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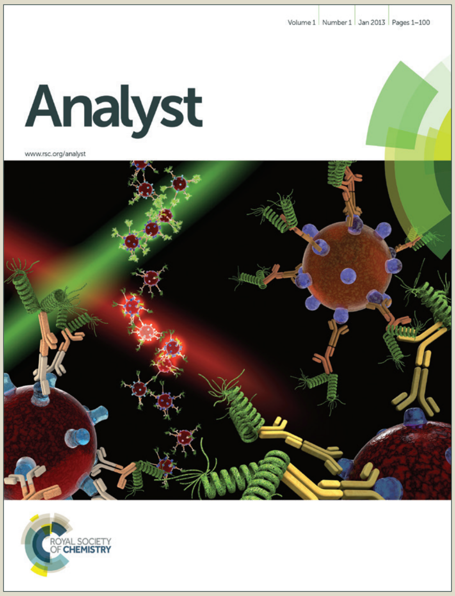

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A novel method for identification of inorganic and organic gunshot residue particles of lead-free ammunitions in the hands of shooters using Scanning Laser Ablation- ICPMS and Raman micro-spectroscopy

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ABSTRACT

A method based on scanning laser ablation and inductively coupled plasma-mass spectrometry (SLA-ICPMS) and Raman micro-spectroscopy for the detection and identification of compounds consistent with gunshot residue particles (GSR) has been developed. The method has been applied to the characterization of particles from the discharge of firearms using lead-free ammunition. Modified tape lifts were used to collect the inorganic and organic residues from skin surfaces in a single sample. Using SLA-ICPMS, aggregates related to the composition of the ammunition, such as Cu-Zn-Sn, Zr-Sr, Cu-Zn, Al-Ti, or Al-Sr-Zr, were detected, but this composition is only consistent with GSR from lead-free ammunition. Additional evidence was provided by micro-Raman spectroscopy, which identified the characteristic organic groups of the particles as centralite, diphenylamine or their nitrated derivatives, which are indicative of GSR.

KEYWORDS: Gunshot Residue Particles; lead-free ammunitions; SLA-ICPMS, Raman micro-spectroscopy

1. INTRODUCTION

Gunshot residue (GSR) is a set of burned and unburned particles formed as a result of the combustion process of ammunition and contains components from the firearm, projectile and cartridge case [1, 2]. The detection and identification of compounds consistent with GSR provides physical and chemical forensic evidence about a crime scene being indicative of an association with the use of firearms.

Currently, conventional forensic GSR analysis methods are based on the detection of lead-, barium- and antimony-containing spherical particles using scanning electron microscopy coupled with energy dispersive spectroscopy (SEM-EDX) [3, 4]. However, characterization of GSR has been hindered due to the introduction of so-called “lead-free”, “non-toxic”, “green” or “clean” ammunitions by some major handgun manufacturers. This ammunition was mainly developed to alleviate the health hazards caused by the exposure of instructors and competitive shooters to high lead levels as well as to avoid environmental damage [5]. The reduction in the generation of airborne lead was achieved through several manufacturing procedures: enclosing the base of the projectile or the entire projectile with brass, copper or gilding metal; fabricating the projectile from a sintered metal that does not contain lead [6]; or replacing lead, barium and antimony from the primer mixture with other ingredients, such as strontium, potassium-based oxidizers, diazodinitrophenol or calcium silicide 5, 7, 8. Consequently, the introduction of lead-free ammunition makes the identification of GSR unfeasible using the methods established for lead-containing ammunitions.

To date, several studies have been performed to elucidate the elemental profiles of GSR particles originating from lead-free ammunition cartridges. Based on SEM-EDX analysis, spherical particles composed mainly of Ti and Zn were identified in GSR discharged from lead-free Sintox ammunition [9]. In contrast, the analysis of the residues from CCI Blazer® Lead Free ammunition determined that the only metal present was Sr [10]. Oommen et al. examined four commercially available lead-free handgun ammunitions and concluded that combinations of Al, Si, Cu and Zn were very common [6], while Martiny et al. found irregular particles composed mostly of K, Al, Si and Ca in second-generation primers [5]. In 2008, the ENFSI (European Network of Forensic Science Institute) considered particles with one of the following elemental profiles “characteristic of GSR”: type 1: Gd, Ti, Zn and type 2: Ga, Cu, Sn [4]. However, this criterion was based on the analysis of tagged ammunitions introduced to regulate the technical demands of the German police and to identify GSR associated with the discharge of police firearms in shooting incidents [11].

From the above, it can be induced that, despite the elemental profiles of GSR, the particles from lead-free primers are generally in agreement with the composition of their respective primers; none of them could be considered exclusive to firearm handling. These particles share similarities with residues found in association with automobile repair and maintenance activities (mechanics, auto electricians and petrol station attendants), which may present not only Ba-Sb particles but also irregular particles composed of different concentrations of Al, Si, Mg, K, S, Fe, Ti, Cu, Ni and/or Zn [12]. As an alternative, some authors have recently suggested the inclusion of luminescent markers in ammunition for visual identification of GSR [13, 14]. However, current tagging techniques are not considered feasible and chemical compositional analysis is recommended [15].

The results from previous works imply that the identification of the organic components in GSR may allow for discrimination between GSR and other deposits [16, 17]. Organic GSR mainly originates from the primer mixture and the propellant powder, and their original chemical compositions have barely changed over time. Additives, such as stabilizers, plasticizers, flash inhibitors, coolants, deterrents, surface lubricants, dyes and other components, are added and may contribute to the composition of organic GSR (OGSR)[2, 18]. The proposed methods for OGSR analysis are based on bulk analysis using gas chromatography - mass spectrometry (GC-

MS) [16, 19], liquid chromatography tandem mass spectrometry (LC-MS/MS) [20], desorption electrospray ionisation-mass spectrometry (DESI-MS) [21] and micellar electrokinetic capillary electrophoresis (MICE) [22], even though these techniques do not allow for the identification of individual GSR particles.

In recent years, several studies have indicated Raman spectroscopy as a noteworthy screening tool for inorganic and organic GSR. In 1998, Raman microscopy was used to identify the anions of lead and barium in GSR deposited on cellulosic substrates[23]. More recently, a methodology based on Raman spectroscopy for ammunition identification using OGSR analysis from clothing was introduced[17]. In this work, OGSR spectra were linked to the spectrum of the propellant, allowing the GSR to be traced to the ammunition used. Combined with advanced statistics, Raman spectroscopy has also been used for ammunition caliber differentiation on the basis of the organic and inorganic residues deposited in cloth wipes[24]. Unfortunately, a “case by case” assessment procedure is required for each ammunition, which would not be feasible.

Although the information provided by the analysis of OGSR using several techniques is not conclusive, it would provide complementary information that may strengthen the evidence based on the inorganic residues. However, and as far as the authors are aware, these published analyses were performed on lead-containing primers. Therefore, further research into the analysis of the organic compounds in GSR originating from lead-free ammunition is an important task for forensic science.

The present work is focused on the development of a procedure for the proper interpretation of GSR analysis to prevent false negative conclusions resulting from the use of lead-free cartridges. To achieve this goal, the authors propose a new protocol based on the direct analysis of inorganic and organic particles from a single sampling device using surface analysis techniques for the identification of GSR. The main interest of forensic scientists is the availability of fast, selective, sensitive and non destructive examination methods. In this respect, LA-ICPMS and Raman spectroscopy are well suited. On the one hand, SLA-ICPMS has been demonstrated to be a suitable alternative to the standard SEM-EDX technique for the analysis of inorganic GSR from different lead-containing ammunition discharges[25]. The significant advantages of this technique rely on the limited sample preparation, the drastic reduction in the analysis time and the removal of the potential problem of skin cells hiding the lead in the GSR particles. Besides, Raman spectroscopy is a suitable screening tool for organic and inorganic GSR analysis because it requires minimal sample manipulation. The application of this technique would provide additional reliable information for the confirmation of lead-free ammunition.

Both SLA-ICPMS and Raman spectroscopy require a sampling device that should enable the application of both techniques to the same sample. Carbon adhesive devices designed for tape lifting have been proven to effectively collect GSR from the surface of the sampling area [2, 19]. However, a modification of the tape lift surface was required to adapt this device for the Raman instrumentation.

2. EXPERIMENTAL

2.1 Gunshot Residue Collection

The GSR particles were collected directly from the shooter´s hands using the tape lift as the collection device. Commercially available tape lift kits with a 12.7-mm diameter, designed for the SEM-EDX analysis of GSR (Adhesive Lifts GRA 200, Sirchie Finger Print Laboratories, Youngsville, NC, USA), consisting of carbon adhesive tape mounted on an aluminum stub and inserted into a plastic vial with a tightly fitted cap were used. However, they were modified for the simultaneous analysis of inorganic and organic residues by covering half of the carbon adhesive with 0.2-mm thick polytetrafluoroethylene (PTFE) (ATMOS, Bělá pod Bezdězem, Czech Republic). First, double-sided adhesive tape was used to cover half of the device and trimmed using a cutter. Once the protective cover of the double-sided adhesive tape was removed, a strip of PTFE was placed on the surface to reduce roughness. Finally, the PTFE layer was cut to match the tape shape. The procedure for modifying the sample holder is shown in Figure S-1 (Electronic Supplementary Information).

For the sampling process, stub was removed from the vial, applied to the thumb and index finger of the hand that held the weapon, and finally covered with the vial. The GSR collection was performed by 20 dabbings from the hands within 5 min of shooting to ensure no cross-contamination of the sample [26].

2.2 Firearm and ammunition

The samples were produced by discharging four different lead-free ammunitions: F1: Fiocchi 9 mm Luger ZP (Lecco, LC, Italy), F2: Fiocchi 9 mm Luger Gas Check (Lecco, LC, Italia), F3: Fiocchi 9 mm Luger 9x19 Leadless (Lecco, LC, Italia) and R1: Remington/UMC Leadless TM 9 mm Luger (Madison, NC). The conventional ammunition used in the reference assays was SB1: Sellier&Bellot 9 mm Luger FMJ (Vlašim, Czech Republic). All of them came from individual and unopened ammunition boxes and were discharged from a 9 mm Heckler & Koch pistol model USP Compact (Oberndorf/Neckar, Germany), one of the most commonly used by the police forces in Spain, which was carefully cleaned with a cotton cloth to eliminate any contamination from previous discharges. Based on the safety data sheets, the compositions of these ammunitions are given in Table S-1 (Electronic Supplementary Information).

Test shots were carried out on the test-firing field at the Basque Academy of Police and Emergencies (Arkaute, Basque Country, Spain). Five volunteers were asked to fire, and each one used a different ammunition to avoid cross-contaminations. To avoid contamination and carry over effects between experiments, the volunteers scrubbed their hands properly with tap water and liquid soap and rinsed again with tap water before each assay.

2.3 Scanning Laser Ablation - ICPMS

All experiments were performed on a New Wave Research UP266 ablation system (Huntingdon, UK) coupled to an Agilent 7500ce series ICP-MS (Agilent Technologies, Palo Alto, CA, USA) equipped with an octopole collision cell. Laser ablation of the carbon adhesive was performed using a frequency-quadrupled pulsed Nd:YAG laser operated in the Q-switch mode with a wavelength of 266 nm, 10 Hz repetition frequency, pulse duration of 4 ns, and with an energy density of 20 %. The ablated material was transported through a 1 m long polyurethane tube (i.d. of 4 mm) by a helium gas stream, used as the carrier gas due to its good characteristics as an ablation medium and enhanced transport efficiency for the ablated material [27]. During the laser ablation analyses, the stability of the plasma was monitored by continuously nebulizing a 1 ng mL^{-1} thallium standard solution via a dual-flow introduction system equipped with a Y-connector specially designed and manufactured for the physical requirements of our ICPMS system. Using this experimental arrangement, helium carrier gas (0.44 L min^{-1}) and argon nebulizer gas (0.75 L min^{-1}) flows were selected because they provide the highest sensitivity and lowest fractionation, which minimizes the occurrence of nonstoichiometric effects in the transient signal [27].

The ICPMS was tuned daily by nebulizing 1 ng mL^{-1} of a tuning solution using the developed dual-flow introduction system. The ion detection and counting system consists of an electron multiplier used in a pulse counting mode. The optimization of the laser ablation conditions was performed using a glass standard reference material (NIST SRM 612) to ensure maximum sensitivity, low oxide formation (assured by an m/z $^{232}Th^{16}O^+/^{232}Th^+$ ratio less than 0.3 %) and low fractionation effect (Th/U ratio close to one).

Twenty-two isotopes corresponding to 20 analytes, ^{13}C, and ^{205}Tl were qualitatively analyzed. The intensities of these isotopes were systematically normalized after subtracting out the mean background signal against the ^{13}C signal because it is homogeneously distributed throughout the polymer of the adhesive matrix. Normalization in LA-ICPMS allows for compensation of the variations in the ablation yield because of the laser energy drift and sample density.

A raster template based on the analysis of continuous lines on the carbon adhesive surface of the tape-lift device was used as the scanning-ablation pattern. The laser operated with a scan speed of 20 μm s^{-1} and it was focused with a spot size diameter of 160 μm. The helium background was measured before each analysis for the 40 s prior to ablation.

2.4 Micro-Raman spectroscopy

The Raman measurements were performed using an inVia confocal Raman microscope (Renishaw, Gloucestershire, UK). The spectrometer was coupled to a Leica DMLM microscope (Wetzlar, Germany). The excitation light was delivered to the sample, and the scattered light was collected from the sample through a Leica microscope long-range lens. The microscope was equipped with a motorized XYZ positioning stage with integrated position sensors on the X and Y axes. Renishaw WIRE 3.2 software controls the system and collects the data.

The microscope was set to 100x magnification, although some measurements were acquired using a 50x objective. The excitation wavelength was usually 514 nm but 785 nm in some cases.

The 514-nm Modu-Laser has a nominal power at the source of 50 mW and approximately 20 mW at the surface of the sample. This power can be controlled and attenuated using density filters. For the analysis of the standards, gunshot residues (GSR) and ammunitions, and to avoid thermal decomposition, the laser power was set to no more than 2 mW. The 785-nm NIR excitation laser has a nominal power at the source of 350 mW and approximately 150 mW at the surface of the sample. In this case, the laser power was also controlled, and it was set at no more than 2-3 mW. In all cases, the spectral resolution was approximately 1 cm^{-1}. Taking into account the heterogeneity of the GSR samples, more than 80 spectra were acquired from each sample to extract the spectral information. Each spectrum was an average of 2-15 accumulations for 10-15 s over a spectral range of 100 to 4000 cm^{-1}. Some spectra were acquired in a shorter wavelength range.

Spectral assignment was conducted using the Raman spectra of pure standards (minimum purity of 98%): 2,4-dinitroanisole (DNAN), 2-amino-4,6-dinitrotoluene (2-ADNT), 4-amino-2,6-dinitrotoluene (4-ADNT), 2,4-dinitrodiphenylamine (2,4-DNDPA), 2-nitrodipheylamine (2-NDPA), N-nitrosodiphenylamine (N-NDPA) and 4-nitrodiphenylamine (4-NDPA) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Octogen (HMX), cyclonite (RDX) and pentaerythritol tetranitrate (PETN) were provided by AccuStandard&Inc. (New Haven, USA). 2,4,6-trinitrotoluene was obtained from the Institute of Industrial Organic Chemistry from Warsaw (Poland). Diethyl phthalate (DEP) was supplied by Alfa Aesar (Karlsruher, Germany) and methyl centralite (MC) was from MP Biomedicals (Illikirch, France). Diphenylamine (DPA), dimethyl phthalate (DMP), ethyl centralite (EC), 2,4-dinitrotoluene (2,4-DNT) and nitroguanidine (NQ) were provided by Sigma-Aldrich (Steinheim, Germany). Quartz, calcite, gypsum and lazurite mineral standards were obtained from Kremer Pigmente

(Germany), as well as amorphous carbon and graphite powder black. $PbSO_4$ (anglesite: lead (II) sulphate 98 %) and $Ba(NO_3)_2$ (nitrobarite: barium nitrate ACS reagent) were purchased from Sigma-Aldrich (USA). Sb_2S_3 (stibnite: Ichinokawa Mine, Japan) was obtained from SPI Supplies (West Chester, USA). The spectrometer was calibrated before the Raman spectra acquisition using a silicon reference standard (520 cm^{-1}). The analysis of the Raman spectra was undertaken using GRAMS/AI 7.02 software package (Thermo Fisher Scientific Walthan, USA) and OMNIC 7.2 (Nicolet, Madison, Wis., USA).

3. RESULTS AND DISCUSSION

3.1 Modifying the sampling device

A good sampling support is important for the collection of the maximum number of GSR particles from a shooter´s hand. In a previous work,[25] the efficiency of the tape lifting devices designed for SEM-EDX and used with SLA-ICPMS was demonstrated. As previously mentioned, the modified tape lift device has two types of surface: adhesive carbon on which the SLA-ICPMS measurements are performed and a polymeric material on which the Raman spectroscopy is performed. It is important to select a surface that offers a low background (low fluorescence emission) for the analysis using Raman spectroscopy. If the sampling support offers a background with high fluorescence, Raman bands of interest coming from the GSR particles can be masked by the background of the sampling support. In this work, different sampling layers were tested for use as a sampling support for the GSR particle collection: polydimethylsiloxane (PDMS), polyvinylidene difluoride (PVDF), polyurethane (PUR), Parafilm® and polytetrafluoroethylene (PTFE).

As can be observed in Figure 1, PDMS and PTFE are the potentially usable materials for the sampling support because they have fewer Raman bands and low background across the spectrum. Because a PDMS-based surface must be freshly prepared in the lab, it could be more time consuming to choose this polymer as the sampling support; therefore, PTFE was selected.

Once the most suitable polymer for OGSR analysis was selected, commercially available tape lifts were modified by covering the carbon adhesive of the tape lifts with 0.2-mm thick PTFE. To analyze both the inorganic and organic residues on the same sampling device, only half of the carbon adhesive was covered with PTFE. The particles deposited on the carbon adhesive and on the PTFE layer were analyzed using SLA-ICPMS and micro-Raman spectroscopy, respectively.

3.2 SLA-ICPMS analysis

The analytes (^{27}Al, ^{29}Si, ^{31}P, ^{33}S, ^{35}Cl, ^{39}K, ^{44}Ca, ^{49}Ti, ^{57}Fe, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{71}Ga, ^{88}Sr, ^{90}Zr, ^{118}Sn, ^{121}Sb, ^{137}Ba, ^{157}Gd and ^{208}Pb) were chosen on the basis of the ENFSI criterion[4] and previously published works on the elemental composition of different lead-free ammunitions [5, 6, 9, 10]. However, the monitoring of ^{27}Al from the carbon adhesive surface required optimization of the laser energy to avoid the ablation of the aluminum stub below. The optimization procedure was performed by ablating a carbon adhesive sampling stub. Laser energies between 20 and 55% were tested, and the ^{27}Al and ^{13}C signals were monitored (Figure S-2, Electronic Supplementary Information). As shown, the ^{27}Al signal increased with increasing laser energy, indicating that the aluminum stub was being ablated. For energy values above 50%, saturation of the signal acquisition was observed. The ^{13}C signal remained constant assuring the efficiency of the ablation process. Therefore, a 20% energy density enabled the efficient ablation of the carbon adhesive without affecting the aluminum surface. However, the sensitivity of the method could be compromised when the laser energy is decreased. To determine the sensitivity, a study of the number of Pb-Sb-Ba particles detected was conducted using SB1 conventional ammunition. Five samples were obtained after 3 shots and were analyzed using 20 and 55% energy densities. The number of detected Pb-Sb-Ba GSR particles was not affected; it was similar at both energy values (24±7 and 22±8 particles, respectively). Thus, using 20% laser energy assures the ablation of the particles deposited on the carbon adhesive, and the presence of Al in the GSR could be confirmed .

As reported above, the modified tape lifts developed for this study use half of the carbon adhesive sampling area for inorganic GSR, which yields a surface area of 63.3 mm^2, and its analysis becomes prohibitively time-consuming. Because the particles do not tend to cluster, the distribution of particles collected on an adhesive surface is random, and it is reasonable to analyze a portion of the carbon adhesive surface. As reported in a previous paper [25], a raster ablation pattern provides an appropriate sampling protocol and guarantees GSR particle detection in a positive sample. Using this ablation pattern, 11.2 mm^2 (17.7 %) of the carbon adhesive surface was ablated in approximately 1 hour, assuring the effectiveness of the procedure.

The criterion established for the unambiguous identification of GSR particles by SLA-ICPMS was based on the appearance of signals corresponding to the isotopes of Pb, Sb and Ba at identical ablation times. However, this criterion was only acceptable for lead containing ammunition. The lack of consensus of the elemental profiles of GSR particles in these new ammunitions made the adoption of a less restrictive criterion necessary, and any positive peak corresponding to the monitored isotopes, chosen on the basis of previous works, was considered a possible positive signal (Figure 2). The criterion established for positively identifying a peak was based on a minimum signal defined as 10 times the mean background signal of the corresponding blank sampling stub normalized to the ^{13}C signal.

Under the selected sampling and scanning laser ablation conditions, GSR samples from the four ammunitions investigated were analyzed. These ammunitions were selected because they all cover, to a great extent, the different toxicity degrees of currently marketed ammunitions: heavy metal free ammunition (F1), and three lead-free ammunitions (F2, F3 and R1). Table S-2 (Electronic Supplementary Information) shows the elemental profiles and amount of particles detected after the analysis of samples taken after 1, 3 and 6 shots. As can be observed, these results confirm the suitability of the proposed method for GSR detection because a significant number of particles were observed when samples were obtained after a single shot. These results also indicate that particles of Pb, Sb and/or Ba were found without exception, when the lead-free or heavy metal free ammunitions were fired. Therefore, it is reasonable to assume that there was some degree of contamination produced by preceding firings and that the memory effect of the firearm plays an essential role. Previous works revealed that these particles adhere strongly to the barrel and inside the bullet chamber and neither mechanical cleaning with copper borebrushes nor chemical cleaning with acid solutions completely remove these deposits from the firearms[10, 28].

It is interesting also to note that R1 and F2 ammunitions with jacketed lead bullets generated Pb-Sb-Ba particles in greater amounts than F1 and F3. It seems quite reasonable that Pb was released from the open base of the bullet as a consequence of the high pressure generated from the hot gases produced when the primer and propellant are ignited.

In the same way, the results from the monitoring of other elements revealed the presence of type of particles other than Pb-Sb-Ba aggregates. However, the chemical composition and abundance of the GSR particles generated from lead-free ammunitions do not remain constant. This observation confirms that it is not feasible to establish a universal criterion for the identification of GSR derived from lead-free ammunitions and, unfortunately, a “case by case” assessment procedure should be performed. Figure 3 shows the percentage of each particle type out of the total particles found for each of the ammunitions assayed.

As expected, the presence of Cu (6-11.4%), Al (4.5-10.7%), Zr (4.1-9%) and Ti (8.3-13.1%) particles as well as Cu-Zn particles (3.3-8.1%) was noticeable for their statistical significances, but they are not relevant because they are not exclusive to GSR and could have an environmental or occupational origin [12, 29, 30]. Ti could be significant because it was not declared by the manufacturers, although it has been detected in these primers in previous works [31]. It should be noted that non-typical metallic aggregates of Al-Sr-Zr particles were found associated to F1 and F3 ammunitions. However and to the best of the authors’ knowledge, the occurrence Al-Sr-Zr particles in GSR have not been reported in the literature and further studies should be carried out to establish their origin and obtain supporting evidence about their correlation with lead-free ammunition.

It is worth mentioning that this technique does not provide information about the morphology of the GSR particles. However, as reported by other authors, when lead-free ammunitions are discharged, spherical and irregularly shaped particles are generated [5, 6] .

Therefore, it can be stated that, in the case of lead-free ammunition, there is not a type of GSR particle whose composition can be considered characteristic, except in the case of tagged ammunition. The confirmation of a shot with lead-free ammunition will require additional data.

3.3 Raman spectroscopic analysis

To obtain additional information for GSR identification in lead-free ammunition, Raman micro-spectroscopy was also applied to the analysis of GSR from the PTFE on the modified sampling device. An examination of the particles on the modified tape lift was performed with the help of the microscope at 100x magnification. Then, each particle was individually analyzed, and a Raman spectrum was obtained. Several Raman spectra were obtained for each particle in order to ensure the representativeness of the final spectral results (3 spectra at least). Using microscope observation times of 60 min and based on the experience gained throughout this work (288 samples analyzed), the number of particles ranged between 2±2 (1 shot sampling), 4±2 (3 shot sampling) and 12±4 (6 shot sampling), depending on the type of ammunition.

GSR particles were identified from all of the studied ammunitions and contained inorganic and organic particles. In Table 1, the inorganic and organic compounds found in the GSR particles from the five ammunitions are summarized. In the following paragraphs, some examples of these results are discussed in detail.

Centralite, diphenylamine and nitro-diphenylamines. In Figure 4A, a Raman spectrum (in blue) of a 2-µm GSR particle from F1 ammunition is shown. Several organic compounds were detected. Methyl centralite (MC) and ethyl centralite (EC), the most common centralites in gunshot residues, have similar Raman features. The broad band at approximately 2793-3010 cm^{-1} in the blue spectrum suggests the presence of centralites. Moreover, the main bands (1001, 1606 and 3061 cm^{-1}) associated with centralites were also observed in the diphenylamine (DPA) spectrum; thus, centralite and diphenylamine distinction was difficult. It is worth mentioning that the use of EC and MC is exclusive of ammunition[22, 32-34]. Therefore, their detection in GSR provides confirmatory evidence of firearm discharge.

Although DPA is one of the most predominantly used stabilizers in smokeless powders and explosives, it could not be considered as evidence of GSR because it is a widely used industrial compound and might induce false positives[35]. However, during the stabilizing reactions and firing process, nitrated derivatives of DPA (predominantly mono-, di- and trinitro-DPA) are formed [19, 36]. Thus, nitrate derivatives, such as 2-NDPA, 4-NDPA or 2,4-DNDPA, and their association with DPA on a suspect´s hands involve him in manipulating firearms or explosives[37]. Figure 4B shows a Raman spectrum (in blue) of a 2-µm F3 GSR particle. In addition to DPA, bands related to nitro-DPA compounds are also detected. The band at 1349 cm^{-1} confirms the presence of nitro-DPA as 2-NDPA or 2,4-DNDPA, as shown in the spectrum of the nitro-DPA standard.

Nitrotoluenes. Other mixed organic-inorganic particles were also identified. Nitrate compounds were identified in particles of approximately 3-4 µm from R1 ammunition (see the microscopic details in Figure 5) where the main band at 1043 cm^{-1} could be assigned to ammonium nitrate (NH_4NO_3). Raman bands related to DPA, N-NDPA and centralite were also observed (see band at 1601 cm^{-1} in Figure 5). In addition to these compounds, some unknown bands at 1339, 1357 and 1544 and a shoulder at 1611 cm^{-1} were detected in the same spectrum. According to the Raman spectra of the standards, the bands at approximately 1357, 1544 and the shoulder at 1611 cm^{-1} could be related to the NO_2 stretching vibrations of 2,4-dinitrotoluene (DNT), used in the propellant.

These results indicate that Raman spectroscopy is a suitable screening tool for the analysis of inorganic and organic compounds of GSR and also provides valuable complementary information when there are doubts about the elemental profile of GSR obtained by SLA-ICPMS. Unlike metallic GSR, the analysis of organic GSR is not influenced by the memory effect of the firearm when different ammunitions are discharged because their persistence time is finite [37]. Therefore, by taking into account that the firearms were meticulously cleaned before firing, it could be confirmed that the organic compounds detected correspond to the fired ammunitions and not to the previously used ones.

In addition to the mentioned identification of compounds expected in GSR, other particles were identified.

Unburnt graphite powder and amorphous carbon. It is well-known that propellant grains are coated with varying amounts of graphite [38], usually used to prevent static electricity from causing undesired ignition. Because graphite powder burns at 1093 ºC, below the temperatures reached in the explosion of the ammunition, the unburnt graphite powder is expelled as soot and can be detected in GSR as graphite dust (D band at 1352 cm^{-1}, G band at 1576 cm^{-1} and 2D (overtone of D band) band at 2715 cm^{-1}). In Figure S-3 A (Electronic Supplementary Information), different spectra of carbon crystallized as graphite and amorphous carbon can be observed. The green spectrum represents the graphite covering of F1 ammunition (the left microscopic image represents the graphite covering of the ammunition under a 20x objective lens). The blue spectrum can also be assigned to graphite, and it belongs to a particle detected on the PTFE used for F1 GSR sampling (not completely spherical particle of approximately 7 µm, see the microscopic detail under a 100x objective lens in the middle of Figure S-3-A). The bands of the blue spectrum coincide in symmetry and position with those of the green spectrum; thus, it can be confirmed that the non-spherical particle is unburnt graphite due to the graphite covering of the ammunition. In contrast, the red spectrum from Figure S-3-A shows the representative bands of amorphous carbon, more broad than those belonging to graphite. Moreover, the position of the amorphous carbon bands does not fit exactly with those of the graphite covering and non-spherical particle. This last spectrum was acquired on the blackened spherical particle (approximately 10 µm, see the microscopic detail under a 100x objective lens in the top-right of Figure S-3-A). This observation suggests that the spherical particle is a GSR particle from the carbon powder present in the ammunition. In the Raman spectra shown in Figure S-3-B (Electronic Supplementary Information), the D and G bands of the carbon can be observed. These bands belong to a 2-µm spherical black particle also from the F1 ammunition. If we compare the width of these bands with the ones of the blackened spherical particle (red spectrum and microscopic image in the top right of Figure S-3-B), the latter are narrower than those from Figure S-3-A red spectrum. Therefore, different crystallization degrees of the carbon can be found in the burnt GSR carbon particles.

$Ba(NO_3)_2$, $PbSO_4$ and Sb_2S_3. Other inorganic GSR particles, possibly related to the use of standard ammunition in previous uses of the weapon used in the test, were also found. As an example, Figure S-4-A (Electronic Supplementary Information) represents Raman spectrum of an elongated particle 1-2 µm in width and 5 µm in length detected after firing R1 ammunition with bands corresponding to graphite, $Ba(NO_3)_2$ and $PbSO_4$. In Figure S-4- B, a Raman spectrum corresponding to a 3-4 µm size particle detected after firing the F2 ammunition is shown. It should be noted that, despite the presence of additional bands that do not correspond to pure Sb_2S_3, there is no doubt about the presence of this sulfide mineral in the particle.

External contaminants. A first analysis under the optical microscope revealed the presence of some brownish spots (see the details of these spots under a 100x objective lens in Figure S-5, Electronic Supplementary Information). Initially, these spots could not be considered GSR particles. By analyzing the bands in the Raman spectrum of Figure S-5, excluding the PTFE bands (labeled with a T) additional bands were distinguished. The bands at 1447 and 1731 cm^{-1} could be assigned to CH_2 and CH_3 scissoring vibrations [δ (CH_2 and CH_3)] and C=O stretching vibrations [υ (C=O)] of skin proteins and lipids, respectively [39, 40]. The bands at 2873 and 2936 cm^{-1} are also associated with skin, but, in this case, they are related to the asymmetric stretching vibration of CH_2 and CH_3, respectively[41]. The organic compounds present in the GSR particles can also present bands in the 1400-1800 and 2000-3200 cm^{-1} spectral regions; thus, it is important to consider contamination derived from the skin during the GSR sampling for Raman spectroscopy. However, the band at 3061 cm^{-1} is indicative of centralite, and DPA does not appear in the skin blank and it is perfectly distinguishable when the sampling is performed on a glove or on the skin (Figure S-5 A and B). If the particle is constrained to be spherical (ensuring it is GSR), the interference from skin cells is very limited.

In addition to the influence of skin, additional external contaminations should be considered before performing the Raman assignments for the GSR particles. The primary example of external contamination could be the deposition of crustal particulate matter, formed by the erosion caused by the wind over the surface of the Earth. The granulometry of these particles is variable, and their emission wavelength of the compounds varies depending on the nature of the soil, humidity, wind speed, etc. A very common crustal particle identified in all PTFE sampling supports was quartz (SiO_2), and the size of most of the quartz particles detected was between 30-60 µm (see the microscopic detail of the biggest quartz particle identified under a 50x objective lens in Figure S-6-A, Electronic Supplementary Information). Calcite ($CaCO_3$) was another important crustal particle. Most of the calcite particles identified ranged from 2 to 5 µm (see the microscopic detail of a 2-3-µm calcite particle under a 100x objective lens in Figure S-6-B, Electronic Supplementary Information). Both quartz and calcite were identified in most of the PTFE sampling supports used for the GSR sampling of the different ammunitions. Gypsum ($CaSO_4 \cdot 2H_2O$) was also detected in some samples, and like calcite, the gypsum particle size ranged from 2 to 3 µm. In Figure S-6-C, in addition to the PTFE Raman bands, two additional bands at 3403 and 3492 cm^{-1} were also detected. These bands can be assigned to the gypsum water molecule stretching mode (I) and (II), respectively [42].

Finally, the appearance of particles of aluminum and calcium silicate, lazurite type, can be mentioned; although, on this occasion, they are directly related to the composition of heavy-metal free primers. Figure S-6-D shows the complete overlap of the bands of the lazurite pattern and a 4-µm F2 GSR particle.

4. CONCLUSIONS

The characterization of GSR particles from lead-free ammunition on the hands of a shooter presents serious challenges. In this study, a novel procedure based on SLA-ICPMS and Raman micro-spectroscopy for the analysis of inorganic and organic GSR is proposed. To achieve this goal, a modified sampling device based on a tape lift that combines carbon adhesive and PTFE surfaces has been developed, enabling the analysis of inorganic and organic GSR using a single sample.

The suitability of the proposed method has been shown via the analysis of GSR samples taken from a shooter´s hand after the discharge of lead-free ammunition. The SLA-ICPMS analysis of 20 elements enabled the detection of aggregates related to the ammunition: Al-Ti, Cu-Zn, Cu-Zn-Sn and Sr-Zr. The presence of Al-Sr-Zr particles has not been previously reported. In addition, the presence of Pb-Sb-Ba particles has been detected in all of the samples, indicating a memory effect in the firearm, even when it has been meticulously cleaned. Although these aggregates are consistent with lead-free ammunition, it cannot be said unequivocally that they are characteristic of this type of ammunition. These results highlight the necessity of further research into the presence of these particles to establish a common criterion for lead-free ammunition.

The analysis on the same sampling device using Raman micro-spectroscopy shows the presence of organic compounds indicative of GSR, such as centralites and nitrated derivatives of DPA in combination with DPA. The detection of the organic components in the GSR provides supporting evidence to the analysis of the inorganic particles in GSR detected by SLA-ICPMS. The entire analysis process for both techniques does not exceed two hours. The laser ablation scan is automatic. In this paper, the observation of the particles via optical microscopy for subsequent analysis by Raman spectroscopy is performed manually. It is expected that this last step can be automated using image recognition software, which will further decrease the analysis time.

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Figure Captions

Figure 1. Raman spectra of the potentially usable materials as the sampling support for the GSR particle collection.

Figure 2. Characterization of ten GSR particles (GSR1 to GSR10). The colored vertical bands show the abundance peaks of the different metals at the same ablation time. The ablated stub comes from a sample of the hand of a shooter who fired three shot with a 9 mm pistol using R1 ammunition.

Figure 3. Elemental profile of the particles found, represented as the percentage of each particle type out of the total particles found for each of the ammunitions assayed: 45 tape-lifts analyzed in the different tests with a total of 1381 particles (350 from R1, 384 from F2, 315 from F3, 332 from F1).

Figure 4. A) Raman spectrum of a representative F1 GSR particle in blue (see the microscopic detail of the particle under a 100x objective lens) and the Raman spectra of the methylcentralite and DPA standards. B) Raman spectrum of a representative F3 particle (see the microscopic detail of the particle under a 100x objective lens) and Raman spectra of DPA, MC, 2,4-DNDPA and 2-NDPA standards.

Figure 5. Raman spectrum of a representative R1 particle (see the microscopic detail of the particle under a 100x objective lens) and Raman spectra of the DPA and 2-NDPA standards

Table 1. Inorganic and organic compounds identified in GSR particles coming from the five ammunitions using micro-Raman spectroscopy.

Ammunition	Inorganic compounds	Organic compounds
F1	Graphite, amorphous carbon, nitrobarite ($Ba(NO_3)_2$), (KNO_3), calcite ($CaCO_3$)	DPA, centralite
F2	Graphite, Sb_2S_3	DPA, 2,4-DNDPA,
F3	Graphite, amorphous carbon, lazurite	DPA, 2-NDPA, centralite
R1	Graphite, amorphous carbon, nitrobarite, niter, calcite	DPA, N-NDPA. 2,4-DNT, centralite
SB1	Graphite, amorphous carbon, nitrobarite, niter, (NH_4NO_3), calcite, ($PbSO_4$)	DPA, 4-NDPA, 2,4-NDPA, N-NDPA, centralite, TNB, TNT, methyl cellulose

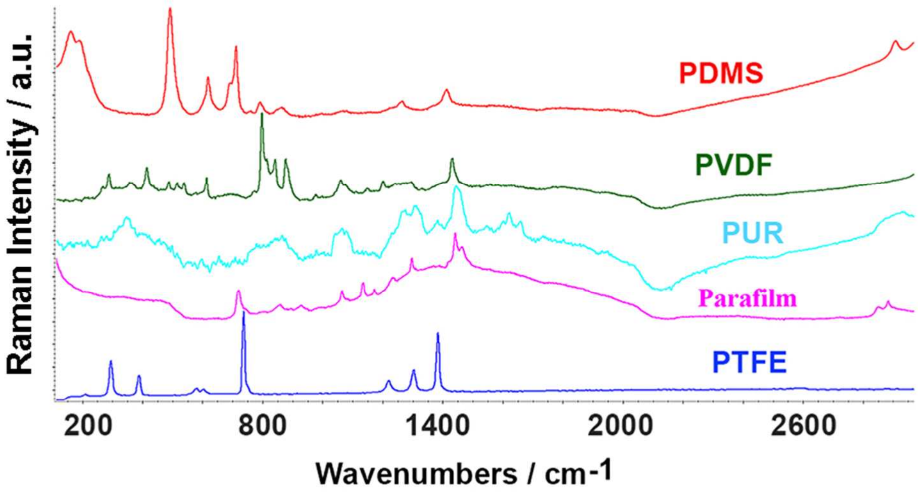

Figure 1. Raman spectra of the potentially usable materials as the sampling support for the GSR particle collection
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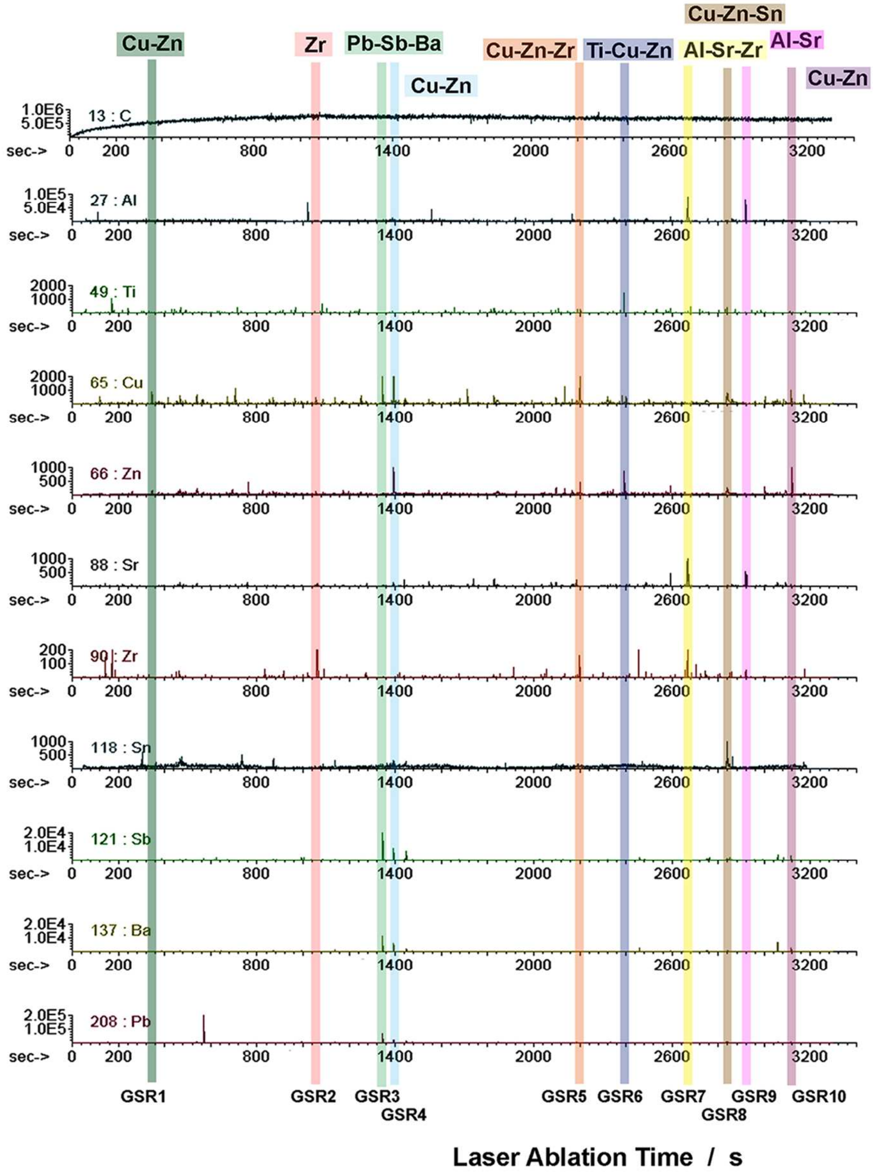


Figure 2. Characterization of ten GSR particles (GSR1 to GSR10). The colored vertical bands show the abundance peaks of the different metals at the same ablation time. The ablated stub comes from a sample of the hand of a shooter who fired three shot with a 9 mm pistol using R1 ammunition.
105x139mm (300 x 300 DPI)

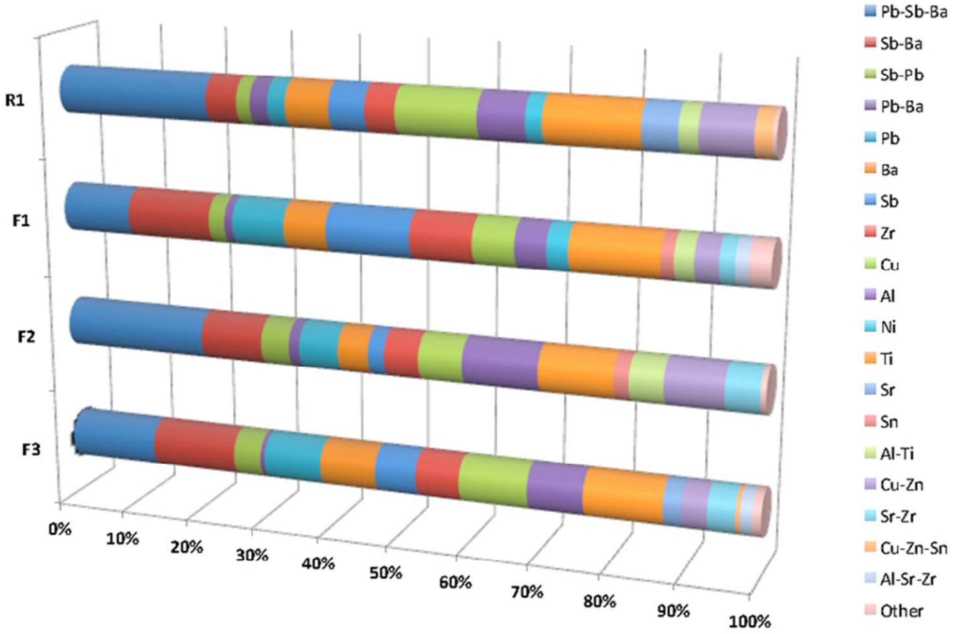


Figure 3. Elemental profile of the particles found, represented as the percentage of each particle type out of the total particles found for each of the ammunitions assayed: 45 tape-lifts analyzed in the different tests with a total of 1381 particles (350 from R1, 384 from F2, 315 from F3, 332 from F1).
74x50mm (300 x 300 DPI)

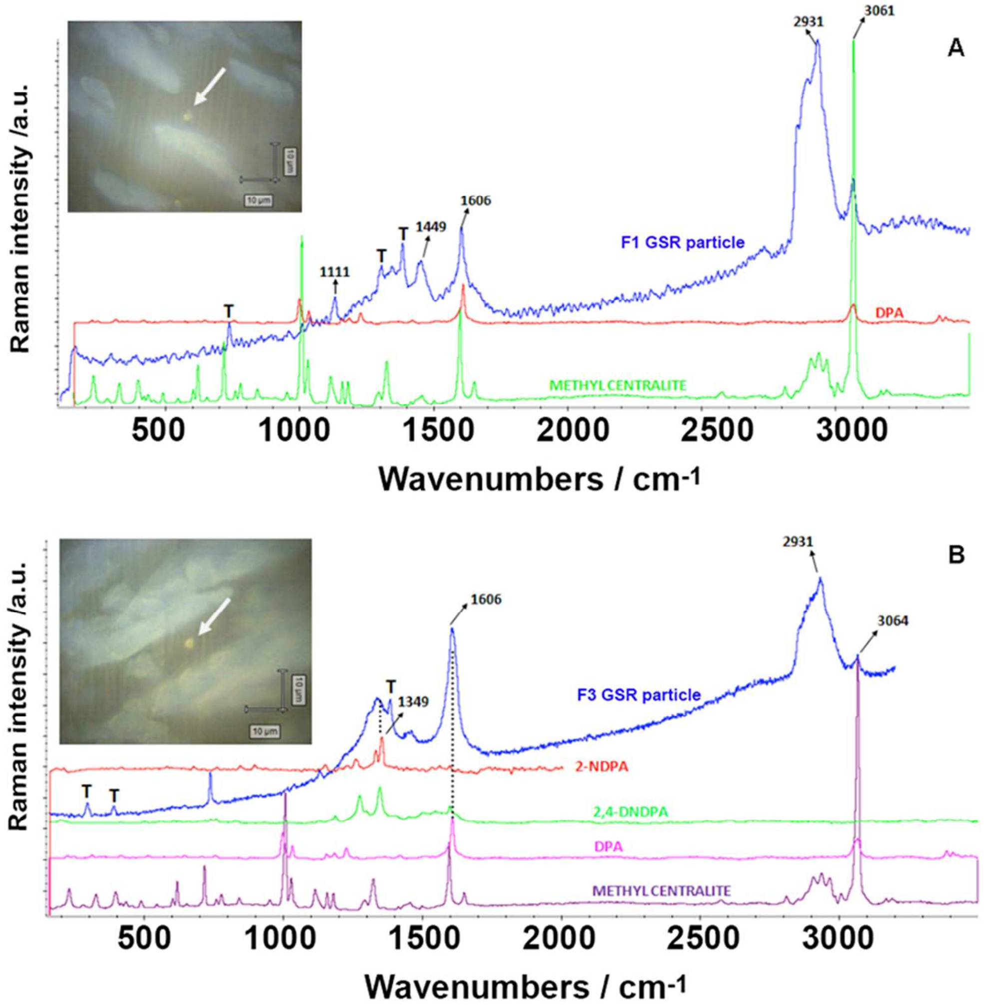


Figure 4. A) Raman spectrum of a representative F1 GSR particle in blue (see the microscopic detail of the particle under a 100x objective lens) and the Raman spectra of the methylcentralite and DPA standards. B) Raman spectrum of a representative F3 particle (see the microscopic detail of the particle under a 100x objective lens) and Raman spectra of DPA, MC, 2,4-DNDPA and 2-NDPA standards.
73x76mm (300 x 300 DPI)

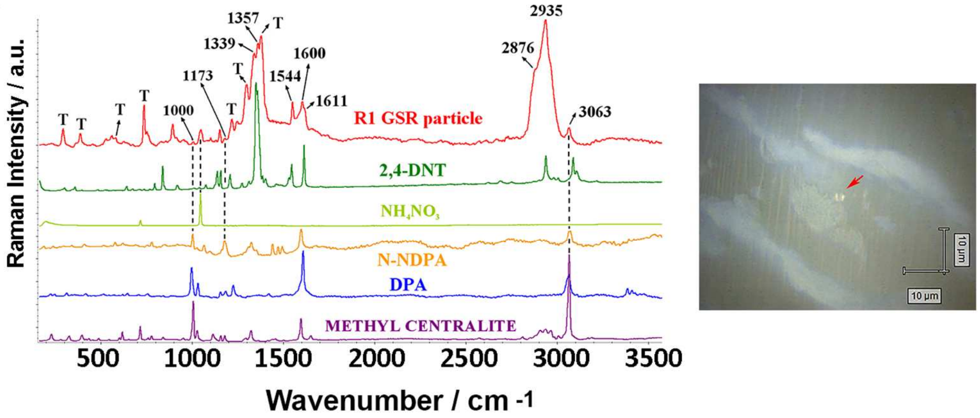
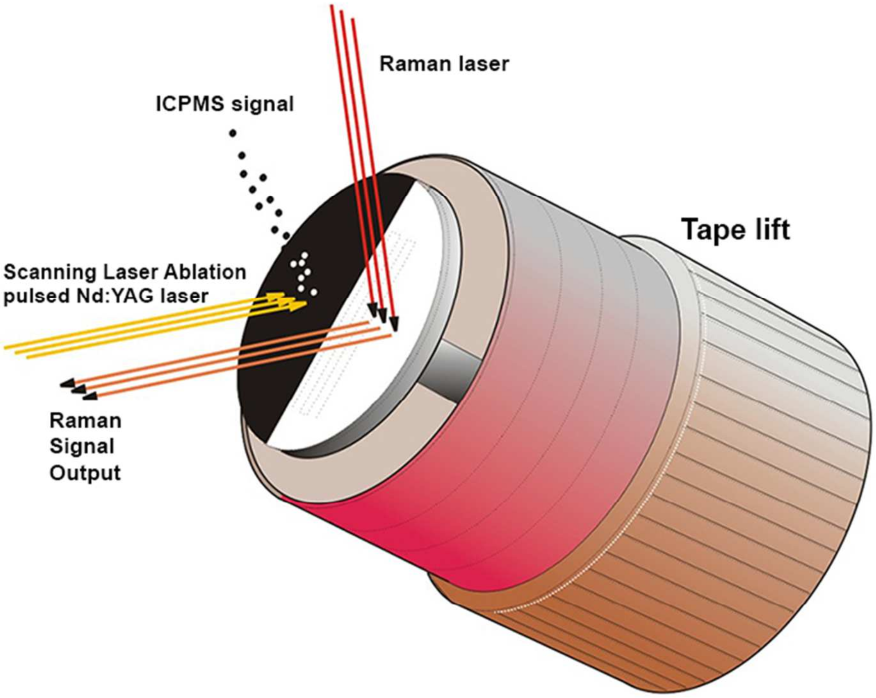


Figure 5. Raman spectrum of a representative R1 particle (see the microscopic detail of the particle under a 100x objective lens) and Raman spectra of the DPA and 2-NDPA standards
123x55mm (300 x 300 DPI)



A simultaneous method based on SLA-ICPMS and Raman micro-spectroscopy has been applied to the characterization of GSR using modified tape lifts
66x47mm (300 x 300 DPI)