

## Gunshot Residue Analysis—A Review

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**ABSTRACT:** A review of all aspects of analysis associated with gunshot residues is provided. This includes a summary of what both inorganic and organic residues are, how they arise and how they may be collected from a variety of substrates. Ways of processing samples to the point of analysis and the means by which they may be analyzed using a variety of techniques is discussed. The techniques described include SEM/EDX, flameless atomic absorption spectroscopy, neutron activation analysis, energy dispersive X-ray fluorescence and some other less common methods for inorganic residues and gas, high performance liquid and super critical fluid chromatography with speciality detectors including mass spectrometry together with capillary electrophoresis, for organic residues. A summary of how the results of such analyses may be interpreted is discussed under the different analytical techniques.

**KEYWORDS:** forensic science, criminalistics, gunshot residue, gunshot residue analysis, review

The growing use of firearms in acts of violence associated with robbery, as well as increasing acts of terrorism has prompted this review of the analysis of firearms residues which we hope the forensic science community will find of use in the pursuit of their profession.

### General Considerations

When a firearm is discharged, an assortment of vapors and particulate material are expelled in the area around the firearm. These products of firearm discharge can be collectively referred to as gunshot residues (GSR) or sometimes cartridge discharge residues (CDR).

Gunshot residues have been used in criminalistics to estimate firing distances (1–7), identify bullet holes (2,8), and most importantly, to determine whether or not a person has discharged a firearm (9–24). The ability to provide evidence, based on analysis of gunshot residues, that a person has discharged a weapon has been one of the most persistent goals of forensic scientists since the turn of the century. Several factors serve as motivation for this intensity of concern. Crimes associated with firearms are particularly serious and require perhaps the greatest investigative effort. It is often possible to recover spent bullets or cartridge casings and associate them with a particular gun. However, the remaining link in the chain of evidence is to associate a suspect with the firing of the gun in question. Gunshot residue evidence that provides proof that an individual has recently fired a gun has been

of substantial help in the investigation of many of these cases. Gunshot residue detection methods are based on analysis of the chemical residues produced by discharge of the firearm's cartridge. Normally, only trace amounts are deposited on the back of the hand, face, hair, and clothing of the firer, and this material can be used to determine if a person has discharged a weapon.

### Sources of Gunshot Residues

#### General

When a gun has been fired, gunshot residues can arise from the primer, propellant, lubricants, and metals that are found in the bullet, bullet jacket, cartridge casing, and gun barrel. The first step in the firing of a bullet is the detonation of the primer. The primer is detonated when it is crushed by the force of the firing pin of the firearm. This drives hot gases and hot particles into the propellant and ignites it. The ignition of the primer mixture and the propellant occurs in a matter of a few ten thousandths of a second (25). The ignited propellant decomposes and forms gaseous products. Simultaneously, heat, in enormous quantity, is released by this reaction. The high pressure from the heated gases forces the projectile out of the cartridge casing into the barrel and out of the muzzle of the gun.

The heat generated on ignition of the primer causes the inorganic ingredients of the primer mixture to vaporize. Because of supersaturation, these vapors recondense into droplets (26), which are further subjected to high pressure and temperature arising from the burning propellant powder. Some of these droplets will grow by coalescence. With the expansion and cooling on leaving the barrel many of these droplets freeze in their existing form. The gunshot residues originating from the primer contain elements of primer components mainly lead, antimony, and barium (26). Elements such as copper, iron, and some other nonspecific elements such as aluminum, silicon, sulfur, potassium, and calcium are also often found associated with them. These latter elements can originate from other sources such as etched bullets, cartridge casings, or barrels. In addition to the "primer particles," lead particles, when abundant, can arise from the lead bullets themselves (27).

Under ideal circumstances it would be expected that all of the propellant powder would be consumed in the burning process and would be converted into gases. However, in practice this is not the case because the whole powder charge is never totally burnt. When a firearm is discharged, unburnt and partially burnt powder granules are propelled out of the barrel along with the projectile toward the target. The size of these particles range from large visible particles to a fine dust. Gunshot residues are mainly composed of these organic and inorganic particles. A simplified probability equation assessing the probability of finding gunshot primer residues when only a portion of a specimen is to be searched, has been described by Halberstam (28).

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### Primers

Ammunition primers consist of four basic chemical components: the initiating explosive, oxidizing agent, fuel, and sensitizer. Each component can be expected to contribute gunshot residues after a gun has been fired. The standard explosive initiator in primers is lead styphnate. Formerly, lead azide and mercury fulminate had been used as initiator in primers, but are no longer commonly used because the intensity of flame produced is insufficient and a corrosive effect is imparted by mercury fulminate to gun barrels.

Oxidizing agents are used in primers to increase the heat of ignition. Barium nitrate is most commonly used in small-arms ammunition, but barium peroxide, lead nitrate, or lead peroxide may also be encountered. Antimony sulfide is commonly used as fuel in primers, but calcium silicide, lead thiocyanate, powdered aluminum, and powdered zirconium, magnesium, and titanium have also been used (9).

The standard sensitizing material used in small-arm primers is tetracene (1-(5-tetrazolyl)-4-guanyltetrazene hydrate). Pentaerythritol tetranitrate, trinitrotoluene, and tetryl are also used as sensitizers in primers (9).

### Gunpowder's

The use of black powder as a propellant for bullets has largely been discontinued. Black powder is composed of 75% potassium nitrate, 15% sulfur, and 10% charcoal. Smokeless powders, which are commonly used as propellants, are composed of nitrocellulose in single base powders and nitrocellulose together with nitroglycerine in double base powders. In some double base powders a portion of nitrocellulose and nitroglycerine has been replaced by nitroguanidine. These are sometimes referred to separately as triple base powders but they are not commercially available in the USA. Typical single based powders are found in rifle cartridges and some rim fire rifle and revolver cartridges, whereas double based powders are used in revolver and pistol as well as shotgun cartridges. Rim fire rifle and revolver cartridges may also be double based (29).

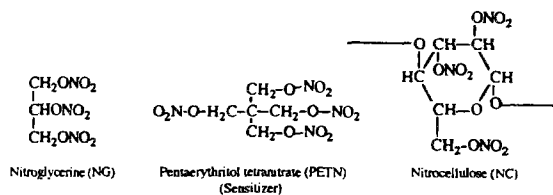
Nitrocellulose is used, not only in propellants and explosives, but also in lacquers, varnishes, celluloid films and the printing and pharmaceutical industries (30). Nitroglycerine occurs in both pharmaceutical preparations and explosives.

All smokeless powders, in addition to explosive ingredients, contain a number of additives. These additives fulfill the role as stabilizers, plasticizers, flash inhibitors, coolants, moderants, surface lubricants, and antiwear additives. A particular propellant powder will contain one or more of these additives depending on its use.

Common stabilizers used are diphenylamine, the centralites, and resorcinol. Diphenylamine (DPA) is the most common stabilizer used in smokeless powders, especially in single base powders. In smokeless powders the diphenylamine content is usually about 1% (29). In addition to DPA, the main reaction products of nitric oxide (NO<sub>2</sub>) with DPA, 2-nitrodiphenylamine, 4-nitrodiphenylamine, and N-nitrosodiphenylamine have also been reported to be frequently encountered in gunpowder's (31–33). Another group of stabilizers used in smokeless powders are the centralites. The most common is ethyl centralite, but sometimes methyl centralite may also be used. Ethyl centralite is usually found in double base powders. Another stabilizer found in some smokeless powders is resorcinol. The chemical structures of some of these compounds are given in Fig. 1.

In the process of making powder grains, plasticizers are mixed

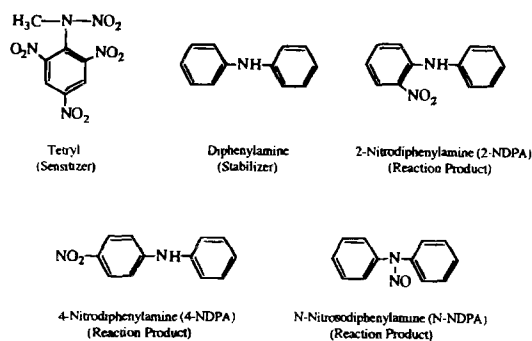
### Nitrate Ester Explosives.



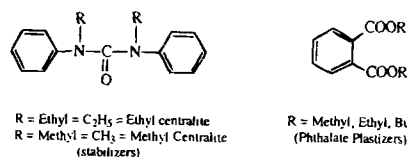
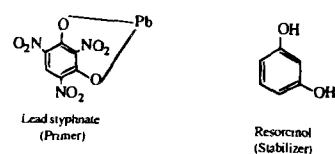
### Aromatic Explosives.



### Aromatic Additives.



### Aromatic Additives.



### Other Additives.

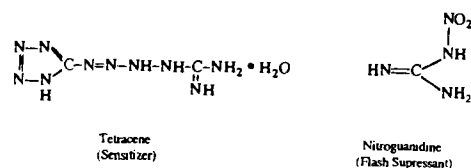


FIG. 1—Chemical structures of some compounds found in firearm ammunition.

with the powder components. These provide strengthened flexibility to the grains. Some of the plasticizers used are glyceryl triacetate (triacetin), dimethyl phthalate, diethyl phthalate, and dibutyl phthalate. Dinitrotoluene is used as a flash suppresser in some smokeless powders. Nitroguanidine fulfills the same role. The role of a flash suppresser is to produce nitrogen gas to dilute the muzzle

gases (29). The powder grains are also coated with graphite which reduces any hazards which could arise from static electricity but it also acts as a surface lubricant to improve the flow properties of powder during cartridge manufacture.

The compositions of some single base and double base smokeless powders are shown in Tables 1 and 2.

**Bullets**—The bullet in an automatic pistol cartridge contains a lead core that is covered with a full metal jacket of copper alloyed with 5 to 10% zinc. Revolver bullets are generally composed of lead, or lead plated with a thin layer of copper, or the lead is alloyed with antimony or tin, or with both. In some bullets, such as hollow-point and soft-point bullets, a copper jacket covers the base and cylindrical portion, leaving a soft metal at the tip.

**Other Sources of Residues**—Cartridge cases are usually composed of brass but the brass case may sometimes be coated with nickel which leads to this element being present in some residues. Materials present on the interior surface of a gun barrel can give rise to gunshot residues. Iron and oil as well as rust and fouling from previous shots can be found inside a gun barrel. Bullet lubricants are common in cartridges providing a smooth release of the bullet from the cartridge casing.

## Characteristics of Gunshot Residues

### Inorganic Components

Harrison and Gilroy (9) first suggested that inorganic constituents of residues produced by the primer discharge might be the most characteristic components of gunshot residues. Most primers produce residue deposits that contain lead, antimony, and barium. These elements normally become more abundant on the hand after a handgun is fired. Lead and antimony are potentially present in

the form of free metal from the bullet or as chemical compounds formed during combustion. Barium is present in compound form. The chemical composition of these compounds can have great influence on both potential residue collection and elemental analysis techniques. However, methods for the determination of inorganic molecular composition are more difficult to apply than those for elemental analysis. It is unlikely that extensive information regarding the molecular composition of inorganic gunshot residues will be available in the near future.

Several techniques have been used to determine the elements in gunshot residues. Neutron activation analysis (NAA) indicates that a hand sampled for residues after discharging a handgun usually contains on its surface about 1 $\mu$ g of barium and 0.1 $\mu$ g of antimony. Copper is often found in residue hand samples, but its frequent presence as a hand contaminant would make it a poor candidate for an indicator of gunshot residues. Atomic absorption spectroscopy (AAS) has been used to measure, in addition to barium and antimony, lead residues. Approximately 1 $\mu$ g of lead is commonly deposited from a handgun discharge (34). These techniques use a bulk quantitative elemental analysis approach which measures total content of barium and antimony (NAA) or barium, antimony, and lead (AAS) that has been collected from specific regions of the hands. The deficiency of the bulk analysis methods lies in the lack of specificity for gunshot residues because total quantification includes environmental and occupational level contributions. Because inorganic gunshot residue particles are formed during rapid cooling from extreme temperatures and high pressures, in order to minimize their surface area they are predominantly spherical in shape. Scanning electron microscopy combined with energy dispersive X-ray analysis (SEM/EDX) has therefore been used to observe the characteristic spherical shape of inorganic gunshot residue particles and to determine their unique elemental composition (lead, antimony, and barium). Because SEM/EDX characterizes individual gunshot residue particles through both morphological and elemental characteristics, it is superior to the bulk analysis methods. The main disadvantage of SEM/EDX is that it is very time and manpower consuming, but this has been overcome to some extent by the advent of automated instruments (15,16). However, SEM/EDX was reported to be unable to identify inorganic residue particles in several test firings where the results of organic residues detection were all positive (35). Furthermore, there is no antimony used in some brands of caliber .22 ammunition and additionally in a number of newly developed cartridges, lead and other toxic elements were eliminated from the primers and the lead cores of bullets are fully jacketed with other metals (7). The major constituents of gunshot residues derived from these types of ammunition are not specific and as a consequence, the applicability of SEM/EDX to the detection of these types of gunshot residues is restricted.

Two interesting studies have been carried out by Zeichner et al. (36,37) one reporting on the levels of mercury on the hands of the firer from ammunition containing mercury fulminate as primer, and the second study on the residues obtained when using different ammunition in the same firearm. In the former report the amount of mercury found on the hand of the firer was much less than expected whereas for the latter the exceptional compositions of the residues was reported as being different from the classical expectations. This is explained by the fact that mercury would be expected to vaporize more from GSR particles moving with the burning propellant and ejected onto the firer than particles remaining in the cartridge case.

TABLE 1—Compositions of some single-base powders (% w/w) (29).

NC	Ba(NO <sub>3</sub> ) <sub>2</sub>	KNO <sub>3</sub>	DPA	DNT	MC	DBP	Others
80.00	8.00	8.00	0.75	—	—	—	3.25
89.00	6.00	3.00	1.00	—	—	—	1.00
87.00	6.00	2.00	1.00	—	—	—	4.00
84.00	7.50	7.50	1.00	—	—	—	—
99.00	—	—	1.00	—	—	—	—
97.70	—	—	0.80	—	—	—	1.50
90.00	—	—	1.00	8.00	—	2.00	—
85.00	—	—	1.00	10.00	—	5.00	—
79.00	—	—	1.00	—	—	—	20.00
98.50	—	—	1.00	—	—	—	0.50
87.9–96.9	—	—	1.00	1.00–10.00	—	—	1.10

NC: nitrocellulose; DPA: diphenylamine; DNT: dinitrotoluene; MC: methyl centralite; DBP: dibutyl phthalate.

TABLE 2—Compositions of some double-base powders (% w/w) (29).

NC	NG	EC	DEP	DMP	DNT	DPA	Others
77.45	19.50	0.60	—	—	—	—	2.45
52.15	43.00	0.60	3.00	—	—	—	1.25
51.50	43.00	1.00	3.25	—	—	—	1.25
56.50	28.00	4.50	—	—	11.00	—	—
60.00	37.80	0.90	—	—	—	0.2	1.10
47.00	37.70	1.00	—	14.00	—	—	0.30
58.60	24.20	1.00	—	9.60	6.60	—	0.10
58.5–83.5	15.4–40.0	1.00	—	—	—	—	0.50

NC: nitrocellulose; NG: nitroglycerine; EC: ethyl centralite; DEP: diethyl phthalate; DMP: dimethyl phthalate; DNT: dinitrotoluene; DPA: diphenylamine.

## Organic Components

Earlier gunshot residue detection methods were based on analyses for nitrates and nitrites in the gunpowder combustion products. The exact chemical compositions of the residue materials responsible for the reactions in these tests are still unknown, although it is likely that unburned gunpowder particles were present in the residues.

Particles 0.07 to 0.0001 cm in size have been observed in residues deposited from a Browning .380 semiautomatic pistol. When particles 0.05 to 0.01 cm in size are examined by optical microscopy, they resemble the larger smokeless powder particles of the unfired ammunition (38). These particles have been shown to produce the streaking blue reaction used to detect nitrate and nitrite-bearing compounds in the dermal nitrate test (34). If they are produced from unburned smokeless powder fragments, they may contain substantial amounts of nitrocellulose and nitroglycerine as well as the other constituents of smokeless powders mentioned previously (see Section Gunpowder's).

Bratin and Kissinger (19) have used high performance liquid chromatography with electrochemical detection to detect gunshot residues especially nitroglycerine and 2,4-dinitrotoluene recovered from cotton swabs taken from the back of a firer's hand. Diphenylamine also was detected by electrochemical detection in the oxidative mode but this was more difficult than the determination of nitroglycerine because the concentration of the latter in smokeless powder is much smaller than that of the former.

Using size-exclusion and high performance liquid chromatography with electrochemical detection, Lloyd (35) analyzed gunshot residue samples from both the firing hand and firer's clothing. He found that nitrocellulose, nitroglycerine, and diphenylamine could all be sensitively detected, but importantly, he also found that some swabs collected from people with no known firearms contact contained various amounts of diphenylamine.

Dahl et al. (24), using the same technique as the previous author, detected both diphenylamine and ethyl centralite in gunshot residue samples discharged from 20 different handguns of various caliber's using 20 different ammunition types. These workers found that 19 out of the 20 gunshot residue samples contained at least one of these two stabilizers, with 14 samples containing diphenylamine and 10, ethyl centralite.

Kee et al. (39) used gas chromatography/mass spectrometry to detect the gunshot residue particles from the discharge of a Wesson .357 magnum revolver. These residues were found to contain both nitroglycerine and diphenylamine.

Northrop et al. (40) used adhesive film to lift gunshot residue particles from a firer's hand. The lifts were examined under a stereomicroscope and suspect particles removed and extracted with ethanol. The extracts were then subjected to micellar electrokinetic capillary electrophoresis analysis after evaporation to dryness and reconstitution in buffer solution. Gunpowder constituents such as nitroglycerine, diphenylamine, N-nitrosodiphenylamine, 2-nitrodiphenylamine, ethyl centralite, and dibutylphthalate were detected on residue particles from four different types of handgun ammunition. These workers also found that there were compositional differences between gunpowder's from different manufacturers. Their results also demonstrated that both the unfired gunpowder and the gunshot residue particles from the same gunpowder were similar in composition.

Nitrocellulose is the main component of all smokeless powders and as a consequence is present in organic gunshot residues. It has been reported that the amount of nitrocellulose on hands and

clothing of the firer can be as high as 11  $\mu\text{g}$  (41). Nitrocellulose is however, also in widespread use in many consumer products such as lacquers and paints. Although, because of its relatively high molecular mass range, nitrocellulose from smokeless powders can be differentiated from those from environmental sources by size-exclusion chromatography (42), nitrocellulose in gunshot residues tends to be degraded in molecular mass, and therefore may not be distinguishable in the presence of environmental nitrocellulose (35). Thus, as evidence of firearms contact, traces of nitrocellulose are of little value.

Nitroglycerine is not only used in double and triple base gunpowder's but also in pharmaceutical products in which its role is as a cardiac stimulant for those suffering from angina pectoris. Although the presence of nitroglycerine in hand swab samples is of evidential value, such evidence is of much greater value when 2,4-dinitrotoluene is also present. The evidential value of the presence of nitroglycerine in samples vacuumed from clothing is higher than that taken from hand swabs because it is subjected to less environmental interference.

Several workers (19,24,41) have considered the possibility that the detection of diphenylamine may be taken as evidence of the presence of gunshot residues and diphenylamine can be detected on hands uncontaminated by firearms residues, occasionally in very large amounts. However, Lloyd (43) thought that relevance might be attached to the degradation products such as nitrated diphenylamines formed on storage and on firing. Unfortunately, the amounts of these products in residues are well below the detection limits of currently available techniques in all but rare practical circumstances.

Ethyl centralite was recommended by Mach et al. (44) as the most characteristic material in smokeless powders, with 2,4-dinitrotoluene being the second most characteristic material. Other known uses of ethyl centralite and 2,4-dinitrotoluene are scarce but in addition to its use in smokeless powder, ethyl centralite is used as an additive to solid rocket propellants (45).

## Persistence of Gunshot Residues

Gunshot residues deposited on a person are continuously lost as a result of normal activities and as a consequence, it is difficult to generalize as to the period over which the residues would be retained. However, the length of time during which gunshot residues remain on hands and clothing of a firer is an important factor in evaluating evidence. If the residues are retained over a period of days, then gunshot residues found on a person's hands or clothing might have resulted from a firing several days prior to the incident under investigation.

Kilty (46) has reported the effect of hand activity and time on the persistence of gunshot residues found on the hands. Persons who test-fired guns had their hands examined for antimony and barium at various timed intervals after shooting. The shooters' activity was unrestricted after firing, except that hand washing was forbidden. This study led to the conclusion that 2 h following firing, substantial amounts of antimony and barium were removed.

Importantly, the same worker (46) reported no evidence of gunshot residue deposition remaining on the hands of a shooter after the hands were washed with soap and water and then dried with paper towels. Activities shown to remove substantial amounts of antimony and barium include rinsing the hands under low-pressure aerated water for 3 s, wiping the hands on clothing, and placing the hands in pockets three times. In this study, a significant amount of primer residue still remained on the hands of the shooter after

they placed their hands in their pockets three times. A transfer of antimony and barium from the shooting hand to the nonshooting hand was noted when hands were wiped with towels following a shooting and when the shooter was handcuffed with his hands behind his back and then transported in an automobile.

It has also been reported that nitroglycerine residues on the shooter's face, throat, and hands may be retained up to 7 h (35). For unwashed hands of suicides, deposits may be present for 48 h and perhaps for a much longer period (18).

A contradictory result has also been reported by Douse (17). In this author's study, no nitroglycerine was detected on hands 0.5 h after 11 test firings carried out with a variety of weapons and ammunition. The persistence of gunshot residues on cloth is much greater than that on skin. In test firings which were carried out with a revolver, nitroglycerine, nitrocellulose, and diphenylamine have been detected on a variety of clothing types, 6 h after the firing had taken place. A jumper removed just after firing and stored, undisturbed, was found to retain readily detectable amounts of gunshot residues when examined the following day. No gunshot residues could be detected on the firer after the same time period. This suggests that the loss of gunshot residues is due to physical disturbance rather than any chemical degradation (41). It is further reported that the residues deposited on a cotton sheet placed one metro from a revolver which fired five shots, remained detectable for a period of two months providing the sheet was undisturbed during this period (41).

Lloyd (35) reported that nitroglycerine could be detected on garments which were worn with unrestricted activity for as long as 5 days after test firing. Whether or not the prolonged persistence of the residues on clothing is of value depends on the availability of firearms in the relevant community. Clearly, much greater significance attaches to the results if access to firearms is restricted.

**Gunshot Residue Deposition**—When a weapon is discharged, gunshot residues are mixed with propulsive gases and deposited on nearby objects, including the person who fired the weapon. The amount of deposition is determined by several factors such as the type and condition of weapon, number of shots fired, the amount of oil, moisture or perspiration material on the deposited surface, and the direction and force of air currents (34). The following discussion of the deposition of gunshot residues considers deposits from handguns and long guns on the firer and the target.

#### *Gunshot Residue Deposition on the Target*

If the muzzle of the discharging firearm is sufficiently close, gunshot residues will deposit on the target in an approximately circular area around the bullet hole. The residues include not only those burned and unburned primer and propellant materials ejected forward with the bullet, but also some material ablated from the bullet or jacket. The deposition of gunshot residues around holes in tissue, clothing, wood, metal, and glass permits identification of the bullet entrance holes. However, it has been reported that when the target is backed with a glass pane, another residue pattern is produced on the reverse side of the target (47). This may also be true for other backing materials but this is not commented upon by the authors.

If the muzzle of the weapon is in contact with the target or within approximately 0.5 in., gunshot residues are generally absent (25). This is due to the lack of space available for expansion which results in any gunshot residues penetrating the target through the entrance hole. When the muzzle to target distance increases, gunshot residues will disperse and this distribution will permit an

assessment of the distance from which the weapon was fired. A more precise assessment can be made from repeated firings at described distances from a target and observation of the spread of the residues. To obtain satisfactory results for comparison, testing firings have to be performed with: (a) the suspect weapon; (b) the suspect ammunition or the same type, brand, and year of manufacture; and (c) material of the same nature and quality as the material of the target (7). Any comparison to be made may relate to the geometric area and the concentration of residue. A study using porcine flesh as target material demonstrated that the three elements antimony, barium, and lead were deposited on the target up to 4 and 3 ft from a pistol and 38 caliber revolver respectively (48).

### **Gunshot Residue Deposition on the Firer**

#### *Handguns*

During discharge, revolvers and pistols leak residues from parts of the gun near the firing hand. There are three sources of deposit: (1) Leakage from gaps in the firing mechanism. Revolvers have a small gap between the cylinder and the rear end of the barrel. Some gunshot residues are leaked from this gap and deposited on the firer during discharge. (2) Emission at ejection of the cartridge case. In semiautomatic pistols, as the bullet moves out of the barrel, another mechanism opens the breech and ejects the spent cartridge case. During this process, some gunshot residues are released and deposited on the firer. (3) Blowback from the muzzle cloud. The major portion of gunshot residues are emitted from the muzzle of the weapon and some of these residues may flash back and be deposited on the firer. Krishnan (49) concluded that pistols and revolvers deposit more residues on the firing hand compared to the nonfiring hand, but this may not always be the case because the concentration and/or location of the residue depends on the shooting stance and grip used. Generally for this type of weapon, the larger the caliber, the larger is the likely amount of gunshot residue to be deposited on the hand. The gunshot residues on the firing hand are mostly deposited in the web area (34). This is the V-shaped part of the hand between the thumb and forefinger facing upward when the handgun is fired and is the area in closest contact to gases escaping along the side or back of the gun during discharge. Residues may also be deposited on the forearm or sleeves and the front of the chest from both revolvers and pistols (26,34). Other skin surfaces such as face and throat have also been reported to be useful sources of gunshot residues (35). Mere handling or loading of a firearm, as opposed to firing, is inferred from the detection of gunshot residues on the palm or inside of the hand (34).

**Long Guns**—After firing some shotguns and rifles, gunshot residues can be detected on the hands and cheek of the firer but the amount of the residues is usually less than that of common handguns. When both hands are used to fire the long guns, because the nonfiring hand is much closer to the muzzle than the firing hand, then more gunshot residues, due to flash back, may be deposited on the nonfiring hand than the firing hand. The shorter the barrel, the closer the nonfiring hand is to the muzzle. Hence, more gunshot residues may be deposited on the nonfiring hand in the case of shotguns and rifles (49).

#### *Criminalistic Uses of Gunshot Residues*

The most common objective of gunshot residue analysis is to determine whether or not a suspect fired a gun. Information from

this determination is usually applied as an investigative aid to confirm or negate an alibi, to decide if a suspect should be detained, to induce an admission or confession, to discriminate between homicide or suicide, and to distinguish among several suspects. However, gunshot residues can also be deposited on the bodies of persons other than the firer of the weapon. For example, in a struggle when a gun is discharged, any hands close to the muzzle of the weapon or anyone handling a wound or clothing containing a close range bullet hole may become contaminated (49). Thus the interpretation and use of gunshot residue analytical results should be carried out with care.

Gunshot residues are also often used to answer the question of whether or not the hole in a piece of material is a bullet hole. Additionally, whenever possible, it is also used to determine the firing distance. Neutron activation analysis by Capannesi et al. (50) for determining both the distance and the firing angle using antimony as the target element. Test firings enabled distances to be assessed within  $\pm 2$  cm and the angle to within  $\pm 5^\circ$ . Nag and Sinha (51) have proposed two different methodologies for estimating firing distance using discharge residues. Little success has been achieved in using gunshot residues to provide investigative leads other than those described. The use of gunshot residues to estimate the weapon caliber or weapon type has been shown to be unreliable (34).

#### *Choice of Approach to Organic Gunshot Residue Analysis*

There are a number of different analytical methods used for the identification and characterization of gunshot residues. Which analytical procedure is to be used depends on whether organic residues derived from the propellant or the inorganic residues derived from the primer components of a cartridge, are to be analyzed. Highly specific results may be obtained by scanning electron microscopy/energy dispersive X-ray analysis (SEM/EDX) of the inorganic residues. If all the requirements for the SEM/EDX method of identification of inorganic residues are met, the additional contribution towards the evidence of organic residues is relatively small. However, when the inorganic residues have not been recovered, or when their characteristics are poorly specific, evidence may be forthcoming from an examination for organic residues. Furthermore, for unknown reasons, it is found that there is a rough reciprocity between the amounts of inorganic and organic residue detected (43). That is to say when large amounts of inorganic material are found, organic residues may add little further information but where they are not found or are only found at very low levels, then organic residues may be informative. When used together the two approaches substantially increase the number of cases in which firearms contact may be substantiated.

### **A Surveys of Methods for the Identification and Characterization of Organic Gunshot Residues**

#### *Inorganic Gunshot Residue Components*

The identification and characterization of gunshot residues (GSR) has always been of great interest to forensic scientists. Because Harrison and Gilroy (9) drew attention to the fact that gunshot residues contain trace amounts of Ba, Sb, and Pb, a number of analytical methods have been developed for the detection of these characteristic elements. Surveys by DeGaetano et al. (52) and Singer et al. (53) revealed that gunshot residue detection methods used by forensic science laboratories in the United States are scanning electron microscopy/energy dispersive X-ray analysis

(SEM/EDX) (10,11), flameless atomic absorption spectroscopy (FAAS) (12,54,55), neutron activation analysis (NAA) (13), anodic stripping voltammetry (ASV) (14), and chemical tests. Although Hellmiss et al. (56) have reported on the use of Auger spectroscopy, there seems to have been little general application of this technique. FAAS, NAA, and ASV represent quantitative analyses of the bulk content of Ba, Sb, and Pb collected from specific area of a hand. The amounts of these elements, beyond certain established threshold limits, are considered to be characteristic of gunshot residues. However, the environmental and occupational contributions of these elements to any measured values severely restrict the ability of bulk analysis to conclusively identify gunshot residues. The observation that the quantity of these elements present on skin surface decreases with the lapse of time also leads to inconclusive results.

Scanning electron microscopy/energy dispersive X-ray analysis (SEM/EDX) is potentially superior because it characterizes individual gunshot residue particles both morphologically and elementally. Its ability to detect as little as a single gunshot residue particle also makes conclusive determination of gunshot residues possible after longer lapses of time. Even the main disadvantage of SEM/EDX, the excessive time required for search and identification, has been overcome by the advent of automated instruments (15, 16,57,58). SEM/EDX offers many advantages over other methods and is reported to have success rates for homicide/assault cases, suicide/homicide decision, and suicide verification of up to 79%, 89%, and 89% respectively. See also the report by Denis et al. (59). An important application of this technique to lead free ammunition has been reported by Gunaratnam and Himberg (60) where they find that the most important particles arising from Sintox ammunition contain titanium and zinc. Additionally, the surface morphology of the particles is an essential discriminator. Lloyd (35) has reported that after several test firings organic gunshot residues could be detected on a number of occasions where SEM/EDX was unable to find any inorganic gunshot residue particles. When the inorganic residues have not been recovered, or when their element characteristics are poorly defined, it is necessary to analyze for organic gunshot residues to provide complementary data and additional confirmation for SEM/EDX results.

The organic constituents of gunshot residues which have been discussed in depth above will be briefly reviewed. The remainder of this section will describe and assess in detail, sampling methods, sample pretreatment techniques, and analytical methods for organic gunshot residue detection. Some of the techniques are no longer in use, but for completion they have been included.

#### *Organic Gunshot Residue Components*

The sources of gunshot residues include every part of the ammunition used, but the main contribution comes from propellant and cartridge primer. In connection with its work on a mass spectrometric approach to the analysis of gunshot residues, the FBI laboratory has compiled a list of 23 organic compounds that may occur in smokeless gunpowder's (61). These compounds are listed in Table 3 together with nitroguanidine. Nitrocellulose is the main ingredient of all smokeless powders (62) and will invariably occur in GSR's. Nitroglycerin is present in all double-base gunpowder's (48). Diphenylamine (DPA) and its derivatives (63-65), the centralites (64), and resorcinol (63,65) are used as stabilizers in smokeless powders. Although triacetin (TA), dimethyl phthalate, diethyl phthalate, and dibutyl phthalate (63,65,66) are mixed with the powder components to act as plasticizers. Some smokeless gunpowders contain dinitrotoluene (67) which has the role of a flash

TABLE 3—Organic compounds that may be found in smokeless gunpowder.

Cresol	RDX (Cyclonite)
Resorcinol	Diethyl phthalate
Carbazole	Nitroglycerin
Diphenylamine	Trinitrotoluene
Dimethyl phthalate	Dimethylsebacate
N-Nitrosodiphenylamine	N,N-Dimethylcarbanilide (Methylcentralite)
Dinitrocresol	2,4-Dinitrodiphenylamine
Carbanilide	N,N-Diethylcarbanilide (Ethylcentralite)
Nitrodiphenylamine	Dibutyl phthalate
Triacetin	PETN (Pentaerythritol Tetranitrate)
Nitrocellulose	N,N-Dibutylcarbanilide (Butylcentralite)
Dinitrotoluene	Nitroguanidine

suppressor. Nitroguanidine (67) is another flash suppresser used in smokeless powders. Although it is not included in the FBI list, it is contained in Table 3 for completeness.

**Sampling Methods**—Considerable effort has been directed toward the development of satisfactory techniques for the collection of gunshot residue samples. The decision to collect residues is often made by the arresting officers, who must collect samples immediately after apprehension of a suspect because residue deposits normally “wear off” within a few hours. Therefore, the collection procedure must be simple and fast, and the possibility of external contamination must be avoided. The current techniques used for organic residue collection, include swabbing and vacuum, and tape lifting are summarized below. Washing and film lifts are generally used to collect inorganic gunshot residues from hands (See also 68,69) and will not be discussed here. The use of human nasal mucus as a media for the recovery of GSR’s has been reported by Schwartz and Zona (70). Successful recovery was achieved 48 h after the firearm was discharged.

### Swabbing

Swabbing is the most common technique used for collecting organic compounds from hands. For swabbing, sampling is conducted by repeatedly scrubbing the appropriate area of skin with certain material. Recommended materials include a ball of cotton (18,71–73), synthetic wool (74), filter paper (49,75), a piece of cloth (9,76), filter cloth (35), or acrilan fiber (77). The material used is moistened with an appropriate solvent before swabbing. The typical solvents recommended by different authors for organic gunshot residue sampling are acetone (78), ether (72), methyl tertbutyl ether (79), alcohol (809), isopropanol (34,77), and isopropanol/water (8:2 V/V) (81). Whichever solvent is chosen, the aim must be to maximize the transfer of gunshot residues to the swabs using a minimum amount of solvent. Seven different solvents (eight solvent systems) have been studied by Twibell et al. (82) for use in the removal of nitroglycerine from hands with 30 mg cotton swabs. Their results indicated that: (1) Acetone or even water alone produces the best recovery; the water extract being further extracted with ether. This was followed immediately by cyclohexane and ethanol as the next best. Very similar recoveries were obtained for aqueous buffer (pH 7), and detergent (both followed by ether extraction). The lowest recoveries were obtained for ether on its own and water, the latter being followed by cyclohexane extraction (2). Water and detergent extracted the least interfering materials. Ethanol was satisfactory, but acetone and ether coextracted interfering materials. (3) The use of distilled

water as the swabbing solvent is simple and safe. Moreover, organic and inorganic materials can be simultaneously removed from the hands and then separated by solvent extraction. Unfortunately, microorganisms can grow in aqueous media and degrade the explosive. Extracted nitroglycerine was more stable in ethanol than water and acetone. (4) On the basis of the above mentioned factors, ethanol appeared to be the best compromise organic solvent for removing nitroglycerine from hands using cotton swabs.

The most common technique used to recover organic residues from the swabs is to leach out the swabs with solvents. In order to avoid problems associated with concentration of the sample, impurities present in the solvent, and loss of the more volatile explosives during concentration, a minimum amount of solvent should be used. The commonly used recovery methods are: (1) Direct extraction (72): With the help of a glass rod, swabs are extracted by successive washing with small portions of the solvent. (2) Constricted tube technique (83): A test tube is drawn to produce an extension tube with a narrow hole at the bottom. The swabs are compressed into the tube using a glass rod and compounds in the swabs are eluted by repeated washings with a small volume of solvent. (3) Squeeze method (83): Solvent is added to the swab in a storage vial and the swab is squeezed and pressed against the inside wall of the vial using forceps. As much liquid as possible is removed by squeezing the swab on the inside of the vial neck. This solvent is then removed to another vial and the same procedure repeated with further volume of solvent and the extracts pooled. (4) Syringe elution (83): The swabs are compressed into the base of a 5-mL glass syringe and the plunger is pressed down to expel any solvent. A portion of solvent is drawn up into the syringe and forced out through the swabs which are again compressed. This procedure is repeated and the extracts pooled. (5) Centrifugal microfilter extraction (83,84): All parts of a microfilter are pre-cleaned by Soxhlet extraction and assembled. The swabs are gently inserted into the sample compartment and centrifuged at 4000 rpm for 5 min to remove as much solvent from the swabs as possible. This is collected as the first fraction. Two or three subsequent centrifuge fractions are collected after adding further quantities of solvent.

Twibell et al. (83) have carried out a study to compare the relative efficiencies of some of these techniques for extracting some common explosives. They found that the constricted tube extraction gives more variable recovery efficiency than other methods because of inconsistency generated in packing the swab. Although efficient extraction can be obtained by every studied technique using the correct manipulation procedure, with the exception of centrifugal microfilter extraction the final extract volume will be of the order of 3 to 5 mL. The use of centrifugal microfilters gives extraction volume less than 1 mL thereby reducing the amounts of solvent impurities which will be concentrated prior to analysis. The technique will therefore diminish the loss of the more volatile explosives on evaporation.

**Vacuum Lifting**—Vacuum lifting is widely used for collecting organic gunshot residues from clothing (18,21,35,41,85). Clothing debris is vacuumed on to a filter disc, and then the filter is extracted with solvent to recover the organic gunshot residues. Its main advantages are its ease of operation and the fact that a larger area can be sampled within a short time. In the case of vacuum sampling from hands, a considerable amount of nitroglycerine was detected following a subsequent swabbing. This indicates that nitroglycerine is deposited both as particulate matter and as a condensing vapor

(41). The vacuum lifting technique removes only the particulate matter portion.

**Tape Lifting**—For the tape lifting method, sampling is carried out by pressing a single sided adhesive tape or a double sided adhesive tape onto the substrate surface. This technique has mainly been used to sample inorganic residues for SEM analysis. Northrop and MacCrehan (40) used tape lifts to collect organic gunshot residues for micellar electrokinetic capillary electrophoresis (MECE) analysis. Masking tape in 1 in. square sections used to collect the gunshot residues were observed under stereomicroscope and any suspect particles removed with tweezers and placed in a glass microvial. The particles were then extracted with 50  $\mu$ L of ethanol. A second approach made use of a direct ethanol extraction of a 2 cm<sup>2</sup> section from a tape lift in a microvial. These workers compared the tape lifting with the swabbing methods and found that swabbing recovered unwanted quantities of skin fats and oils causing matrix interference in analysis when the extract was concentrated. Only minute quantities of the interfering fats and oils were found using tape lifts. They concluded that adhesive tape lifting is an effective sample collection procedure for organic gunshot residues when using MECE as the analytical method.

### Sample Pretreatment

In many chromatographic analyses, the analytical systems are able to detect pure standards of organic gunshot residues down to low nanogram levels but such sensitivity cannot be achieved with extracts from handswabs or vacuumed clothing samples. This problem arises because such extracts are substantially contaminated with lipids and other co-extracted materials which result in deterioration of the separation column and the detector. A number of authors (18,35,72,79,84,86–88) have suggested that 'clean-up' is an essential pretreatment to minimize column deterioration and detection noise, even where the system used possess a high degree of selectivity.

Twibell et al. (88) used thin-layer chromatography as a clean-up procedure prior to analysis. The swab extracts were applied to the base line of a silica gel plate prior to development in a mixture of cyclohexane and toluene (70:30 V/V) under chamber-saturated conditions. After development the area of the plate containing nitroglycerine was removed and extracted with acetone. The clean-up procedure gave an order-of-magnitude improvement in detecting nitroglycerine in handswab extracts and reduced the contamination problems.

Douse carried out a series of studies on clean-up procedures for gas chromatographic analysis. In 1982 (72), he recommended using Amberlite XAD-7 porous polymer beads to remove interfering lipid material. As the first part of the process, the handswab extract was evaporated to dryness, redissolved in pentane and gently shaken with Amberlite XAD-7 beads. The beads were subsequently rinsed with pentane and extracted with ethyl acetate. At the second stage, the ethyl acetate extract was evaporated to dryness and redissolved in pentane. The Amberlite XAD-7 extraction was then repeated using fresh beads.

In a second report (79), Douse made use of an Amberlite XAD-7 column constructed from 18 mg of the beads in a silanized soda glass tube. The column was washed, in turn, with ethyl acetate and pentane just prior to use. The handswab extracts were evaporated, redissolved in pentane, and passed through the XAD-7 column. The column was then washed with pentane and eluted with ethyl acetate to desorb the organic explosives. The ethyl acetate extracts

were concentrated and analyzed by gas chromatography. He reported that the improved method resulted in a faster clean-up procedure, considerably less sample manipulation and higher recoveries of a range of explosives. In 1986 (17) and 1987 (73), he used a mixture of methyl tert-butyl ether and n-pentane instead of pentane to both dissolve the evaporated handswab extracts and to wash the XAD-7 column. The change of eluting solvent resulted in better selectivity of the XAD-7 procedure and much cleaner extracts.

In his study of 1989 (18), Douse used a dynamic headspace method to improve the clean-up procedure. Handswabs were placed in a luer-lock glass syringe fitted with filters. The methyl tert-butyl ether (MTBE) solvent on the swab was removed by a current of nitrogen and the swab was moistened with acetone and allowed to stand for 5 min. The acetone was then removed by a stream of nitrogen before the syringe was inserted into the dynamic headspace apparatus. The syringe was connected to an XAD-7 column and the syringe barrel was plugged. Nitrogen was passed through the syringe and XAD-7 column. The apparatus was then inserted into an aluminum block held at 100°C which was mounted in a heated unit. After 7 min, the column was removed, mounted vertically, and eluted in the following sequence: (a) pentane to elute nitrobenzene and 4-nitrotoluene; (b) pentane-MTBE (1:1, V/V) to elute unwanted coextractives; (c) ethyl acetate to elute nitroglycerine and other explosives. The glass syringe containing less volatile explosives was washed with MTBE. This solution was then passed through a freshly prepared XAD-7 column. Explosives were eluted with ethyl acetate.

It was found that although the headspace procedure was effective in separating nitroglycerine from less volatile compounds it was still necessary to selectively elute volatile impurities from the XAD-7 beads. The two-stage clean-up process permitted the final extract to be reduced to a very low volume and as a result a detection limit of about 100 pg of nitroglycerine per sample could be obtained.

Lloyd conducted another series of studies in clean-up procedures for high performance liquid chromatography. He used a mixture of octadecylsilylsilica and alumina adsorbent in a centrifugal microfilter assembly to clean-up swabs (84). The swab was inserted into the top of a microfilter in which the mixed adsorbent had already been placed. The assembly was attached to a vacuum manifold, and a silica desiccant trap was attached to the entrance of the filter. The solvent was completely removed from the swab by a flow of air. The outlet tip of the microfilter was washed with acetone and fitted with a tapered receiver tube. The swab was then treated with methanol-water (100:35 V/V) and centrifuged to collect the eluent. There are three important functions performed by the mixed adsorbent in the extraction assemblies. The first is the removal of lipids and strongly lipophilic materials. The second is the removal of the large amounts of highly polar reducible species present in some handswabs, which give rise to an intense broad peak that obscures the early part of the chromatogram when electrochemical reductive detection was used. Third, the adsorbent traps any explosive components that volatilize during the solvent removal stage.

In 1985 (87), Lloyd reported two kinds of microcolumns used in recovery and clean-up procedures. A reusable column was made from stainless-steel tubing in which the end-fittings contained a steel mesh. The tube was packed with Porapak T adsorbent. The sample, in aqueous solvent, was transferred to the column by way of the sample loop and washed through with aqueous methanol. Inorganic components and other highly polar compounds appeared



in the effluent at this stage. The elution was continued with solvents in a sequence depending on the compounds sought. A typical sequence was: (a) isopentane to desorb lipophilic materials, nitrobenzene, and nitrotoluenes; (b) diethyl ether to remove the remainder of the latter compounds and most other explosives except tetryl and HMX; (c) acetonitrile to complete the desorption.

A disposable column, made from PTFE tubing, was plugged with cotton wool at the outlet end. The extracts of handswabs were diluted with water and to them was added the adsorbents Porapak T, Chromosorb 104, and charcoal. The mixtures were shaken intermittently over 10 min, and the adsorbent then allowed to sediment and the supernatant removed. The adsorbent was washed with methanol-water (1:3 V/V) and drawn into a prepared empty microcolumn, the outlet of which was attached to a filter pump. The column was eluted according to the procedure described above for a reusable columns. The disposable columns were applied to heavily contaminated samples.

In 1986 (20), Lloyd reported a study of the adsorption and exclusion characteristics of nitrocellulose on 12 representative adsorbents under conditions relevant to the trace analysis of nitrocellulose by size exclusion chromatography. He found that the recovery of nitrocellulose in acetonitrile from Porapak T was higher than those from the other adsorbents. He also found that nitrocellulose was strongly adsorbed onto Porapak T when a mixture of acetonitrile and diethyl ether was used as eluent. He therefore suggested a microcolumn clean-up technique that enabled nanogram amounts of nitrocellulose, in contaminated firearms propellants residues, to be characterized. The microcolumn was made from a 40 by 0.6 mm PTFE tube into which a Porapak T slurry in acetonitrile was packed. The acetonitrile handswab extracts was diluted with a 1.5-volume ratio of diethyl ether, centrifuged to remove precipitated material, and pumped through the column. The column was rinsed with 200  $\mu$ L of further solvent mixture, and then eluted with acetonitrile to recover the nitrocellulose.

In his other works (21,35), Lloyd used the above mentioned microcolumn techniques (20,87) to analyze for nitroglycerine, 2,4-dinitrotoluene, and nitrocellulose in gunshot residue samples. In 1990 (81), he used amberlite XAD-4, instead of Porapak T, and Chromosorb-104 as adsorbents packed in microcolumns. The adsorbed nitroglycerin or 2,4-dinitrotoluene from gunshot residues were recovered by eluting with acetonitrile/water (25:12 V/V) at a flow rate of 1  $\mu$ L/s, and the fraction eluting between 35 and 90 s was retained for HPLC analysis.

## Analytical Methods

### Chemical Tests

The earliest technique used in gunshot residues detection is the well-known "dermal nitrate test" or "paraffin test." Detection is based on the color reaction produced by nitrites and nitrates present in gunshot residues to diphenylamine in sulfuric acid. This diphenylamine reaction is not specific to nitrates and nitrites because other oxidizing agents such as chlorates, dichromates, iodates, bromates, permanganates, higher metal oxides etc. may also cause the reaction and lead to "false positive" results. For reasons not well understood, negative results can be obtained from the paraffin test, even where it is known that a person has fired a gun and has not subsequently washed his hands. Because of its unreliability, the paraffin test has been abandoned by forensic scientists.

Another chemical test used to detect the nitrites in gunshot residues is the Griess reagent which is based on the formation of a diazonium salt when nitrite in acid solution reacts with a primary

aromatic amine. A common example is a solution of  $\alpha$ -naphthylamine and sulfanilic acid in acetic acid. The sulfanilic acid reacts with nitrous acid to yield a diazonium ion which then couples with  $\alpha$ -naphthylamine to produce a red azo dye. The Griess test is specific for nitrites but not specific for gunshot residues.  $\alpha$ -naphthylamine is little used now because it is a powerful carcinogen but N-(1-naphthyl)ethylenediamine is a suitable replacement agent (89,90).

In 1937, Walker proposed a method to produce a permanent graphic reproduction of the powder residues pattern from a garment without damaging or significantly altering the fabric containing a bullet hole. For this purpose, a desensitized photographic paper which had been immersed in a warm 5% solution of "C acid" (2-naphthylamine-4,8-disulfonic acid) was used. A piece of this treated paper was laid face up on a table and the fabric containing the bullet hole was laid face down on top of the treated paper. On the top of this was placed, respectively, a thin layer of dry towel, a layer of towel moistened with 20% acetic acid and another layer of dry towel. The whole pack was then pressed with a warm iron for 5–10 min. A number of dark red spots which corresponded to the position of the partially burnt powder grains around the bullet hole appeared on the treated paper (29).

Sinha and Misra (91), in 1971 used a filter paper which was treated with a mixture of  $\alpha$ -naphthylamine and sulfanilic acid to detect gunshot residues on the surface of skin and clothing. Tewari (92), in 1974 used an acid washed Whatman filter paper to remove nitrates and nitrites arising from gunshot residues from suspected areas. The paper was then cut into three sections. One section was treated with 1% solution of antazoline hydrochloride in 5 N HCl. The development of a bright yellow color indicated the presence of nitrites. The second piece of paper was sprayed with 1% aqueous antazoline hydrochloride followed by concentrated sulfuric acid. Development of a deep red color indicated the presence of nitrates. The initial appearance of an orange color indicated the additional presence of an excess of nitrites. The third section was sprayed with 5% sulfamic acid which was followed by 5% antazoline hydrochloride in concentrated sulfuric acid. The development of a deep red color indicated the presence of nitrates.

Steinberg (89) et al. in 1984 described the use of a modified Griess reagent for the qualitative and quantitative spectrophotometric determination of nitrites originating from gunshot residues. The reagent used was sulfanilamide and N-(1-naphthyl)ethylenediamine dissolved in phosphoric acid. For the qualitative determination of nitrites on cotton swabs, the swab was treated with hydrolysis reagent (8% KOH) at 100°C for 5 min and then treated with a modified Griess reagent. The appearance of a pink-violet color indicated the presence of nitrites. For the quantitative determination of nitrites, the acetone extract of a cotton swab was evaporated to dryness and then treated with hydrolysis and modified Griess reagents. The reaction mixture was diluted with deionized water and subjected to spectrophotometric determination. The characteristic absorbance of the modified Griess reaction was found to be at 540 nm. These workers found that the hydrolysis step preceding the application of Griess reagent was crucial for the detection of gunpowder nitrite residues. The detection limit of the modified Griess reagent was found to be 0.1  $\mu$ g which was better than that of the classic Griess reagent (0.5  $\mu$ g).

Cole and Thorpe (93), in 1992 proposed a method for detecting nitrites, lead, and copper from gunshot residues using a single paper lift. Gunshot residues deposited on cotton denim fabric were transferred to a Whatman filter paper dampened with Griess reagent. After the Griess test, the same paper was sequentially

tested for lead using sodium rhodizonate and for copper using ammonia and benzoinoxime solutions. Detection limits obtained from analyte standards directly deposited onto filter papers were  $26 \text{ ng cm}^{-2}$ ,  $1.3 \text{ } \mu\text{g cm}^{-2}$ , and  $1.1 \text{ } \mu\text{g cm}^{-2}$  for nitrites, lead, and copper, respectively. These workers found that lead and nitrite from muzzle gases were detected at short range test firings only. At longer ranges, the deposits detected were lead and copper wipe from bullets.

The use of lead free ammunition has demonstrated a requirement for additional chemical screening tests. One such test (94) makes use of zincon reagent to detect copper, zinc, and titanium which occur after the discharge.

### *Thin Layer Chromatography*

Thin layer chromatography (TLC) is one of the simplest and most widely used chromatographic techniques and procedures for its use are well documented in standard texts (95).

Using TLC Booker (96) analyzed dichloromethane extracts of 37 smokeless powders together with solvent washings from fired cases and compared these with gunshot residues which involved the same powders. Although the individual components were not identified, it was found that the chromatograms of unfired powder, a grain of fired powder, and washings from a fired cartridge case were identical.

Peak (30) used sequential TLC to separate nitrocellulose (NC) from nitroglycerin (NG) and to distinguish propellant-grade nitrocellulose from nonpropellant-grade products by differences in the length of the cellulose polymer chain. The unburned or partially burnt flakes of smokeless flakes were removed under a microscope using fine forceps and extracted with acetone. The concentrated acetone extracts were then subjected to a sequential TLC separation. The TLC plate spotted with acetone extract was firstly chromatographed in a methyl cellosolve/ethanol (15:85) solvent for 2.5 cm. The solvent was allowed to dry, and the plate was rechromatographed in a toluene/petroleum ether/ethyl acetate (12:12:1) solvent for 5.0 cm. The developed plate was treated with 0.1 N NaOH, heated at  $100^\circ\text{C}$  for 6 min, and visualized with Griess reagent. In ethanolic solutions of methyl cellosolve, most of the propellant-grade NC remained at the origin, with a small amount moving with the solvent front. For nonpropellant NC, the process was reversed. NG also moved with the solvent front at this stage. For the second separation NG migrated with an  $R_f$  value of 0.3 whereas NC remained stationary.

Douse (72) used TLC to detect nitrocellulose on the upper surface of a firer's hand. Gunshot residues were sampled from the hand with cotton swabs moistened with ether which were then extracted with ether and centrifuged. The insoluble residues removed from the solution of the handswab extract in ether were then extracted with acetone, concentrated, and analyzed by TLC using Griess reagent spray to detect the nitrocellulose. Jane et al. (41) using TLC separated and identified nitroglycerine and nitrocellulose in gunshot residues obtained from a firer's hands and on clothing. It was reported that nitrocellulose on clothing could be detected by TLC after a time lapse of up to several hours after firing.

In addition to gunshot residue analysis, the TLC technique has been used by different workers to separate and identify minor components present in smokeless powders. Yasuda (97) separated 19 N-nitroso- and nitrodiphenylamines using a two-dimensional TLC system. A spray reagent, p-dimethylaminobenzaldehyde, used in conjunction with silica gel G/Zn plates, offered a sensitive

detection method for each diphenylamine. He also separated and identified the impurities in 2,4,6-trinitrotoluene by a two-dimensional TLC (98).

Kohlbeck (99) analyzed for nitroglycerine and resorcinol in double-base propellant by TLC whereas Parihar et al. (100) described a quick method of quantification of six explosive nitrates including nitroglycerine when presented as mixtures. Barnes (101) reported the separation and identification of nitroglycerine and four other nitrate esters by TLC.

A combination of TLC and spectroscopy was used by Macke (65) to analyze for typical plasticizers and stabilizers in aged double-base propellants. Nitroglycerine, triacetin, 2-nitrodiphenylamine, and resorcinol and their derivatives were well separated.

Using TLC Archer (31) analyzed the minor additives and stabilizers present in a range of 42 samples of smokeless powders from various manufacturers. This author found that the most commonly used stabilizer was diphenylamine and this appeared always to be associated with N-nitroso- and 2- and 4-nitrodiphenylamine. Nitroglycerine, 2,4-dinitrotoluene and the N,N'-dialkyl-N,N'-diphenylureas (centralites) were also present in the majority of the samples examined.

Espinoza and Thornton (33) used a TLC method to characterize 118 different gunpowder's based on the presence of diphenylamine (DPA) and its nitrated derivatives. Only 5 propellants from 118 samples could not be differentiated. The authors explained that the similarities in these gunpowder's could be attributed to samples having experienced similar environmental conditions, or they could be propellants that originated from a common source and were distributed to various cartridge loader manufacturers. The authors found that the most frequently detected stabilizers were DPA, N-nitroso-DPA, 2-nitro-DPA, and 4-nitro-DPA. This is consistent with the findings of Archer (31).

TLC is simple, rapid, moderately sensitive, and inexpensive. However, it suffers from a number of disadvantages such as offering poor quantification, it is time consuming in visualization and differentiation, and requires a relatively large amount of sample.

### *Gas Liquid Chromatography*

Gas liquid chromatography (GC) is one of the most extensively used analytical techniques for the separation of complex mixtures and its use and application have been detailed in standard texts (102). Flame-ionization, electron-capture, and thermal energy analysis (TEA) are the basis of some common detector systems used for gunshot residue analysis. The TEA has been demonstrated to be a very selective detector for explosives (18,79,103-107). In TEA, the explosive compound from the column is directed into a heated chamber maintained at reduced pressure. Catalytic pyrolysis of the nitro compound takes place here to yield nitrogen dioxide ( $\text{NO}_2$ ). This  $\text{NO}_2$  is readily converted to nitrosyl radical ( $\text{NO}\cdot$ ) under the same catalytic conditions. The pyrolyzed effluent then flows through a cryogenic trap, where most contaminants are condensed, but the nitrosyl radicals are swept into a reaction chamber containing ozone ( $\text{O}_3$ ) gas. When the ozone is mixed with  $\text{NO}\cdot$ , electronically excited nitrogen dioxide ( $\text{NO}_2^*$ ) is formed. This excited species then undergoes radiative decay to the ground state, emitting wavelengths of light within a rather broad range of between 600 nm and 1600 nm. However, as a result of response characteristics of the photomultiplier used, only light between 600 nm and 800 nm is detected, thereby making this device quite selective for analytes which can be pyrolyzed to form  $\text{NO}\cdot$  or  $\text{NO}_2$  (108).

Using gas chromatography-chemical ionization mass spectrometry Mach et al. (44) carried out a feasibility study of gunshot residue detection making use of organic constituents. In their work, two packed columns were used, one of which was operated isothermally at 175°C and the other was programmed from 160°C to 250°C at 15°C/min rate. In the first part of their study, they analyzed smokeless powders from 33 sources and showed that nitrocellulose, nitroglycerine, 2,4-dinitrotoluene, diphenylamine, dibutyl phthalate, and ethylcentralite were common major components. In the second part, they analyzed samples collected from firers' hands and found, using both morphology and organic composition, a suspect particle could easily be identified as a smokeless powder residue. Furthermore, there were shown to be no other volatile or volatilizable organic compounds in the residues other than those originally present in the propellant. They also identified ethylcentralite as the most characteristic material found in smokeless powders, followed by 2,4-dinitrotoluene and then diphenylamine.

In 1982 Douse (72) used a silica capillary column to analyze precleaned handswab extracts. The column was temperature programmed from 25°C to 240°C at 40°C/min rate and the eluate was detected by a tritium based ECD. The presence of nitroglycerine in gunshot residue handswab extracts taken from the upper surface of a firer's hand immediately after discharging three rounds of double-base propellant ammunitions, was demonstrated.

Using the method reported by Douse, Jane et al. (41) analyzed gunshot residues collected from firers' hands and clothing. They found that nitroglycerine could be detected on a woolen sweater and hands 6 and 2 h, respectively, after firing. However, because of insufficient sensitivity and the lack of selectivity, the usefulness of GC/ECD is reported as being limited.

In 1985, Douse (79) compared thermal energy analysis (TEA) and electron capture detection (ECD) for the analysis of explosives in handswab extracts. He reported that TEA approached the sensitivity of ECD but was more selective, enabling low nanogram levels of explosives to be detected.

Using capillary GC-ECD Douse and Smith (17) analyzed precleaned handswab extracts of gunshot residues sampled from both firer's hands and clothing. Various weapons and different types of ammunition were used in their study. They found that no nitroglycerine was detected on hands 0.5 h after firing. They also reported that the deposition of nitroglycerine on clothing was very variable (0–364 ng). In one instance residues were detected 5 h after firing. In 1987, Douse (56) used GC/TEA to determine nitroglycerine in gunshot residues vacuumed from clothing and found that the selectivity of the system was such that vacuumed samples could be analyzed for traces of organic residues without the need for clean-up of the samples. His work of 1989 (18) further verified the high selectivity of GC/TEA for organic gunshot residue detection.

In addition to using direct GC, Newton and Booker (109) have used pyrolysis GC to identify smokeless powder residues. They examined 40 different smokeless powders and found that the chromatograms of each powder was distinguishable from all others and that there was no difference between the partially burned powders, residues taken from the barrels of fired weapons, and the original powders.

In 1990, Kee et al. (39) reported a two-stage approach to the identification of single propellant particles. This involved a preliminary infrared microspectrophotometric examination of the suspect propellant grain to detect nitrocellulose, followed by a gas chromatography-mass spectrometry analysis of an extract. They found the IR technique was able to successfully determine nitrocellulose, but was of limited use in determining minor constituents such

as stabilizers in propellant grains. They observed a variation in analytical results between fired and unfired grains for some propellant brands. The GC-MS results revealed that the unfired propellant grains contained nitroglycerin, diphenylamine, and ethylcentralite, whereas the fired grains contained only nitroglycerine and diphenylamine. The reason for this analytical variation was not satisfactorily explained.

In 1991, Lloyd (107) reported a procedure for the analysis of forensic explosives and firearms traces using GC/TEA as a confirmatory technique. Trace amounts of explosive compounds, separated from cleaned-up extracts of handswabs and clothing debris by high-performance liquid chromatography (HPLC), were trapped from the chromatographic effluent onto a porous polymer (acrylonitrile or methacrylate copolymer) microcolumn for confirmatory examination by GC/TEA. The microcolumn eluate was injected directly into a GC retention gap of an unmodified silica capillary column. This enabled explosives with as low a volatility as HMX to be chromatographed. The retention gap was used not only as a guard column to reduce the effects of nonvolatile sample by-products on chromatographic performance, but also as a precolumn to reconcentrate solute bands that were broadened in the column inlet and thus to allow on-column injection of large sample volumes (110). The overall technique enabled the elimination of evaporative concentration procedures in the sample work-up, and minimized any question of whether the confirmation was responding to irrelevant components. Using this technique, Lloyd was able to detect nitroglycerine from articles of clothing which were known to have been in contact with firearms discharge residues.

GC is rapid, sensitive, and highly selective especially when TEA is used. It is able to analyze gunshot residues both qualitatively and quantitatively. The separating power of capillary column GC is unparalleled. However, the nitrate esters which are frequently found in gunshot residues are incompatible with the usual GC conditions because of their thermal instability and their tendency to decompose on improperly prepared columns. This is particularly true of PETN even for capillary column systems in which the response to PETN is seen to decline with use. This can be restored by proper cleaning of the injection system and removal of the front end of the capillary column. Furthermore, the main ingredient of smokeless gunpowder's, nitrocellulose, is a nonvolatile compound, and cannot therefore be analyzed by GC. GC was also reported to be inappropriate for the analysis of stabilizers such as N-nitrosodiphenylamine because it may be denitrosated to diphenylamine in the heated zones of the chromatograph (32).

#### *High Performance Liquid Chromatography*

The technique of high performance liquid chromatography (HPLC) has been described in standard texts (111) and will not be detailed here.

#### **Electrochemical Detection**

In 1981, Bratin et al. (19) used HPLC with electrochemical detection for the analysis of smokeless powders and gunshot residues. In the first part of their study, they analyzed eight smokeless powders and found that nitroglycerine and 2,4-dinitrotoluene could be detected when using the reductive mode, but diphenylamine, 2-nitrodiphenylamine, and 4-nitrodiphenylamine need to be analyzed in the oxidative mode. In the second part of their study, they applied these techniques to gunshot residue analysis. They reported that the determination of diphenylamine in gunshot residues was

more difficult than the determination of nitroglycerine because the concentration of diphenylamine in smokeless powders was much smaller than the latter compound. In addition, oxidative mode chromatograms were more complex than the reductive mode chromatograms because of interference from phenolic compounds which were present in the lipid layer of the skin. They concluded that this method provided a simple, rapid and inexpensive tool for determining components in explosives and gunshot residues. An advantage of this approach over metal residue analysis was the fewer false positive results arising from the fact that the only alternative source of nitroglycerine is as a pharmaceutical preparation. Detection of nitroglycerine and other components of smokeless powder was therefore indicative of firing a weapon or handling nitroglycerine-based explosive.

In 1983, Lloyd (84) used HPLC to analyze precleaned handswab extracts obtained from hands after firing a revolver. By using a pendant mercury drop electrode (PMDE) detector, he was able to detect down to 1 ng/swab of nitroglycerine.

In 1984, Lloyd (42) reported an approach to detect and differentiate nitrocellulose using HPLC and a size-exclusion column in combination with PMDE. He reported that nitrocellulose could be detected in amounts as small as 100 pg. The linear range of the detector extended to approximately 200 ng. Nitrocellulose in trace amounts was detected and differentiated when present in propellants, explosives, paints, lacquers, celluloids, adhesives, and a wood-filler composition.

In 1986 (20), Lloyd used a microcolumn clean-up procedure to remove interferential materials from gunshot residues samples, the pretreated extracts then being analyzed by size-exclusion chromatography with PMDE detection. This provided an approach to GSR analysis which enabled nitrocellulose to be characterized at a sensitivity approaching those of other explosive and propellant components.

In three reports (21,35,86), Lloyd used HPLC/PMDE to determine diphenylamine and nitroglycerin, and size-exclusion chromatography /PMDE to determine nitrocellulose in gunshot residue samples. Because diphenylamine is a widely used industrial intermediate and is used in the foodstuffs industry as a post-harvest treatment for apples against scald, he suggested that diphenylamine detected in the absence of other propellant components may not be attributable to firearms contact.

He also reported that nitrocellulose remaining after the discharge of a weapon tends to degrade, and may not then be distinguishable from the large amounts of environmental nitrocellulose sometimes present in clothing debris. Less interference is obtained from handswabs. Because of the considerable speed with which they are obtained, the nitrocellulose chromatograms can provide useful information in multisample work such as plotting the surface distribution of residues. In this respect they could be evidentially significant. For nitrocellulose detected alone, the position is similar to the case of diphenylamine.

Nitroglycerine is a characteristic component of double base propellants and is subject to less environmental interference than nitrocellulose. Lloyd found that traces of nitroglycerine on clothing could be detected by HPLC/PMDE many days after a weapon had been discharged. This is consistent with the GC work of Jane et al. (41) who detected nitroglycerine on clothing up to 6 h after discharging a firearm. He also found that the skin surfaces other than hands, such as face and throat might be more useful sources of residues.

Lloyd recommended that GC/TEA be used as an independent

confirmatory technique for HPLC/PMDE in organic gunshot residue detection. He reported a procedure (107) in which a four-port sampling valve was inserted between the HPLC column and the detector. This enabled selected peaks to be diverted from the detector line to a microcolumn installed in one of the two remaining ports. The microcolumn was packed with either Chromosorb-104 or Porapak-T to retain explosive components. To the 4th port of the valve was connected a microsyringe fitted with a micrometer screw gage. This enabled microliter amounts of an eluent to be delivered to the microcolumn while the HPLC effluent was directed to the detector after collection of an HPLC peak. The eluate of the microcolumn was then subjected to confirmatory examination by GC/TEA.

Dahl and Lott reported in 1987 (22–24) a series of works on gunshot residue determination. First, they proposed an approach to distinguishing the residues of black and smokeless gunpowder's (22). This process included chemical spot tests, microscopic examination, X-ray diffraction, and HPLC with electrochemical detection. They found that X-ray diffraction conclusively showed the presence of black powder whereas the HPLC procedure, in conjunction with spot tests was considered as conclusive for the determination of diphenylamine in smokeless powder residues. Because ethylcentralite is another stabilizer frequently used in smokeless powders, its detection would also be deemed indicative of smokeless powders.

In their second report (23), Dahl and Lott described a method for the determination of both organic and inorganic components in gunshot residues. They used HPLC with oxidative electrochemical detection to analyze the gunpowder stabilizers diphenylamine, ethylcentralite, and 2-nitrodiphenylamine and graphite furnace atomic absorption spectrometry to determine the trace metals Sb, Ba, and Pb. From their results they concluded that diphenylamine can be obtained from the handling of tires. Thus, it is important to have additional confirmation for a gunshot residue analysis by a second independent means such as metal analysis. They suggested that analyses for the metals Sb, Ba, and Pb alone cannot provide conclusive evidence as to the presence of gunshot residues. This is a direct consequence of the wide variability of hand blank values and from the possible absence of certain metals in some primers. However, coupling the HPLC analysis of the stabilizers with that of a metal content analysis can greatly reinforce the conclusions as to the presence of gunshot residues.

Finally, using 20 hand guns and 20 different types of ammunition of various calibers to perform test firings, they carried out an applicability study for gunshot residue analysis (24). The handswab samples from the firers' hands were analyzed by methods mentioned above (23). Results based on stabilizer content alone revealed positive identification in 19 of 20 residues (95%). Results based on metallic determination alone revealed positive identification in 14 of 20 residues (70%). The method examined in this study for the determination of organic stabilizers and metallic elements showed a high degree of reliability in the identification of gunshot residues.

In their later work, Leggett and Lott (45) used HPLC with electrochemical detection to search for other possible substances producing a peak at the same retention time as either diphenylamine or ethylcentralite. Test surfaces of fruit, vegetables, rubber, plastics, and clothing were swabbed with cotton swabs moistened with 2.5% glycerol in methanol. The swabs were then extracted with ether. The extracts were evaporated to dryness and reconstituted in acetone. Half of the acetone extract was evaporated to dryness, reconstituted with HPLC mobile phase, and subjected to HPLC

separation. The remainder of the acetone solution was used for the analysis of NC by fourier transform infrared spectrometry (FT-IR) over the wavelength range 400–4000  $\text{cm}^{-1}$ . The HPLC results revealed that commonly eaten fruits purchased at local grocery stores gave peaks at the same retention time as diphenylamine and/or ethylcentralite. Virtually all of the rubber products tested showed peaks at the same retention time as diphenylamine, whereas practically none of the plastic products did. Neither the rubber nor the plastic products tested gave positive results for ethylcentralite, except a "Pentel" white pencil eraser.

To provide discrimination, these workers proposed an FT-IR approach for the determination of nitrocellulose in gunshot residues. The remaining acetone/residue solution mentioned above was dried over anhydrous sodium sulfate and the dried acetone solution applied to a small portion of a heated potassium bromide IR pellet and its IR spectrum recorded using an instrument setting of 1000 scans.

They concluded that the HPLC determination of diphenylamine and ethylcentralite can serve as a rapid screening method for gunshot residues. A negative result would be highly conclusive that GSR was not present but a positive finding would require confirmation by a second method, such as analysis for nitrocellulose by FT-IR.

#### *Mass Spectrometric Detection*

Mass spectrometry (MS) is a highly specific and sensitive detection method. The combination of HPLC and MS would constitute a powerful technique for the analysis of explosives and explosive residues.

In 1977, Vouros et al. (112) reported an off-line combination of HPLC and MS for the analysis of explosives. Explosive mixtures were separated by HPLC and fractions were collected and then introduced by solid probe into the mass spectrometer. The off-line LC-MS approach has been applied to the analysis of simple residues from test explosions. However, this method is tedious and requires a human interface to collect the HPLC fractions.

Yinon and co-workers used on-line HPLC/MS for the analysis of explosive mixtures. At first, a direct liquid introduction interface which allowed about 1% of the solvent/sample effluent into the ion source was used (113,114). When chemical ionization (CI) was utilized, a characteristic positive ion spectra was produced (113). Although high  $m/z$  ions, such as  $\text{MH}^+$ ,  $\text{M}^+$ , and  $(\text{M} + \text{CH}_3\text{OH} + \text{H})^+$ , were well represented in the spectra of the nitroaromatics, with the nitramines and nitrate esters such as nitroglycerine, the spectra were complex and did not show the simplicity normally expected of chemical ionization processes. The production of an identifiable spectrum required the introduction of 10–100 ng of compound into the source. Although an order of magnitude increase in sensitivity was obtained by the negative ion CI (NICI) technique using a direct liquid insertion probe interfaced to a quadrupole spectrometer, the fragmentation problem of nitramines and nitrate esters was not improved (114). The introduction of a thermospray (TSP) interface to explosives work by Voyksner and Yinon (115) has transformed this position. The TSP interface appears to offer the greatest capability with high sensitivity for analyzing explosives separated by HPLC. The interface allows high flow-rates of aqueous solvents and volatile buffers into the system and provides the gentle ionization necessary to obtain the molecular ion. There were three ionization modes available for use with the TSP interface: thermospray (filament-off), filament-on (CI), and discharge ionization. With TNT as the reference

compound, a sensitivity ratio of 1:3:1.4 was observed for these ionization modes, respectively (116). Both positive and negative ion spectra were acquired in the thermospray and CI modes. The explosive detection proved most sensitive in the negative ion mode with filament-on ionization. Detection limits varied from 200 pg for trinitrotoluene to 5 ng for ammonium picrate. They found that TSP/HPLC/MS was useful in separating and identifying components in commercial explosive mixtures without interference's from plasticizers as well as in detecting explosives from hand swabs with excellent sensitivity and specificity. The plasticizers were detected in the positive ion mode whereas the explosives were most sensitive with negative ion detection.

Later work by Berberich et al. (116) is in broad agreement with that of Voyksner and Yinon. Also, under positive ion conditions the water gel component monomethylammonium nitrate and the firearms propellant stabilizers diphenylamine and ethyl centralite were readily characterized. Thus, both nitroglycerine and diphenylamine could be detected in the residues of a pipe bomb that had contained a double-base smokeless powder. Other application examples included intact military explosives, a water gel explosive, and a smokeless powder. With selected ion monitoring the detection limits in the negative ion mode with filament-on ionization were less than 2.5 pg. This is the highest sensitivity claimed for the detection of explosives by HPLC. As most of the characteristic components in gunshot residues have been detected by HPLC with MS, the application of the technique to the analysis of gunshot residues seems to merit investigation.

#### *Fluorimetric Detection*

Fluorimetry is one of the most sensitive and selective modes of detection commonly used in HPLC. In 1974, Wessel et al. (34) suggested that it was possible that organic constituents of gunshot residues could be identified by molecular luminescence. Therefore, additional development to extend this technique to the detection of organic gunshot residues appeared worthwhile. However, because explosives in general are not intrinsically fluorescent no practical application of fluorimetric detection to explosives or gunshot residues has been reported.

Prime and Kerbs (117) have used dansyl chloride for the derivatization of the monomethylamine traces left by a water gel explosive. However, the high fluorescence of this class of derivatives was not exploited using reversed phase HPLC, the effluent being monitored by UV absorption. Presumably the amounts detected, down to the low nanogram level, would be reduced to the picogram level by fluorescence detection. In organic gunshot residues, possible candidates for fluorescence detection are ethyl centralite (EC), 2,4-dinitrotoluene (2,4-DNT), 2-nitrodiphenylamine (2-NDPA), and 4-nitrodiphenylamine (4-NDPA). As a compound, ethyl centralite is similar to the urea herbicides and fluorescence detection of these has been reported by several workers (118–122). The urea herbicides were hydrolyzed in various ways to their corresponding anilines, which were then labeled with dansyl chloride to yield fluorescent derivatives. Accordingly, ethyl centralite can also be hydrolyzed to *N*-ethylaniline. 2,4-Dinitrotoluene, 2-nitrodiphenylamine, and 4-nitrodiphenylamine are all aromatic nitro compounds which can be reduced to their corresponding aromatic amines (123). The aromatic amines derived from EC, 2,4-DNT, 2-NDPA, and 4-DPA are capable of reacting with a fluorescent agent to yield fluorescent derivatives. This approach had been illustrated in a series of three papers by Meng and Caddy (124–126). The first reported on the hydrolysis of ethyl centralite to

ethylaniline and reaction of this in situ on a TLC plate with dansyl chloride followed by two dimensional TLC chromatography. The second paper used this same derivatization procedure but analyzed the fluorescent derivative by HPLC. The third paper of the series used a different fluorophor, 9-fluorenylmethylchloroformate, to derivatize the ethylaniline for the HPLC analysis. The sensitivities for ethyl centralite using these different techniques were 1 ng, 670 pg, 200 pg, respectively. Although these procedures were acceptable for those laboratories infrequently involved in this type of work, they were not readily adapted to the busy forensic laboratory.

#### *Supercritical Fluid Chromatography*

In supercritical fluid chromatography (SFC) the mobile phase is a supercritical fluid such as CO<sub>2</sub>, N<sub>2</sub>O, NH<sub>3</sub>, or SF<sub>6</sub>. The mobile phase is pumped as a liquid, and the pressurized fluid is preheated above the critical temperature before passing into a column via an injection valve, and hence into the detector. A pressure restrictor is located either after the detector or at the end of the column to ensure super critical conditions. Both packed and capillary columns can be used for SFC. Supercritical fluids (SF) are dense gases with liquid-like densities and gas-like diffusivities and viscosities. These properties result in a good solvation power for SF and higher separation efficiency in SFC than for HPLC. In SFC, the retention of a solute can be controlled by the density and composition of the mobile phase and the temperature of the system. This is analogous to temperature programing in GC and gradient elution in HPLC. Accordingly, pressure-density-temperature programing has become a standard feature of retention control in SFC. A major advantage of SFC versus other more established separation techniques is its compatibility with a large number of chromatographic detectors. Virtually all HPLC and GC detectors can be used with SFC, with detection principles ranging from universal to very selective.

Because SFC appears ideally suitable for analysis of some thermally labile or nonvolatile explosives, there are a number of reports on the determination of explosives by SFC using UV absorption, FID, TEA, FTIR, or MS detection (32,127–133). However, only one of them relates to the characterization of smokeless powders and gunshot residues (131). In 1991, Munder et al. (131) reported a method which allowed the sample to be extracted with supercritical fluids, and the extract preconcentrated and analyzed by capillary supercritical fluid chromatography in a single coupled unit. To achieve greater selectivity and sensitivity for selected species, they used three different detectors (UV absorption, FID, and ECD) which were all connected on-line. In addition to the determination of explosive residues in soil, they analyzed firearm propellants and gunshot residues. Several smokeless gunpowder's were characterized through their extractable organic constituents, and they found that the two basic types of propellants were easily distinguished. Extracts from double-base powders exhibited a major signal from nitroglycerine, which was missing in single-base powder extracts. Furthermore, the composition of the minor components in single-base powders was less complex than in double-base powders: 2,4-dinitrotoluene was the only detected minor constituent of single-base powders investigated. Although several minor constituents such as ethylcentralite, diphenylamines, dinitrotoluenes, and dibutylphthalate were generally detected and identified in double-base powders, the lot-to-lot variation of these constituents were approximately the same order of magnitude as the brand-to-brand differences. Thus

the possibility of identifying the brands of the double-base powders by their extractable organic constituents was very low.

In gunshot residue analysis, the sample was scratched from the inner surface of the spent cartridge case with a spatula. A portion of the residues was packed in the extraction cell and extracted with supercritical CO<sub>2</sub>. After the propellant residues in the spent cartridge cases had been directly extracted and analyzed by SFC, they found that all extractable organic constituents were recovered and even the relative intensities of most of the signals were still preserved after shooting. Thus, a fingerprint of the propellants used for charging the ammunition could be recovered after firing it. However, it is important that the differences in the extractable organic pattern of the propellants in question be pronounced enough to allow a meaningful correlation between a specific lot of propellant and the gunshot residues under investigation.

#### *Capillary Electrophoresis (Micellar Electrokinetic Capillary Electrophoresis)*

Capillary electrophoresis (CE) is one of the most important analytical techniques that can provide rapid, high-resolution separations of complex mixtures. In CE separation is carried out by the two related electrokinetic effects, electrophoresis, and electroosmosis. Although electrically neutral substances such as organic gunshot residues cannot be separated by conventional CE, micellar electrokinetic capillary electrophoresis (MECE) permits the separation of these neutral substances. In 1984, Terabe and co-workers (134) introduced an important development in the use of micelles to carry out separation of neutral species in CE. When a high voltage is applied to a capillary tube filled with a sodium dodecyl sulfate (SDS) micelle solution, the negatively charged SDS micelles can migrate at a velocity  $V_{ep}$  toward the positive electrode by electrophoresis and the aqueous solution can flow at a velocity  $V_{eo}$  toward the negative electrode by electroosmosis. Because  $|V_{eo}| > |V_{ep}|$ , the micelles will move slowly toward the negative electrode. When a neutral analyte is added into the micellar solution, some portion of the solubilize may be solubilized in the micelles. When inside the micelles, the solubilize will migrate with the micelles, whereas in free solution the solubilize will migrate with the bulk flow. Thus selective partitioning of the analytes into the micellar phase causes them to migrate at different rates from that of the bulk electroosmotic flow rate. The micelles can be considered as the "stationary phase," and the free solution is the "mobile phase." MECE may be classified as a type of liquid-liquid partition chromatography. In conventional elution chromatography, a totally retained compound is never eluted. Conversely a compound that is totally solubilized by the micellar phase in MECE is eluted in a time that is equivalent to the effective retention time of the retarded micelles. Hence, MECE is characterized by a limited elution range.

In 1991, Northrop et al. (135) reported the use of MECE in separation and identification of organic gunshot and explosive constituents. The instrument used was a commercially available capillary electrophoresis system. Sodium dodecyl sulfate (SDS) was used to form micelles. The running voltage was constant at 20 kV. Electroinjection at 5 kV for 2 s was used to introduce samples. A multiple-wavelength UV absorbance detector was used to select an ideal monitoring wavelength for different compounds. Standard solutions of 11 gunpowder constituents and 15 high-explosive constituents, reloading powders, plastic explosives, and gunshot residues swabbed from spent ammunition casings, were analyzed. Qualitative identification of the components in each of

the samples was made by comparison to the capacity factors of standard solutions, by sample spiking, and by monitoring at selected wavelengths. Eleven gunpowder constituents were completely separated. In the analysis of a mixture of 26 gunpowder and high-explosive components, coelutions were limited to two of the mononitrotoluene isomers and two of the dinitrotoluene isomers. In analysis of gunshot residues collected from two spent ammunition casings, both casings showed the presence of ethylcentralite and nitroglycerin. The plasticizer dibutyl phthalate (DBP) was also found in all gunshot residue samples and swab blanks. The swabbing solvent was obtained from a plastic storage container. The reloading powders and plastic explosives have also been successfully analyzed and identified.

These same workers also reported that a separation of 11 components in a gunshot residue test mixture was achieved in under 10 min with baseline resolution of the four isomers of dinitrotoluene, a signal-to-noise ratio of better than 100 to 1, and separation efficiencies of between 200,000 and 400,000 theoretical plates. Munder et al. (131) also examined these same gunpowder and explosive components using SFC. Due to the lack of complete chromatographic resolution, unambiguous identification of all components by SFC required three different detectors (UV, FID and ECD). For instance, 2,4-dinitrotoluene, diphenylamine, and N-nitrosodiphenylamine all coeluted in the system described. The MECE system described by Northrop provided better resolution using a single detector (UV). The potential for the application of MECE to organic gunshot residue detection is very high.

The gunshot residue samples analyzed by Northrop were swabbed from spent ammunition casings that are abundant in organic gunshot residues and free from contamination. However, gunshot residue samples collected from a firer's hand are usually heavily contaminated and of low concentration. It becomes necessary therefore to carry out a further investigation to verify the feasibility of using MECE for the analysis of gunshot residues sampled from a firer's hand. In 1992, these same authors reported their further work in gunshot residue detection by MECE (40). The samples were collected by adhesive tape lift from the firer's hands immediately after discharging a handgun three times. Suspected particles were either directly removed from the adhesive tape or a section of the tape was extracted with ethanol. Ethylene glycol was added to the extracts to prevent the sample from going to dryness during the evaporative concentration which resulted in the losses of analyte. The extracts were then evaporated under a stream of nitrogen and reconstituted with the MECE buffer. The reconstituted extracts were introduced to the MECE by gravity injection to avoid the small bias when electrokinetic injection was used. The running voltage was constant at 25 kV. They found that the tape lift sampling technique was able to circumvent the analyte losses by adsorption during collection and handling, and minimized coextraction of sample matrix interferants associated with solvent swabbing collection methods. In this work, three different weapons and ammunition were used for the test firing. They successfully detected the characteristic gunshot residue constituents from both collected particles and small sections of tape in all test firing hand samples. They also found that both the unfired gunpowder and the post-firing gunshot residues from the same gunpowder were similar in composition.

*Comparative Instrumental Studies on Gunshot Residues Analysis*—A comparative study by Casaccia et al. (136) using SEM/EDX and NAA was undertaken during a casework examination to determine firing distance. No preference was offered concerning

the individual techniques. A similar study was provided by Degae-tano et al. (137) for the analysis of GSR's on the hand of the firer. The advantages and disadvantages of the three sampling techniques were tabulated with the tape lift procedure being identified as the best.

## Conclusions

Although highly specific results may be obtained by the analysis of inorganic gunshot residues using scanning electron microscopy, the analysis of organic residues to provide complementary and additional information is required especially when inorganic residues have not been recovered or their characteristics are non-specific.

The choice of approach for organic residue sampling depends on the substrate, that is the material on which the sampling is to be carried out, and the analytical techniques to be used. Swabbing is the commonest technique used for collecting organic residues from hands, whereas vacuum lifting is widely used in sampling organic residues from clothing. Because the extracts from hand-swabs or vacuumed clothing samples are substantially contaminated with lipids and other extraneous materials which result in interference for the detection of analytes and deterioration of the equipment used, a clean-up procedure is an essential pretreatment to preserve the performance of equipment and to enhance sensitivity and selectivity. In addition to the most widely used solid phase extraction techniques, TLC has also been used as a clean-up procedure.

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