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Determination of efficacy of fingermark enhancement reagents; the use of propyl chloroformate for the derivatization of fingerprint amino acids extracted from paper☆

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ABSTRACT

The analysis of the constituents of fingerprints has been described numerous times, mainly with the purpose of determining the aging effect on fingerprints or showing the differences between donors or groups of donors. In this paper we describe the use of derivatized amino acids to determine the efficacy of the visualization reagents 1,8-diazafluoren-9-one (DFO) and ninhydrin. At present certain conditions are used for the application of these reagents, as determined by trial-and-error investigations, to the effect on fingerprints. The recovery of amino acids from a porous surface can be used as a measure for the efficacy of a visualization agent.
In this paper we describe a method for the determination of the amount of amino acid left after reaction with well known fingerprint visualization reagents. This will allow a more scientific approach to method development for fingermark enhancement techniques. Furthermore, investigations on the influence of the concentration of fingermark amino acids, the order of application of and exposure time to reagents and the influence of age of the amino acids were carried out. These studies have resulted in a broader understanding of the mechanism involved in visualization of fingermarks using DFO and ninhydrin.

1. Introduction

The use of amino acids in deposited sweat for the enhancement of fingermarks is well known and described [1]. The identification of amino acids as an excretion product of the sweat glands appearing in the hands was described by Oden and von Hofsten in 1954 [2] and Hamilton in 1965 [3] and many publications latter, comprehensively published by Ramotowski [4].

Amino acids are typically found in fingerprints in a concentration range between 0.3 and 2.6 mg/l, making the analysis of these materials rather difficult.

A common approach to reagent development comprises the modification of the formulations [5,6] used or the chemical altering of reagents [7–9] and subsequently testing these analogs on fingerprints. This mode of operation has been successful, since it has delivered compounds that can readily be applied to the visualization of fingermarks on porous surfaces. However, it does act on a basis of serendipity, since the underlying processes are not well understood. An increased knowledge of these processes and in particular the interaction between amino acids and DFO, ninhydrin and 1,2-indanedione will provide a more rational ground for method development.

The reaction mechanisms of these three reagents have been investigated frequently and are well known [4,5]. However, in previous research the differences in the reactivity of the reagents towards fingerprints were observed and the origin of this phenomenon is not yet explained.

We provide evidence that the reactivity of the reagents can be investigated by analyzing a standard solution of amino acids before and after treatment with DFO, ninhydrin or 1,2-indanedione. More specifically, knowledge of the influence of certain moieties of amino acids on the effectiveness of the reagents could be applied to the development of novel reagents on a rational basis. The analysis of a selection of amino acids has been described and problems with the isolation of arginine have been proved to be difficult in this forensic context [10].

In order to make the right choice for a reagent, it is important to understand what the efficacy of the applied method is. For this, it is crucial to understand the mode of action of the reagent and the interaction with the constituents of a fingerprint or fingermark. Until now the choice is based on numerous comparisons between fingerprints developed with the different reagents available [10].

It is unknown to what extent the nature of the composition of the fingerprint affects the performance of the enhancement reagent:

(1) what are the differences in reactivity between the amino acids,
(2) does the concentration of amino acids have an effect or
(3) to what extent does the age of a fingermark or -print influences the reaction.

We envisioned that if we would be able to reproduce the method described by Croxton et al. for the GC–MS analysis of amino acids [11]

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we would be able to measure the amount of amino acid left after treatment of a deposit, with the reagents DFO, ninhydrin and 1,2-indandion. In this study we have chosen to limit the scope to DFO and ninhydrin.

Standard solutions of amino acids will be deposited on (filter) paper to mimic the deposition of sweat from a finger on a porous substrate.

The consumption of amino acids by reaction with DFO [12] or ninhydrin [13] will decrease the amount available for derivatization. As such the amount of the amino acid PCF-derivative determined by GC–MS is a measure for the performance of the reaction.

With the approach described above the following questions can be answered:

- Is there a concentration dependency in the reaction between amino acids and DFO and ninhydrin?
- What is the effect of the order of application of DFO and ninhydrin?
- Is there an effect of the age of the amino acid stain? and
- What is the effect of the exposure time of an amino acid to DFO and ninhydrin?

Treatment of porous materials with current reagents is insufficient in many cases. Fingermarks may develop only partially or only with particular reagents, depending on the composition of the deposition left by a donor.

For example, it is known from casework that fingermarks that are invisible after DFO treatment can be visualized by ninhydrin [14]. This observation indicates that ninhydrin either reacts with a different type of amino acids or a different amount of amino acids, which is a factor that has not been investigated yet.

As the structural differences between DFO and ninhydrin are rather large in comparison to the differences between ninhydrin and 1,2-indanedione, we envisioned that the electronic effects (polarity and/or polarizability) of these molecules might affect the reactivity towards amino acids.

The successful visualization of a fingermark is strongly dependent on its composition and the environmental conditions the mark has been exposed to before treatment. Marks may have been left by children [15], or have been exposed to light or heat [16,17], which appears to make a difference in the development. Recently Dorman and co-workers [18] have described the differences in fatty acid composition of finger depositions illustrating the problem.

These conditions are not of trivial influence on the amount of the constituents present in fingermarks [1]. It is predicted that a more thorough understanding of the fundaments of the mechanism involved in the visualization of fingermarks using these reagents will give us an insight into the problems involved in the visualization of fingermarks after unusual circumstances [15,16] or different donors [15,19].

In the protocols found in the literature it is advised to treat samples with ninhydrin, DFO or 1,2-indandion reagent for only a very short period of time, by what is called the dipping method [6]. From an organic chemistry perspective, the reaction time is a crucial parameter for the success of a reaction, which makes the choice for the so-called dipping method rather strange. Even though the reaction carries on after the exposure of the exhibit to the solution in the oven, a prolonged exposure time to the solution might have a positive influence on the recovery of the amino acids.

It has been shown [10] that leaving materials for a prolonged period of time at ambient temperature and pressure, after treatment, the amount of marks for individualization purposed is increased. The yield in useable marks[1] is increased in a shorter time span at elevated temperatures with a higher relative humidity. These effects are irrespective of the length of exposure to the reagents.

As items under investigation might be under scrutiny for other forensic disciplines, the exposure to chemicals will disturb the traces possibly observed here. An example thereof is that inks will run when papers with handwritten text are treated in this fashion.

Secondly, in some judicial systems it is not allowed to perform destructive methods on exhibits and the application of the dipping method is less destructive in that sense.

Various studies on amino acid analyses by GC–MS have been carried out and therefore, using the knowledge available in the literature, the approach described in this paper was thought to be feasible for the goals we have set. The main issue in chromatographic analysis of amino acids appears to be their lack of separation due to their boiling point and polarity [20]. Derivatization of the amino acids could provide the separation that is necessary.

Ethyl chloroformate (ECF) has been used to protect amines by transforming them to a carbamate functionality [21], thereby changing the polarity of the reaction product and improving the separation with GC–MS. As described previously [20], the derivatization of amino acids with alkyl chloroformates, in the presence of an alcohol and pyridine as a catalyst can essentially produce several products and mixtures thereof. Additionally, ECF has been used to derivatize human bodily fluids [22] and was applied to the derivatization of amino acids associated with the deposition of fingermark residue by Croxton and co-workers [11,23].

As described by Croxton et al. most amino acids produced a linear calibration curve by GC–MS analysis of ECF derivatized samples. Lysine, ornithine and tyrosine were detected, but did not produce acceptable calibration curves. Arginine, histidine, and cystine could not be detected at all after ECF derivatization [11].

Van den Akker and co-workers [24] described the use of propyl chloroformate (PCF) for the derivatization of amino acids, which play a role in the metabolism of unborn children. Phenomenex® also applies PCF derivatization in their EZ:Faast™ solution for amino acid analysis [25]. Additionally, the EZ:Faast™ kit was applied to the photo- and thermal degradation studies on fingerprint constituents executed by De Paoli et al. [16]. Most amino acids can be analyzed by GC–MS after PCF derivatization, except for arginine [20]. Therefore, PCF was applied to the analysis of amino acids in these studies. Obviously, amino acids containing a functional side group reactive towards PCF will be derivatized as well, leaving tri-substituted end product. The final product will only be formed if the reaction conditions are optimal, meaning the reagents and catalyst are present in excess. If the reaction conditions are suboptimal, the reaction will not complete and various mixtures of the intermediate products may be found. Therefore, the presence of an excess of the chloroformate, alcohol and pyridine is of crucial importance.

2. Experimental details

To test the hypothesis that the efficacy of fingerprint reagents can be measured with GC–MS, a representative mix of amino acids will be used in several experiments. The following amino acids belong to the class of non-polar amino acids: glycine, alanine, valine, phenylalanine, proline and leucine. These examples include aromatic entities and aliphatic derivatives.

Furthermore, the acidity of the amino acids might play a considerable role in the affinity for the reagents used in these experiments. Therefore amino acid examples with both acidic and basic side chains will be examined in the experiments.

Glutamic and aspartic acid make up the group of acidic amino acids used in these experiments and lysine is the representative of the class of basic amino acids.

Calibration curves were produced within a concentration range that represents the amount of amino acids in fingerprints. The effect of the PCF derivatization on the recovery of the amino acids was not determined, as procedure is the same for all amino acids in the determination of the recovery, we assume that for the comparisons the effect will even out.

[1] The terminology used here, useable marks, can be explained in several manners. With useable marks we refer to marks with sufficient quantitative information for individualization purposes.