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Identification of Firearms Holders by the [Fe(PDT)$_3$]$^{+2}$ complex. Quantitative Determination of Iron Transfer to the Hand and Its Dependence on Palmar Moisture Levels

ABSTRACT: Quantitative data are reported for the first time on the amounts of iron, which are transferred to the hand upon holding a firearm. Iron levels between 21–315 ng/cm^2 were found on volunteers' palms after a single holding of a handgun. Determination of the iron traces was accomplished spectrophotometrically using 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine (PDT) as a complexing agent. The transfer of iron from firearms to the palm was found to be, by and large a chemical (dissolution) rather than mechanical dislodgement. The prime factor that determines the amount of iron transferred from the firearm to the hand is the moisture level on the palm. These findings are likely to be of importance in courts of law, as well as in the war against terrorism and serious crime.

KEYWORDS: forensic science, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine (PDT), iron determination, palmar sweat, firearms

In view of the increasing need to identify firearm holders, particularly suspects involved in terrorist acts, it is necessary to broaden our understanding of the processes involved in the chemical detection of firearms contact impressions on the hands. These magenta-colored marks are formed by the reaction of iron traces that are left on the hand by contact with the metallic part of firearms and the chemical reagent 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine (PDT). Thornton and co-workers were the first to suggest the use of PDT for visualizing firearms impressions (1,2).

The technique is based on the formation of a colored complex with the stoichiometric ratio of 1:3 (Fe^{+2}:PDT) as shown in Fig. 1. In the late 1970s, a working formulation was developed that comprises spraying a PDT solution, which contains ascorbic acid, onto the suspect's hand and observing the instantaneous development of a magenta stain (3).

The ascorbic acid is necessary to reduce Fe(III) to Fe(II), which selectively reacts with PDT and produces the color as a result of forming a charge transfer complex (4). Consequently, spray cans containing PDT and ascorbic acid have become operational tools for the crime scene technicians in the Israeli Police and in other law enforcement agencies. A number of cases that have been solved using this technique have been reported recently (5).

Several parameters that are involved in the visualization of firearm holding as well as sensitivity comparisons with alternative reagents have been reported (4,6). Studies aimed at sensitivity enhancement of this technique by applying PDT analogues for detection of iron in solution also have been reported (7–9). The influence of factors, such as the time lapse since the contact on the quality of marks, the transferability of the latent mark to adhesive tapes, and the effect of washing the hands, also have been studied (10). It was reported in a following study that a considerable percentage of the population, male and female, respond positively to PDT after holding a handgun (11). Nevertheless, deeper understanding of the chemical and physical processes involved in the application of PDT to human skin (mostly hands) is still desirable for two important reasons:

- Court argumentation: it is still unclear why some persons develop clear marks after holding an iron-made object and being sprayed with PDT, while others develop very faint marks or do not develop visible marks at all. In other words, what are the individual factors that govern the development of a mark?
- Performance improvement: understanding the processes involved in the uptake of iron and its detection by PDT will allow further improvement of the protocol currently used.

So far, the intensity of the [Fe(PDT)$_3$]$^{+2}$ impressions on the palms has been assessed only qualitatively and compared solely by visual means (11). There have been no data concerning the amounts of iron transferred to the hand and the threshold levels that can be detected by the PDT technique. We wish to report here the first quantitative determination of the iron amounts that are transferred to the hand by a single holding of a firearm and its relation to palmar moisture (sweat).

The study comprised three stages: extraction and quantitative determination of the magenta complex from the palms (expressed in terms of iron density on the skin); palmar moisture measurements and correlation with iron density levels; and an initial attempt to clarify the relative importance of the two processes which are likely to control iron transfer to the hand: mechanical dislodgment versus chemical dissolution.

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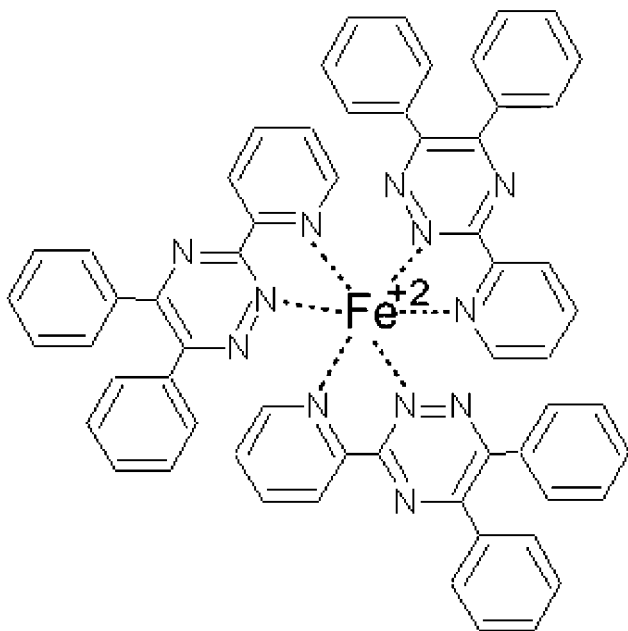
FIG. 1—*Schematic representation of the complex [Fe(PDT)₃]⁺².*

Materials and Methods

Apparatus

A Corneometer CM 825® was used for measuring the moisture of the palm. The measurement is related to the capacitance (see below under Results and Discussion). Measurements are in arbitrary units between 0–130. Absorbance measurements were carried out with a HITACHI (Tokyo, Japan) U-2000 model spectrophotometer.

Reagents

All reagents (Aldrich) were of analytical grade unless otherwise specified. $FeSO_4.7H_2O$ was purchased from Merck. PDT solution from Ferrotrace™ spray cans was applied according to the manufacturer's instructions (Mistral Detection Ltd., Herzlia, Israel). Highly pure water (Milli-Q, 18 MΩ·cm) was used in all experiments. The laboratory glassware was kept overnight in 5% nitric acid, after which it was rinsed thoroughly with water and dried.

Procedures

A calibration curve for iron(II) was constructed by measuring 10 analytical standards ranging from 0.1–80 μmole/L. The limit of detection (LOD) was determined as the signal corresponding to 3 times the noise and the limit of quantification (LOQ) as 5 times the LOD (12). The iron concentration in each sample was calculated from the absorbance of the solution after adding PDT using absorptivity of 24 400 $M^{-1} \cdot cm^{-1}$ at 555 nm (8,13).

Initially, it was necessary to develop a reliable and reproducible procedure for extracting the iron-PDT complex from the palms. This included the selection of an extraction solvent and swabbing materials.

The efficiency of the iron-PDT complex extraction from the palm was evaluated in the following way. The palm surface was pre-cleaned prior to each test. A 50 μL $FeSO_4 \cdot 7H_2O$ solution containing 10 ppm Fe(II) was pipetted onto the palm. After evaporation in air, the hand was sprayed with PDT solution, which resulted in the formation of a clear magenta stain. The stained areas were washed

with the following solvents or their mixtures: water, ethanol, isopropanol, and acetone, for testing the dissolution of the complex.

Three swabbing materials (cotton cloth, cotton-wool, and Watmann filter paper) were examined for their complex extraction-efficiency. Each swab was wetted by 50 μL of $FeSO_4 \cdot 7H_2O$ solution and sprayed with PDT. The solvent showing the best dissolution properties (see above) was used (10 mL) for extracting the magenta color, which was determined spectrophotometrically. For each of the swabbing materials, 15 measurements were performed, and the recovery was determined.

The most efficient solvent and swabbing material determined by the above method were used in the following experiments:

- One volunteer, donning latex gloves, grabbed the pistol butt, which was wrapped by a dry paper towel, for 1 min. The same experiment was repeated with a wet paper towel. Each experiment was carried out ten times. All paper towels, dry and wet, were sprayed with PDT.
- Twenty-two healthy Caucasian adult volunteers (12 males, 10 females) without dermal diseases washed their hands with tap water and gently wiped the excess water with paper towels. The moisture levels on their palms were measured using the Corneometer CM 825®. The mean values and standard deviation (S.D) were calculated based on ten readings of each palm. After each person, the probe was cleaned and recalibrated on dry paper. During the experiment, room temperature was $18.8 \pm 1.2°C$, and the relative humidity (RH) was $66 \pm 3\%$.

Then, each volunteer gripped the handgun's butt for one min. Ten min later, the hand was sprayed with PDT from a Ferrotrace™ can. The hands bearing the colored stains were held for 10 s over a beaker with boiling water to accelerate the complex formation. Mark intensities were assessed by three experienced crime-scene experts and graded as *weak*, *average*, or *good* (11). The area of the stain, which corresponded to the metallic parts of the handgun, was measured by a ruler (Fig. 2).
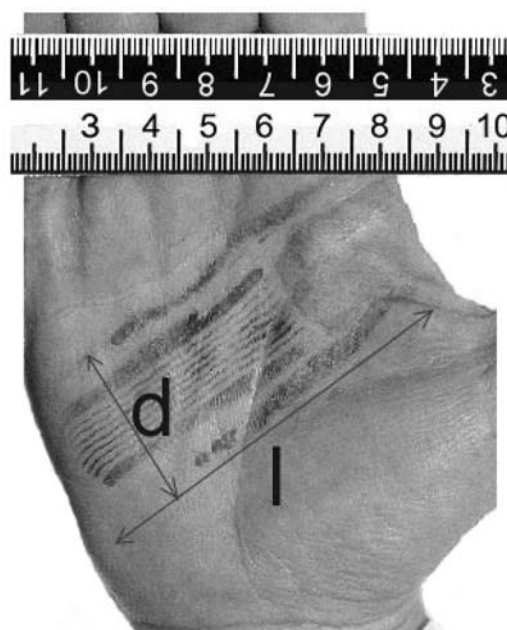

FIG. 2—*A Colt 45″ stain of 6.5 cm length (l) and 2.5 cm width (d).*

The stains were extracted by gently rubbing with cotton-wool swabs soaked in the extraction mixture, until color was no longer observed on the palms. The swabs were squeezed into a glass vessel and filtered through a filter paper into a small volumetric tube. The iron concentration was measured spectrophotometrically. Iron densities in the stained areas (ng/cm^2) were calculated by dividing the total iron by the area of the stained rectangle (l * d in Fig. 2).

Results and Discussion

In spite of the fact that the PDT technique is already used as a routine procedure for identifying persons who held iron-made tools, the fundamental parameters that affect the development of a stain on the palm have never been studied fully. To correlate between such parameters and the amount of iron that is transferred, it was first necessary to develop a reliable and reproducible protocol for measuring the amount of iron transferred to the palm. A solvent mixture of ethanol:water 1:1 (v/v) gave the best results for the collection of the magenta complex from the palms. This mixture was therefore used as an extraction solvent for sample dilution and for the absorption measurements. The highest recovery of the iron-PDT complex from the different swabbing materials ($86 \pm 7\%$) was obtained using cotton-wool swabs.

Therefore, we established a procedure in which the magenta complex is transferred upon dissolving in a 1:1 (v/v) ethanol:water mixture. The stains were repeatedly extracted using a cotton wool swab until the stain was no longer observable. We found that the recovery of the iron from the palm following this procedure was $70 \pm 7\%$.

The calibration curve for Fe(II) is linear in the range of 0.9–36 μmole/L. The graph passes through the origin with an apparent absorptivity of 24 721 $M^{-1} \cdot cm^{-1}$ at 555 nm. The analytical sensitivity, detection limit (14), and other analytical characteristics are summarized in Table 1.

This allowed correlation of the intensity of the stains and the levels of iron determined spectrophotometrically. Iron densities between 21–315 ng/cm^2 with average of 116 ± 78 ng/cm^2 were found in the stained areas. On the other hand and independently, the stain quality, which was evaluated by visual inspection, was divided into three groups: *weak* (n = 5), *average* (n = 4), and *good* (n = 13). We found a clear correlation between the intensity of the stains and the levels of iron measured:

- Hands with weak stains: 55 ± 8 ng/cm^2
- Hands with average stains: 102 ± 30 ng/cm^2
- Hands with good stains: 190 ± 80 ng/cm^2

It should be noted that due to the uneven distribution of iron in the stains, these are average values, calculated for the entire area of each stain (Fig. 2).

TABLE 1—*Analytical data of the spectrophotometric determination of Fe(II) by PDT.*

Analytical Parameter	Value at 555 nm
Slope of analytical curve	$24,721 \pm 300$
Intercept	0.0001
Analytical sensitivity (S_A)	188.6 mL μg^{-1}
Inverse of the analytical sensitivity ($1/S_A$)	5.3 ng mL^{-1}
Limit of detection (3δ)	0.179 μmole/L
Limit of quantification (15δ)	0.895 μmole/L

Evidently, there may be several parameters that can affect the efficiency of iron uptake by a human hand. The amounts of iron extracted from the palms vary by a factor of 4–5 as can be seen from the above measurements. This does not necessarily mean that this is exactly the uptake factor, since it might well be that not all the iron transferred to the hand is available for chelation. Hence, the absorbance of iron into the tissues can be quite different from hand to hand. Nevertheless, as the time that elapsed between the firearm holding and spraying was relatively short (always 10 min) we assume that the variation found in the levels of iron, indeed, primarily represents differences in the uptake efficiency of iron. A study of iron extractability from the palm over time is underway.

The uptake of iron can involve two mechanisms: a physical dislodgement caused by mechanical friction, and a chemical dissolution assisted by perspiration. In our preliminary experiments, only wet towels, which contacted an iron object, produced visible magenta marks upon spraying with PDT. The dry towels did not develop any color. This observation alludes to a chemical (dissolution) mechanism in which moisture is essential for iron transfer from the firearm to the hand. These observations are in accordance with previous indications that have not been established quantitatively (1,2,4,11). Subsequently, palmar sweat measurements were made in an attempt to correlate moisture levels with levels of extracted iron.

Palmar sweat is measured with a Corneometer CM 825® (CK electronic Gmbh, Koln, Germany). This is an analytical device, which is used experimentally and clinically for measuring the moisture on the upper 18–20 μm layer of the skin, mainly the stratum corneum. It measures the electric capacitance of the sample, which depends on the dielectric constant of the medium between its tracks. Since the capacitance of the skin depends on its water content, capacitance measurement can determine the amount of moisture on the skin. The influence of ground capacitance and other sweat components on the measured capacitance is insignificant, due to the fact that the dielectric constant of water ($\varepsilon = 81$) differs greatly from that of most other substances ($\varepsilon < 7$) (15–18).

The readings are in arbitrary units and can vary between 0–130. Two participants were rejected from the statistical analysis, due to their extreme inter and intra variance in their moisture levels. The difference between the lowest and highest reading on the same person (intra-variability) did not exceed 10%. The values measured on the palms of the 22 volunteers varied between 22–118. The data can be grouped into three clusters: 27.7 ± 5.5 (n = 5), 56.1 ± 39.8 (n = 4), and 92.9 ± 26.1 (n = 13), which also correspond to the Corneometer manufacturer's scale of "*very dry*," "*dry*," and "*sufficiently moistured*" skin, respectively.

Interestingly, the grouping derived from palmar moisture levels correlated extremely well with that based on stain intensity. In other words, all individual persons categorized, for example, in the "very dry" palms group, also belonged to the "weak stain" group, and the same applies to the other groups. The correlation between the levels of palmar moisture and the transfer of iron is even more obvious upon plotting the extracted iron levels against palmar moisture levels (Fig. 3). The significance found while performing Pearson's correlation between iron densities and moisture values was 0.05 (R = 0.484). Therefore, the 22 points could be grouped into the same three clusters. The prime factor that determines the impressions quality appears to be the amount of moisture on the palm.

Consequently, this study provides a reasonable explanation of the differences between the PDT stain intensities observed as a result of holding a firearm between individual persons. Under normal physiological conditions, an irregular liquid layer (0.4–4.0 μm
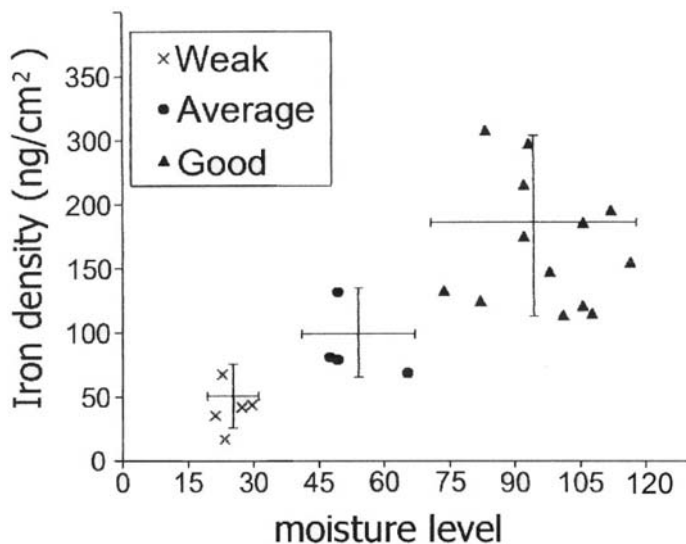
FIG. 3—*Iron densities as a function of palmar moisture levels of 22 persons.*

thick) covers the skin of the hand. This layer is composed of sweat excreted from the sweat ducts and the sebaceous glands (19). The perspiration rate depends on the activity and density of the sweat glands (20). Differences of up to 1.5 orders of magnitude prevail in the amounts of palmar sweat and sweat components between individual persons (21), which could well account for the differences in impression intensity measured in this study.

Nevertheless, there is some overlapping of iron densities between the three groups (Fig. 3). This can be explained as follows:

- Sweat constitution—not only the amounts of sweat, but also several of its chemical components, such as chloride ions and amino acids, may have an effect on the rate of iron dissolution in aqueous medium. Some ingredients accelerate the dissolution while others have an inhibitory effect on this process (22,23). The results of a study of these factors will be presented in a forthcoming paper.
- Gripping strength—of all the parameters involved in the PDT process, this is the least measurable. It is conceivable that increasing the pressure exerted by the palm would increase also the amount of iron that is transferred to the hand. Thus, a relatively "dry" hand that contains at least some moisture could produce stronger marks than a more humid hand, depending on the pressure applied.
- Errors in stain area measurements—there is a relatively large error in determining the actual area of contact (Fig. 2). In particular, the edges of the contact area are not always well defined, yet these regions probably contribute significantly to the total amount of iron that is transferred.

It is noteworthy that our results agree very closely with the observations of Jensen et al., who investigated a similar problem, but from the opposite angle (24,25). 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.

The fact that dissolution rather than mechanical process is responsible for transferring traces of iron from the weapon to the palm suggests that iron is transferred in an oxidized form, i.e.,

Fe(II) or Fe(III). This is not surprising, as iron surfaces exposed to oxygen are always coated with a native oxide layer that is composed of iron oxides, such as FeO and Fe_2O_3. Most of the iron-containing parts in arms, including Colt 45, are traditionally made of stainless steel, which is an iron alloy and contains, in addition to the iron, carbon, chromium, and other additives. The oxidation of stainless steel by oxygen and formation of an oxide layer has been studied (26). The thickness of the oxide layer, which is of the order of tens of nanometer, depends on the corrosive conditions to which the metal was exposed and on the specific composition of the stainless steel. We examined the composition of freshly polished stainless steel 316L by XPS (X-ray photoelectron spectroscopy) and found that the outermost layer was FeO. However, thermodynamic considerations would favor the more stable oxide, namely, Fe_2O_3 that is formed either at higher temperatures or after a longer time of exposure to ambience. Hence, it is very likely that a mixture of FeO and Fe_2O_3 is transferred to the palm upon dissolution by chloride containing palmar sweat.

Conclusions

This study demonstrates that the transfer of trace amounts of iron from an iron-made tool to the hand is primarily a chemical process in which iron is dissolved by palmar moisture. Furthermore, we clearly show that the stain intensity of the Fe(II)-PDT correlates well with the amounts of iron extracted from the hand. Finally and most importantly, the amounts of iron transferred to the hand depend on the palmar moisture levels.

A study of the influence of other physiological factors (pH, amino acids, and chloride concentrations) on the iron transfer is underway.

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