

TECHNICAL NOTE

Eric W. Bohannon¹ and Dean A. Van Galen,¹ Ph.D.

A Sensitive Electrochemical Method for the Analysis of Nitrite Ion and Metals in Gunshot Residue

REFERENCE: Bohannon, E. W. and Van Galen, D. A., "A Sensitive Electrochemical Method for the Analysis of Nitrite Ion and Metals in Gunshot Residue," *Journal of Forensic Sciences*, JFSCA, Vol. 36, No. 3, May 1991, pp. 886-893.

ABSTRACT: A method for the analysis of nitrite ion and metals in a single gunshot residue sample using electrochemical methods of detection is described. In this method, nitrite ion present in the sample is chemically converted to a diazonium ion, which is then detected by differential pulse voltammetry. The peak current for the reduction of the diazonium ion is proportional to the nitrite concentration up to 400 ppb nitrite ($r = 0.9995$), with an absolute detection limit of 0.01 μg nitrite. Lead ion present in the gunshot residue sample is detected in the same voltammetric scan, and antimony is subsequently detected by anodic stripping voltammetry. The method has been successfully applied to a number of samples obtained in test firings.

KEYWORDS: criminalistics, gunshot residues, nitrite, chemical analysis, electrochemical analysis, lead, antimony

It is well established that when a firearm is discharged, small amounts of material originating from the ammunition are expelled from the weapon and are often deposited on the shooter's hands [1]. These deposits, commonly referred to as gunshot residues (GSR), consist of metals from the primer material or bullet (barium, antimony, lead, or some combination of these), and nitrite (NO_2^-) compounds as well as organic compounds such as nitrocellulose, nitroglycerin, and stabilizers from the ammunition gunpowder. There has been much interest in developing methods to analyze residues that have been removed from the hands of a person suspected of firing a weapon, since the detection of certain levels of metals or compounds can be interpreted to be indicative that a suspect has fired a weapon.

A commonly used sampling procedure is to employ plastic-stemmed cotton swabs that have been moistened with 5% nitric acid to remove the residue from the hands [2]. The swabs are then placed in an acidic solution so as to leach the residue materials from the swab, and the resulting solution is analyzed for the metals that are present in common primers. Previous studies have shown that the amount of residue deposited on the shooter's hand depends on the caliber, condition, and type of weapon fired; the case situation;

Received for publication 4 April 1990; revised manuscript received 9 July 1990; accepted for publication 23 July 1990.

¹Undergraduate student and assistant professor chemistry, Division of Science, respectively, Northeast Missouri State University, Kirksville, MO.

and the elapsed time and activity between firing and sampling of the hand [1,3]. The analytical method used to detect components in GSR must be extremely sensitive since the metals and nonmetals of interest are present at microgram to nanogram levels. Gunshot residue analysis for metals is commonly carried out using flameless atomic absorption spectroscopy (FAAS) [4-6]. Other methods that have been applied to GSR metals analysis include neutron activation analysis [7,8], inductively coupled plasma atomic emission spectrometry [9], anodic stripping voltammetry [8,10-13], and scanning electron microscopy [14-16]. The nitrite ion that is present in GSR has been determined both qualitatively and quantitatively by reacting nitrite with an aminobenzene sulfonate and then naphthylamine (or a derivative thereof) [17]. The final product of these reactions is a highly colored compound that is visible to the eye and can be detected spectrophotometrically. The sensitivity of this method was reported as 0.1 μg of nitrite.

In considering the present status of GSR analysis, the authors felt that a number of practical advantages could be realized by the development of procedures that rely on electrochemical methods as a means of detection. Specifically, electrochemical methods are able to detect both metals and nonmetals (ions and organic compounds) in a single sample with very high sensitivity, their application is quite rapid and simple, and the necessary instrumentation is relatively inexpensive. One drawback in the application of electrochemical methods to GSR analysis is that it is presently not possible to detect barium at the microgram level since the very negative reduction potential of barium overlaps with the cathodic limit of water [18]. The goal of this work was to develop a procedure for the analysis of nitrite ion and metals from a single GSR sample using electrochemical methods of detection.

Previously reported electrochemical methods for nitrite using a standard mercury electrode are not sufficiently sensitive or are too difficult to apply to be useful for GSR analysis, which typically requires the detection of between 0.05 and 4 μg of nitrite in a forensic setting [17]. If a solution volume of 10 mL is used for electrochemical experiments, the detection of 0.05 μg of nitrite corresponds to a concentration detection limit of 5 ppb. One common method for nitrite detection at a mercury electrode involves its polarographic reduction as nitrous acid in 2M citrate buffer at pH 2.5 [19]. Because of the instability of nitrous acid and because its reduction potential is close to that of oxygen, this method has a lower concentration limit of 500 ppb. A detection limit of 2300 ppb for nitrite has been reported when using a molybdenum-modified electrode [20], but the use of such electrodes, besides lacking sensitivity, can be difficult and time-consuming for the nonexpert to prepare. Improved detection limits have been obtained by detecting species that have been formed through the reaction of nitrite with organic reagents. For example, Chang and co-workers report a practical working limit of 4.6 ppb for a method involving the reaction of nitrite with diphenylamine to form diphenylnitrosamine, which is detected [21]. Also, Sulaiman [22] detected the diazonium ion formed upon the reaction of nitrite with orthonilic or sulfanilic acid at a mercury electrode and reported a detection limit of 5 ppb. One concern with these methods is that the detected species are known to adsorb to a mercury electrode, that is, they act as surfactants. The problem in this case is that oils that are present on the human hand also adsorb strongly to a mercury electrode and can therefore affect the adsorption of other species in an unpredictable way. It was therefore necessary to develop a sensitive, reliable electrochemical method for nitrite that was compatible with GSR samples as well as the well-established electrochemical behavior of lead and antimony, allowing all three species to be quantitated.

Experimental Details

Apparatus and Reagents

All electrochemical experiments were performed using a Princeton Applied Research Model 174A Polarographic Analyzer and a Metrohm Model 663 VA Electrode Stand.

Potential-current curves were recorded using a Houston Instruments Model 2000 XY recorder. Electrochemical experiments were performed in a glass cell, and the electrode configuration consisted of a hanging mercury drop working electrode, a silver/silver chloride reference electrode, and a platinum wire auxiliary electrode. A fresh mercury drop was dispensed for each scan. All solutions were deoxygenated with argon prior to analysis and between scans.

Deionized water obtained from a Nanopure II water system was used for all dilutions. Acids were of the highest purity commercially available: Nitric acid [HNO_3] from Aldrich, redistilled; hydrochloric acid [HCl] from GFS Chemicals, 99.999 + %). Standard additions of lead and antimony were made using 1000-ppm commercial standards (Aldrich). All other chemicals were reagent grade or of the highest purity commercially available. The hydrolysis reagent used in the conversion of nitrate esters to nitrite was composed of 0.8 g of potassium hydroxide (KOH) in 20 mL of water.

GSR Sampling Procedures

Shooters or control persons' hands were swabbed with plastic-shafted Johnson and Johnson swabs that had been moistened to saturation with a swabbing solution (either 5% concentrated nitric acid in water or acetone). The back of each hand was swabbed, including the web of the hand between the thumb and forefinger [2]. After swabbing, the used end of the swab was cut off and placed in a capped, 5-mL polyethylene vial. For each subject, a swabbing of the right and left hand was performed with separate swabs, and a third swab was moistened with swabbing solution to serve as the blank.

Analytical Procedures

In order to extract the GSR from the collection swab, 3.0 mL of acetone was added to the vial that contained the collection swab. The swab was gently swirled and then allowed to rest in the acetone for 15 min. The swab was then pressed against the side of the vial to remove excess acetone and removed from the vial, and the acetone was evaporated just to dryness in a 80°C water bath. The vapor pressure of nitroglycerin at this temperature was calculated to be 0.15 mm Hg using the Clausius-Clapeyron equation, $\Delta H_{\text{vap}} = 57.5 \text{ kJ/mol}$, and $P = 0.31 \text{ mm Hg}$ at 93°C for nitroglycerin [23,24]. With this low vapor pressure at 80°C, the loss of nitroglycerin due to volatilization should be minimal. Using a procedure modified from that of Steinberg et al. [17], the nitrate ester functional groups that were present in the nitroglycerin and nitrocellulose were converted to nitrite by hydrolysis. This was accomplished by adding 1.0 mL of hydrolysis reagent to the vial. In order to assure complete reaction, this mixture was heated for 25 min in a 80°C water bath. Next, the pH of the solution was adjusted to 2.4 with nitric acid, the solution was quantitatively transferred to a 10-mL volumetric flask, and 10.0 mg of sulfanilamide and 250 μL of 2400-ppm cetyltrimethylammonium bromide (CTAB) was added to the flask. Finally, the solution was diluted to volume with nitric acid at pH 2.4. The solution was then transferred to an electrochemical cell for analysis. Approximately 50 min was required after the addition of the diazotization reagent (before analysis) to assure that the conversion of nitrite to diazonium ion was complete.

Electrochemical Procedures

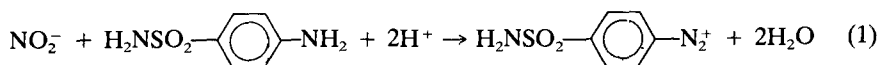
The determination of diazonium ion (which is proportional to the nitrite concentration) and lead ion was accomplished in a single scan by differential pulse voltammetry at a hanging mercury drop electrode. During this scan, diazonium and lead ions were reduced at the electrode at characteristic potentials, yielding a peak current that was proportional

to their concentration in solution. Next, 10.0 mL of 6M HCl was added to the analysis solution and antimony ion was determined by differential pulse anodic stripping voltammetry. The electrochemical parameters are listed in Table 1.

Results and Discussion

Electrochemical Method for Nitrite and Metals Determination in GSR

Our method for the determination of nitrite ion in GSR is based on the detection of the diazonium ion ($R-N_2^+$), which is formed from the reaction of nitrite with sulfanilamide in acidic media (Eq 1). The reduction of the diazonium ion formed



by this reaction can be detected by differential pulse voltammetry in pH 2.4 nitric acid at a hanging mercury drop electrode. In order to prevent adsorption of diazonium ion and perhaps hand oils to the electrode, all voltammograms were obtained in the presence of 60-ppm cetyltrimethylammonium bromide (CTAB). CTAB is a common surfactant that adsorbs strongly to a mercury electrode, thereby preventing adsorption of other species. It was found that in the absence of CTAB, the diazonium ion peak for GSR samples was not reproducible, and therefore CTAB was added to all solutions.

A differential-pulse voltammogram showing the reduction of the diazonium ion formed by the reaction of a 2-ppb nitrite solution with sulfanilamide in pH 2.4 nitric acid is shown in Fig. 1. The peak at -0.65 V is due to the reduction of the diazonium ion, the height of which is directly proportional to the concentration of nitrite ion before its reaction. Six repetitive scans for a single GSR sample whose nitrite concentration was 140 ppb gave a relative standard deviation of 0.7% for the cathodic peak current. The stability of the diazonium ion in pH 2.4 HNO_3 was tested by measuring the peak height as a function of time after its formation. Two hours after the addition of nitrite (about 1 h after the nitrite reaction was complete), the peak height decreased 3%, while a 10% decrease was observed 4 h after nitrite addition. Thus, the best accuracy is obtained by performing voltammetry shortly after diazonium ion formation is complete.

The detection limit for nitrite by this method is about 1 ppb. This concentration gave a peak current of 19 nA with our particular electrode, which is of sufficient magnitude to be readily measured with standard electrochemical instrumentation. The voltammetric

TABLE 1—*Electrochemical parameters.*

Parameter	Value
Differential pulse voltammetry (NO_2^- , Pb, and Sb)	
Scan rate	10 mV/s
Pulse height	50 mV
Clock time	0.5 s
Determination of NO_2^- and Pb	
Initial potential	-0.30 V
Final potential	-1.00 V
Determination of Sb	
Initial potential	-0.65 V
Deposition time	5 min
Final potential	-0.20 V

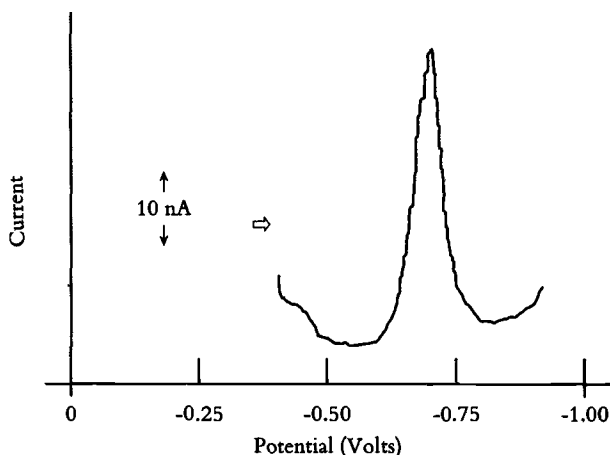


FIG. 1—Differential pulse voltammogram corresponding to 2 ppb nitrite in pH 2.4 HNO_3 in the presence of 60-ppm CTAB.

peak height was found to be linear with the nitrite ion concentration over the range of 1 to 400 ppb, with a correlation coefficient of 0.9995. For nitrite concentrations that are greater than about 400 ppb, the observed current is less than expected, perhaps because of blocking of the electrode by adsorbed diazonium ion even in the presence of CTAB. Samples with nitrite concentrations greater than 400 ppb should be diluted into the linear working range.

The peak reduction potential for lead ion in pH 2.4 nitric acid is -0.46 V. Since this potential is well separated from the reduction potential of the diazonium ion ($\Delta E_p = 190$ mV), it is possible to detect lead and nitrite ions in a single differential pulse voltammetric scan. Detection of lead by this method is well documented and will not be discussed further here. Finally, it is possible to detect antimony by anodic stripping using differential pulse voltammetry for quantitation. In this method, a constant, relatively negative potential is applied to the electrode, thus reducing the antimony ion to antimony amalgam, that is, the reduced metal “dissolves” in the mercury electrode. Following the deposition step, the electrode potential is scanned positively, resulting in the oxidation of antimony with a peak potential of -0.31 V.

Determination of Nitrite, Lead, and Antimony in Test Firing Samples

The method described for the quantitative determination of nitrite, lead, and antimony in a single sample was applied to GSR samples obtained in test firings. After sample collection, the residue from each swab was extracted with acetone, and the acetone was evaporated. Next, hydrolysis in KOH was carried out to convert any nitrate esters to nitrite ion [17]. Following pH adjustment and dilution, a differential-pulse voltammogram of the solution was obtained. The results of these analyses are shown in Table 2. Figure 2 shows the electrochemical response for the analysis of Sample 1R which was obtained from the right hand of a person who fired two rounds from a Heckler and Koch 9-mm semiautomatic weapon. For each analysis, the current response for the blank (from a swab moistened with swabbing solution and treated identically to the sample swab) was subtracted from the current response for the sample. Figure 2a shows the detection of lead and diazonium ions (corresponding to nitrite) in a single scan. Subsequent standard additions allow these ions to be quantitated. Next, the solution volume is doubled by the addition of 6M HCl, and antimony is determined by anodic stripping voltammetry

TABLE 2—Test firing results.^a

Sample	Type of Weapon (No. of Rounds)	NO ₂ ⁻ , μg	Pb, μg	Sb, ng
1R	Heckler and Koch 9 mm	1.5	2.8	150
1L	semiautomatic (2)	2.5	5.2	<9.8
2R	Beretta P21 (2)	1.3	2.9	<1.6
2L	Beretta P21 (2)	2.4	2.9	52
3R	Heckler and Koch 9 mm	1.3	1.2	83
3L	semiautomatic (1)	0.54	...	67
4R	Smith and Weston 0.32	1.0	0.47	60
4L	caliber long (1)	0.15	0.59	<5.1
5R	Heckler and Koch 9 mm	0.14	0.46	35
5L	semiautomatic (2)	0.24	0.33	5.5
6R	12-gauge Mossberg New	1.3	0.44	1.5
6L	Haven shotgun (2)	...	0.11	<17
7R	hand blank (0)	<0.01	0.005	<4.3
7L	hand blank (0)	<0.01	0.049	<4.3
8R	hand blank (0)	0.01	0.084	<1.4
8L	hand blank (0)	<0.01	0.33	<1.4

^aThe swabbing solution was 5% HNO₃ for Samples 1–4 and 6–8. Acetone was used for Sample 5. Three dots (. . .) indicates that the peak currents for the sample and blank were indistinguishable.

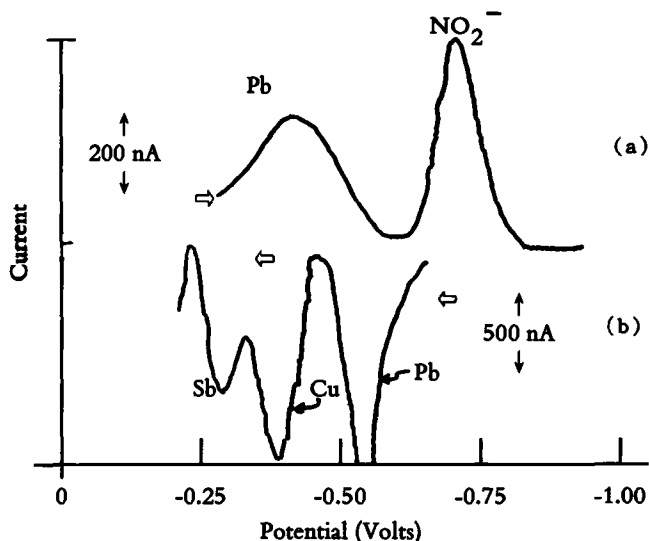


FIG. 2—Electrochemical analysis of GSR Sample 1R.

in the same cell (Fig. 2b) utilizing a standard addition method. As shown in Fig. 2b, copper and lead are also detected in the anodic stripping experiment, and, in fact, the levels of these metals present in the solution determines the detection limit for antimony in a given GSR sample. The amount of nitrite found in these samples is consistent with those amounts reported for the spectrophotometric method for nitrite (0.05 to 4.0 μg). The results of analysis of residues collected from persons who had not recently fired a weapon (hand blanks) are also presented in Table 2. The amounts of nitrite and metals were typically at least 10 times smaller than the corresponding amounts from the hands of the shooters sampled.

In summary, the procedure described in this paper constitutes a sensitive, inexpensive, and relatively rapid method to quantitate nitrite, lead, and antimony in GSR samples. Clearly, it is necessary to determine the residual time of nitrites on hands after they have fired a weapon, as well as the frequency of occurrence of nitrites in a control population, before this method can be applied to case situations. Future work will address these issues as well as focus on developing procedures that will also allow the electrochemical detection of barium.

Conclusions

The electrochemical method for nitrite that we have developed has been shown to possess the necessary sensitivity to be applied to GSR analysis. The absolute detection limit of this method is approximately 0.01 μg of nitrite, which is ten times smaller than that of the previously reported spectrophotometric method for nitrite in GSR samples. The method has been incorporated into an analytical procedure that makes possible the analysis of metallic components and nonmetallic components (originating from the powder) in a single GSR sample.

Acknowledgment

We thank Joy Pugh of the Northeast Missouri Crime Laboratory for assistance in sample collection and Northeast Missouri State University for financial support.

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Address requests for reprints or additional information to
Dr. Dean A. Van Galen
Department of Chemistry
Division of Science
Northeast Missouri State University
Kirksville, MO 63501