

# **Review Article**

## **SIGNIFICANCE OF USING SCANNING ELECTRON MICROSCOPY WITH ENERGY DISPERSIVE X-RAY SPECTROMETRY (SEM-EDX) FOR ANALYSIS OF GUN SHOT RESIDUE**

### **Abstract**

Forensic application of SEM are found mostly in areas where there is a need for good imaging at relatively high magnifications in combination with elemental analysis. SEM enables the forensic scientist to examine specimens at much higher magnification than those possible with optical microscopy and without the difficulties of specimen preparation associated with conventional electron microscope. Physicochemical examinations of gunshot residues, called also chemical ballistics, are helpful, e.g. in identification of damages and injuries as the effect of the use of firearms (with indicating the entrance and exit of projectile), estimation of the shooting distance and also establishing, whether a person has used a firearm. It is discussed in details that SEM-EDX can serve as a powerful tool for forensic scientists to classify and discriminate evidence material because they can simultaneously examine the morphology and the elemental composition of objects.

Keywords: gunshot residues, elemental analysis, light microscope, forensic science

### **1. Introduction**

The electron microscope uses electron beams to generate an image of the sample, unlike the compound light microscope, which uses light to image the sample. Two important parameters of any microscope are the resolution and magnification. The electron microscope has a much greater resolution capability than the light microscope because the wavelength of the electrons is about  $10^5$  times smaller than the wavelength of light. The best resolution with the light microscope is around 200 nm whereas with electron microscope it is 5 nm. Thus, the resolution with electron microscope is ~100 times higher than the light microscope. Similarly, the magnification of electron microscope is almost 300 times more than the optical microscope.

Scanning Electron Microscopy (SEM) has been an important forensic tool since the 1970s (Pilkington, 2022). SEM enables the forensic scientist to examine specimens at much higher magnification than those possible with optical microscopy and without the difficulties of specimen preparation associated with conventional electron microscopy (Taylor, 1973). One of the most striking properties of SEM is its ability to combine imaging with the elemental analysis together with its suitability for digitisation and automation of complete tasks. SEM can also be a

**Comment [User1]:** The title of the review article is not comprehensive, as the authors are referred only to the analysis of gun shot residue, although in the main body of the manuscript they are also referring to car paint residue and glass residue.

**Comment [User2]:** The authors are requested to take care of the grammar.

**Comment [User3]:** SEM standalone could not be used for elemental analysis. The authors are requested to rephrase it.

**Comment [User4]:** I suggest scanning electron microscopy instead of light microscope, as the authors are focusing on the significance of SEM in the forensic applications.

**Comment [User5]:** The authors are kindly requested to provide appropriate references for the magnification, wavelength and resolution of SEM and light microscope.

useful tool for solving the forgeries, and to interpret the damage of textile materials ([Vermeijetal, 2009](#)).

Forensic application of SEM are found mostly in areas where there is a need for good imaging at relatively high magnifications in combination with elemental analysis ([Yashoda etal, 2021](#)). The concept of trace evidence in forensic science originates from Locard's exchange principle, which states that "every contact leaves a trace." The trace evidence is typically in the form of particles of skin, hair, fibers, clothing, soil, paint, and glass, among other materials ([Mummery, 2021](#)). Fragments of various materials such as glass, paints, fibres and gunshot residues (GSR) are frequently present at the scene of such events as car accidents, burglaries, fights or crimes committed with the use of fire arms. Very small fragments of those materials that arise during these events can be recovered from hair, hands, clothing and shoes of participants and witnesses of the events and provided as an evidence at a court ([Zadora & Brožek-Mucha, 2003](#)). Traces such as fragments of glass, paints or gunshot residues most often occur in very small quantities and are of relatively heterogenic character and with a complex composition. Thus, sensitive analytical methods like SEM are required in order to obtain satisfactory results from small amounts of sample.

When SEM is combined with energy dispersive X-ray spectrometry it is known as SEM-EDX. It is a powerful tool for forensic scientists to classify and discriminate evidence material because they can simultaneously examine the morphology and the elemental composition of objects. Moreover, the obtained results could be enhanced using some methods of chemometric analysis ([Zadora & Brožek-Mucha, 2003](#)).

## 2. Experimental details of SEM-EDX

The SEM is an instrument that produces a largely magnified images by using electrons beams. A schematic diagram of the SEM is shown in figure 1. Firstly, a beam of electrons is produced at the top of the microscope by an electron gun mounted in an enclosure and maintained at high vacuum. The electron beam follows a vertical path through the microscope. The beam travels through electromagnetic fields and lenses, which focus the beam towards the sample. There are three main signals that occur when an electron beam meets the sample (figure 1). Secondary electrons, backscattered electrons, and X-rays. These signals are produced from interactions between electrons and the sample material, and each signal provide different information about the sample. Secondary electrons emanate from atoms on the surface of the sample material and recording them enables SEMs to output detailed, topographic imagery with high spatial resolution. Backscattered electrons are refracted incident electrons that have pierced below the sample surface and interacted with atoms inside the material. Atoms of more massive elements in the sample bounce incident electrons further away, and these scatter patterns can reveal information about the sample's internal elemental makeup ([Pilkington, 2022](#)).

**Comment [User6]:** The authors are requested to take care of the grammatical number in this sentence.

**Comment [User7]:** The authors are requested to follow either UK English or USA English in the whole text.

**Comment [User8]:** Rephrase of the sentence is required.

**Comment [User9]:** The examples (glass, paints, gunshot residues) have been repeated two sentences before and they could be omitted.

**Comment [User10]:** The sentence is not specific; thus, the authors are requested to explain why they have selected SEM as a sensitive analytical method and which are other methods that could be used for such applications.

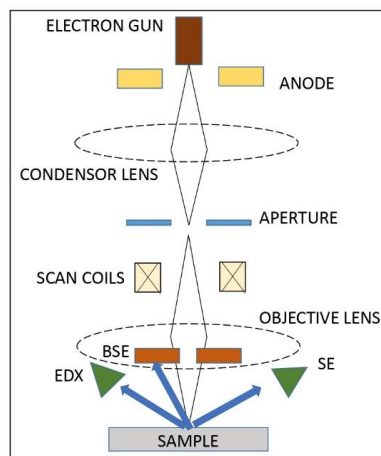
**Comment [User11]:** The phrase is general and could mislead the readers. The authors are requested to refer to specific method of chemometric analysis.

**Comment [User12]:** The article "the" is not required before the technique.

**Comment [User13]:** I would propose "technique" or "microscope" instead of "instrument" and then rephrase the sentence accordingly.

**Comment [User14]:** The authors are requested to take care of the grammar.

**Comment [User15]:** I suggest "penetrated" rather than "pierced".



**Comment [User16]:** The authors are kindly requested to add the meaning of the abbreviations presented in the figure in the caption.

**Figure 1:** Schematic diagram of SEM-EDX. A beam of electrons is produced at the top of the microscope by an electron gun mounted in an enclosure and maintained at high vacuum. The beam travels through electromagnetic fields and lenses, which focus the beam towards the sample. All electromagnetic lens and detector are maintained at high vacuum. There are three main signals that occur when an electron beam meets the sample.

X-rays are formed when electrons in the electron beam replace electrons from atoms in the sample. Designated detectors mounted in vacuum collect these X-rays, backscattered electrons, and secondary electrons and convert them into a signal that is sent to a screen. This produces the final image. The wavelengths of X-rays produced in SEM are related to the elements that electrons have interacted with. Energy-dispersive spectrometry (EDX) is combined with SEM in SEM-EDX to record this information. EDX can be used for both qualitative and quantitative analysis, enabling users to identify both the type of elements that are present as well as the percentage of each element's concentration within the sample.

**Comment [User17]:** The authors are requested to provide appropriate reference about the generation of the X-rays in SEM-EDX.

**Comment [User18]:** The authors are requested to provide appropriate reference for the qualitative and quantitative analysis through EDX.

It is worth mentioning that SEMs can achieve magnification higher than 100,000x with a resolution below 1 nm. The samples need to either be conductive themselves or be coated with a conductive material such as gold-plated nanoparticles before putting it in SEM. SEM-EDX is considered a relatively rapid, inexpensive, and basically non-destructive approach to surface analysis. So, the sample may be reused for other analyses. This is particularly helpful in the case of forensic investigation as often the sample obtained is of micro or nanoscale range.

**Comment [User19]:** In the introduction section the authors have stated that the resolution of SEM is 5 nm, while here it is stated <1 nm. They are asked to correct to the right one and provide an appropriate reference.

**Comment [User20]:** The authors are requested to take care of the grammar of the sentence.

**Comment [User21]:** The authors are requested to provide a more formal vocabulary.

### 3. Result and discussion: SEM-EDX in forensic analysis

#### A) Gunshot Residue:

The first scanning electron microscope with very high resolution came in 1937 by Manfred von Ardenne (Von Ardenne et al., 1921). In 1968, research for detection of GSR elements was done using SEM/EDX first carried out in England (Ward, 1982). GSR, which is also sometimes called Firearm Discharge Residue, can be collected from various surfaces such as parts of the human

**Comment [User22]:** The authors are requested to consider if it is really an inexpensive technique according to the purchase cost, maintenance cost and the cost of sample preparation material, especially if gold-plated nanoparticles are required.

**Comment [User23]:** I suggest this sentence to be transferred in the previous section, as it is about the first SEM and not about gunshot residue.

**Comment [User24]:** The abbreviation should be placed in parenthesis after the first time the complete phrase is found in the text.

**Comment [User25]:** The English in this point is very simplified.

body, clothing, parts of an automobile, or the vicinity of the suspected gun discharge. The efficiency of collecting the samples will greatly affect the detection and analysis. A simple tape lift-off method is the most common method for collecting samples for SEM analysis and is used to collect GSR from various surfaces. An SEM aluminium stub with a carbon adhesive is used to collect the samples ([nanoscience.com](http://nanoscience.com)).

**Comment [User26]:** It should be "a" instead of "an".

Anywhere between 0 and 1,000 particles, or even in excess of 10,000 particles, can be found on a GSR sample. It depends largely on the circumstances, such as which gun has been used, which ammunition, and which location (whether outdoors or indoors). While suspects are the main source of samples, it is still common practice to take samples from victims. In instances of potential suicide, it is possible to gather evidence of whether or not the victim has used the firearm in order to take their own life. Significantly, more GSR particles will be present on the victim as compared to cases where another individual has fired the gun. It is hard to determine exactly what a positive GSR outcome means as it is generally accepted that GSR particles do not only come from firearms. They may, for instance, be picked up via secondary processes. Shaking the hand of a person who has shot a firearm recently may deposit GSR particles on a person's hand.

**Comment [User27]:** I suggest "more than" instead of "in excess of".

**Comment [User28]:** The authors are requested to provide an appropriated reference about the statistics of the particles and the referred circumstances.

The presence of GSR alone is not sufficient – it is always presented as supplementary evidence. For instance, if investigators locate GSR-particles on a person's hand and that person's neighbour was killed the previous night, this does not guarantee that the person in question murdered their neighbour ([azom.com](http://azom.com)). French et. al. (2013) have reported that while varying between runs, over 100 particles were transferred via a handshake in one instance, and it was found that even very large particles (60–100+  $\mu\text{m}$ ) were transferred from the shooter to the second individual via a handshake. The findings have implications for forensic investigations, including highlighting the need to sample from individuals who might have been involved in transfers and underscoring the importance of achieving accurate particle counts using the SEM-EDX method. Most importantly, the findings suggest that the presence of GSR (especially in small quantities) may not always indicate that a person discharged a firearm and that the possibility for misidentification of the shooter exists, as does the potential to distinguish shooters from those who have acquired GSR through secondary transfer.

**Comment [User29]:** This is a very simplified sentence and the authors are requested to rephrase it significantly.

The gunshot residue are mainly of two types, Organic and Inorganic. Organic residues originate from the propellant: unburned and partially burned gun powder particles, some products of their transformation and also particles of lubricants. Inorganic residues, mostly metallic, originate from the primer as well as from metallic parts of cartridge and the weapon itself ([Gunaratnam, Himberg 1994](#)). Among them only particles originating from the primer reveal specific chemical content and characteristic morphology. Their morphology reflects the kinetics of the processes undergoing during a gunshot, especially rapid cooling of droplets of the molten metals present in the expanding plum of products of the primer detonation and the propellant combustion. The analysis of gunshot residue of inorganic nature, is always preferable using scanning electron microscopy

**Comment [User30]:** The authors are requested to take care of the grammar number.

**Comment [User31]:** The authors are requested to rephrase the sentence.

coupled with the X-ray microanalysis (SEM/EDX) ([Brozek-Mucha, 2014](#); [Taudte et al., 2014](#)), because it is nondestructive and allows the chemical and morphological identification of mineral particles ([DeGaetano & Siegel, 1990](#); [Romolo & Margot, 2001](#)).

Neutron Activation Analysis (NAA) assess with high sensitivity the traces of antimony (Sb), Barium (Ba), Bromine (Br) and iron (Fe) on the graphite sampling support. Unfortunately, Lead, which is a key element in the composition of GSR is undetectable by NAA. Also, NAA requires a relatively large sample size and a nuclear reactor. This allows to say that the revelation of GSR using only INAA is insufficient. In addition, the technique is unable to decide whether these elements come from a GSR or another source of pollution.

Atomic absorbance spectroscopy (AAS) detects Pb, Ba, and Sb in trace samples but is an expensive and destructive technique. Inductively coupled plasma (ICP) combined with mass spectrometry (MS) can rapidly detect various elements, but is also an expensive and destructive technique. All these techniques are slow, lack spatial specificity, and require highly trained personnel to run the instruments and interpret the results ([Hellmissetal, 1987](#)).

The non-destructive nature of the SEM-EDX permits the retention of evidence, allowing for re-examination if necessary, making it a reliable and invaluable tool for forensic firearm investigations. The requirement for highly trained personnel has also been overcome with the introduction of automated SEMs ([nanoscience.com](#)). Many automated SEM are compact, has a great speed, thus saving floor space and time. The automated SEM comes with an automated particle analysis software built onto the platform, offering a dedicated GSR analysis solution that is compliant with instrument. The workflow of a typical GSR run involves defining a scan area for each sample stub, scanning the scan area frame by frame, using the backscattered electron detector to detect particles, and determining the elemental composition of each particle. The automated SEM also comes with various user friendly features like customize and save workflows, Auto Video function to optimize brightness and contrast at any point throughout the GSR run and Dual Thresholding feature ([azom.com](#)).

The revelation of ballistic gunshot residue (GSR) is commonly performed with the aim of determining whether or not a suspect handled a firearm and or to estimate a firing distance ([Gallusseretal, 2002](#); [Kersh et al., 2014](#)). Most frequently, gunshot residues are collected from the suspect's hands, where the concentration of these traces is maximal immediately after shooting. In case more time has elapsed since the event traces should be collected rather from the hair, face and clothes where they usually remain for longer than on hands. It has been established from empirical studies that only three-component particles containing lead, antimony and barium are unique primer residues ([Basu, 1982](#); [Woltenetal, 1980](#)). But according to Gunshot Residue Subcommittee of Chemistry Scientific Area Committee in the organization of Scientific Area Committees (OSAC) for Forensic Science classifies Lead, barium, calcium, silicon combination as well as the combinations of Gadolinium, titanium, zinc; Gallium, copper, tin; Titanium, zinc and Strontium as Gunshot Residue. Particles containing iron, chromium, nickel, copper, zinc and other

**Comment [User32]:** Indeed, Lead could be detected through NAA as described in the following reference:  
[10.1007/BF02042613](#)

**Comment [User33]:** I suggest to omit this sentence as it can mislead the readers.

**Comment [User34]:** Is AAS more expensive than SEM-EDX. The authors are requested to comment on this on the manuscript and provide an appropriate reference.

**Comment [User35]:** The authors are also requested to comment on the rest elemental techniques that are not included in these two paragraphs. TXRF for example is a non-destructive technique, less expensive than SEM-EDX and it could be used for the detection and quantitation of Pb, Ba, Sb, Br and Fe simultaneously. It is quick, simple in use and there is also the possibility of imaging if micro-TXRF is employed.

**Comment [User36]:** The authors are requested to take care of the grammar number.

**Comment [User37]:** It should be one word with a hyphen.

**Comment [User38]:** The authors are requested to provide the respective reference.

elements are typical for the case, projectile and its jacket as well as the barrel. However, they can also originate from subjects of everyday use or pollution and thus are considered as false positives and cannot be considered as an evidence of firearm shooting ([Mosher et al., 1998](#); [Romolo & Margot, 2001](#), [Chohra, 2015](#)).

Physicochemical examinations of gunshot residues, called also chemical ballistics, are helpful, e.g. in identification of damages and injuries as the effect of the use of firearms (with indicating the entrance and exit of projectile), estimation of the shooting distance and also establishing, whether a person has used a firearm. Only few particles, of total mass no greater than 100 **pico-grams**, can be accepted as the evidence relating an individual with a shooting incident. Fulfilling these tasks is very helpful for the reconstruction of an investigated crime. However, new challenges for gunshot residues examiners arise. With a growing frequency the administration of justice asks about the type of ammunition, and so the firearm used in cases when the only accessible for examinations evidence are gunshot residues. Thus one more task can be formulated, i.e., the identification of an ammunition from the gunshot residues detected. **The uses of morphological and elemental indicators help to differentiate one ammunition from another and to make a difference between firearms used to discharge a round, case, and bullet material was reported by Johnson et al.** ([Lebiedzik & Johnson, 2000](#)).

**Comment [User39]:** It should be replaced with the abbreviation of the unit.

**Comment [User40]:** The sentence should be rephrased.

**Comment [User41]:** The authors are requested to take care of the grammar number.

In an investigation by [Brożek-Mucha Z.](#) (2014), it was reported that the distribution of particles in the surroundings of the shooting gun is not uniform. **The numbers of particles**, their chemical composition, and their morphological features depend on the distance from the muzzle of the shooting gun and the type of the substrate the particles sediment on. Results of this study gave rise to working out the method of shooting distance estimation from the physical and chemical examinations of GSR pattern around the gunshot wounds and damages—extending the possibilities of shooting distance estimation with range of about 50–100 cm, in addition to the three categories commonly used until now: (i) contact or a nearest vicinity shot (about 0-1 cm), (ii) close distance shot (about 1–50 cm), and (iii) distant shot.

[Collins et al. \(2003\)](#) performed for the analysis of gunshot residue along with glass fragments from the hands of shooters using SEM/EDX and according to him, these glass fragments when found along with these inorganic GSR particles, that is, fusion of Pb and Ba, help to discriminate between the two. [Hellmiss et al.](#) in 1987 equipped an SEM instrument with Auger electron spectroscopy for the analysis of gunshot residue instead of using EDX.

**Comment [User42]:** Rephrase of the sentence is required.

### **B) Car Paint Residue:**

Vehicular accidents and crimes involving automobiles are increased nowadays. Paint smears or chips are found on the crime scene as trace evidences leading to establishing the identity of vehicle involved in the crime by analysing with control sample of suspected vehicle. Investigation of such cases are influenced by many factors, such as information from CCTV footages, **eye witness other possible physical evidences.**

**Comment [User43]:** A conjunction is missing.

According to the Forensic Paint Analysis and Comparison Guidelines by Scientific Working Group on Materials Analysis (SWGMA<sup>T</sup>) Forensic paint analyses and comparisons are typically distinguished by sample size that precludes the application of many standard industrial paint analysis procedures or protocols. The guidelines instruct that when paint evidence is recognized, every effort should be made to manually remove it before using tape lifts to collect other types of evidence. If paint is collected with tape lifts, the collector should be aware of the possible difficulty encountered when attempting to manipulate paint samples bearing adhesive residues. In addition, components of the adhesive could contaminate the paint sample and change its apparent chemistry. When contact between two coated surfaces is indicated, the possibility of cross transfers must be considered. Therefore, if available, samples from both surfaces should be collected. Paint flakes can be removed from the parent surface by a number of methods. These include but are not limited to the following: lifting or prying loosely attached flakes, cutting samples of the entire paint layer structure using a clean knife or blade, or dislodging by gently impacting the opposite side of the painted surface. When cutting, it is important that the blade be inserted down to the parent surface. It should be noted that no one method of sampling should be relied upon exclusively (SWGMA<sup>T</sup>, 2000).

**Comment [User44]:** The authors are requested to take care of the grammar and syntax of the sentence.

**Comment [User45]:** The authors are requested to correct the phrase.

For the forensic analysis of multi-layered paint chips of hit-and-run cars, detailed compositional analysis, including minor/trace chemical components in the multi-layered paint chips, is crucial for the potential credentials of the run-away car. The number of layers, painting process, and used paints are quite specific to the types of cars, colour of cars, and their surface protection depending on the car manufacturer and the year of manufacture, and yet overall characteristics of some paints used by car manufacturers might be quite similar. (Malek et al., 2019). The composition includes one or more under coats, topcoat and clear coat on the surface and each layer have organic pigments, additive and binder. Automotive paints generally consist of three or four layers and may vary from manufacturer company. The paint coat of a car body consists of a number of successively overlaid paint layers. These layers differ from each other in terms of their ingredients, i.e., resin, pigments and fillers. The number of layers making up a car covering depends on its type. In brand new cars and in those that have not been repainted there are only three to four layers. The components present in the various layers can be identical and thus, individualization of vehicle by its automotive paint is the prime information for investigators for discriminating. Characterizing and individualizing of automotive paints using instruments analysis enabled breakthrough in scientific investigation (Lavine et al., 2015).

**Comment [User46]:** The authors perhaps mean instrumental analysis.

Paint coverings of renovated cars consist of a larger number of layers (sometimes even more than a dozen), including not only enamels, but also putties, painters' putties and ground undercoats. In identification and comparative studies of paint chips, scientists define their macroscopic properties—colour, shade and texture—and their microscopic properties relating to their morphology (the number and sequence of layers, their thickness and colour). The next stage is a detailed analysis of the chemical content of each layer, including identification of the binder, pigments and fillers. SEM–EDX can be very useful in the case, when the compared paint samples

are similar in the microscopic properties relating to their morphology (the number and sequence of layers, their thickness, colour) and the results of infra-red spectroscopy analysis. Thus, the identification of a particular paint layer can be carried out comparing the contents of elements since they are characteristic for a given layer and do not repeat.

**Comment [User47]:** The sentence seems to be half-finished. The authors are requested to complete the sentence.

In a study done by [Kaur et al](#) (2022), examination has been done on the layers of automotive paint chips collected from Maruti Suzuki from Kottayam region of Kerala using SEM-EDX. The result shows the presence of Thallium in white and grey paint samples and Aluminium in the red paint samples of automobile paint. Thus, profiling chemicals and elements in the sample may lead to individualization of the automobiles and can be included in the paint database.

In a study done by [Malek et al](#), SEM/EDX were applied in combination with ATR-FTIR imaging and RMS for a detailed characterization of three samples of car paint chips. The molded and polished cross-sections of the car paint chips maintaining their layered structures were specially prepared for the multi-modal analysis. Unambiguous molecular speciation of the chemical components within the layers as well as a determination of the physical layered structures were possible using this multi-modal approach. Although elemental composition information from SEM/EDX analysis is insufficient for molecular speciation, the detection of chemical elements in the layers is consistent with and supportive of the ATR-FTIR and Raman data for polymer resins, inorganics, and pigments. Five types of polymer resins, such as alkyd, alkyd-melamine, acrylic, epoxy, and polybutadiene resins, were clearly distinguished along with  $\text{TiO}_2$ ,  $\text{SnO}_2$ ,  $\text{FeS}_x$ ,  $\text{Fe}_3\text{O}_4$ ,  $\text{CuCl}_2$ ,  $\text{ZnO}$ , and  $\text{Al}_2\text{O}_3$  as pigments, and kaolinite, talc, pyrophyllite,  $\text{BaSO}_4$ ,  $\text{Al}_2(\text{SO}_4)_3$ ,  $\text{Zn}_3(\text{PO}_4)_2$ , and Al flakes as fillers. This study provides detailed information on the chemical identities of every layer of three car paint chips. This is useful for tracing the origin of the car manufacturer when compared to the coating history database of the respective company, which clearly provides the potential credentials of cars involved in hit-and-run accidents. ([Malek et al, 2019](#))

**Comment [User48]:** Although the authors provide information about ATR-FTIR and Raman spectroscopy for the detection of car paint residues, the significant advantages of the micro-techniques (micro-Raman and micro-IR) are not expressed thoroughly by the authors. Moreover, the advantages of the SEM-EDX towards micro-Raman and micro-IR for the identification of car paint residues are not presented in the manuscript.

### C) Glass Residue:

Glass is an amorphous translucent or transparent material that is made up of a mixture of silicates. It is made by fusion of molten silicate and then solidification without crystallization. Most commercially produced glasses utilize sand ( $\text{SiO}_2$ ), and normally other oxides like  $\text{CaO}$ ,  $\text{Na}_2\text{O}$ , and  $\text{K}_2\text{O}$  are added, which help reduce the viscosity and melting point of  $\text{SiO}_2$  ([SWGMAT, 2004](#))

Other materials are also added depending upon the required properties of the glass; for example,  $\text{PbO}$  increases refractivity,  $\text{B}_2\text{O}_3$  reduce thermal expansion,  $\text{Al}_2\text{O}_3$  increase durability etc. Therefore, there are various elemental components in glass that help determine properties. Elementary analysis of glass using SEM determines the ratio of concentrations of  $\text{Na/Mg}$ ,  $\text{Ca/K}$ ,  $\text{Mg/Al}$ ,  $\text{Ca/Na}$ , and  $\text{Na/Al}$ . Through these ratios, the glass is categorized, and properties are determined ([Khan, 2022](#))

**Comment [User49]:** It should be changed to "reduces".

**Comment [User50]:** It should be changed to "increases".

Although glass is most commonly associated as evidence in property offenses, it may be encountered in almost every type of criminal investigation. In fact, glass fragments have been reported as being among the various types of physical evidence most frequently encountered at crime scenes. ([Parker and Peterson, 1972](#)).

**Comment [User51]:** The paragraph should be rephrased, as it is not clear to the reader.

The large frequency of occurrence of glass as forensic evidence is attributable to many factors. Glass is ubiquitous, produced in large quantities and for a wide variety of applications, and due to the way in which it fractures, small particles of glass are common to the environment. These tiny glass fragments, either generated during the commission of a crime or pre-existing at the scene, can be carried away from the crime scene unwillingly by the perpetrator by adhering to clothing or shoes. Further, glass is chemically stable and resistant to environmental factors and therefore can be analyzed by the forensic laboratory with meaningful results even after a considerable amount of time has lapsed between the occurrence of the crime and the collection of questioned glass samples associated with the suspect ([Buscaglia, 1994](#)).

Glass as the evidence material often occurs in very small quantities. Thus, investigations of glass samples require sensitive analytical methods providing satisfactory results from small amounts of the examined material such as the quantitative elemental analysis using an SEM-EDX method. The elemental composition of glass strongly depends on the properties of glass products.

**Comment [User52]:** The authors are requested to take care of the use of "a" and "an" in front of SEM in the whole manuscript.

[Almiralletal](#) (2012) performed elemental analysis of Glass residues with a number of methods like  $\mu$ XRF, LIBS and LA-ICP-MS and they observed that SEM-EDS should only be used either for classification between glass types or for the exclusion of an association when the glass samples have an obvious compositional difference. This is due to the extremely limited utility of SEM-EDS in differentiating between different glass samples as a result of the poor sensitivity (LOD of ~ 1000 ppm) and the fact that SEM-EDS is a qualitative analysis method that suffers from differences in analytical results depending on sample morphology (flat vs irregular surface). Thus, the researchers have used this technique as a mere comparison tool in glass analysis.

**Comment [User53]:** The authors are requested to provide the full name of the techniques prior to their abbreviations, the first time they appear in the manuscript. In addition, it is suggested that the authors use in the whole manuscript the abbreviation EDX and not EDS, so as to be clearer to the reader.

**Comment [User54]:** The authors have stated in the section 2 of the manuscript that SEM-EDX is also used for quantitative analysis. They are requested to comment on this.

In most published studies, scanning electron microscopy-energy dispersive X-ray spectrometry measurements of element intensity ratios have been applied to classification of glass types ([Ryland 1986](#); [Terry et al. 1982](#)). An analytical scheme that combines measurement of Ca/Mg intensity ratios obtained using scanning electron microscopy-energy dispersive X-ray spectrometry with Ca/Fe ratios obtained using X-ray fluorescence spectrometry has been used with good success by several forensic laboratories to classify glass fragments into sheet and container categories ([Keeley and Christofides 1979](#); [Ryland 1986](#)).

**Comment [User55]:** The abbreviation could be used in this part of the manuscript.

**Comment [User56]:** The meaning of the sentence is not clear.

**Comment [User57]:** The abbreviation should be used.

For discrimination among glass sources, a scanning electron microscopyenergy dispersive X-ray spectrometry protocol was reported for determining the ratios of the intensities of Na/Mg, Na/Al, Mg/Al, Ca/Na, and Ca/K in glass fragments ([Andrasko and Machly, 1978](#)). Measurement of these ratios by scanning electron microscopy-energy dispersive X-ray spectrometry was incorporated

**Comment [User58]:** The abbreviation should be used.

**Comment [User59]:** The abbreviation should be used.

into a scheme with refractive index, density, and emission spectrography. Thirty-eight out of 40 window glasses analyzed by this scheme were found to be distinguishable. The variation in the measured element intensity ratios by scanning electron microscopy-energy dispersive X-ray spectrometry was found to be consistent across a new sheet, an old sheet, and within a single fragment of glass.

**Comment [User60]:** The abbreviation should be used.

**Comment [User61]:** The authors are requested to present a figure or a table with the meaning of these ratios for the discrimination of different glass sources.

[Jack Mershon](#), an electrical engineer has reported a simulated trace-evidence scenario subjecting an iPhone 4S to a gunshot, generating glass particles from the iPhone's front and back covers. The report says that as SEM, EDS, and micro-CT are complementary tools for forensic science, so the combination of these tools have helped to establish a match between a glass particle and its source on the surface of an iPhone. The use of variable pressure imaging conditions, as well as wide field, depth, and resolution scan modes were critical for image acquisition. The creation of panorama images enabled the correlation of SEM, micro-CT, EDS and optical data. SEM 3D reconstruction and micro-CT imaging were used to correlate the surface and topography, as well as to give insight into the damage within the phone. Finally, EDS microanalysis data in the form of spectra, line scan, mapping, and large area mapping were acquired from samples to characterize the compositional variations within the glass, which provides corroborating evidence of the origin of the glass. ([Mershon, 2021](#)).

**Comment [User62]:** The authors are requested to provide why SEM-EDX is superior to the other analytical techniques (XRF, micro-CT, etc.) in the identification of glass residues.

#### 4. Conclusion

The concept of trace evidence in forensic science originates from Locard's exchange principle, and trace evidence is typically in the form of particles of skin, hair, fibers, clothing, soil, paint, and glass, among other materials. The effectiveness of the SEM-EDX as a non-destructive technique, over the other available instruments, makes it the most suitable technique for the analysis of forensic evidences from a crime scene. Many scientist have carried out the investigations and analysis of such commonly retrieved forensic evidence from the crime scene using SEM-EDX.

**Comment [User63]:** The article is not required.

**Comment [User64]:** The authors are requested to take care of the grammar numbers.

The analysis of evidence collected from a crime scene, such as GSR, car paint and glass residues, are discussed and explained with illustrations pertaining to the working principle and guidelines of the techniques employed. The obtained results are expected to be useful for the reconstruction of a crime scene under investigation, which can also be used as a tool in the subsequent judicial proceedings.

**Comment [User65]:** The conclusions should be more informative and more comprehensive and not just a repeat of the main body of the manuscript. The authors are requested to rephrase the conclusions focused on the significance of SEM-EDX in the identification of evidence material for forensic sciences in comparison to other available methods.

It is discussed in details that SEM-EDX can serve as a powerful tool for forensic scientists to classify and discriminate evidence material because they can simultaneously examine the morphology and the elemental composition of objects.

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