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## Concentration Techniques for the Detection of Gunshot Residues by Scanning Electron Microscopy/Energy Dispersive X-Ray Analysis (SEM/EDX)

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**ABSTRACT:** The efficiency of the filtration process for concentrating small populations of gunshot residue (GSR) particles (for detection by scanning electron microscopy/energy dispersive X-ray analysis [SEM/EDX]) was examined with a novel method using GSR particle suspensions. Methods of concentrating and segregating GSR particles on small areas (for example, 7 mm<sup>2</sup>) using both filtration and heavy liquid separations with centrifugation were examined from the practical point of view. It is shown that, in real cases, there may be an intrinsic difficulty in concentrating GSR particles as a result of a pile up of extraneous material or clogging of the collecting filter or both.

Collection of GSR particles from clothes using a suction method was compared to the direct glue-lift technique. The suction method involved trapping the GSRs in an organic solvent and recovering them on a membrane filter. The method proved less efficient than using the glue-lift technique.

From the findings in this study, it seems that the most efficient way of detecting GSR particles by SEM/EDX is by using the recently developed automated systems directly on glue-lift samples without any pretreatment.

**KEYWORDS:** criminalistics, ballistics, gunshot residues, chemical analysis, scanning electron microscopy/energy dispersive X-ray

The use of the scanning electron microscopy/energy dispersive X-ray (SEM/EDX) technique for the identification of gunshot residue (GSR) particles has been thoroughly investigated by Wolten et al. [1-5]. In brief, these authors showed that not only do GSR particles have a definite chemical composition (mostly, but not always, lead, antimony, and barium), but they also often have a distinctive morphology, that is, they are generally spherical particles of from 0.5 to 10  $\mu$ m diameter.

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Since the pioneering work of Wolten et al., the mechanism of formation and the morphology of GSR particles have also been studied by Basu [6] and their mineralogy by Tassa et al. [7].

The ability of the SEM/EDX technique to visualize the individual GSR particles and to determine their elemental composition has led to its widespread adoption in forensic science laboratories in spite of the relatively high cost of purchasing the necessary equipment and maintaining it.

The search for GSR particles is usually carried out by using a backscattered electron image (BEI). A backscattered electron detector can be adjusted to detect preferentially heavy elements (for example, lead) which give a brighter image than light elements. The specimen is scanned at low magnification (say  $\times 1000$ ), and only those particles whose BEIs are sufficiently bright are examined at higher magnification to determine their elemental composition. The brightness of a BEI is influenced primarily by the composition, but also by the topography of the particle producing it [8].

A major disadvantage of the SEM/EDX technique is the length of time taken to carry out a test. The time required to scan a single 13-mm-diameter stub can be up to 8 h [9]. During this period both a highly trained operator and an expensive instrument are tied up. Furthermore, this type of work quickly fatigues the operator who is then liable to miss a GSR particle. One solution to this problem has been to automate the search and analysis procedures [10]. Recently, a number of fully automatic methods based on commercially available equipment have been described [11-14]. The method developed by Keeley and Nolan [12] is used by the Israel Police and is briefly described below. These methods do reduce to some extent the amount of time required to carry out a search. However, more important, they free the operator for other work and enable the microscope to run both unattended and out of working hours. An alternative and complementary approach to reduce the search time is to segregate and concentrate the GSR particles on to a small area as discussed below.

### Sample Collection for SEM/EDX

GSR samples for SEM/EDX are collected in a number of ways. They may be taken directly onto a specially treated sticky SEM stub kept in uncontaminated conditions. This is the method used by the Israel Police where a special kit using 25-mm-diameter stubs has been developed [15]. Other similar methods have been described in the literature [10, 11, 16, 17] and may be collectively described as the "glue-lift" technique. These samples are then examined in the SEM.

An alternative to direct sampling and examination is to collect the GSRs by using a swab or dabbing with a sticky surface to remove them from the subject's clothes or skin, or both. The GSRs are then transferred (after dissolution of the collecting medium or ultrasonic release of the particles) to a filter which is in turn mounted on a SEM stub for examination [18, 19]. This type of procedure also allows a pretreatment of the sample to remove interfering substances that hinder the detection process in the SEM. However, any pretreatment of the samples brings with it danger of contamination and loss of GSRs.

A promising approach to cutting down the time needed to carry out a test for GSRs on the SEM has been to concentrate the GSR particles onto a small area and thus cut down the search time significantly. It is this approach that we examine in detail in this paper.

A number of authors have proposed methods for depositing the particles on a 13-mm-diameter membrane filter after some form of filtration [18, 19]. The particles may also be segregated from interfering substances by virtue of their greater density and then concentrated onto a small area of filter: Ward [20] used 3 mm<sup>2</sup> and Gonzales<sup>3</sup> 5 mm<sup>2</sup>. Another method for

<sup>3</sup>G. Gonzales, "A Simple Method of Concentrating Gunshot Residue Particles," private communication, 1984.

collecting and concentrating the particles is due to Sild and Pausak [21] who used a vacuum device to collect the particles which were deposited onto a 2-mm-diameter area on a sticky stub.

### Processing Efficiency

The efficiency (that is, percentage recovery after the collection step) of some of these sample processing techniques has been tested. Wallace and Keeley [18] used an atomic absorption spectrophotometry for this purpose and demonstrated recoveries of 85 to 90%. The population of GSR particles used was very large indeed, running into many thousands. It is not obvious that the recovery rates obtained by these authors also apply to small populations of particles. Ward [20] used a direct counting method in the SEM to evaluate the efficiency of his concentration technique, but it is not clear on what particle populations he was working.

The object of the present work was to evaluate various concentration techniques for *small* populations of GSR particles in an effort to reduce search times in the SEM and to develop a method for estimating possible losses in processing. It must be stressed that any additional manipulation of the samples involves taking extra precautions against contamination which complicate the procedure.

### Experimental Procedure

#### *Preparation of Calibrated GSR Particle Suspensions*

Suspensions of GSR particles of known concentration in an organic solvent were prepared [22]. These suspensions were used to test both the loss and recovery of GSR particles in the various manipulations described below.

The suspensions were prepared by extracting three (fired) W-W 5.56 by 45 cartridge cases with 40 mL of trichloroethylene (or a nonpolar organic solvent such as petroleum ether, toluene, or hexane) in an ultrasonic bath for 1 min. The resulting suspension was filtered through a 20- $\mu\text{m}$  stainless steel mesh and made up to 50 mL with the appropriate solvent. Using a rough counting technique with the SEM it was determined that this suspension contained about 15 000 GSR particles/mL, most of these were between 0.5 and 5  $\mu\text{m}$  in diameter. From this suspension a working suspension containing about 15 GSR particles/mL was prepared by multiple dilution with the organic solvent.

These working suspensions may be sampled reproducibly since they are quite stable [18], in our experience, for at least several months. Reproducibility and stability were checked as follows: the suspensions were thoroughly shaken and 2.0 mL removed using a disposable plastic syringe fitted with a stainless steel needle. The suspensions were filtered through a 1.2- $\mu\text{m}$  membrane filter using a special apparatus. This apparatus is essentially similar to that of Wallace and Keeley [18] and McQuillan [19] which consists of two 13-mm-diameter filter holders connected in series. The holders are mounted vertically in a filter flask and liquid is drawn through them by application of a vacuum. A funnel is fitted to the top filter holder which is normally used with a coarse prefilter while the lower holder is fitted with a membrane filter.

The apparatus used for this work was fitted with a specially constructed polytetrafluoroethylene (PTFE) lower filter holder with a 3-mm-diameter aperture.

The apparatus was swilled three times with 10-mL portions of hexane and the whole filter surface examined under the SEM. The results of these experiments (which were carried out without a prefilter) are shown in Table 1.

It can be seen that the spread of results is quite narrow. Blank solutions were also run using the same hexane. The blank solutions occasionally contained lead-bromine particles.

TABLE 1—Particle count and composition of 2-ml standard GSR suspensions on 3-mm-diameter filter.<sup>a</sup>

	Col. 1	Col. 2	Col. 3	Col. 4	Col. 5	Col. 6	Col. 7	Col. 8
Test	Pb	Pb-Ba-Sb	Pb-Sb	Pb-Ba	Ba-Sb	Pb-Br	Pb-Cl	Total GSRs (Col. 1 to 5)
1	1	16	7	0	0	0	1	24
2	4	15	14	0	0	2	0	33
3	4	18	11	1	0	1	0	34
4	7	7	10	0	0	4	0	24
5	3	19	3	1	0	0	1	36
6	5	21	7	1	2	4	0	36

<sup>a</sup>Average = 31 and Std. dev. = 6.

Lead-chlorine- and lead-bromine-containing particles are well-known environmental contaminants originating from the use of leaded gasoline. Similar recoveries are obtained when working with a 20- $\mu$ m mesh prefilter as described below.

This method of testing recoveries is much more sensitive than bulk analysis.

#### Choice of Solvents and Filters

A number of organic solvents have been recommended for work with GSRs. Wallace and Keeley [18] recommended trichloroethylene (TCE) or petroleum ether. Because TCE attacks various plastic materials, we prefer to use paraffinic hydrocarbons such as petroleum ether (60 to 80°C) or hexane in most of this work.

Many analytical grade solvents are badly contaminated with various particles, most of which consist mainly of iron. However, this is not too serious a problem, since in case work, the hands and clothes of the subjects usually contribute more extraneous particles than the solvents. These extraneous particles may be skin debris, fibers, dust, and also particles containing heavy elements which hinder the search for GSR particles. We have tried to remove these materials with a variety of prefilters: 12- $\mu$ m cellulose nitrate membrane filters (Sartorius), 12- $\mu$ m polycarbonate membrane filters (Nucleopore), and 20- $\mu$ m stainless steel mesh. The cellulose nitrate material has a honeycomb structure and lets no GSR particles through at all. The polycarbonate membrane has cylindrical holes through which GSR particles pass easily, but its filtering capacity is small and the filter itself is delicate. Stainless steel sieve cloth (625 mesh, hole size 20  $\mu$ m) is very suitable and is easily cleaned with dilute mineral acid if required.

Final (collecting) filters of cellulose nitrate (Sartorius, 1.2  $\mu$ m) and polycarbonate (Nucleopore, 1.0  $\mu$ m) were used in the PTFE apparatus described above. Of these, the polycarbonate filters gave the best results for visualizing the GSR particles in the SEM, but are more easily blocked than the other type. Both types of filters were used in the various experiments.

#### Concentration of GSR Particles in Real and Simulated Samples

*Filtration onto a Small Area*—In this section of the work we describe the use of the filter apparatus previously described to concentrate GSR particles onto a small (3-mm-diameter) area to expedite the search procedure in the SEM. An attempt has been made to simulate the conditions encountered in real cases. In our experience, samples of GSRs taken in case work are almost always contaminated with large amounts of extraneous materials. These materi-

als are both light, such as fibers, skin debris, dust, and so forth and heavy, such as lead-, gold-, and iron-containing particles, as well as lighter flint, barium sulfate, and so forth. To reflect such conditions, a simulated suspension of extraneous material was prepared in the following way: one of the authors' hands (up to the wrist) was rubbed against a dirty surface and then was swilled with 50 mL of hexane. An attempt was made to filter this suspension through the combined filtration apparatus. After 5 mL of liquid had passed, the apparatus was completely blocked. Microscopic examination showed that a thick mat had built up on the membrane filter. The stainless steel prefilter was not blocked.

Attempts were made to remove extraneous matter from the simulated suspension both by heavy liquid separation (using bromoform, specific gravity 2.88) and by heavy liquid separation followed by centrifugation [20]. These treatments were followed by filtration through the two-stage filtration apparatus. With centrifugation, the filtration was completed (but with difficulty), however a thick mat of extraneous material still formed on the final membrane filter. This mat must bury many GSR particles and thus prevent their discovery. It may be assumed that the material trapped on the upper (20- $\mu\text{m}$ ) filter also traps many GSRs.

This simulation illustrates one of the great dangers of preconcentration on a small area of a filter. If pile-up or clogging of the filters occurs during the processing stage the whole test is put in jeopardy. In the best case, further manipulations may save part of the sample; in the worst, an unretrievable piece of evidence has been destroyed.

Thus it can be seen that any attempt to concentrate GSRs taken by swab, washing, or glue-lift techniques, onto a small area, is fraught with intrinsic difficulties.

*Collection of GSRs by Suction*—Another way of concentrating GSRs is to sample large areas by suction into a small volume of liquid. This method seems particularly suitable for articles of clothing where the GSRs may be assumed to be retained below the outer surface of the sample. Because of this effect, clothing is generally considered to retain GSRs longer than skin.

The authors attempted to collect GSRs from clothing using a suction technique and to compare the results to those obtained by glue-lift.

The apparatus used was a 250-mL filter flask fitted with a 6-mm (inside diameter [id]) PVC suction tube. The end of the suction tube has four small notches which allow a continuous stream of air to pass through. The collecting liquid is 40 mL of petroleum ether (60 to 80°C). The air flow through the apparatus is supplied by suction from a water pump and regulated with a flow meter. The apparatus is shown schematically in Fig. 1.

The air flow was adjusted so that it was fast enough to suck in sand grains but not fast enough to cause splashing of the collecting liquid. These air flow limits were 3.0 and 4.5 L/min, respectively, and all further work was carried out at a flow rate of 4.0 L/min.

After sampling GSRs by repeatedly passing the collecting tube over the surface to be tested, the collecting tube is swilled out by sucking two further portions of petroleum ether (20 and 10 mL) through it. The liquid in the collecting flask is now passed through a two-stage filtration apparatus with a 13-mm final membrane filter of cellulose nitrate (1.2  $\mu\text{m}$ ) or polycarbonate (1.0  $\mu\text{m}$ ) as described by Wallace and Keeley [18]. The effective filter area is approximately 9 mm in diameter. The prefilter is made of 20- $\mu\text{m}$  stainless steel mesh. After filtration the flask and filter apparatus are swilled with two successive portions of solvent (20 mL). The collecting membrane filter is then removed, dried, mounted on a stub, coated with carbon, and examined on the SEM. Sometimes charging interference was encountered even when using a relatively thick carbon layer. In these cases a conductive aerosol was used.

SEM/EDX searches in this series of experiments were carried out using an automated GSR system attached to a Camscan 4 SEM with motorized stage drive and a four-sample holder. A Tracor Northern TN 5500 EDX system was used. The Camscan automated GSR system has been described by White and Owens [11]. The software used in the authors'

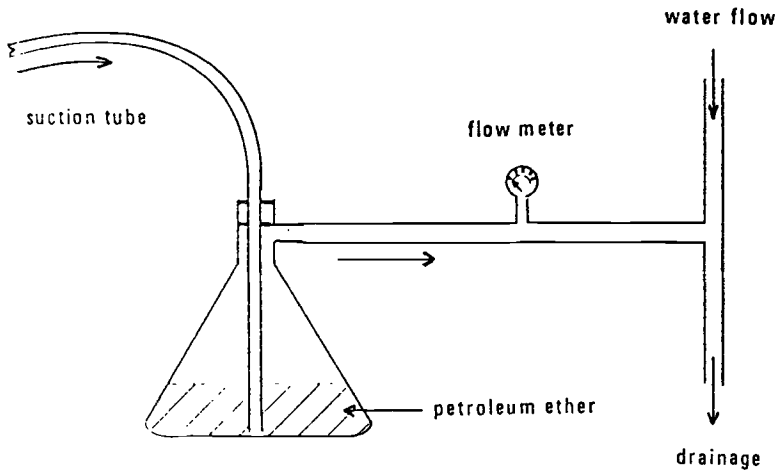


FIG. 1—A schematic view of a suction apparatus for collecting GSR particles from clothes.

laboratory is an improved version developed by Keeley and Nolan [12] in the laboratories of the London Metropolitan Police. This software sorts the analyzed particles into various categories (for example, GSRs, lighter flints, petrol pollution, and so forth) and produces a continuous printout of the results as well as a summary at the end of the run. Final positive identification of the presence of GSRs is always made by bringing the system to the coordinates of the alleged GSR particle and confirmation by an experienced operator.

The recovery efficiency of collecting GSRs by suction is difficult to determine. An attempt was made to do this by dropping calibrated GSR suspensions onto swatches of clean cotton cloth and recovering the particles. However, wet penetration is not a good simulation of the natural deposition of GSRs onto an object. Therefore, we compared the relative efficiency of the suction and glue-lift methods in the following manner.

Subjects wearing various types of clothing (as detailed in Table 2) were allowed to fire three rounds, right-handed, from a 9-mm FN semi-automatic pistol. Winchester 9-mm Luger ammunition was used.

Tests were carried out in pairs at various times after firing. Between firing and sampling the subject carried out his usual work in the laboratory (in an area not exposed to GSRs). Each pair of tests was comprised of first sampling the test area by one method and then, immediately afterwards, repeating the sampling by the second method, for example, first by suction and then by glue-lift. The glue-lift technique used a 25-mm-diameter stub coated with glue (Scotch tape No. 465, 3M Company) [15]. The suction samples were collected by applying suction for 2 min while gently moving the collecting tube over the sample area. In all cases the subject's right sleeve was sampled from wrist to elbow starting at the wrist. The sleeve of the firing arm is one of the natural locations of GSRs.

The results of these experiments are shown in Table 2.

In all cases the search area in the automated SEM was about 10 by 10 mm<sup>2</sup>. This is equivalent to the whole filter but only about one quarter of the glue-lift surface.

The results in Table 2 show that neither of the sampling methods have a high recovery efficiency of GSRs, since in most of the experiments the number of particles found in the second stage of sampling is similar to the one found in the first stage. Furthermore, except in Experiments 4 and 5, there was no clear advantage in favor of either technique for the same search area. However, since in the suction technique there are many more handling stages

TABLE 2—Comparison of suction and glue-lift collection of GSRs from clothes.

Experiment	Cloth Type <sup>a</sup>	Time After Firing	Sampling Sequence	Number of 3 Elements GSRs on Search Area
1	1	15 min	glue-lift	20
			suction	19
2	1	15 min	glue-lift	7
			suction	5
3	2	15 min	suction	290
			glue-lift	215
4	2	15 min	suction	10
			glue-lift	56
5	2	2 h	glue-lift	0
			suction	1
6	2	2 h	glue-lift	6
			suction	10
7	2	5 h	glue-lift	75
			suction	3
8	2	5 h	glue-lift	8
			suction	10
9	3	15 min	glue-lift	5
			suction	3
10	3	15 min	glue-lift	11
			suction	11

<sup>a</sup>Cloth types: 1 = cotton laboratory coat, 2 = polyester/cotton flannel shirt, and 3 = acrylic/wool knit (70:30).

involved in the sample preparation, it is obvious that, from a practical point of view, the glue-lift technique is better. Moreover, in the glue-lift technique only about 25% of the stub area was searched, so that in all cases the overall efficiency of the glue-lift technique was much greater.

Note that the glue-lift technique is more efficient on cloth samples than the suction method. This is in spite of the fact that the glue surface only samples the outermost surface of the fabric and also that it rapidly loses its tackiness.

The relatively low efficiency of the suction technique may be due to the following factors:

- (1) nonoptimal suction rate—it is possible that GSRs pass right through the collecting liquid,
- (2) trapping of GSRs in the extraneous material on the prefilter (see Fig. 2), and
- (3) pile-up of extraneous matter on the collecting membrane itself leading to burying of GSRs (see Fig. 3).

Note that as a result of pile-up it is not advantageous to increase the collecting time to more than about 2 min. It is possible that heavy liquid separation with centrifugation might increase the efficiency of detection by removing light materials such as fibers. However, this line of experiment was not pursued due to the dangers involved in extra manipulation.

## Conclusions

There is an apparent advantage in concentrating GSRs onto a small area to accelerate their detection by SEM/EDX. In practice, we have found that there is an intrinsic difficulty in achieving this goal because of pile-up and clogging of the filters used. A suction method for collecting GSRs was developed and compared to the direct glue-lift technique. This method is less efficient than the glue-lift technique.

All the concentration methods considered in this article involve considerable additional

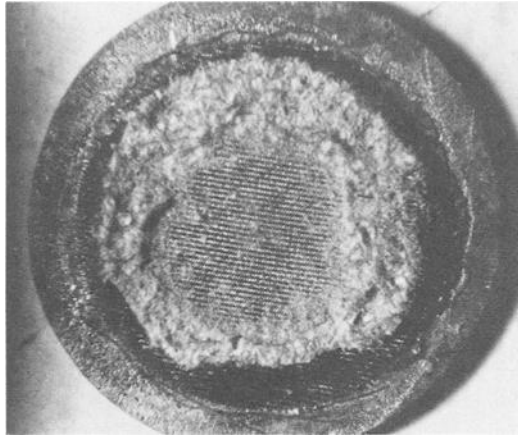


FIG. 2—An extraneous material collected on prefilter in the suction Experiment 9 (Table 2).

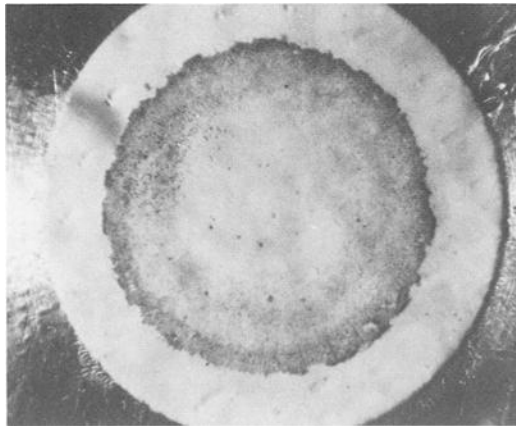


FIG. 3—Pile-up of extraneous material collected on the final membrane filter in the suction Experiment 9 (Table 2).

manipulation of the sample, which is time-consuming, requires skilled manpower, and may well lead to contamination.

It is our opinion that the direct examination of glue-lift samples by SEM/EDX is superior to all the alternative techniques tested. The obvious improvement in GSR detection techniques lies in the use of the automated SEM/EDX systems that have recently been developed. Such a system has been successfully used in the present work.

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