use.

4. *Determination of viscosity*

Hydrogel viscosities were measured using a Brookfield DV1 Digital Viscometer equipped with the RV-4 spindle. The viscometer was calibrated using a silicone oil of known viscosity prior to taking experimental measurements. All measurements were performed on samples cooled to a temperature of 25 °C, with a spindle setting of 50 RPM. Torque values greater than 10% were observed for all samples. The spindle was allowed to rotate at least five times prior to taking measurements. Measurements are reported here in centipoise (cP).

5. Calculated viscosities with Brookfield DV1 Viscometer parameters [1]

Sample	Spindle	Temp	RPM	% Torque	Viscosity (cP)
2% Xanthan/ 10% Glycerol	RV-4	25 ℃	50	92.4	3688
1% Xanthan/ 10% Glycerol	RV-4	25 ℃	50	38.6	1554
4% Starch Gel	RV-4	25 ℃	50	55.8	2224
2% Starch Gel (Method 1)[2]	RV-4	25 ℃	50	17.6	704
2% Starch Gel (Method 2)[2]	RV-4	25 ℃	50	34.6	1248

Note 1: Starch hydrogels containing less than 2% starch lacked the requisite viscosity to be measured accurately with a viscometer setup that would allow for meaningful comparison to the more viscous gels. Consequently, the 1% and 0.5% starch hydrogel values have been excluded from this table.

Note 2: Two variants of the 2% starch hydrogel were made in this work, a less viscous variant that was heated to a lower temperature of 70 °C (Method 1) and a more viscous variant heated to 100 °C (Method 2). The 2% starch gel described in the manuscript was made according to Method 1 to closely match an earlier forensic formulation. The viscosity value for Method 2 is included here as a validation of our viscometer results with other reports of this kind.[2]

6. Deposition and treatment of fingermarks

Throughout this study, the fingermarks presented fall into one of two categories: groomed or natural prints. Groomed fingermark specimens are made by adding sebaceous residue and then typically used fresh (*i.e.* without aging). These may show enhanced performance compared to routinely encountered operational samples. By contrast, natural fingermarks have no increased amounts of sebum when laid, and all fingermarks are aged prior to assessment to better replicate evidence encountered during casework.

In this work, groomed fingermarks were used to test and optimise foundational elements of a new fingermark visualization methodology. For these experiments, the inter-donor variability expected from natural fingermarks could complicate interpretation of the experimental results, thereby hindering accurate optimisation of the new method. Other than for the Phase 1 study (see below), this research used groomed fingermarks obtained from one donor aged 20-25 years. Fingermarks were laid on either Reflex® Ultra White Premium Paper or starch-free paper. Prior to application of hydrogels (or water) fingermarks were fumed with iodine for 30 seconds, or until visualization of the fingermarks was seen with minimal background development. These fingermarks were next brushed with the chosen hydrogel using a squirrel-hair brush. Unless otherwise specified, the results were photographed 5 minutes after application.

Only natural prints were used when comparing indicative performance of the gel fixation method to iodine benzoflavone fixation under approximated casework conditions (*i.e.* the Phase 1 study). During the study, three fingermark

donors took part, including one weak, one medium, and one strong donor, with these designations deriving from past performance assessments from other studies. The donors comprised one male, and two female volunteers, two of which were aged 20–24 years, one of which was aged 35–40 years. Donors were screened to ensure that they had not handled food or chemicals, worn gloves, or washed their hands in the hour preceding deposition of fingermark specimens. Homogenization of fingermark residue on the finger pads by rubbing them together was not undertaken prior to deposition so as not to supress variation, and regular activities had been carried out by the donors beforehand. Donors were instructed to sequentially apply their fingerpads to separate areas of the dictated surface type using light pressure.

7. Phase 1 validation study details

The validation study comprised a total of 720 fingermarks placed on three paper substrates (starch-free newspaper, Reflex copy paper, and J. Burrows 100% recycled copy paper) to evaluate the relative performance of 4% starch gel and iodine benzoflavone solution. This pool of fingermarks can be subdivided into two aging periods: fingermarks aged for 1 day (360 fingermarks) and 7 days (360 fingermarks). Within either age category, fingermark development performance was further subdivided into three substrate categories: starch-free newspaper (120 fingermarks), Reflex copy paper (120 fingermarks), and J. Burrows 100% recycled copy paper (120 fingermarks). Within each category, the performance of experimental 4% aqueous starch treatment was compared to that of iodine benzoflavone solution (60 fingermarks each). While being aged, fingermarks were stored in a darkened drawer, under standard laboratory conditions (25 °C, ± 2 °C).

8. Iodine benzoflavone solution

An all-in-one iodine-naphthoflavone developer solution was used as a comparison for the effectiveness of 4% starch gel in the Phase 1 study. This specific formulation is used operationally by the Queensland Police Service in Australia, and the formulation can be found in the literature.[3] Details of the iodine solution are provided below:

Reference	Fingerprints and Other Ridge Impressions[3]
Type of solution	All-in-one iodine developer solution
Components of work solution. These are combined prior to use.	*Iodine stock* 1 g iodine 1000 mL AK-225 *α-naphthoflavone stock* 2.4 g α-naphthoflavone 20 mL dichloromethane
Standing time	5 minutes
Stability (if known)	30 minutes

9. Documentation of treated fingermarks

Treated fingermarks were photographed using a Nikon D3500 camera coupled with a TAMRON SP AF 90 mm f/2.8 Di Macro Lens without the use of filters. Treated fingermarks were illuminated under ambient lighting, or using a LUMATEC Superlite M05 LED portable forensic light source set to the white light setting (400–700 nm).

10. Expanded results and statistical analysis drawn from the Phase 1 study

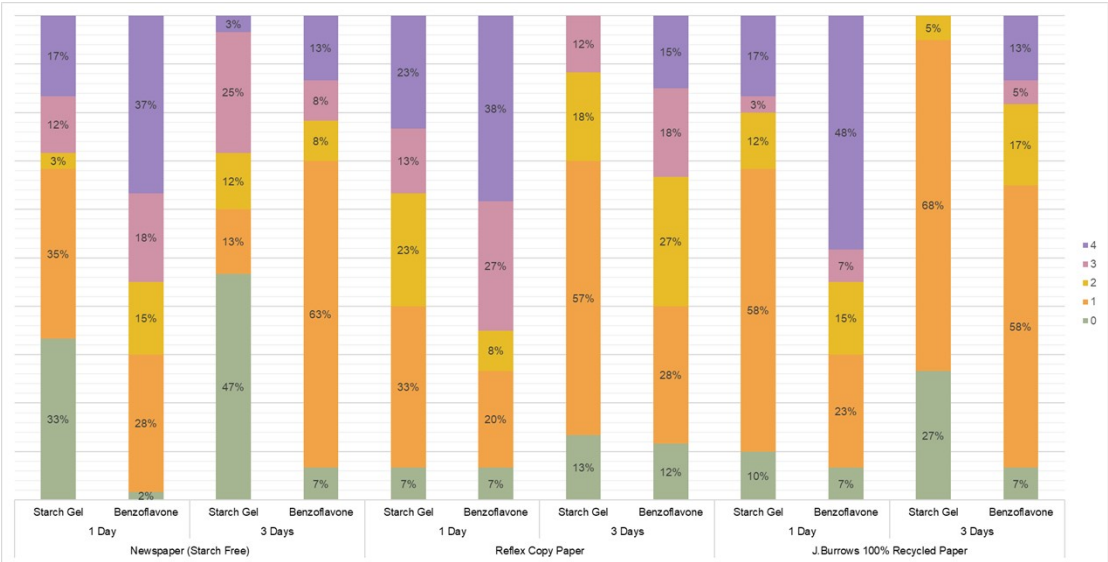

Fig. S1 Full CAST grading scores assigned during the Phase 1 study.

	Newspaper (Starch Free)			
	1 Day		3 Days	
	Starch Gel	Benzoflavone	Starch Gel	Benzoflavone
R	2802	4458	3236	4024
n	60	60	60	60
R^2/n	130853.4	331229.4	174528	269876
n (Total)	120		120	
k	2		2	
H	18.88661157		4.276473829	
df	1		1	
p-value	0.00001		0.03864	
Significant? (95%)	Yes		Yes	

Fig. S2 Full Kruskal-Wallis test parameters calculated for starch-free newspaper.[4]

	Reflex Copy Paper			
	1 Day		3 Days	
	Starch Gel	Benzoflavone	Starch Gel	Benzoflavone
R	3208	4052	3055.5	4204.5
n	60	60	60	60
R^2/n	171521.0667	273645.0667	155601	294630
n (Total)	120		120	
k	2		2	
H	4.905895317		9.092293388	
df	1		1	
p-value	0.02677		0.00257	
Significant? (95%)	Yes		Yes	

Fig. S3 Full Kruskal-Wallis test parameters calculated for Reflex copy paper. [4]

	J. Burrows 100% Recycled Paper			
	1 Day		3 Days	
	Starch Gel	Benzoflavone	Starch Gel	Benzoflavone
R	2897.5	4362.5	2875.5	4384.5
n	60	60	60	60
R^2/n	139925	317190	137808	320397
n (Total)	120		120	
k	2		2	
H	14.78116391		15.68237603	
df	1		1	
p-value	0.00012		0.00007	
Significant? (95%)	Yes		Yes	

Fig. S4 Full Kruskal-Wallis test parameters calculated for J. Burrow Recycled paper. [4]

11. References

1) R. D. Olsen, *Scott's fingerprint mechanics*, Thomas, 1978.
2) N. Castanha, M. L. Rojas, P. E. D. Augusto, *Scientia Agropecuaria*, 2021, **12**, 203.
3) (a) C. Champod, C. Lennard, P. Margot and M. Stoilovic, *Fingerprints and Other Ridge Skin Impressions*, CRC Press, Boca Raton, 2004. (b) M. Stoilovic, C. Lennard, *Fingermark Detection and Enhancement*, 6th ed., Canberra, Australian Capital Territory, Australia: National Centre for Forensic Studies, 2012.
4) D. Hockey, A. Dove, T. Kent, *Forensic Sci. Int.*, 2021, **318**, 110604.