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## The Detection of Iron Traces on Hands by Ferrozine Sprays: A Report on the Sensitivity and Interference of the Method and Recommended Procedure in Forensic Science Investigation

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**ABSTRACT:** Spectrophotometric studies and controlled laboratory tests showed that ferrozine alone is inefficient in revealing iron traces on hands. However, when a reducing agent capable of converting Fe(III) to Fe(II) is used in conjunction with the ferrozine spray, the sensitivity of the detection is greatly enhanced. Several reducing agents including sulfite, hydroxylamine hydrochloride, thioglycolic acid, and ascorbic acid were studied, and ascorbic acid was found to be the best choice in terms of the reduction efficiency and nontoxicity. The effects of copper, zinc, nickel, aluminum, tin, lead, and chromium on the detection of iron by ferrozine with and without the reducing agents were studied, and ways to remove the interference or to interpret the results in the presence of interference are suggested. Finally, a procedure is outlined for spraying hands and gloves with a ferrozine-ascorbic acid mixture and deducing from the revealed iron traces the kinds of metal objects with which they have been in contact. A procedure is also outlined for transferring iron traces from hands onto filter paper for those persons who refuse to have their hands sprayed with chemicals like ferrozine whose toxicity is unknown.

**KEYWORDS:** forensic science, trace evidence, ballistics, ferrozine

In the investigation of a crime involving the use of a firearm or tool of theft like a spanner, the forensic scientist is often asked to demonstrate any physical link between suspect and firearm or tool. The technique frequently used involves spraying the hands of the suspect with a 0.2% solution of 8-hydroxyquinoline and observing the sprayed area under ultraviolet light [1] or with a 0.1% methanolic solution of ferrozine (that is, 3-(2-pyridyl)-5, 6-diphenyl-1, 2, 4-triazine-p, p'-disulphonic acid, disodium salt) and observing the sprayed area in daylight for magenta stains [2]. While the superiority of ferrozine over 8-hydroxyquinoline has been demonstrated [2], there are still limitations to its use. The first of these is that although it strongly complexes Fe(II) to form the magenta complex [3], it has no reaction with Fe(III). Rust is basically Fe(III) oxide and often Fe(III) is the predominating state of the iron traces transferred. Consequently, a significant level of iron traces have to be transferred before a sufficient quantity of Fe(II) is present for the color development. This limitation is especially prominent in well used or poorly cared for weapons and tools. If all the

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transferred iron traces, Fe(II) and Fe(III) alike, can be made to react with ferrozine, the sensitivity of the detection will be greatly enhanced.

Another limitation is chemical interference on the iron-ferrozine interaction. While the inhibitory effect of oxalate, cyanide, and nitrite in the color formation has been described [3], there is surprisingly very little mention in the literature on the effects of metal ions, especially Cu(II), Ni(II), Zn(II), Pb(II), Sn(II), Al(III), and Cr(III) on the reaction. These metal ions are to be expected on hands from contact with everyday objects such as coins, window frames, and water pipes.

The present study was initiated in an attempt to eliminate these limitations, first by reducing the Fe(III) in the transferred traces to Fe(II), and then to remove interference (if any) by various metal ions. The study was divided into two parts. The first part was solution chemistry and spectrophotometric measurement to establish the effectiveness of the reducing agents tested and to quantify the extent of interference from the various metal ions. The second part involved controlled laboratory tests where metal objects such as a revolver, a spanner, a hacksaw, and coins were gripped using gloved and ungloved hands. The resulting metal transfers were revealed using both ferrozine and a ferrozine-reducing agent mixture. The feasibility of transferring the acquired metal traces onto a piece of filter paper before detection was also studied to provide a potential solution to the problem of suspects who refuse to have their hands sprayed directly.

## Experimental Procedure

### Reagent

The Fe(II) and Fe(III) stock solutions (1000 ppm) were prepared by dissolving about 7.002 g of  $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$  (CE 451457) and 8.607 g of  $\text{NH}_4\text{Fe}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$  (BDH 27164) in deionized water and made up to 1 L in volumetric flasks. Their actual concentration was determined by titration as described by Vogel [4]. The Fe(II) and Fe(III) working solutions (10 ppm) were freshly prepared from their respective stock solutions by suitable dilution with 95% ethanol (BDH).

The stock solutions of the interferents (1000 ppm), namely, Cu(II), Ni(II), Zn(II), Pb(II), Sn(II), Al(III), and Cr(III) were prepared according to the procedure given in Ref 5.

The ferrozine solution (0.1% w/v) was prepared by dissolving 0.10 g of 3-(2-pyridyl)-5,6-bis(4-phenylsulphonic acid)-1,2,4-triazine, monosodium salt (Trade TCI-GR) in 100 mL of 95% ethanol (BDH). The solution was found to be stable for months if kept away from light and heat.

The sulfite and thiourea solutions were prepared by dissolving 10 g of sodium sulfite ( $\text{Na}_2\text{SO}_3$ ) (BDH, AR) and thiourea (Peking, AR), respectively, in 100 mL of deionized water. The hydroxylamine hydrochloride, thioglycollic acid (TGA), and ascorbic acid solutions were made up by dissolving, respectively, 10 g of  $\text{NH}_2\text{OH} \cdot \text{HCl}$  (CE 348307), 10 mL of thioglycollic acid (BDH), and 3 g of L(+)-ascorbic acid (E. Merck, extra pure) in 100 mL of 95% ethanol.

### Spectrophotometric Measurement

A Hitachi 200-00 UV-VIS spectrophotometer with digital display was used for the spectroscopic study with the following instrumental settings: slit, 2 nm; gain, normal; and response, medium.

The reagents under study were mixed in 25 mL of volumetric flasks and made up with 95% ethanol before spectrophotometric measurement against the reagent blanks. Where heating was required, the reagents were mixed in conical flasks and the cooled solution was quantitatively transferred to a 25-mL volumetric flask and made up with 95% ethanol before spectrophotometric measurement.

*Controlled Laboratory Tests*

The metal object of interest was held firmly in the hand, but without imparting undue force for 1 min. The acquired metal traces on the hand were either revealed *in situ* by spraying with the test reagents or transferred onto filter paper as follows: a clean filter paper was slightly moistened by spraying with deionized water. The hand was pressed on a clean moistened filter paper for about 15 s while its outline was traced with a soft pencil (6B). The paper was then dried with an air blower and could be stored until it was convenient to spray the paper with the test solutions. Ball pens and fountain pens are not recommended for tracing the hand's outline as their inks often contain iron and they may also diffuse on spraying the test solution.

As culprits often wear gloves, the spray procedure was also performed on gloves which have been used to grip the metal objects in the foregoing manner.

**Results and Discussion**

The results of the spectrophotometric studies are summarized in Tables 1 to 10.  $A_{560}$  stands for absorbance at  $\lambda_{\max}$  of 560 nm and the values in brackets are the uncertainties ( $2\sigma$ ) of triplicate determinations. The color of the solutions was magenta, unless otherwise stated.

Table 1 shows that there was no color complex formation between Fe(III) and ferrozine even with the aid of ultraviolet (UV) irradiation or heat: it must be reduced to Fe(II) for the color complex formation with ferrozine. Four reducing agents were tested and the results are given in Table 2. Thus,  $\text{Na}_2\text{SO}_3$  was ineffective in reducing Fe(III).  $\text{NH}_2\text{OH} \cdot \text{HCl}$  required heating to complete the reduction. TGA and ascorbic acid were efficient reducing agents. However, TGA has a strong unpleasant odor and can cause headaches and skin blistering [6], while  $\text{NH}_2\text{OH} \cdot \text{HCl}$  is a mutagenic agent and corrosive to skin [6]. Ascorbic acid is therefore the most suitable of the reducing agents tested, since it is odorless and nontoxic.

Another factor influencing the choice of reducing agent is whether it promotes or reduces the interference effects of other metal species on the Fe(II)-ferrozine complex formation. Tables 3 and 4 indicate that Zn(II), Sn(II), Pb(II), and Cr(III) did not interfere with complex formation, whereas Cu(II) and Ni(II) inhibited the complex formation. Al(III) reacted with ferrozine to form a magenta complex similar to that of Fe(II)-ferrozine and therefore had a positive interference effect. The interference was not serious as the molar absorptivity of Al(III)-ferrozine complex was about 350 times smaller than that of Fe(II)-ferrozine complex.

As shown by Tables 5 and 6, the use of  $\text{NH}_2\text{OH} \cdot \text{HCl}$  as the reducing agent had no effect on the aforementioned metal species' interference except that it removed the inhibitory effect of Cu(II) permitting the analysis of Fe(III) in the presence of Cu(II).

The use of TGA or ascorbic acid as the reducing agent promoted the interference of Cu(II)

TABLE 1—Reaction between Fe(III) (1 ppm) and ferrozine ( $4 \times 10^{-3}\%$ ).

Treatment	$A_{560}$
Stand 2 min	0.004 (0.004)
Stand 1 h	0.005 (0.004)
Stand 24 h	0.005 (0.006)
Stand 1 week	0.006 (0.006)
Irradiate with UV light for 1 min	0.004 (0.004)
Irradiate with UV light for 30 min	0.004 (0.004)
Irradiate with UV light for 1 h	0.003 (0.005)
Heat at about 100°C for 15 min	0.006 (0.005)
Heat at about 100°C for 30 min	0.006 (0.006)

TABLE 2—Effectiveness of various reducing agents in reducing Fe(III) (1 ppm) to Fe(II) for reaction with ferrozine ( $4 \times 10^{-3}\%$ ).<sup>a</sup>

Reducing Agent		A <sub>560</sub>				
		Stand			Heated	
Species	Concentration, %	2 min	1 h	1 week	15 min	30 min
TGA	0.4	0.402 (0.004)	0.408 (0.004)	0.411 (0.006)	0.410 (0.006)	0.409 (0.006)
	1.6	0.408 (0.005)	0.408 (0.005)	0.410 (0.006)	0.410 (0.007)	0.409 (0.010)
NH <sub>2</sub> OH · HCL	0.4	0.061 (0.008)	0.207 (0.008)	0.348 (0.008)	0.390 (0.007)	0.404 (0.008)
	1.6	0.104 (0.012)	0.220 (0.010)	0.350 (0.012)	0.408 (0.009)	0.415 (0.009)
Ascorbic acid	0.12	0.408 (0.004)	0.408 (0.004)	0.410 (0.004)	0.410 (0.007)	0.414 (0.007)
	0.6	0.408 (0.004)	0.408 (0.005)	0.410 (0.006)	0.406 (0.006)	0.405 (0.008)
Na <sub>2</sub> SO <sub>3</sub>	2.5	0.069 (0.008)	0.076 (0.008)	0.080 (0.012)	0.089 (0.016)	0.090 (0.016)

<sup>a</sup>Remarks: A<sub>560</sub> for the reaction between Fe(II) (1 ppm) and ferrozine ( $4 \times 10^{-3}\%$ ) after standing for about 2 min was  $0.401 \pm 0.006$ . Thus TGA, NH<sub>2</sub>OH · HCL, and ascorbic acid, as the results showed, could reduce Fe(III) completely to Fe(II). From the A<sub>560</sub> value it was calculated that the molar absorptivity was 22 460, which roughly agreed with the literature value [3].

TABLE 3—Reaction between nonferrous metal species (100 ppm) and ferrozine ( $4 \times 10^{-3}\%$ , excess).

Species	A <sub>560</sub>		
	Stand 2 min	Stand 24 h	Heated 15 min
Cu(II)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
Zn(II)	0.004 (0.002)	0.012 (0.006)	0.015 (0.006)
Ni(II)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
Al(III)	0.260 (0.004)	0.260 (0.005)	0.266 (0.008)
Sn(II)	0.002 (0.004)	0.018 (0.006)	0.016 (0.006)
Pb(II)	0.002 (0.006)	0.029 (0.006)	0.030 (0.006)
Cr(III)	0.004 (0.003)	0.005 (0.003)	0.005 (0.004)

TABLE 4—Effect of nonferrous metal species (100 ppm) on the reaction between Fe(II) (1 ppm) and ferrozine ( $4 \times 10^{-3}\%$ , excess).

Species	A <sub>560</sub>		
	Stand 2 min	Stand 24 h	Heated 15 min
Cu(II)	0.015 (0.007)	0.015 (0.007)	0.020 (0.008)
Zn(II)	0.413 (0.004)	0.418 (0.006)	0.418 (0.007)
Ni(II)	0.042 (0.010)	0.048 (0.008)	0.045 (0.010)
Al(III)	0.664 (0.006)	0.664 (0.006)	0.665 (0.009)
Sn(II)	0.416 (0.005)	0.428 (0.012)	0.424 (0.010)
Pb(II)	0.401 (0.002)	0.402 (0.002)	0.405 (0.005)
Cr(III)	0.406 (0.005)	0.401 (0.008)	0.410 (0.008)

TABLE 5—Reaction among nonferrous metal species (100 ppm), ferrozine ( $4 \times 10^{-3}\%$ ), and  $\text{NH}_2\text{OH} \cdot \text{HCl}$  (1.6%).

Species	Stand 2 min		Stand 24 h		Heated 15 min	
	Color	A <sub>560</sub>	Color	A <sub>560</sub>	Color	A <sub>560</sub>
Cu(II)	colorless	0.000 (0.000)	colorless	0.005 (0.002)	pink	0.030 (0.007)
Zn(II)	pink	0.010 (0.003)	pink	0.040 (0.008)	pink	0.055 (0.008)
Ni(II)	colorless	0.000 (0.000)	colorless	0.004 (0.001)	colorless	0.004 (0.001)
Al(III)	magenta	0.256 (0.003)	magenta	0.260 (0.003)	magenta	0.260 (0.006)
Sn(II)	pink	0.014 (0.004)	pink	0.021 (0.005)	pink	0.040 (0.007)
Pb(II)	colorless	0.008 (0.003)	colorless	0.012 (0.004)	colorless	0.008 (0.003)
Cr(III)	yellowish green	0.015 (0.003)	yellowish green	0.016 (0.003)	yellowish green	0.016 (0.004)

TABLE 6—Effect of nonferrous metal species (100 ppm) on the reaction among Fe(III) (1ppm), ferrozine ( $4 \times 10^{-3}\%$ ), and  $\text{NH}_2\text{OH} \cdot \text{HCl}$  (1.6%).

Species	Stand 2 min		Stand 24 h		Heated 15 min	
	Color	A <sub>560</sub>	Color	A <sub>560</sub>	Color	A <sub>560</sub>
Cu(II)	colorless	0.000 (0.000)	colorless	0.005 (0.001)	magenta	0.414 (0.005)
Zn(II)	magenta	0.400 (0.004)	magenta	0.410 (0.005)	magenta	0.414 (0.005)
Ni(II)	colorless	0.000 (0.000)	colorless	0.000 (0.000)	colorless	0.000 (0.000)
Al(III)	magenta	0.659 (0.005)	magenta	0.658 (0.005)	magenta	0.652 (0.007)
Sn(II)	magenta	0.401 (0.004)	magenta	0.402 (0.005)	magenta	0.420 (0.008)
Pb(II)	magenta	0.404 (0.005)	magenta	0.406 (0.004)	magenta	0.409 (0.005)
Cr(III)	magenta	0.406 (0.006)	magenta	0.406 (0.006)	magenta	0.409 (0.009)

by the formation of a golden brown complex, as illustrated by the results of Tables 7 to 10. The effects of Cu(II) could, however, be easily removed by thiourea with an effective concentration of 0.5% w/v in water. Unlike ascorbic acid, TGA caused Pb(II) to interfere by the formation of a pink colloid.

Whichever reducing agent was used, Zn(II), Sn(II), and Cr(III) remained inert whereas Ni(II) remained inhibitory to the iron-ferrozine interaction and the inhibition could not be removed by thiourea, ethylenediaminetetraacetate (EDTA), glyoxime, or other common complexing agents. Therefore, it must be absent or present at only a low level before the analysis of iron by ferrozine could be successful. The positive interference of Al(III) was

TABLE 7—*Reaction among nonferrous metal species (100 ppm), ferrozine ( $4 \times 10^{-3}\%$ ), and TGA (0.4%).*

Species	Stand 2 min		Stand 24 h		Heated 15 min	
	Color	A <sub>560</sub>	Color	A <sub>560</sub>	Color	A <sub>560</sub>
Cu(II)	golden brown	0.304 (0.012)	golden brown	0.298 (0.016)	golden brown	0.160 (0.020)
Zn(II)	colorless	0.004 (0.001)	colorless	0.006 (0.002)	pink	0.020 (0.006)
Ni(II)	colorless	0.000 (0.000)	colorless	0.000 (0.000)	colorless	0.000 (0.000)
Al(III)	magenta	0.265 (0.005)	magenta	0.265 (0.006)	magenta	0.264 (0.009)
Sn(II)	pink	0.021 (0.005)	pink	0.025 (0.003)	pink	0.031 (0.006)
Pb(II)	pink	0.640 (0.105)	pink	0.085 (0.013)	pink	0.070 (0.016)
Cr(III)	turbid yellowish green	0.015 (0.003)	yellowish	0.015 (0.005)	yellowish	0.015 (0.005)

TABLE 8—*Effect of nonferrous metal species (100 ppm) on the reaction among Fe(III) (1 ppm), ferrozine ( $4 \times 10^{-3}\%$ ), and TGA (0.4%).*

Species	Stand 2 min		Stand 24 h		Heated 15 min	
	Color	A <sub>560</sub>	Color	A <sub>560</sub>	Color	A <sub>560</sub>
Cu(II)	reddish brown	0.568 (0.070)	pink turbid	0.427 (0.095)	violet turbid	0.876 (0.114)
Zn(II)	magenta	0.414 (0.006)	magenta	0.418 (0.005)	magenta	0.430 (0.008)
Ni(II)	colorless	0.007 (0.002)	colorless	0.007 (0.004)	colorless	0.006 (0.004)
Al(III)	magenta	0.659 (0.006)	magenta	0.660 (0.006)	magenta	0.656 (0.008)
Sn(II)	magenta	0.431 (0.006)	magenta	0.430 (0.005)	magenta	0.442 (0.008)
Pb(II)	pink turbid	0.968 (0.120)	pink turbid	0.684 (0.098)	pink	0.465 (0.077)
Cr(III)	magenta	0.429 (0.007)	magenta	0.445 (0.009)	magenta	0.440 (0.007)

likewise not affected by the reducing agent or common complexing agents including sodium fluoride (NaF).

From the above results it can be seen that ascorbic acid is the best reducing agent not only because of the reduction efficiency and nontoxicity, but also because it does not promote the interference of nonferrous metals [except Cu(II)] on the iron-ferrozine reaction. Interference by copper can be removed by thiourea.

Tests simulating the actual forensic sciences cases as described in the Experimental Procedure section were performed. Figures 1 to 4 show the iron traces left by a revolver on a hand or glove as revealed by spraying with ferrozine (0.1% w/v in ethanol) and by spraying with ascorbic acid (3% w/v in ethanol) following the ferrozine spray. The enhanced detection of

TABLE 9—Reaction among nonferrous metal species (100 ppm), ferrozine ( $4 \times 10^{-3}\%$ ), and ascorbic acid (0.12%).

Species	Stand 2 min		Stand 24 h		Heated 15 min	
	Color	A <sub>560</sub>	Color	A <sub>560</sub>	Color	A <sub>560</sub>
Cu(II)	golden brown	0.140 (0.008)	colorless	0.002 (0.001)	colorless	0.002 (0.001)
Zn(II)	pink	0.010 (0.003)	pink	0.031 (0.006)	pink	0.035 (0.006)
Ni(II)	colorless	0.000 (0.000)	colorless	0.000 (0.000)	colorless	0.001 (0.001)
Al(III)	magenta	0.248 (0.006)	magenta	0.248 (0.005)	magenta	0.249 (0.008)
Sn(II)	pink	0.018 (0.004)	pink	0.038 (0.004)	pink	0.044 (0.007)
Pb(II)	colorless	0.008 (0.002)	colorless	0.015 (0.002)	colorless	0.015 (0.003)
Cr(III)	yellowish green	0.016 (0.004)	yellowish green	0.016 (0.004)	yellowish	0.016 (0.005)

TABLE 10—Effect of nonferrous metal species (100 ppm) on the reaction among Fe(III) (1 ppm), ferrozine ( $4 \times 10^{-3}\%$ ), and ascorbic acid (0.12%).<sup>a</sup>

Species	Stand 2 min		Stand 24 h		Heated 15 min	
	Color	A <sub>560</sub>	Color	A <sub>560</sub>	Color	A <sub>560</sub>
Cu(II)	golden yellow	0.141 (0.012)	golden yellow	0.062 (0.009)	golden yellow	0.060 (0.009)
Zn(II)	magenta	0.424 (0.007)	magenta	0.428 (0.005)	magenta	0.439 (0.007)
Ni(II)	colorless	0.007 (0.002)	colorless	0.007 (0.002)	colorless	0.008 (0.003)
Al(III)	magenta	0.654 (0.006)	magenta	0.660 (0.006)	magenta	0.662 (0.007)
Sn(II)	magenta	0.431 (0.004)	magenta	0.448 (0.004)	magenta	0.445 (0.007)
Pb(II)	magenta	0.404 (0.005)	magenta	0.410 (0.006)	magenta	0.408 (0.006)
Cr(III)	magenta	0.402 (0.005)	magenta	0.404 (0.006)	magenta	0.404 (0.008)

<sup>a</sup>Remark: When thiourea was incorporated in the mixture with an effective concentration of 0.5% w/v, the A<sub>560</sub> was  $0.402 \pm 0.005$  and the color of the solution was magenta, indicating that the copper interference was removed.

the iron traces when using ascorbic acid can be clearly seen. The same result was obtained when the iron traces were transferred from the hand onto a moistened filter paper and the spraying was done on the filter paper, as shown by Figs. 5 to 6. The beneficial effect of ascorbic acid on iron detection was found to be even greater for rusty objects such as spanners and crowbars, whether the detection was performed on the hand, glove, or the filter paper.

Coins are mostly copper alloys. When spraying with ferrozine/ascorbic acid mixture was done on a hand that had gripped coins and a spanner sequentially, the magenta stains outlining the shape of the spanner was smudged with the brown stains due to the copper traces

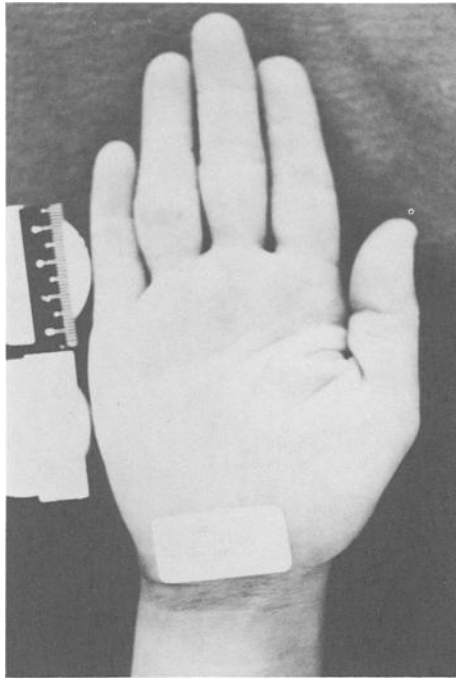


FIG. 1—Iron stains left on a hand by a revolver as revealed by spraying with 0.1% w/v ferrozine solution.



FIG. 2—Same as Fig. 1, but the stains were revealed by spraying with 0.1% w/v ferrozine solution and then 3% w/v ascorbic acid solution.





FIG. 3—Iron stains left on a glove by a revolver as revealed by spraying with 0.1% w/v ferrozine solution.



FIG. 4—Same as Fig. 3, but the stains were revealed by spraying with 0.1% w/v ferrozine solution and then 3% w/v ascorbic acid solution.

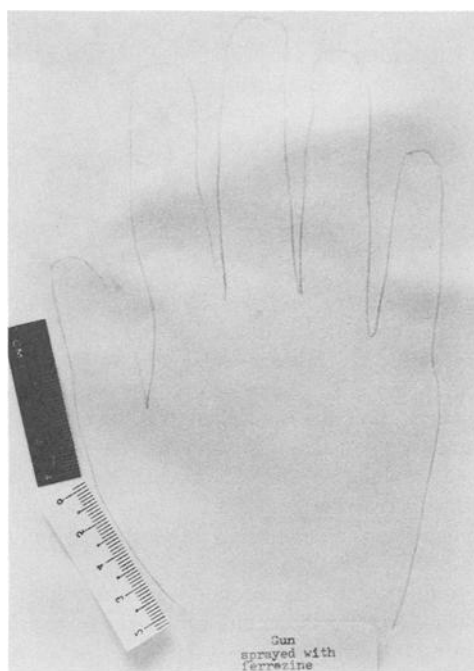


FIG. 5—Iron stains on the filter paper pressed from the hand which had gripped a revolver, as revealed by spraying with 0.1% w/v ferrozine solution.



FIG. 6—Same as Fig. 5, but the stains were revealed by spraying with 0.1% w/v ferrozine solution and then 3% w/v ascorbic acid solution.

transferred from the coins. The brown interfering stains were removed by a spray of 2% w/v thiourea solution. In many of the simulated tests with burglars' tools, it has been shown that a final spray of thiourea following the ferrozine/ascorbic acid sprays will help to make the backgrounds on the hands or gloves clean, making the interpretation of the magenta stains easier.

### Recommended Spraying Procedure

From this study the following guidelines are recommended in detecting iron traces with ferrozine spray.

1. Three sequential sprayings should be used. The first spray is 0.1% ferrozine in ethanol. Any magenta stains revealed could be due to Fe(II) or Al(III). The second spray is 3% ascorbic acid in ethanol. If the magenta stains revealed by the first spray are intensified, the stains are caused by iron compounds; otherwise they are probably a result of aluminum compounds. The third spray is 2% thiourea in water. This removes any dirty brown discoloration caused by Cu(II) and does no harm if this interference is absent. The three spraying reagents may be combined into one, but then interference by Al(III) may not be detected.

2. Photographs should be taken after each stage of spraying for documentation. Flash or available light color photography is recommended. Distribution and dimension of the magenta stains should be recorded.

3. Since it is difficult to transfer all the acquired traces from hands, especially from the curved surfaces of the fingers, spraying the hands is preferred to spraying the filter paper for better accuracy in measuring the distribution and dimension of the stains.

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