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Applications of Raman Spectroscopy in Forensics for In-Depth Examinations of Pigments and Dyes in Evidence from Ink and Paint

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Introduction

Due to its high potential level of selectivity using structural information, the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) designated it as a Category A method. Raman spectroscopy is increasingly common in the realm of forensic analysis. Raman spectroscopy is a non-destructive, non-invasive technique that can be used to analyse even the smallest sample with very little sample preparation. While water can dwarf the signals of genuine analytes in the infrared, it does not exhibit substantial signals in the Raman spectroscopy. When NIR light sources are utilized (to reduce fluorescence interference), Raman signals may be significantly weaker because scattering is inversely proportional to the fourth power of the incident light's wavelength. At least through some proof-of-concept studies, forensic scientists have begun to use a novel technique called shifted-excitation Raman difference spectroscopy (SERDS) to get around fluorescence interference in the Raman analysis of some forensic evidence [1].

Description

The traditional backscattering Raman spectroscopy strategy is reasonable for surface investigation. Although conventional confocal Raman microscopy can be used to analyze subsurface layers if upper layers are transparent, using it to directly analyze a subsurface material through a turbid surface layer can be very challenging. Spatially offset Raman spectroscopy (SORS) has been developed to retrieve subsurface Raman information regardless of whether the surface layer is transparent. This technique has found use in forensic science [2].

Application of conventional Raman spectroscopy in forensic science and investigation

Conventional Raman spectroscopy also referred to as normal or ordinary Raman spectroscopy is an effective and useful tool for forensic analysis. It has been demonstrated that it can distinguish between a variety of bodily fluids, including gender, race, chronic age, and blood samples. It can also distinguish racial semen samples. Paints, ink/questionable documents, fibers, gunshot residue, bones, and other evidence have all been analyzed forensically using Raman spectroscopy. Using a 785 nm laser as the excitation source, conventional Raman spectroscopy was used to distinguish numerous types of seized cocaine. It was also found to perform better than FT-IR in identifying inorganic adulterants and benzoic acid in seized cocaine. In preparation for use in real-world forensic analysis, Raman spectroscopic techniques have also been developed to identify and quantify cocaine concealed in food matrices and a cocaine analog imbedded in textiles. A recent study yielded Raman spectra for 21 phenethylamines. Using statistical techniques, it has been demonstrated that conventional Raman spectroscopy can distinguish between all of these phenethylamines' regioisomers, structural analogs, and even homologs. A very high percentage (95 percent) of the 59 seized phenethylamine samples were correctly identified with minimal sample preparation, demonstrating the potential of this non-destructive method in forensic field investigations. Conventional Raman spectroscopy for forensic analysis has been thoroughly examined. The application of SERS, SERDS, and SORS in forensic investigation and research will be the primary focus of this article [1].

Surface-Enhanced Raman Spectroscopy (SERS) for the sensitive detection of drugs Despite the fact that Raman scattering is typically very weak, it has been discovered that many molecules that are adsorbed on certain rough metal surfaces—particularly metal nanoparticles—and other nanostructures—or porous structures—give greatly boosted Raman scattering signals, with up to a 1011 fold increase. Surface-enhanced Raman spectroscopy (SERS) was the result of this. It comes as no surprise that the field of forensic science has adopted this approach for the more delicate analysis of evidence, particularly controlled substances. Researchers at a measurable science lab worked by US FDA fostered a SERS technique involving handheld Raman spectrometers for fast and touchy identification (as low as 100 ng/mL) of fentanyl and other narcotics in low-measurement pills after microextraction, with the assistance of monetarily accessible colloidal silver nanoparticles and the totaling specialist KBr, straightforwardly through boring glass vials. A 10% aqueous solution of methanol was generally the best solvent for this kind of analysis because it could dissolve the active pharmaceutical ingredients (APIs) (or simply the active ingredient) more effectively than pure water [3].

Fentanyl was one of the opioids examined in the aforementioned SERS study. Because of its extremely high potency and toxicity, fentanyl is rarely found in relevant street drugs. It is necessary to develop highly sensitive detection techniques for it, particularly field detection techniques. Fentanyl's normal Raman and SERS spectra were found to be very similar, with only minor differences in the shift wavenumbers of the same vibration modes. At 1002 and 1030 cm^{-1}, the fentanyl SERS Raman spectrum's strongest peaks were associated with some phenyl ring vibration modes. Other SERS studies of fentanyl with gold or silver nanoparticles made similar findings. The SERS spectrum did not contain the amide carbonyl peak that is located at 1647 cm^{-1} in the conventional Raman spectrum.

Shifted-Excitation Raman Difference Spectroscopy (SERDS) for overcoming fluorescence interference

Fluorescence is generally regarded as the archenemy of Raman spectroscopy if any component is present. Shifted-Excitation Raman Difference Spectroscopy (SERDS) can be used to overcome fluorescence interference. is fluorescent in the sample. Consequently, numerous efforts have been made to resolve this issue. A relatively new approach known as shifted-excitation Raman difference spectroscopy (SERDS) has been demonstrated to be quite effective in removing fluorescence interference and other background interference from the Raman analysis of some samples. The Kasha-Vavilov rule is used to remove fluorescence background from Raman spectra with SERDS.

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This rule states that molecular luminescence—also known as fluorescence or phosphorescence—can only occur when the lowest excited level relaxes to the ground state. To put it another way, despite the fact that molecules can be excited to a variety of levels in an excited state (or excited states) by (slightly) different excitation wavelengths, all excited molecules must first relax non-radiatively to their lowest excited level (the lowest vibration level in the first excited state) before they can fluoresce. As a result, even minor adjustments to the excitation wavelength (or wavenumber) have no effect on fluorescence emission. However, while the Raman shifts—differences in wavenumbers between excitation photons and inelastically scattered photons—do not change with the change in excitation wavelength (or wavenumber), the wavenumbers of Raman-scattered photons do. Theoretically, if there is no photobleaching, subtracting the two spectra of the same sample acquired with slightly different excitation wavelengths can remove fluorescence interference, producing a Raman difference spectrum that can be transformed into a clean Raman spectrum without background using reconstruction operations. In some proof-of-concept studies, forensic scientists have begun to use this relatively new Raman method to analyze difficult forensic evidence [4].

Spatially Offset Raman Spectroscopy (SORS) for subsurface investigation

For subsurface investigation, Spatially Offset Raman Spectroscopy (SORS) is used. The majority of Raman methods employ the backscattering technique, in which the incident photons hit the sample and turn 180 degrees at the point of illumination to be focused on the detector. Therefore, surface investigation greatly benefits from it. This method, on the other hand, lacks the ability to effectively extract information from a subsurface material through a surface layer that is opaque. In the scientific field, many medications are as cases or covered tablets, or hid in apparently authentic items for carrying. Preferably, examination of such examples ought to be managed without changing the type of proof for various reasons, including yet not restricted to saving proof, watching out for them for additional examination, forestalling measurable agents/researchers from being hurt by the substance inside the bundling/holder, and abstaining from harming/annihilating authentic product. Analyses of potentially explosive or highly toxic substances should ideally be carried out directly through the packaging or container from a sufficient distance for safety reasons. However, even when confocal Raman microscopes are utilized, it is extremely challenging to detect scattered photons from depth in conventional Raman backscattering geometry due to the fact that the majority of commonly used packaging materials and containers are opaque enough to host a significant number of scattering events. If the materials in question are fluorescent, the packaging or container's Raman or fluorescence signals may also pose a significant challenge. Spatially offset Raman spectroscopy (SORS) is a method for retrieving subsurface Raman data from diffusely scattering media. This method can be used in all of the aforementioned forensic investigation scenarios and more. In point of fact, the majority of materials exhibit diffuse scattering because they are neither completely transparent to light nor completely able to block or absorb light. These materials scatter light at least to a certain depth and area, where some photons will be in elastically dispersed and travel in a zigzag pattern. A Raman detector strategically placed directly above the corresponding offset position can be used to detect some of these photons if and when they exit the sample at an offset from the illumination spot on the surface. Photons from deeper layers veer further from their initial direction because they experience a greater number of scattering events. As a consequence of this, some of the scattered photons coming from deeper layers will travel longer distances in the opposite direction before reaching lateral offsets. The offset distance is longer the deeper the probed layer. A SORS analysis requires at least two Raman measurements: one at a spatial offset position that is typically a few millimetres away from the illumination spot, the other directly over the spot where the laser illuminates the sample surface (the backscattering setup utilized in conventional Raman). The first measurement primarily collects surface-originating photons if the sample is opaque. However, while a much smaller number of photons from the surface or from closer to the surface can also be detected by the second one, its primary focus is on photons coming from further inside the sample. Two Raman spectra, one for the substance on the subsurface and one for the substance on the surface, can be produced by processing the two spectra with a scaled subtraction [5].

Conclusion

It has been demonstrated that forensic analysis greatly benefits from the use of Raman spectroscopy. Raman analysis of forensic evidence has become even more sensitive, accurate, timely, and practical as a result of the development of some very potent Raman methods like SERS, SERDS, and SORS, among others. We anticipate a growing acceptance and application of these novel Raman techniques in the field of forensic science, facilitating the administration of justice, in conjunction with chemometric methods.

Acknowledgement

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Conflict of Interest

None.

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