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Identification of firearms handling by the $[Fe(PDT)_3]^{2+}$ complex: Chemical and time-dependent factors

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Abstract

Handling of a gun results in the formation of invisible impressions, caused by transfer of iron traces to the skin surface. Visualization of these impressions is possible by spraying the palms with a solution of 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine (PDT), which forms a magenta complex with iron(II) residues. Hence, mark intensity is directly related to the amounts of iron transferred to the palm. Palmar sweat plays a major role in iron transfer from the firearm to the hand. More factors, however, are involved in this process. Three time-dependent factors have been studied with relation to their effect on the developed mark: the gripping duration of the weapon; the time elapsed from the contact; and the rate of iron dissolution in aqueous solutions containing sweat components in physiological concentrations.

We found that the amounts of iron transferred to the palm depend on both, the gripping period and the levels of palmar moisture. Thus, only a few seconds of gripping were required for developing good marks (corresponding to 80 ng cm^{-2} of iron) on highly-moistured hands, much longer gripping periods were necessary for developing marks of similar intensity on relatively dry hands. Experiments that aimed at studying the effect of sweat components on metallic iron dissolution were carried out in aqueous solutions. It was found that chloride ions in physiological concentrations remarkably enhanced the dissolution, while L-serine, the major amino acid in palmar sweat, had a detrimental effect on this process. Urea, another sweat component, had only a minor effect on the dissolution rate.

Keywords: Forensic science; 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine (PDT); Iron determination; Palmar sweat; Dissolution rate; Serine; Urea; Firearms handling

1. Introduction

Latent fingerprints and the invisible impressions of firearms, which are left on the holders' palms, are both distinct cases of Locard's Exchange Principle: "*Every contact leaves traces*" [1]. Both are typical examples of circumstantial evidence that can prove a previous contact between an individual person and an object. The physiological and environmental factors, which are involved in latent fingerprint retrieval, have been studied extensively, for nearly 100 years [2]. Forensic scientists, however, have paid much less attention to the detection of firearms impressions on the hands, and only recently it has attracted considerable attention [3]. Most handguns leave invisible impressions containing di and trivalent iron on the hand. These impressions can be visualized by spraying with the chemical reagent PDT [4,5]. Even brand new weapons leave such marks. From recent experience it is evident that clear, well-defined impressions, which are developed on the hands by the PDT reagent (Fig. 1), can unequivocally prove recent contact with specific firearms [6], and even faint, non-specific marks can at least allude to such contact. The decrease in quality of iron-PDT impressions over time since contact has previously been reported [7,8], but only on a qualitative basis. A qualitative relationship between the length of gripping and the mark intensity has also been reported: the longer the gripping period, the more intense the mark [7,8].

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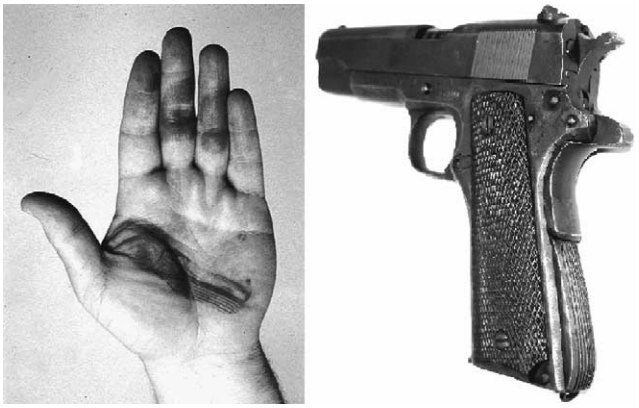

Fig. 1. A typical PDT impression of a Colt 45′ handgun (a) on the palm of a recent holder (b).

The PDT method has been successfully applied in a number of police investigations [6]. This success has resulted in the need for accreditation of the technique in courts of law. Furthermore, the extensive application of the PDT method raised questions and issues regarding the scope and limitations of this technique, such as the effect of time between the contact and the chemical development on mark quality. In spite of the fact that the PDT method has been in use for several years, a quantitative study of the scope and limitations has never been conducted. Such investigation would support both the forensic argumentation in court as well as the work of crime scene officers.

In our recent study [3], we have shown that palmar sweat is essential for successful application of the PDT technique. It was concluded that iron transfer to the palm is governed by chemical dissolution rather than by physical (dislodgement) process. Moreover, we found that the intensity of the developed marks is directly related to the moisture levels of the palms.

Sweat is a complicated mixture of organic and inorganic compounds. Numerous chemical components, organic and inorganic, have been detected so far, only part of which have been identified [9]. Obviously, it would be almost impossible to study the specific influence of each of the components in sweat on iron transfer to the hand. Roughly, 99% of eccrin sweat is water while the major components of the remaining 1% are sodium chloride, amino acids and urea [9]. Therefore, we decided to study the effect of these three components on iron dissolution in aqueous solutions under controlled conditions as a model system for natural sweat. In addition, we studied the effect of the gripping period and the time elapsed since contact with iron on the intensity of the PDT marks.

2. Experimental

2.1. Materials

All reagents were of analytical reagent grade unless otherwise specified. Ultrapure water (Barnstead Easypure UV system, 18 MΩ cm) was used throughout this study. 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine (PDT) was purchased from Aldrich and dissolved (0.1%) in acetone. A 1% (w/v) aqueous solution of L-ascorbic acid was prepared daily. Commercial Ferrotrace® cans containing a similar formulation (Mistral Detection Israel P.O. Box: 4133 Herzliya Israel 46140) were used for spray experiments. Fifteen standard iron tabs, 25 ± 0.1 mm O.D., 3.5 ± 0.1 mm thick with a 6 mm hole (received from Metal Samples Company, Munford, AL), each weighing 13.4 ± 0.05 g were used in the dissolution experiments. A stock solution of iron(III) was prepared by dissolving 360 mg of anhydrous ferric chloride in 100 mL ultrapure water.

2.2. Instrumentation

A Corneometer CM 825® (CK electronic GmbH, Germany) was used for measuring the moisture levels on the upper 18–20 μm layer of the skin. A Mexameter MX® (CK electronic GmbH, Germany) was applied for determining the color intensity of the marks. A pH meter (PHM63, Radiometer A/S Copenhagen, Denmark) equipped with a planar electrode (El-Hamma Ltd., Ramat-Gan, Israel) was used for measuring the skin pH. Absorbance measurements were carried out with a HITACHI (Tokyo, Japan) U-2000, spectrophotometer. Colt 45′ handgun (1976), which leaves a very distinct mark, was used in the gripping experiments. It was disarmed prior to the experiment. The laboratory glassware was kept overnight in 5% nitric acid. Afterwards it was rinsed thoroughly with water and dried.

2.3. Methods

Three experiments were performed in the course of this study.

2.3.1. Effect of time since contact

Fifteen microliter of 22 mM ferric chloride solution were pipetted on glass slides and left until the water evaporated. Nine healthy Caucasian adult volunteers, six females and three males, wiped the slides with their fingers (each one with all 10 fingers, one slide for each finger) until the slides were visibly clean. The fingers of one hand were immediately sprayed with PDT (Ferrotrace®), held for a few seconds above a boiling kettle and dried in air. The intensity of the mark was measured with Mexameter MX 16. This hand was used as “reference”. The fingers of the other hand were sprayed with PDT, each one at a different time-interval since contact, between 1 and 6 h. The fingers were processed similarly and the color intensity measured and recorded as above. The ratio between the Mexameter readings for each finger and the same finger on the reference hand (relative intensity) was plotted against time. Room temperature (26 ± 1 °C) and relative humidity (72 ± 3%) were controlled during the experiments.

2.3.2. Effect of gripping time

Three volunteers identified as "weak", "average" and "good acceptors" (see Section 3) gripped the handgun for periods ranging from 1 to 420 s. The hands were sprayed with PDT after ca. 10 min and left for a few seconds over a boiling kettle to accelerate the mark development. The area of the mark was measured with a ruler. Then, the marks were extracted and the amount of iron was measured spectrophotometrically at 555 nm as previously described [3]. The volunteers washed their hands with hot water and soap after each extraction and repeated the whole procedure at 3 h intervals. At each time, the palm pH was measured.

2.3.3. Iron dissolution in aqueous solutions

Aqueous solutions containing sweat components in physiological concentrations [9] were prepared. The following solutions were tested: ultrapure water, sodium chloride 0.05, 0.1, 1 and 5 mg mL^{-1}; L-serine 0.1 mg mL^{-1} and urea 5 mg mL^{-1}. Mixtures containing: (a) sodium chloride 5 mg mL^{-1} and urea 2.5 mg mL^{-1} and (b) sodium chloride 5 mg mL^{-1} and L-serine 0.1 mg mL^{-1} were also prepared. Nine clean and polished iron tabs were immersed in each of the solutions (80 mL) placed in 200 mL plastic beakers. At intervals ranging from 0 to 130 min, 800 μL samples were transferred to a measurement cell, containing 100 μL of 1 mg mL^{-1} aqueous ascorbic acid. The cells were shaken and left for 5 min at room temperature and then 100 μL of a 1 mg mL^{-1} PDT solution were added and stirred for 1 min. The absorbance was measured at 555 nm against an iron-free blank, prepared in the same way. Iron quantity was calculated according to Beer's law ($\varepsilon = 24{,}400\ M^{-1}\ cm^{-1}$) and dissolution rate was evaluated.

Statistical analysis of the results was carried out using SPSS 11.5 software. Frequencies, *T*-tests, regressions, correlations, one-way analysis and post-hoc tests were generated.

3. Results and discussion

Our recent study clearly showed that the uptake of iron as a result of holding an iron-made object is primarily a chemical process, in which the palmar sweat assists in dissolving the iron from the tool and transferring it to the palm. Moreover, a clear correlation between the amounts of iron on the hand and the moisture levels on the palm was found [3]. In general, it was possible to divide the population into three categories based on their iron uptake. Similarly to "*fingerprint donor*", the person who leaves the latent finger-marks, we wish to define "*iron acceptor*", or simply "*acceptor*", which is the person who absorbs iron traces upon gripping an iron-made object. Accordingly, the three categories mentioned above can be termed "good, average, and week acceptors"; being determined by the intensity of the PDT mark, which in turn, depends on the amount of perspiration on their hands [3].

Evidently, the development of a PDT-iron mark is likely to depend on the time that elapsed between the contact of the iron object and the chelation reaction. Such observations have been reported in the forensic literature, however, only on a qualitative basis [7,8]. The general trend was a decrease in the intensity of the developed marks over time since contact. Based on our previous findings, the rate of decrease may vary from person to person according to his iron accepting nature. Fig. 2 shows the dependence of the mark intensity (%) as a function of time since contact with $FeCl_3$.

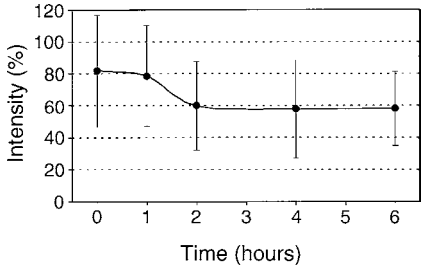

Fig. 2. Average stain intensity ($n = 9$) measured by the Mexameter MX 16 (S.D. = 39–45%). Initial intensity is defined as 80% since this was found to be the efficiency of iron extraction from the palm [3].

It can be seen that there is a decrease in mark intensity over the first 2 h since contact with iron. This phenomenon could be explained by iron permeation through the skin or, perhaps, by iron chelation with skin proteins [10,11]. The color intensities of the stained areas measured by the Mexameter MX 16® in the three groups were as follows: palms with weak stains, 32 ± 7; palms with average stains, 62 ± 9; and palms with good stains, 110 ± 9. The difference between the lowest and highest reading on the same person (intra-variability) never exceeded 5%. Therefore, the Mexameter can serve as an analytical tool for objective assessment of the intensity of iron-PDT marks on hands.

The following mean pH values in the three groups were obtained: palms with weak stains, 4.9 ± 0.3; palms with average stains, 5.1 ± 0.4; and palms with good stains, 5.4 ± 0.6.

A qualitative relationship between the length of gripping of the weapon and the intensity of the colored marks has also been reported. Basically, the intensity of the developed mark increased with the gripping period. Fig. 3 shows the intensity of the mark as a function of the gripping period for

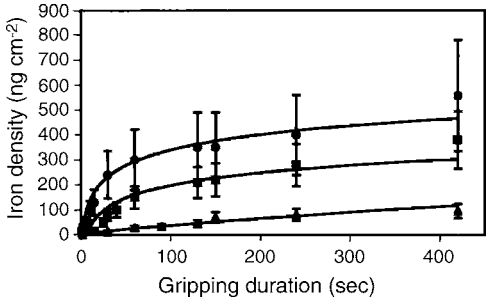

Fig. 3. The effect of pistol gripping duration on the amount of iron transferred to the palm. The stain had been extracted after 60 s: (▲) weak, *very dry* skin (14–33); (■) average, *dry* skin (35–44); (●) good, *sufficiently moistured* skin (52–91).

the same three acceptors. It is evident that persons with very moist hands, i.e., "good" acceptors, produce visible marks already after contact of a few seconds. Much longer gripping periods—5 min or more—are required for "weak" acceptors to produce marks of similar quality, and some may not reach the same intensity even after much longer gripping times. The shape of the curves is also of interest: while on moist palms, the iron levels rise asymptotically with gripping time, and may reach a maximum value after a few minutes, the iron levels on dry palms keep rising almost linearly, for much longer gripping periods. Since the perspiration process continues while holding the gun, it is conceivable that the amount of iron that is transferred reaches a maximum surface concentration, which does not increase with further perspiration. This might suggest that the iron that is transferred to the hand eventually blocks the contact between the sweat and the weapon's surface. Such surface concentration may never be reached on dry palms and hence, the dissolution curve is almost linear with time.

The slope of each curve was calculated by linear regression of the absorbance values between 0 and 30 s, and results in regression coefficient, $R^2 \geq 0.92$. The initial rates of iron transfer are: 0.33, 0.94 and 8.22 ng cm^{-2} s^{-1} for the "weak", "average" and "good" acceptors, respectively. The iron densities measured after 60 and 420 s of gripping for the three acceptors were: 28, 150 and 300, and 96, 380 and 558 ng cm^{-2}, respectively.

Therefore, in respect to iron transfer, handling duration can be regarded as a secondary factor. The sweat level, which is considered a primary factor, can explain the initial rate of iron transfer. The more moistened is the skin the higher is the rate of transfer. After 100–420 s of contact, the rate of transfer decreases to approximately the same level of 0.3 ng cm^{-2} s^{-1} regardless of the acceptor's group.

Virtually, there are two types of factors that govern the quality of the PDT marks: environmental factors, such as time and weapon conditions, and physiological factors that depend on the individual physiology of the holder, e.g., amount and composition of the perspiration. Therefore, besides studying the effect of time of gripping and the time between contact and development, on the quality of the PDT mark, we have also decided to deepen our understanding on the effect of the major sweat substances on the developed mark. The effect of sweat on iron corrosion has previously been studied semi-quantitatively, and from a different perspective. The principal findings showed that the corrosion rate of steel was proportional to the rate of perspiration [12,13].

Since sweat is a complex matrix composed of tens of substances, which differ in their concentration from person to person, we carried out a series of experiments in which iron was exposed to aqueous solutions with known concentrations of three major sweat components, chloride, serine and urea. Following this approach it allowed to isolate the net effect of each of these compounds on the rate of iron dissolution. Chloride ions are the major inorganic component in sweat,

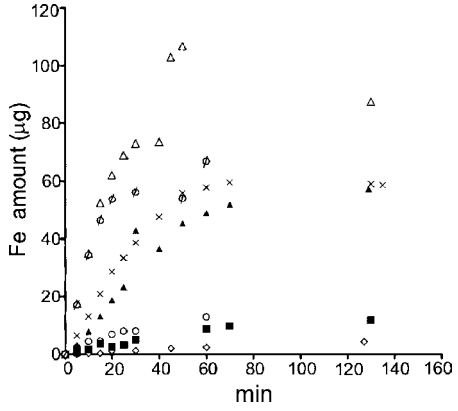

Fig. 4. The effects of sweat components on iron dissolution rate: (◊) DDW; (■) urea, 5 mg mL^{-1}; (◯) L-serine, 0.1 mg mL^{-1}; (▲) NaCl, 0.05 mg mL^{-1}; (×) NaCl, 0.1 mg mL^{-1}; (ø) NaCl, 1 mg mL^{-1}; (△) NaCl, 5 mg mL^{-1}.

while serine is the principal amino acid in palmar sweat [9]. It was thus assumed that among the plethora of sweat components, we ought to begin the study with these two. Urea was added to this trial as another organic component of eccrine sweat. Fig. 4 shows the influence of the three sweat components on the dissolution of iron tabs in aqueous solutions. Duplicates of iron tabs were used and the standard deviation of the absorption measurements was less than 12%. The effect of chloride ions concentration on iron dissolution was also examined.

In our previous work, we reported that the level of palmar moisture was the principal factor responsible for iron transfer to the hand [3]. As can be seen in Fig. 4, chloride ions too have a significant effect on iron dissolution. In solutions containing between 0 and 5 mg mL^{-1} of chloride ions, more iron is dissolved with increasing chloride concentrations. Chloride ions are known to accelerate iron corrosion in aqueous solutions. Iron surfaces are regularly coated with a passive film of oxide, which separates the metal from its environment and which slows down the rate of corrosion. Chloride ions penetrate the oxide film through pores or defects and increase its permeability. Once in contact with the metal surface, chloride ions favor hydration of metal ions and facilitate metal ions entrance into solution [14]. The asymptotic shape of the dissolution curves could possibly be explained by the build up of another protective layer, which prevents further dissolution of the iron [15]. The dissolution is therefore faster at the beginning and after some time it reaches a "steady state". It should be noted that since ascorbic acid was added prior to PDT, the iron that was measured corresponds to the total iron, Fe(II) and Fe(III), that was dissolved and available for complexation. In our experiment, the iron concentrations leveled off after about 60 min. The "gripping time" dependence (Fig. 3), agrees well with this observation, and a similar explanation may prevail for both, the solution model and the handgun experiment. The initial rate of dissolution, which is the slope of the curves, varies between 0.03 and 3.2 μg min^{-1}. Each curve's slope was obtained by linear regression of the

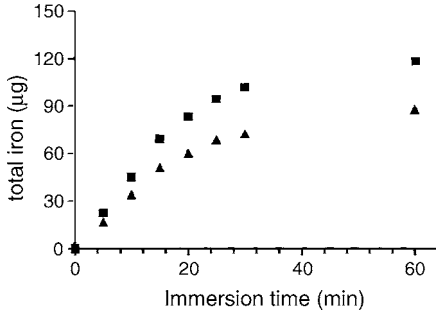

Fig. 5. The concentrations of iron measured in an aqueous solution containing 5 mg mL^{-1} sodium chloride alone (■), and with 0.1 mg mL^{-1} L-serine (▲).

absorbance values between 0 and 30 min, which results with regression coefficient, $R^2 \geq 0.92$.

Of particular interest was the finding on the effect of the amino acid serine, since amino acids are the main organic constituent of palmar sweat. In fingerprint research, serine often represents the entire amino acid content in the perspiration [9,16]. As a single solute, serine slightly increased iron dissolution, in comparison with pure water (Fig. 4), but in presence of chloride ions it significantly decreased the dissolution of iron as compared with chloride only (Fig. 5). The inhibition can be attributed to the adsorption of amino acids molecules on the passivation film. The adsorption process could involve hydrogen bonding between the $-NH_2$ group of one molecule, and the –COOH group of an adjacent one, and this leads to a compact film which inhibits pitting attack by chloride ions that reduces their corrosive activity. It is noteworthy that certain amino acids have been previously reported to inhibit metal corrosion in the canned food industry. No attempt was done to explain this observation [17]. On the other hand, the addition of urea had only a negligible effect on the rate of iron dissolution in the presence of 5 mg mL^{-1} of NaCl (3.7 μg min^{-1}, $R^2 = 0.925$) as shown in Fig. 6.

From experiments in aqueous solutions as a model for palmar sweat it appears that some sweat components, such as chloride ions, accelerate the dissolution of iron, while others, amino acids, for instance, inhibit this process. It was found that L-serine has an inhibitive effect on the dissolution of iron namely; it decreased the rate of dissolution from 3.6 μg min^{-1} ($R^2 = 0.953$) to 2.7 μg min^{-1} ($R^2 = 0.937$). Urea has no significant effect on iron dissolution. The intensity of the iron-PDT impressions thus depends on the sum of all sweat components, the amount of water being the major factor.

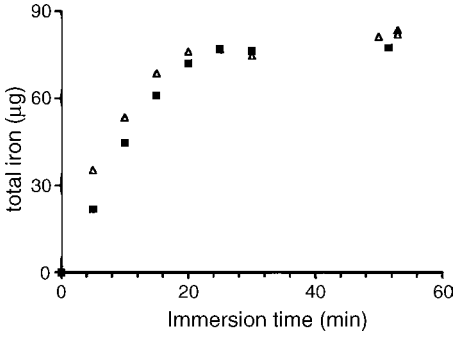

Fig. 6. The concentrations of iron measured in an aqueous solution containing 5 mg mL^{-1} sodium chloride alone (■), and with 2.5 mg mL^{-1} urea (△).

It is noteworthy that our results agree very closely with the observations of Jensen et al., who have investigated a similar problem, but from the opposite angle. They studied the corrosive effect of palmar sweat on steel, measuring not the iron amounts on the palms, but rather the degree of corrosion on the steel. They reported that corrosion increases with increasing sweat rates, and that excessive sweat is the prime cause of corrosion [12,13].

Although the experimental model developed here involves many approximations, it can be applied for a parametric study, such as the influence of a single sweat constituent on the PDT impression.

4. Conclusions

Iron availability for chelation is time dependent, with a marked decrease over the first 2 h since contact. It is, therefore, recommended to perform the PDT test as close to the weapon's handling as possible.

On certain people's hands ("good acceptors") clear PDT marks can be obtained after only a few seconds of contact, while on others ("weak acceptors") marks' intensity may not reach the same quality even after much longer gripping periods.

The "solution model" can represent the dynamics of iron transfer to the palms of "good acceptors" during weapon gripping.

Chloride-rich sweat may bring about stronger PDT impressions, whereas high levels of serine have an opposite effect on marks' quality.

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