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Fast Analysis of Complete Macroscopic Gunshot Residueson Substrates Using Raman Imaging

María López-López, María Ángeles Fernández de la Ossa, Carmen García-Ruiz*

University of Alcalá, Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering, Multipurpose Building of Chemistry and University Institute of Research in Police Sciences, Ctra. Madrid-Barcelona km 33.600, 28871 Alcalá de Henares, Madrid, Spain

Abstract: Raman spectroscopy has emerged as a viable technique for the organic analysis of gunshot residues (GSRs), offering additional information to the well-established analysis using scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDX). In this article, a Raman imaging system with an electron-multiplying coupled-charged device (EMCCD) camera was used to analyze complete GSR particles from both conventional and nontoxic ammunition fired at different cloth targets. The same cloths were then stained with blood to mimic real evidence and measured. The direct analysis using Raman imaging of the GSR particles collected with the stubs used for SEM-EDX analysis (the frequent method used for GSR collection) was evaluated. Multivariate curve-resolution and chemical-mapping methods were applied to the spectroscopic data to identify and highlight the signal corresponding to the GSR particles and differentiate them from the substrates. It was confirmed that both measurement approaches (on the targets and the stubs) could be used for the identification of GSR particles, even under unfavorable conditions such as the presence of blood. The results obtained demonstrate the huge potential of Raman imaging for the fast analysis of complete GSR particles and prove its complementary usefulness in the analysis of the stubs used by the well-established SEM-EDX technique.

Key words: Gunshot residue; Raman imaging; Firearms; Ammunition; Clothing; Blood.

1. Introduction

Gunshot residues (GSRs) are a set of organic and inorganic particles that are transferred to the shooter, the weapon, the victim, and the surrounding environment when a firearm is fired.[1] For this reason, their identification is of extreme importance for crime-scene reconstruction to clarify the facts of the case, link a suspect to a crime, or confirm a suicide hypothesis. To date, the great majority of the analyses performed on the GSR particles have been focused on their inorganic components, which originate predominantly from the primer. In this regard, scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDX) is the reference technique for GSR analysis in the forensic laboratories.[2–5] Using SEM-EDX, it is possible to identify GSR particles on the basis of the presence of lead, barium, and antimony and of their characteristic morphology.[6] For this reason, the identification of GSR from lead-free or nontoxic ammunition using SEM-EDX has generated a debate, which has led to the scientific community proposing alternative analytical approaches. Therefore, analyzing the complementary organic analysis of the particles arising from the unburnt propellants and associated compounds has been reconsidered.[7]

Vibrational techniques, e.g., Fourier transform infra- red (FT-IR) and Raman spectroscopy, have been proposed as ideal approaches for GSR analysis due to their ability to provide immediate and useful information about the identity of the sample in a nondestructive way. López-López et al.[8] compared the Raman spectra of the unburnt propellant from six types of ammunition and the macroscopic GSR particles produced after firing them. The results indicated that the GSR spectra show a high similarity to the spectra of the corresponding unfired propellant, allowing the GSR to be traced to the type of ammunition used; in addition, a GSR-discrimination procedure based on the stabilizers present in the propellant ammunition was proposed. In a second study,[9] the same research group evaluated the weapon memory effect for the analysis of GSRs using Raman spectroscopy and recommended considering at least nine or 10 spectra of different macroscopic particles to discriminate GSRs based on the stabilizers. Recently, Abrego et al. [6] proposed a method based on scanning- laser ablation and inductively coupled plasma-mass spectrometry (SLA-ICPMS) and Raman spectroscopy for the characterization of GSR taken from the hands of the shooters after the discharge of lead-free ammunition. The SLA-ICPMS analysis of 20 elements enabled the detection of

aggregates related to the ammunition, while the Raman measurement gave information regarding the organic compounds indicative of the GSR. Bueno and coworkers[10,11] reported a further step toward the differentiation of GSRs using statistics. In a first article,[10] the authors combined near-infrared Raman microspectroscopy with statistics to differentiate the GSR particles (both organic and inorganic) from two calibers of ammunition. Then, in a second study,[11] they combined the spectroscopic data from FT-IR and Raman spectroscopy into a single dataset to improve the statistical discrimination of different GSRs. The same research group also imaged the GSR particles produced after firing conventional ammunition over cotton clothing using attenuated total reflection (ATR), FT-IR, and confocal Raman spectroscopy.[12,13] In both articles, Bueno and Lednev[12,13] used double-sided-adhesive tape to collect the particles and succeeded in detecting organic and inorganic particles; this presents a considerable improvement over the vibrational approaches proposed to date. The ATR FT-IR images were produced using 102 400 spectra over an area of 500 lm^2, but the authors did not mention the time used in the analysis.[12] In contrast, the Raman approach required 18 h to map (650 spectra, five accumulations of 20 s each) the complete GSR particle (60 lm diameter) presented in the article.[13]

When imaging is done using Raman spectroscopy, the Raman images (normally including thousands of spectra) are generated by acquiring a spectrum at each pixel, making the acquisition time per spectrum a decisive parameter for the total acquisition time. In this sense, the use of conventional confocal Raman systems to image a large number of particles seems unworkable in terms of time. Recently, Raman imaging systems with an electron-multiplying charge-coupled device (EMCCD) have become emerging technologies for the fast and noninvasive analysis of different types of samples. The use of an EMCCD detector instead of a standard charge- coupled device (CCD) camera can considerably reduce the acquisition time to a few milliseconds per spectrum while enhancing the sensitivity. Briefly, the EMCCD is a standard CCD camera with an extra readout register, the electron-multiplying (EM) register. The EM register uses higher clocking voltages than conventional clocking, causing the electron multiplication via impact ionization, and therefore amplifies the signal. In the GSR analysis field, these systems have not been tested to date. In addition, note that, for a real application of this fast imaging technique, it is crucial to consider and assess interferences that can hamper the measurements. For these reasons, our aim here is to study the complete macroscopic GSR particles from conventional ammunition as well as from lead-free

ammunition using Raman imaging and taking into account potential sources of interference, such as clothing color or the presence of blood, effects that have been not studied to date using conventional Raman spectroscopy. In this study, we used two measurement approaches—directly on non- blood-stained and blood-stained cloth targets and on the SEM-EDX stubs used to collect the particles—and Raman imaging for a comprehensive assessment of the technique's potential.

2. Experimental section

Sample Preparation. In this study, we tested four types of ammunition (three conventional and one lead- free), provided by the Ballistics Department of the General Department for Forensic Science Police (Madrid, Spain): G.F.L. 9 mm Luger (Giulio Fiocchi s.p.a., Lecco, Italy), S&B 80 9x19 (Sellier & Bellot, Vlasˇim, Czech Republic), Super X 0.22 inch (Western Cartridge Company, East Alton, United States, and GECO 9x19 Luger SX (RUAG Ammotec, Fu ̈rth, Germany). The cartridges were fired at short distances over white, black, and printed 40x40 cm cotton cloths fixed in cardboard. The barrels of the firearms were not cleaned to provide a more realistic scenario. In addition, some of the targets were stained with a blood sample collected from a volunteer. For the SEM-EDX analysis, we used 13 mm diameter aluminum stubs with adhesive carbon to collect the particles from the non-blood-stained cloth targets.

Instrumentation. A DXRxi Raman imaging micro- scope (Thermo Scientific) with an EMCCD detector and controlled by the OMNICxi Raman imaging software (1.0.0.2427; Thermo Scientific) was used. Measurements were taken using a 455 nm laser (Thermo Scientific; 6.0 mW laser power on the sample) with 1200 lines/mm grating and a slit aperture of 50 lm. The microscope was set to 50x magnification, the wavenumber range measured ranged from 85 to 3500 cm^{-1}, and the step size between two successive measurements was set to 5 lm. The baseline correction option of the software was applied to the spectra. The multivariate curve resolution (MCR) method and chemical maps were applied to the spectral data using the equipment software. The MCR method was applied using two components to identify the GSR particles

from both conventional and nontoxic ammunition on the different cloths studied. The MCR model finds the different components present in the sample based on their calculated pure-component spectra and shows their locations in the chemical image. Chemical maps were generated based on an intensity- based color scale in which white represents high intensity and black represents low intensity, referenced to the intensity of the larger peak area under a specific region (1800–800 cm^{-1}) at each measured point.

3. Results and Discussion

Clothing from the victim or the suspect is evidence commonly submitted to forensic laboratories to have important information extracted from them (e.g., distance determinations and trace evidence). It has been suggested that GSR remains longer on clothing than on the shooter's hands after a firearm discharges.[14,15] Therefore, it is important to study the use of Raman imaging directly on this kind of evidence. Four different types of ammunition (including one nontoxic ammunition) were fired over cloth targets to mimic this kind of evidence. Figure 1 depicts the image of four different macroscopic GSR particles from the four types of ammunition fired over white, black, and printed cotton cloths (Figs. 1a–c) that were measured using Raman imaging spectroscopy for 54, 39, and 51 min, respectively. As can be seen in the Raman spectra of each GSR at the right of Fig. 1, there are two bands present in the spectra, which are from the ammunition propellants.[8] These bands are at about 1347 and 861 cm^{-1}, and they can be attributed to the presence of diphenylamine derivatives and nitrocellulose, respectively.[16] However, because the cloths also present bands in the 1800–800 cm^{-1} region (e.g., see Fig. 1b, in which the black cotton cloth presents bands at about 1592, 1452, 1407, 1342, 1145, and 1100 cm^{-1}), it was necessary to apply MCR to identify and highlight the signal corresponding to the GSR particles and differentiate it from the signal from the cloth. Multivariate curve resolution is one of the most popular resolution methods for image analyses; it offers the contribution values of sample components as fractions (or percentages) of each pure component of the sample without the need to create a calibration set. Instead, the method is based only on the

theory that the spectral information contained in each pixel is the weighted sum of the spectral influences of each component of the sample measured.

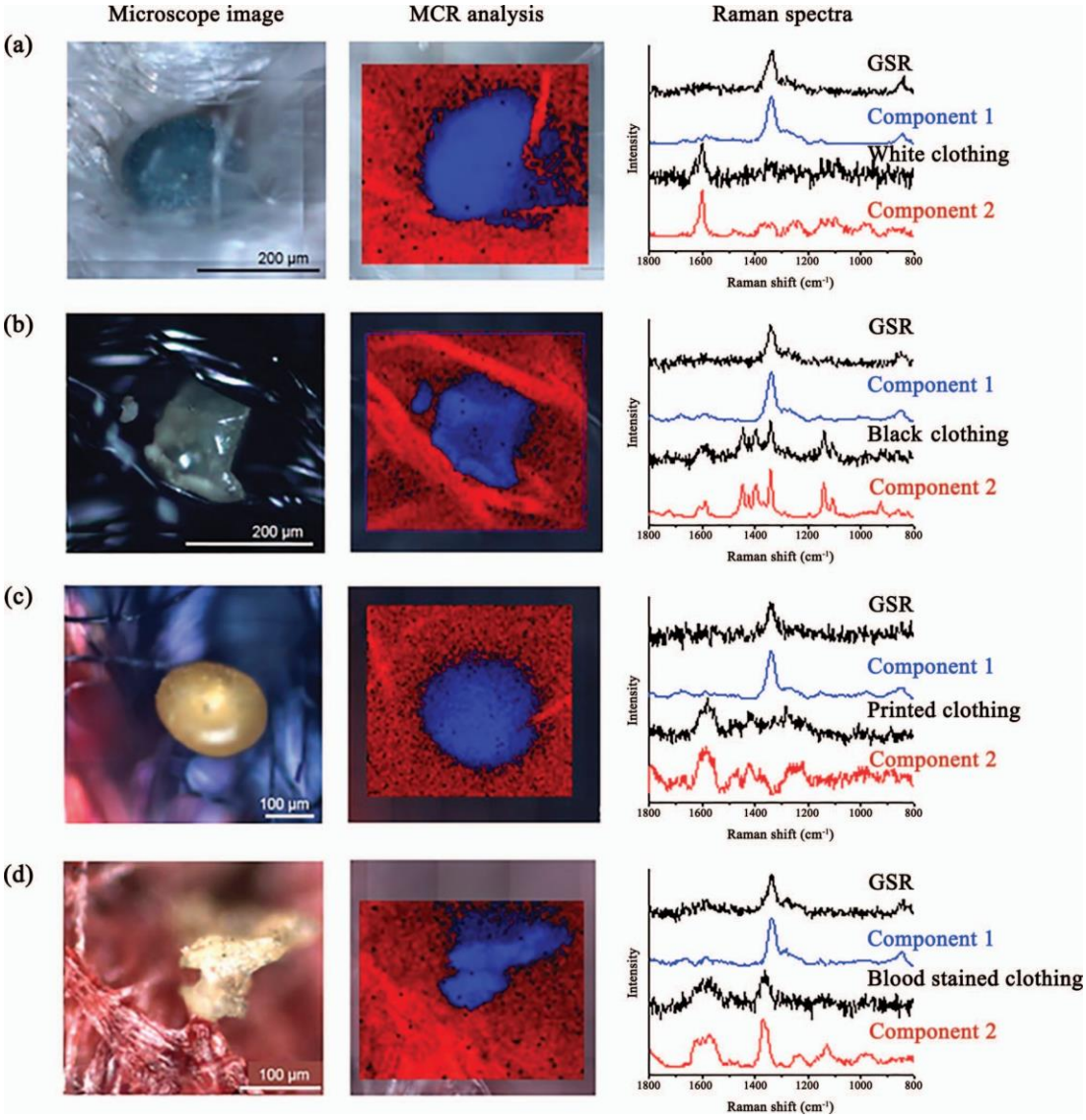

Figure 1. Microscope images of GSR particles from four ammunition cartridges fired over different cloth samples; their corresponding MCR analysis (blue indicates the component assigned to the GSR particles; red indicates the component assigned to the cloth substrates); and the Raman spectra of the GSR particles, the substrates, and the pure components calculated for the MCR model. (a) Super X GSR particle on white cotton cloth. (b) GECO 9 x19 Luger SX GSR particle on black cotton cloth. (c) S&B 80 9 x19 GSR particle on printed (blue and red) cotton cloth. (d) G.F.L. 9 mm Luger GSR particle on white cotton cloth stained with blood. Raman conditions: laser at 455 nm, 6.0 mW, 50x magnification objective lens, slit pinhole size 50 lm, 0.01 s x 30 scans, step size 5 lm (3726, 5829, 7857, and 4248 spectra, respectively).

The dataset is decomposed into the separate components (usually by rank analysis methods that perform a principal component analysis [PCA] repeatedly on small parts of the dataset) in such a way that it is possible to determine the number and distribution of the sample components.[17,18] We applied MCR to the area selected for Raman imaging that included the GSR-particle and cloth spectra. Two pure components were found in the sample, and each of them was assigned a different color. To facilitate the visualization, we assigned blue to the component attributed to the spectra of the GSR particles and red to the component attributed to the spectra of the cloth. As we can see, the MCR analyses obtained (Fig. 1) indicate that the GSR particles from the conventional and lead- free ammunition can be clearly identified even when they are trapped on interfering substrates. However, these samples are not representative of the real evidence submitted to the forensic laboratories, which are usually covered in blood.

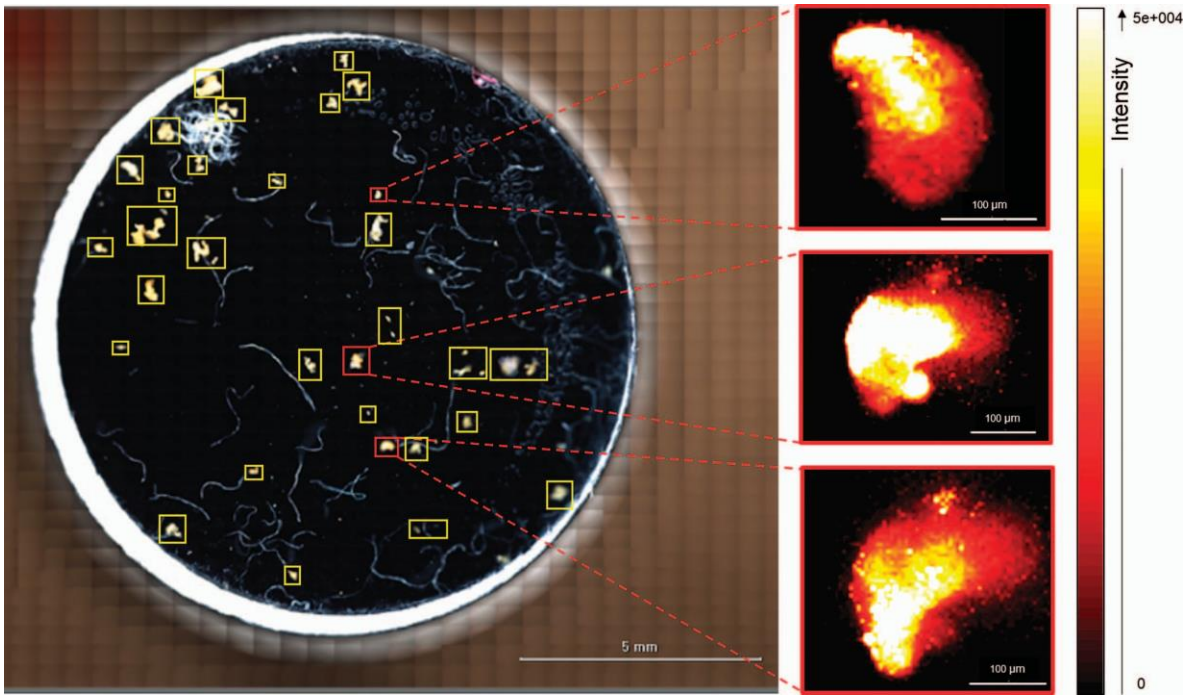

Figure 2. (left) A SEM-EDX stub used to collect GSR particles Super X ammunition was fired over a white cloth sample. Particles from the fired propellant are framed with yellow lines. (right) The chemical maps obtained for three areas with GSR particles (framed in red), given the 1800– 800 cm^{-1} region. Raman conditions: laser at 455 nm, 6.0 mW, 50x magnification objective lens, slit pinhole size 50 lm, 0.01 s x 30 scans, step size 5 lm (6132, 4485, and 1978 spectra, from top to bottom).

Therefore, to evaluate the application of the Raman imaging system to real samples, we stained the target cloths with blood after firing the ammunition; we then recorded the Raman image of the particles when the samples were completely dry. Figure 1d depicts a fired propellant particle on the white cloth covered with blood, which was mapped in 30 min. The MCR analysis obtained for this sample confirms that the presence of blood is not a restraining factor in the identification of GSR particles.

The most widely accepted method for collecting GSRs is to dab the evidence with adhesive lifters or stubs. Forensic investigators press these adhesive items to the face, hair, and hands of suspects; their clothing; and other objects susceptible to retaining GSR particles. Because SEM-EDX is currently the method of choice in practice for the analysis of GSR, aluminum stubs with carbon conductive adhesive are typically used to collect the GSR particles. Therefore, we evaluated the use of SEM-EDX stubs using Raman imaging for a complementary, nondestructive, and fast analysis of the macroscopic particles present on the stubs. The GSR particles on the target cloths were collected by dabbing SEM-EDX stubs on the cloths, and the stubs were then placed under the Raman imaging microscope. Figure 2 (left) depicts the image of a SEM- EDX stub used to collect the GSR particles produced after Super X ammunition was fired over a white cloth. As depicted, the stub shows fibers and yellow particles. Due to the dimensions of the SEM-EDX stub, imaging the totality of the surface would require several days. Thus, we collected images from only the areas where the yellow particles were present (framed with yellow lines in Fig. 2). This approach drastically reduced the image-collection time from days to several hours.

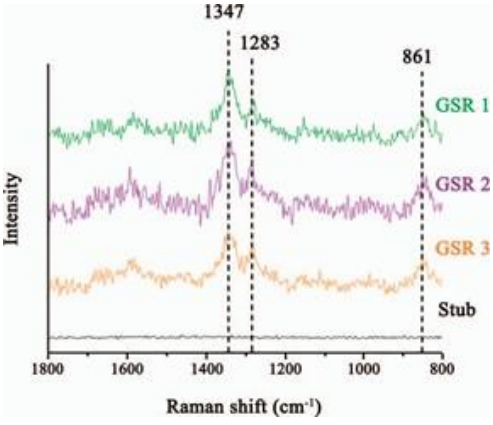

Figure 3. Comparison of the Raman spectra of the three GSR particles framed in red in Fig. 2 (labeled GSR1, GSR2, and GSR3, from top to bottom), and the SEM-EDX stub. Raman conditions: laser at 455 nm, 6.0 mW, 50x magnification objective lens, slit pinhole size 50 lm, 0.01 s x 30 scans.

Figure 3 depicts the spectra extracted from the Raman images collected when the three yellow particles were focused on (measured in 15–45 min). The spectra show bands at about 1347, 1283, and 861 cm^{-1}, which, as stated earlier, are also present in the spectra of the ammunition propellants. The stub itself did not show any spectrum under the measurement conditions used, showing that there is no substrate interference from the stub. Given this, we used a simple approach based on the generation of chemical maps to identify and highlight the signal corresponding to the GSR particles present on the stub. We generated chemical maps of the collected areas, given the most informative region for GSRs (1800–800 cm^{-1}). Figure 2 (right) also depicts the chemical maps obtained for three yellow particles present on the stub, which allows their identification. This confirms that the same stubs used for SEM-EDX analysis can also be used for the direct, fast, and nondestructive analysis of the macroscopic GSR particles using Raman imaging. This analysis offers additional useful chemical and morphological information to that obtained using the SEM-EDX technique.

4. Conclusions

The combination of Raman imaging and the MCR method has been demonstrated to be a powerful tool for the organic analysis of the GSR particles on cloth samples that avoids any type of sample preparation or particle collection, even under unfavorable conditions. Complete macroscopic GSR particles were also directly measured on different colored cloths stained with blood to mimic real evidence. In addition, the macroscopic GSR particles from conventional and nontoxic ammunition were collected by dabbing SEM-EDX stubs on the cloth targets and were then analyzed using Raman imaging. The GSRs collected were measured and successfully identified, and the spectra did not show any substrate interference. Due to the short acquisition times required for their measurement and the nondestructive nature of the proposed technique, a complementary analysis of the SEM-EDX stubs submitted to the forensic laboratories for a SEM-EDX analysis can be performed using Raman imaging to obtain further chemical and morphological information, especially for the nontoxic ammunition.

The Raman images (acquired in the minute time range) were obtained using an EMCCD detector, which dramatically reduces the acquisition time. It is important to

highlight that, although the total measurement time used in this study could be reduced by using shorter acquisition times or larger step sizes, doing so may compromise the quality of the Raman image obtained. However, a shorter total acquisition time can be used in the case of a preliminary analysis of the SEM-EDX stub. In addition, the presence of other environmental contaminants on the cloth sample should be studied to evaluate whether the GSR-particle identification based on the presence of only two bands is enough to avoid false positives. In such cases, to improve the visualization of any small peaks present in the propellants and to increase the selectivity and specificity of the method, much longer measurements are required.

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