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Spectrophotometric Determination of Nitrites in Gunpowder Residue on Shooters' Hands

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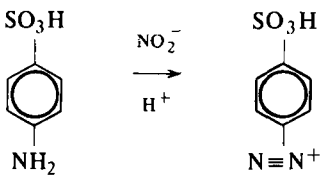
ABSTRACT: Several methods are described for the qualitative and quantitative spectrophotometric determination of nitrites originated from the gunpowder residues formed in firearms discharge. Detectable amounts of nitrites on shooters' hands were found after firing, whereas on nonshooters' hands the amounts were below the limit of detection. Procedures for providing representative samples are also described. Classic and modified Griess reagents were compared for the detection of nitrites. The latter is shown to be more effective and more convenient for identifying individuals which were involved in a firearm discharge.

KEYWORDS: criminalistics, chemical analysis, gunshot residues, nitrite, nitrite analysis, Griess reaction, spot tests, spectrophotometric determination

Smokeless powders, which are commonly used as propellants in small arms ammunition, consist of nitrocellulose in single-base powders and nitrocellulose together with 15 to 40% nitroglycerine in double-base powders. Unburnt or partially burnt powder particles surrounding bullet holes, mainly on clothes [*1*], are analyzed for the estimation of the firing distance.

The Walker test [*2*] is the widespread method for the detection of nitrites derived from the nitro group rich molecules in gunpowder residues. In this test Griess reagent [*3, 4*] consisting of sulfanilic acid and α-naphthylamine in diluted acetic acid, is used. The determination of nitrites is based on the formation of a diazonium salt which couples with α-naphthylamine, in acidic media, to form an azo dye as follows:



(1)

Sulfanilic Acid Diazonium Salt

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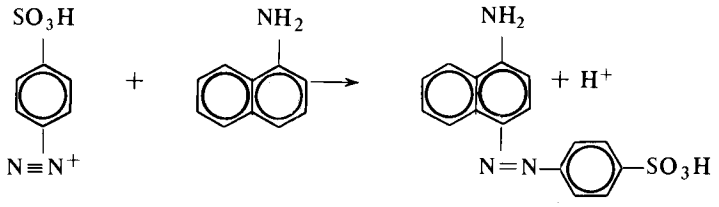


(2)

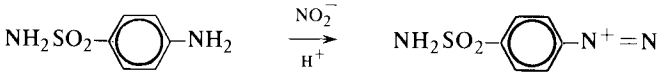
α-Naphthylamine Orange Red Azo Dye

Because gunpowder compounds and hence residues incorporate nitro groups in the form of ester nitrate ($R\text{-}O\text{-}NO_2$), it is essential that reactions of the above described type are preceded by the dissociation of the oxygen-nitrogen bond, to obtain the nitrite anion. This requirement is achieved by hydrolysis with a solution of potassium hydroxide in ethanol, a procedure developed and implemented in the authors' laboratory.[3]

The authors are not aware of any published working method for the determination of nitrites on the hands of individuals who have fired a gun. Furthermore, there is only meager information on the determination of other gunpowder residues [5] in the above situation. It has even been suggested that a meaningful test cannot be made since the majority of the gunpowder particles fall off the shooter's hands immediately after firing [5].

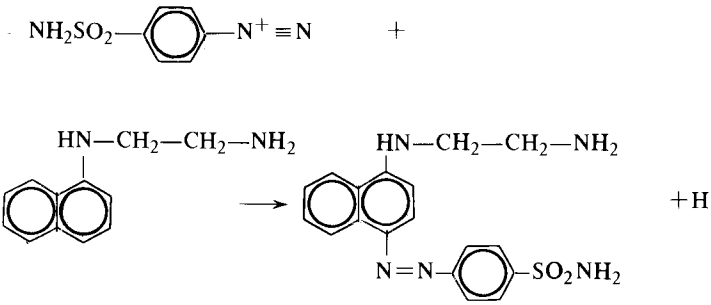
In the present study, the Griess and the modified Griess reactions are applied for qualitative and quantitative determination of nitrites retrieved from the hands of individuals who have shot firearms under various controlled conditions. To evaluate the significance of the results, the same proposed procedure was applied to individuals who had not used a firearm.

Numerous modifications for nitrites detection, have been suggested, based mainly on the application of alternative reagents undergoing diazotization and coupling [*6–10*]. One such sensitive method, henceforth termed "modified Griess reaction," was implemented to forensic science application by Glattstein et al.[4] The pair of reagents used in this method is sulfanilamide and *N*-(1-naphthyl)ethylenediamine dissolved in phosphoric acid [*11*] whose reaction scheme is as follows:



(3)

Sulfanilamide



(4)

N-(1-naphthyl)ethylene Diamine Pink Violet Azo Dye

[3]Goldschmidt, Internal Report, Israel Police Laboratories, Jerusalem, Israel, 1978.
[4]Glattstein, Kraus, and Almog, Internal Report, Israel Police Laboratories, Jerusalem, Israel, 1979.

Experimental Procedure

Sample Collection

Three methods of sample collection were used for the qualitative and quantitative determination of nitrites:

1. Shooters fired the weapon with cloth gloves, which later were submitted for analysis.
2. Shooters fired the weapon with bare hands which were sampled with adhesive tape attached to an aluminum disc (stub) [*12*].
3. Shooters' bare hands were swabbed with cotton swabs [*13*] moistened with analytical pure acetone (it is preferable to use acetone that dissolves the gunpowder particles rather than aqueous solvent). The swabs were stored in polypropylene tubes after sampling.

One to five test shots were carried out with various types of weapons and ammunition with both hands holding the weapon. Samples were collected within 20 min after firing from both sides of the hands. Control samples were similarly taken from persons who had not fired any weapon.

Reagents

Hydrolysis Reagent—Eight grams of potassium hydroxide were dissolved in 100 mL of deionized water.

Griess Reagent—(1) Five grams of sulfanilic acid were dissolved in 1000 mL 30%(v/v) acetic acid; (2) 6 g of α-naphthylamine were dissolved in 1000 mL 30%(v/v) acetic acid; and (3) fresh mixture is prepared by mixing equal volumes of each solution to obtain the working solution.

Modified Griess Reagent—(1) Eighty grams of sulfanilamide were dissolved in 1000 mL of 10%(v/v) A.R phosphoric acid; (2) 4 g of *N*-(1-naphthyl)ethylendiamine were dissolved in 1000 mL of 10%(v/v) A.R phosphoric acid; and (3) fresh mixture is prepared by mixing equal volumes of each solution to obtain the working solution.

Standard Nitrite Solution—One thousand parts per million of nitrite stock solution is prepared by dissolving 1.4997-g anhydrous sodium nitrite in 1000 mL of deionized water. The solution is diluted stepwise to give standards in the range of 0.05- to 1-ppm nitrite.

Instrumentation

A Cary model 15 recording spectrophotometer with 1-cm micro-cuvettes was used. The samples were scanned in the visible range (400 to 700 nm).

Analytical Procedure

Qualitative determination of nitrites on gloves and stubs using the Griess reagent—the hydrolysis reagent is sprayed onto the gloves and the stubs which are then heated at 100°C in an oven for 5 min to complete hydrolysis. Photographic paper is desensitized with sodium thiosulfate solution (photographic "fixer") for 10 min. This paper is immersed in the Griess reagent in a large rectangular dish [*1*] for a few minutes. The gelatinous side of the moistened paper is placed on the sample surface. The upper nongelatinous side of the paper is covered with a cotton cloth and applied with a hot iron for several minutes until the paper is dry. On removing the photographic paper, red-orange spots are observed (see Fig. 1). These spots correspond to the position of the powder particles on the examined surface.

Qualitative determination of nitrites on cotton swabs using Griess reagent—several drops of the hydrolysis reagent are applied to the cotton swabs which are then heated at 100°C in

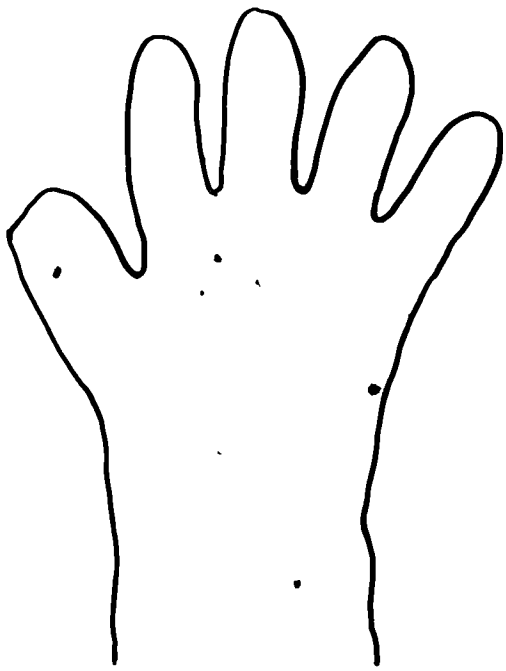


FIG. 1—*Several spots on photographic paper that correspond to the position of gunpowder residue particles on the cloth glove.*

an oven for 5 min. Several drops of the reagent are added to the swabs. The appearance of a red-orange color indicates the presence of nitrites.

Qualitative determination of nitrites on cotton swabs using the modified Griess reagent—several drops of the hydrolysis reagent are applied to the cotton swabs which are heated as in above paragraph. Several drops of the reagent are added to the swabs. The appearance of a pink-violet color indicates the presence of nitrites.

Quantitative spectrophotometric determination of nitrites on cotton swabs—the cotton swab is placed in a polypropylene test tube and treated with a minimum volume (approximately 1 mL) of acetone for 15 min (to extract the gunpowder residues). The swab is then removed from the tube and discarded. The acetone is evaporated from the tube by heating on a hot water bath. Two drops of the hydrolysis reagent are added and evaporation is repeated. One drop (0.1 mL) of the modified Griess reagent is added and followed by dilution of 1 mL with deionized water. The solution is then transferred to the spectrophotometer cuvette.

The characteristic absorbance of the modified Griess reaction was found to be at 540 nm. Standards, blanks, and samples were measured by the same procedure. To avoid decomposition mainly by oxidation of the nitrites, the standard solutions and reagents are prepared within 30 min before sample measurement.

The linear section of the calibration curve ranges from 0.05 to 0.9 ppm. Sample solutions containing high nitrite concentrations were diluted in order to bring them into this working range. The slope of the calibration curve was 1.08 ppm absorbance, the molar absorptivity was 49450 L M^{-1} cm^{-1} and the relative standard deviation was 1.05%.

The hydrolysis solution and the modified Griess reagent did not show any significant absorbance in the visible range, but were found to produce a blank equivalent to 0.05-μg nitrite. This value was subtracted from all the samples measurements.

TABLE 1—*Qualitative nitrite determination on shooters' hands using the Griess and the improved Griess reagents (+++ strong, ++ fair, + weak).*

Reagent	Sampling Surface	Type of Ammunition and Weapon	Number of Shots	Results	
				Right-Hand	Left-Hand
Griess	cloth gloves	.22″ LR[a] Eley semiautomatic pistol	3	++	+
		.22″ LR[a] Winchester Super X semi-automatic pistol	4	+	...
		7.65-mm SBP[b] semiautomatic pistol	3	++	++
		9-mm short Remington semi-automatic pistol	2	+	+
		.38″ Remington-Peters revolver	3	...	+
Griess	stubs	.22″ LR[a] Remington revolver	3	+++	...
		5.56-mm Winchester M-16 rifle	3	++	+
		7.65-mm SBP[b] semiautomatic pistol	3	++	...
		9-mm Parabellum Israeli SMG[c]	3	+	++
		.38″ Kynoch revolver	3	+	+
Griess	cotton swabs	.22″ LR[a] Eley semiautomatic pistol	3	+	+
		5.56-mm Winchester M-16 rifle	3	+	+
		7.65-mm SBP[b] pistol	3	...	++
		9-mm Parabelum Israeli SMG[c]	5	+++	+
		.38″ Remington-Peters revolver	3	++	+
Modified Griess	cotton swabs	5.56-mm Winchester M-16 rifle	3	++	++
		7.65-mm Hirtenberg semiautomatic pistol	5	++	+
		7.65-mm SBP[b] semiautomatic pistol	5	++	+++
		9-mm Parabellum Israeli SMG[c]	5	+++	++
		.38″ Remington-Peters revolver	5	+++	+

[a]Long-rifle.
[b]Silleir-Bellot-Prague.
[c]Submachine-gun.

Results

Qualitative Determination

Data on the qualitative determination of nitrites in 40 samples are listed in Table 1. A (+) mark denotes positive results. The extent of the results, that is, the number of red-orange spots on the photographic paper and the intensity of red-orange or pink-violet color on the cotton swabs, is indicated by the number of the (+) marks.

The data show that nitrites were detected in almost all samples derived from shooters' hands. Nitrites were not detected in the 40 blank samples. Moreover, the data indicate that the modified Griess reagent (used on the cotton swabs) shows better results than the classic Griess reagent.

Quantitative Determination

The sensitivities of the modified and classic Griess reagents were measured and compared spectrophotometrically. The sensitivity of the modified reagent (0.1 μg) was found to exceed that of the classic reagent (0.5 μg). (See also Ref *10.*) Consequently, the modified reagent was used for the spectrophotometric determination of nitrites in 40 samples from hands of individuals who had used different types of weapons and ammunition.

The data obtained are listed in Table 2. Thirty-six samples contained more than 0.5-μg nitrites; four samples did not show a significant nitrite content. The nitrite content of 20 samples from hands of persons who had not used firearms was below the limit of detection.

TABLE 2—*Spectrophotometric determination of nitrites on shooters' hands using the modified Griess reagent. (Results expressed in micrograms and corrected for blank).*

Type of Weapon	Number of Shots[a]	Results	
		Right-Hand	Left-Hand
.22" LR revolver	1	0.09	0.07
	3	0.08	0.08
	3	0.11	0.10
	5	0.10	2.35
M-16 rifle	1	0.12	0.12
	3	0.00	0.00
	3	0.15	0.08
	5	0.21	0.12
7.65-mm semiautomatic pistol	2	3.21	0.15
	2	0.12	0.12
	3	1.90	0.00
	3	0.14	2.54
	5	36.00	0.72
9-mm Parabellum Israeli SMG	1	0.17	0.20
	3	0.18	0.19
	3	0.24	0.19
	5	0.20	0.34
.38" revolver	1	0.15	0.15
	3	0.00	0.12
	5	0.14	0.16

[a]Mixed types of ammunition were used.

Discussion and Conclusions

The qualitative and quantitative nitrites data obtained in this study demonstrate that gunpowder residue particles can be identified on shooters' hands, the nitrites content being between 0.05 to 4 μg in the majority of the samples. The quantity of nitrites on nonshooters' hands was insignificant. No distinct correlation could be observed between the amount of nitrites and the type of ammunition, weapon, and number of shots.

The hydrolysis step preceding the application of both the classic and the modified Griess reagents was found to be crucial for the detection of gunpowder nitrite residues.

The modified Griess reagent is preferable to its predecessor because of both its higher sensitivity and the ease of operation. Furthermore, since the α-naphthylamine used in the classic Griess reaction is a declared carcinogenic substance [*14*], its replacement is of a distinct advantage.

Finally, the method using the modified Griess reagent is proved to be an effective forensic science test for identifying persons who had used a firearm. Therefore, the combination of this method with the identification of metallic gunshot residue, should increase the reliability and conclusiveness of the interpretation of criminal shooting cases.

This method can be applied to the scene of crime, where a rapid screening test is essential. Cotton swabs moistened with acetone or adhesive stubs can be used for this purpose. In cases where a relatively long period of time (exceeding 1 h) has elapsed since the weapon has been fired, other parts of the suspect (hair, face, and clothing) should be sampled, as powder particles might fall off the hands during normal activity.

This procedure can be easily adapted to other areas where nitrite determination is of interest, for example, to environmental pollution and toxicology.

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