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Chapter 32

Non-marking Collection of Amino Acids from Fingerprints Using Hydrogels

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Abstract

The amino acid profile obtained from a fingerprint may provide valuable information on its donor. Unfortunately, the collection of chemicals from the fingerprint is often destructive to the fingerprint ridge detail. Herein we detail the use of cross-linkable solutions of dextran-methacrylate to form hydrogels capable of collecting amino acids from surfaces followed by extraction and quantification with UPLC-MS. This method allows for the amino acid profile analysis of fingerprints while allowing for their increased visualization at a later stage using the standard method of cyanoacrylate fuming followed by basic-yellow dyeing.

Key words Amino acid analysis, Hydrogel, Non-marking, UPLC-MS, Fingerprint

1 Introduction

The amino acid profile retrieved from a donor may contain useful information, such as the sex, age, or health of the donor, and therefore may be of interest to forensic investigators and diagnostic clinicians [1, 2]. Commonly used methods to generate amino acid profiles rely on analysis of either blood, urine, saliva, feces, or cerebrospinal fluid [3]. In recent decades, many mass spectrometry-based methods have been developed for the analysis of amino acids from human sweat and fingerprints [4–7]. Unfortunately, the collection of chemicals from fingerprints is often destructive to the fingerprint ridge detail. Hence, a method which collects analytes and is non-marking to the surface it is applied on would be of value. Forensic investigators primarily use swabs or lifting tape to collect evidence from surfaces. Swabs do indeed absorb analytes very well, but they show poor release of the analytes intended for analysis and are a destructive way of sampling [8]. Lifting tape or gel lifters are commonly used to lift fingermarks of

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surfaces, but these techniques do not allow extraction of components while leaving the fingermark non-marked. The ideal collection material for forensic investigators is non-marking (i.e., it does not physically mark the evidence, yet it is capable of extracting chemical components) to the surface it is applied on. Moreover, adsorption and release of the collected analytes should be fast. Hydrogels, 3D networks of hydrophilic polymers with water as dispersion medium, provide a solution to the sample collection as they can be applied to nonporous surfaces [9]. Herein, we describe the use of cross-linkable solutions of dextran-methacrylate to form hydrogels which are used to collect amino acids from fingerprints in a non-marking manner.

2 Materials

2.1 Chemicals (Also See *Chapter 33*)

1. 21 Amino acids (Sigma-Aldrich, *see* **Note 1**).
2. 21 Isotopically labeled amino acid internal standards (CDN isotopes Pointe-Claire (Canada), Sigma-Aldrich Zwijndrecht (the Netherlands), Isotec Zwijndrecht (the Netherlands), and Cambridge Isotope Laboratories Tewksbury MA (USA), *see* **Note 2**).
3. Methanol (UPLC grade).
4. Acetonitrile (UPLC grade).
5. Ultrapure water (purified deionized water, to attain a sensitivity of 18 MΩ-cm at 25 °C).
6. Formic acid (UPLC grade).
7. Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP, prepared according to the method described by Fairbanks et al. [10], *see* **Note 3**).
8. Dextran-methacrylate (DEX-MA, with a substitution degree of 2.5, prepared according to the method described by De Smedt et al. [11]).
9. 3-(Trichlorosilyl)-propyl-methacrylate (Sigma-Aldrich).

2.2 Amino Acid Standard Stocks

1. MeOH containing 5% (v/v) formic acid: Add 50 ml of formic acid to a 1 L volumetric flask. Add methanol to 1 L.
2. Individual amino acid standard solutions of 100–500 mg/L, depending on their solubility, were prepared in MeOH containing 5% (v/v) formic acid using a volumetric flask and were stored at −20 °C (*see* **Notes 4** and **5**).
3. Individual amino acid internal standard solutions of 100–500 mg/L, depending on their solubility, were prepared in MeOH containing 5% (v/v) formic acid using a volumetric flask and were stored at −20 °C.

4. Master mixture of 2 mg/L of each of the 21 amino acids was prepared from the individual stocks using a volumetric flask and stored at −20 °C.
5. Internal standard mixture of 2 mg/L of each of the 21 amino acid internal standards was prepared using a volumetric flask from the individual internal standard stocks and stored at −20 °C.

2.3 Sample Preparation

1. 26 × 76 mm Glass microscope slides (Thermo Scientific).
2. 15 mL Polypropylene conical tubes (Fisherbrand).
3. Vortex mixer (IKA vortex Genius 3).
4. Evaporator (Pierce model 18,780 Reacti-Vap).
5. 15 mL Tube racks polystyrene (Fisherbrand).
6. Pipettes with volumes of 10, 20, 100, 300, 1000, and 5000 μL (Eppendorf Research Plus).
7. Analytical balance (Sartorius BP2105).
8. 100 ml Glass beakers (Fisherbrand).
9. Coverslips (25 × 25 mm, Borosilicate Glass, Fisher Scientific).
10. Laser pointer (maximum output power <1 mW, wavelength 405 nm ± 10).
11. Plasma cleaner (Harrick Plasma, Ithaca, NY, USA).
12. Vacuum chamber system (using an Edwards E2M1.5 vacuum pump).

2.4 Pre-hydrogel Solutions and Materials

1. Pre-hydrogel solution of LAP was prepared by dissolving 2% w/v LAP in ultrapure water under sonication for 20 min at room temperature (*see* **Note 3**).
2. Pre-hydrogel solution of DEX-MA was prepared by dissolving 20% w/v DEX-MA in ultrapure water under sonication for 20 min at room temperature.
3. Functionalized 25 × 25 mm coverslips: The slips were activated prior to functionalization using a plasma cleaner for 240 s. Then they were transferred to a vacuum chamber containing 100 μL of 3-(trichlorosilyl)-propyl-methacrylate and left under dynamic vacuum for 4 h to functionalize the surface (*see* **Note 6**).

2.5 UPLC-MS

1. 150 mm (length), 2.1 mm (internal diameter), 1.7 μm (particle size) ethylene-bridged hybrid (BEH) amide analytical column (Waters, USA).
2. 5 mm (length), 2.1 mm (internal diameter), 1.7 μm (particle size) ethylene-bridged hybrid (BEH) amide guard column (Waters, USA).

3. Acquity I-class UPLC autosampler and binary solvent pump (Waters, USA).
4. Clear glass 12 × 32 mm screw-neck total recovery vial, with cap and PTFE/silicone septum, 1 mL volume (Waters, USA).
5. Accurate mass TOF with a dual-electrospray source (ESI) (Agilent 6220, USA).

3 Methods

3.1 Calibration Series

1. Prepare a calibration series using the master mixture and internal standard mixture of 0, 100, 200, 400, 600, 1200, and 1600 μg/L of each amino acid with 400 μg/L of each internal standard on the day of the UPLC-MS analysis (total volume of 100 μL) and transfer to injection vials.
2. Put the vials in the sample manager of the Acquity I-class UPLC autosampler (set at 8 °C).

3.2 Hydrogel Fingerprint Amino Acid Extraction

1. Collect fingerprints on 26 × 76 mm glass slides (Fig. 1a, *see* **Note 7**).
2. Add equal volumes (10 μL) of the pre-hydrogel solutions of DEX-MA and LAP to an aliquot and briefly mix using a

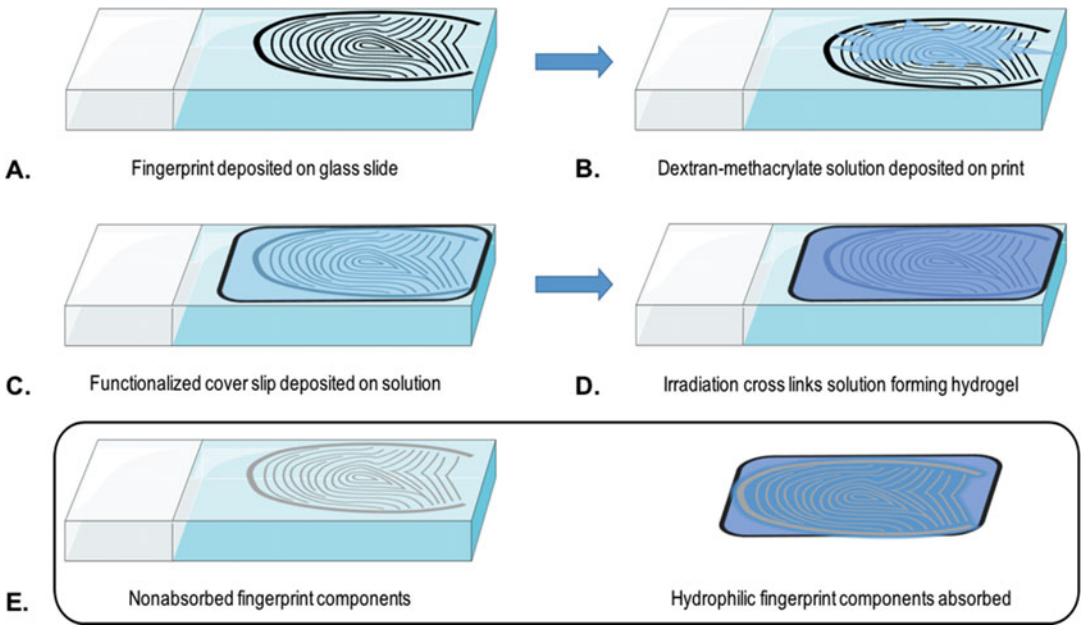

Fig. 1 Schematic representation of water-soluble analyte extraction from a fingerprint by hydrogel formation, including fingerprint deposition on a glass slide (**a**), deposition of the dextran-methacrylate solution on the fingerprint (**b**), deposition of a functionalized coverslip on the fingerprint with solution (**c**), irradiation of the fingerprint sample (**d**), and lastly the removal of the coverslip and hydrogel from the glass slide (**e**)

pipette. Transfer the solution directly onto the glass slide containing the fingerprint (Fig. 1b, *see* **Note 8**).

3. Place the functionalized coverslip over the solution (Fig. 1c).
4. After 3 min, irradiate the sample with a 405 nm laser pen (1 mW) for 30 s to initiate cross-linking, forming the hydrogel (Fig. 1d, *see* **Note 9**).
5. Gently lift the coverslip with the attached hydrogel from the microscope slide and transfer it to a beaker containing 3 mL MeOH:H_2O (1:1) (Fig. 1e, *see* **Note 10**).
6. Transfer the beaker containing the lifted hydrogel to an ultrasonic bath and sonicate for 30 min.
7. Add IS solution (10 μL) and transfer the extract to a 15 mL polypropylene conical tube (*see* **Note 11**).
8. Reconstitute in 50 μL MeOH containing 5% (v/v) formic acid by using the vortex mixer, briefly spin down, and transfer to an injection vial.
9. Put the vial in the sample manager of the Acquity I-class UPLC autosampler (set at 8 °C).
10. The nonabsorbed fingerprint components can be visuzalized using cyanoacrylate fuming followed by basic yellow dyeing (*see* **Note 12**).

3.3 UPLC

1. Prepare solvent A: Acetonitrile containing 0.4% (v/v) formic acid: add about 900 mL acetonitrile to a 1 L cylinder. Add 4 mL of formic acid to the cylinder using a pipette. Add acetonitrile to the cylinder to the final volume of 1 L (*see* **Note 13**).
2. Prepare solvent B: Water containing 0.4% (v/v) formic acid: add about 900 mL ultrapure water to a 1 L cylinder. Add 4 mL of formic acid to the cylinder using a pipette. Add ultrapure water to the cylinder to the final volume of 1 L.
3. Assemble the guard and analytical column in the column oven of the Acquity I-class UPLC and set the oven temperature at 65 °C.
4. Purge and prime the UPLC system.
5. Set flow rate at 0.5 mL/min and condition the column at 95% solvent A and 5% solvent B for 25 min.
6. Program the UPLC gradient as follows: 95% solvent A for 3 min followed by a linear gradient from 5 to 50% solvent B in 19 min followed by 1 min of 50% B. Finally, recondition the column for 2 min with 95% solvent A.
7. Program the method to wash sample syringe with acetonitrile.

8. Generate the sample list including sample name, type, vial position, injection volume (2.5 μL), and UPLC method. Program the autosampler to generate a pulse (contact closure) from 0.01 to 0.2 min using a 9-pole cable (*see* **Notes 14** and **15**).
9. After analysis, store column with both ends capped at 25 °C.

3.4 TOF-MS Parameters

1. Operate the system in the positive ESI mode.
2. Acquire MS spectra from m/z 40–1200 at a rate of 1 spectrum per second, from 1 to 24 min.
3. Set the capillary voltage at 3.5 kV, the source gas temperature at 325 °C, the drying gas flow at 5 L/min, and the nebulizer pressure at 30 psig.
4. Set the fragmentor, skimmer, and octapole 1 RF voltages at 160, 65, and 250 V, respectively.
5. Generate a sample list including sample name, type, and MS method. Make sure to set the run type under worklist run parameters to external start. Start the MS sample list before starting the UPLC sample list (*see* **Note 15**).
6. Process data using Mass Hunter Qualitative Analysis and Quantitative Analysis software and generate calibration curves for each amino acid by plotting the area ratio of amino acid and the respective internal standard on the y-axis and the amino acid concentration on the x-axis, using linear regression.
7. Calculate the amino acid concentration for the amino acids for every sample using the intercept and slope of the calibration curves and the area ratio of amino acid and internal standard in the samples.

4 Notes

1. For this study we used 19 proteinogenic L-amino acids (Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr), the L-cysteine dimer L-cystine (Cys-Cys), and hydroxy-L-proline (OH-Pro). All amino acids except glycine contain a chiral carbon atom. If important for your analysis, be sure to use the enantiomer of interest (for biological purposes usually the L-enantiomer).
2. Ideally, internal standards for mass spectrometric analyses have identical physical and chemical properties except their mass. Hence, the best internal standards in this case are isotopically labeled amino acids, usually containing ^{2}H, ^{13}C, or ^{15}N (or a combination of these). When selecting isotopically labeled internal standards, make sure that the mass difference between the analyte of interest and your internal standard is at least 2 Dalton, to avoid overlapping.

3. Store the LAP as well as the LAP working solution in the dark as much as possible, as they are photosensitive.
4. Consider any hydrates and hydrochlorides which might be present in the amino acids when calculating the amount to weigh.
5. The most convenient way is to dissolve the amino acids after filling the volumetric flask to about 75%. After completely dissolving the amino acids, add until final volume.
6. During this step, the coverslip surfaces are functionalized with methacrylate groups. This enables covalent cross-linking between the coverslip and the hydrogel in the sampling process, thereby facilitating easy lifting of the hydrogel from the sample surface. Handle the functionalized coverslips with extra care after this step to avoid damaging of the functionalized surface.
7. In this study, we used glass slides as substrate because the fact that they are nonporous and flat makes them ideal to be used in the sample preparation. Other nonporous and relatively flat substrates may be used as well. Guidelines on how to collect fingerprints (e.g., the number of donors, whether to use groomed or natural fingerprints, the substrate, and deposition pressure) are described by the International Fingerprint Research Group [12].
8. Execute this step right before applying the hydrogel to your sample. Cross-linking will be initiated by the LAP without the laser pen as well and thus the hydrogel will slowly start to form.
9. In this step, polar components are extracted from the fingerprint. Three minutes is sufficient to achieve high extraction efficiencies for amino acids, but longer extraction times might be needed for other compounds.
10. This is a crucial step in the sample preparation. If not lifted gently, distortion in the fingerprint ridge detail might be generated. It can help to lift the coverslip with a thin and sharp object (e.g., a scalpel).
11. In our study, we chose to add the IS solution at this step, as we wanted to compare the extraction to direct solvent extraction (the same sample preparation, but the glass slide containing the fingerprint is directly transferred to a beaker with solvent for amino acid extraction; the results of this comparison can be found in Fig. 2). Hence, we did not want to IS correct for losses in the amino acid extraction from fingerprint or hydrogel. For other studies, it may be more appropriate to add the IS at an earlier stage.
12. The non-marking nature of hydrogel lifting is displayed in Fig. 3: the comparison of a split fingerprint, where one half is

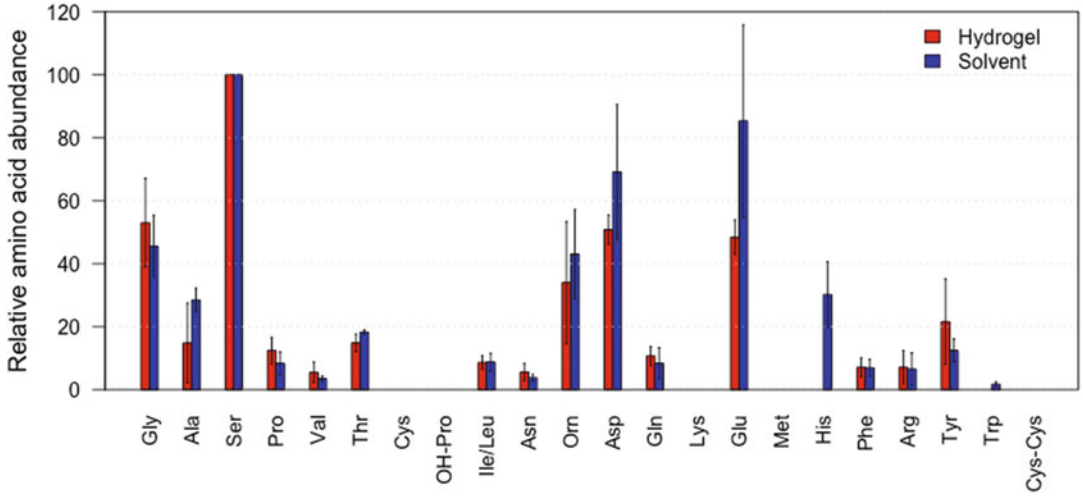

Fig. 2 Comparison of amino acid profiles (i.e., the relative abundance to serine) extracted from hydrogel (red) compared to direct solvent extraction, also *see* **Note 7**, (blue) collected from fingerprints ($N = 5$). Amino acid profiles per extraction method are calculated as an average of the amino acid profiles from the different donors

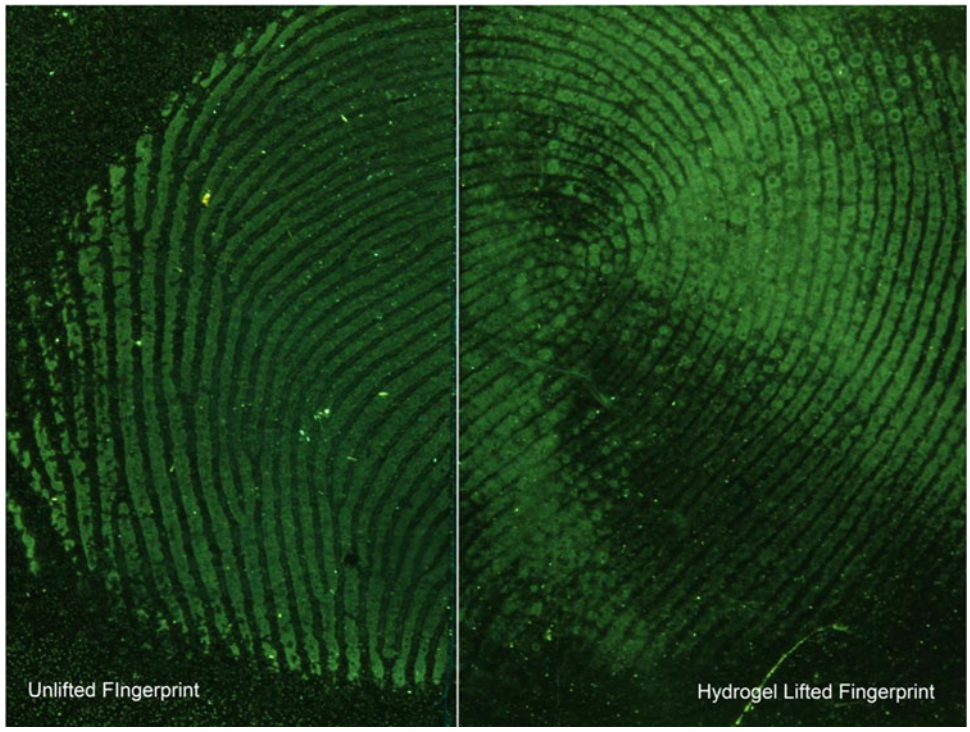

Fig. 3 Comparison of ridge detail from a split fingerprint which was partially extracted using a hydrogel. Left: Treated with cyanoacrylate and basic yellow. Right: Hydrogel extracted and subsequently treated using cyanoacrylate and basic-yellow dyeing

treated with the hydrogel prior to common cyanoacrylate fuming and basic-yellow dyeing, while the other half is visualized using the same techniques without hydrogel lifting.

13. Prepare fresh UPLC solvents at least weekly.
14. Run blanks (MeOH containing 5% (v/v) formic acid) between the calibration series and samples to minimize carryover.
15. Using the UPLC-TOF-MS setup, it is necessary to generate two sample lists, one using the Waters software to specify the sample position, injection volume, and UPLC method. In the UPLC method, program the contact closure at the start of the UPLC run (using a 9-pole cable as contact closure cable). Generate a worklist in the Mass Hunter Acquisition Software, specifying sample name, sample type, and MS method. Set under the worklist run parameters, and run type to external start. Start the MS worklist, wait for the instrument to reach pre-run state, and then start the UPLC sample list. The MS will automatically start recording spectra after the UPLC has injected a sample.

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