Surface Enhanced Raman Scattering (SERS) – Expanding the Limits of Conventional Raman Analysis

Surface Enhanced Raman Scattering or SERS is an anomalous enhancement of Raman scattering when molecules are adsorbed to gold or silver nanoparticles – this enhancement can be as large as 10^7. The advantage of SERS for the analytical chemist lies in its ability to detect analyte concentrations of parts per million and even parts per billion levels, while classical Raman is limited to parts per thousand. Metrohm Raman produces P-SERS assay kits in the form of nanoparticles printed onto substrates using inkjet technology. This method produces inexpensive test strips that exhibit exceptional stability and sensitivity. There are two markets that can be easily addressed with P-SERS: forensic analysis and food safety. This white paper explains the mechanism of SERS and how it can be applied to handheld Raman analysis with Metrohm Raman Mira systems.

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Introduction

Methods for trace detection of analytes can be expensive and time-consuming. Conventional handheld Raman analysis is limited to part per thousand levels in solutions and to solid samples that are easily visible to the naked eye. An extension of Raman spectroscopy, SERS permits analytes in concentrations as low as parts per billion to be measured and non-visible samples to be identified.

A wide range of industries requires highly sensitive detection methods:
- Law enforcement, e.g., illicit drugs or explosive residue detection
- Food quality, e.g. food adulteration, unsafe residual pesticide levels
- Anti-counterfeiting, e.g. counterfeit drugs, foods, and merchandise

Each of these challenges can be addressed with complex analytical laboratory techniques such as GC-MS and LC-MS, but time, skill, and cost requirements can limit their use and effectiveness.

Identification of analytes with SERS can be fast, convenient, and inexpensive. This white paper briefly explains Raman spectroscopy and SERS enhancement, then describes new Printable SERS (P-SERS) substrates from Metrohm Raman. These innovative substrates are featured in a variety of applications that highlight their sensitivity, adaptability, and ease of use.

Raman Spectroscopy

Raman scattering occurs when a photon is inelastically scattered from a molecule's vibrational level. This occurs for about one photon in one million – Raman is a weak effect. Intense lasers that inundate a sample with photons and detectors sensitive enough to register single photons are used to overcome this weakness. With well-designed instruments, Raman spectroscopy can be invaluable for sample identification.

As a material identification method, Raman spectroscopy has several advantages:
- Unique Raman spectra that serve as specific fingerprints for material identification
- Potential multiplexed detection from a single measurement
- Detection and identification can be completed in seconds
- Low signals from water or moisture which creates complementarity to NIR spectroscopy

Traditionally, Raman as an analytical technique has been limited by bulky and expensive laboratory equipment. Efficient diode lasers, rejection filters, and highly sensitive detectors have made handheld Raman possible. This permits noninvasive detection and identification of unknown materials in field settings.

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Drawbacks of Raman Analysis

As stated, Raman scattering is a weak phenomenon: even state-of-the-art Raman instruments only achieve moderate detection levels. Mira applications are limited to pure materials in visible quantities and solutions with concentrations of a few percent or higher.
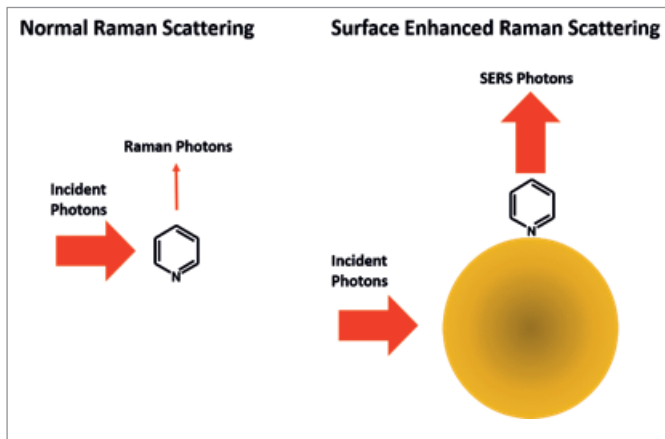

Figure 1. Depiction of Normal Raman and the SERS effect

Figure 1 illustrates the inefficiency of Raman scattering, with perhaps one Raman scattered photon produced by one million incident photons. However, SERS enhancement results in a much stronger signal, enabling detection of fewer molecules.

The second major drawback of Raman spectroscopy is fluorescence. Fluorescence occurs when laser photons are absorbed by a sample and reemitted at longer wavelengths. It is very efficient and can compete with the weaker Raman signal, overwhelming the detector. Fluorescence can be inherent for the target molecule or it can stem from small amounts of impurities. Regardless of its origin, there is no way to distinguish a fluorescence photon from a Raman photon. The result is a large background that can easily occlude the Raman signal.

What is SERS?

When the analyte of interest interacts with gold or silver nanoparticles, the optical electric field can be enhanced by several orders of magnitude. This enhanced electromagnetic field leads to significant enhancement of Raman scattering for molecules adsorbed onto the metal surface (**Figure 1,** right.) SERS enhances Raman scattering by as much as 10 million-fold and enables trace detection of adsorbed molecules.

SERS Substrates

SERS can be performed with nanoparticles in colloidal solutions, with nanoparticles deposited on a surface, or with engineered nanostructured surfaces. Colloidal solutions suffer from instability over time and demanding storage conditions. Engineered nanostructures can give large enhancements, but they are costly to produce. Deposited nanoparticles provide reasonable enhancements and they often aggregate during the deposition process which improves SERS signals.

SERS dramatically increases potential applications of Raman spectroscopy when coupled with a Raman analyzer. Detection of analytes at parts per million (ppm) is readily achievable, *so long as the molecule of interest interacts with the nanoparticles.*

Note This is not an insignificant statement. SERS is NOT a universally applicable method for detection, as the analyte in question must have an affinity for silver or gold. Some illicit materials, food additives, and pesticides are good targets for SERS analysis.

SERS is an extremely attractive technique as it retains all of the appealing features of Raman – portable equipment, multi-analyte detection capabilities, and rapid analysis.

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Metrohm Raman's Mira DS and P-SERS solutions

Our Mira DS system offers rapid Raman spectroscopic analysis:
- Integrated library of over 10,000 materials
- Small size and low weight
- Unique Orbital Raster Scan technology
- Mixture Matching software

Figure 2. Mira DS with P-SERS substrates

P-SERS Substrates: Low Cost, High Enhancement SERS Solution

Unlike commercial nano-fabricated substrates, Metrohm's P-SERS substrates are formed using a innovative patented process to deposit nanoparticles through ink-jet printing, dramatically lowering costs while maintaining uniformity and performance.

An additional advantage of the ink-jet process is that P-SERS substrates can be printed on a variety of membrane materials, not just silicon or glass wafers. Cellulose paper is typically used, but the material can be altered to suit a particular application. These unique substrates enable new ways to use and optimize these substrates:

- Pipetting the sample onto the planar surface (as with «traditional» substrates)
- Dipping to adsorb the sample into the substrate
- Swabbing a surface to collect a trace residue
- On-substrate chromatographic sample cleanup and detection

Analysis with P-SERS is simple – after applying the sample to the SERS substrate, the substrate is inserted into the Mira analyzer, and the sample is identified automatically by the Mira software at the touch of a screen.
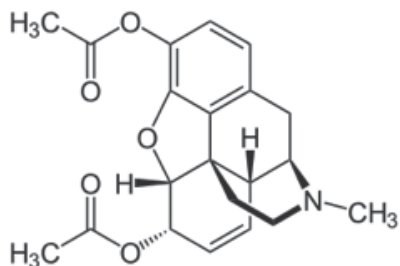
SERS Applications

Heroin

Figure 3. Heroin

Detection of heroin is of much greater importance than most other substances. In the United States, there were 15,466 confirmed deaths from heroin *in 2016 alone.* However, confirmation of heroin in street drug samples is complicated by a lack of fast, reliable, and accurate detection methods

Forensic drug laboratories perform costly, time-consuming, and highly technical analyses of street heroin samples involving sample clean up. Many labs have large backlogs and would benefit from a fast and simple screening method. This application describes a powerful method for detection of heroin in street samples with *minimal sample preparation.*

Heroin presents a unique challenge for Raman spectroscopy: reagent grade heroin does not exhibit fluorescence in Raman spectra, but street heroin is far from pure. Common heroin adulterants often exhibit high levels of fluorescence, which occludes the heroin signal. **Figure 4.** demonstrates this:
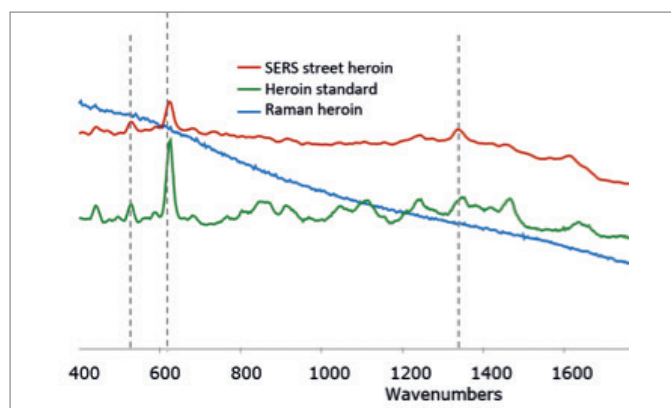

Figure 4. Raman and SERS spectra of Heroin

The blue spectrum in this figure shows the lack of distinct heroin signal in the highly fluorescent Raman spectrum of street heroin. The green curve is a Raman spectrum of pure heroin and the characteristic heroin peaks at 531, 625, and 1336 cm^{-1} can be seen in this and the red SERS spectra.

Method

18 crude heroin street samples, each comprised of 1-5 mg of brown powder or crystalline material, were introduced into solution. Once the solids had settled, the supernatant was applied directly to P-SERS substrates provided with ID Kit and allowed to dry.

Acquisition parameters:
- Raster OFF
- Laser Power 4
- Integration Time 0.25 second
- Averages 2

Figure 5. Metrohm Raman's ID Kit for heroin detection

Results

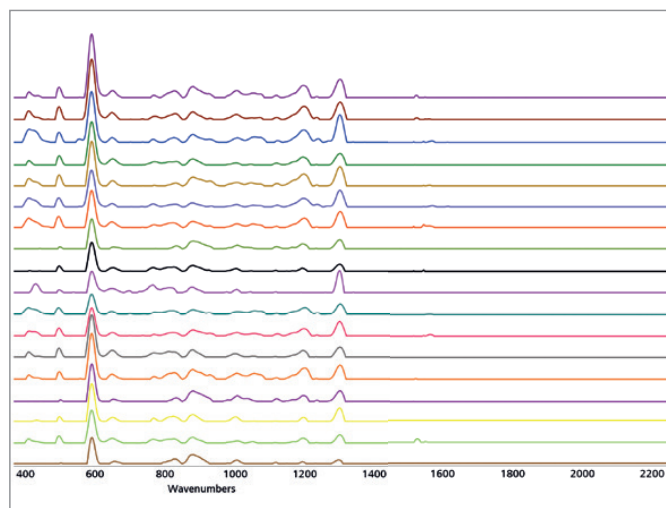Without fail, P-SERS successfully detected heroin in all 18 street samples:

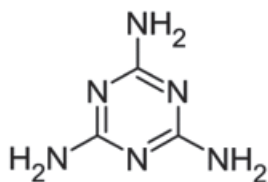**Figure 6.** Analysis of heroin street samples with P-SERS.

Melamine in Milk

Figure 7. Melamine

Melamine is a nitrogen-rich compound that is sometimes added to food products to inflate their apparent protein content. The 2008 Chinese milk scandal was caused by elevated amounts of melamine in milk and milk products, including infant formula. Melamine can cause kidney damage and was responsible for 6 infant deaths and over 50,000 hospitalizations in China. The harmful effects of this chemical food additive were well established, and the FDA in 2006 recommended that melamine at concentrations <2.5 ppm was of no concern. After the 2008 milk scandal, this recommendation was amended to no acceptable level of melamine in food.

Tests for the presence of melamine must be accurate and incredibly sensitive- able to detect trace amounts of melamine in milk. Current techniques used to detect melamine are HPLC and GC/MS. SERS is an excellent trace technique for detection of melamine for two reasons: it is sensitive enough to detect some compounds in part-per-billion concentrations and its many functional groups interact very well with nanoparticles on SERS substrates.

Method

A 2000 ppm stock melamine solution in dilute HNO_3 was added to milk at a final concentration of 100 ppm. To this milk solution, dilute HNO_3 was added dropwise until protein coagulation was observed, pH~ 3.5-4. A centrifuge was used to isolate the solids, the liquid extract was dropped directly onto silver (Ag) P-SERS substrates, and allowed to dry.

SERS spectra were acquired with the following parameters:
- Raster OFF*
- Laser Power 2
- Integration Time 2 seconds
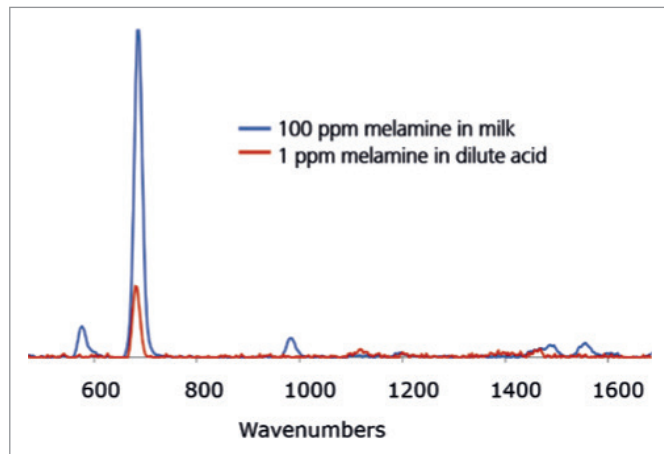- Averages 5

Results

Figure 8. SERS spectra of melamine in milk

Figure 8. clearly shows that melamine gives a strong, clear SERS signal at 100 ppm with P-SERS substrates. Melamine at 1 ppm in dilute acid is used as a reference.

*ORS is designed to increase interrogation area and reduce laser induced degradation of a sample, but SERS benefits from concentrated laser focus.
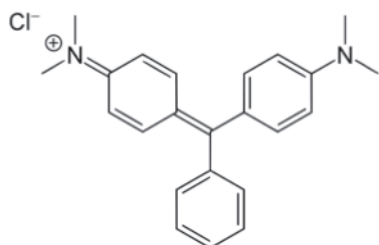
Malachite Green

Figure 9. Malachite Green

Malachite green is a synthetic dye used as a fungicide in aquaculture fishes. Although it has been determined that malachite green has a low public safety and health risk, the FDA banned its use in aquaculture in 1983. SERS was used to establish the concentration of malachite green in water.

Method
An aqueous solution of 1000 ppm malachite green was diluted serially. These solutions were dropped directly onto Ag SERS substrates and allowed to dry.

Acquisition parameters:
- Raster OFF
- Laser Power 3
- Integration Time 1 second
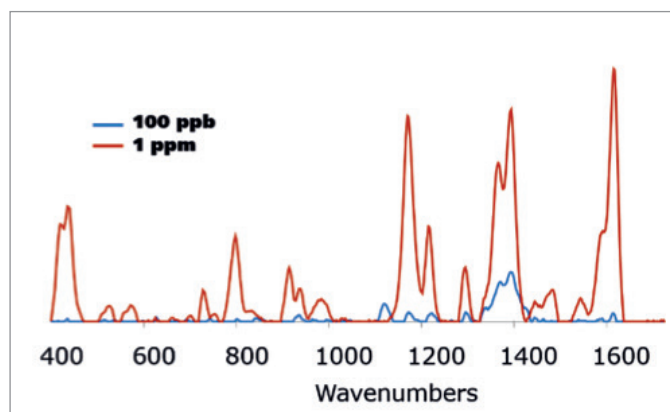- Averages 10

Results

Figure 10. SERS spectra of Malachite Green

It is obvious in **Figure 10.** that malachite green is detectable with P-SERS at parts-per-billion concentrations.
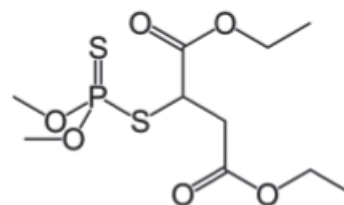
Malathion

Figure 11. Malathion

Malathion is an organophosphate pesticide used both in agriculture and for mosquito control. Malathion itself is known to have low toxicity, but the body converts it to maloxon, a much more toxic compound. It is also converted to maloxon during the chlorination phase of water treatment. For these reasons, sensitive methods for the detection of malathion on fruits/vegetables and in sources of drinking water are essential.

Method
A 10 ppm solution of malathion in water was added directly onto Ag P-SERS substrates and allowed to dry.

Acquisition parameters:
- Raster OFF
- Laser Power 3
- Integration Time 1 second
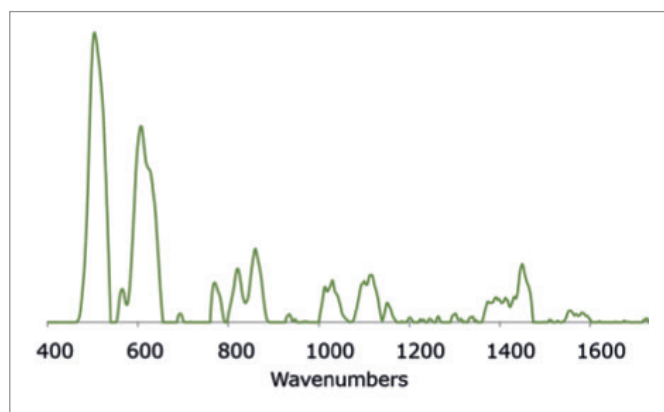- Averages 10

Results

Figure 12. SERS Detection of 10 ppm Malathion

Figure 12. illustrates that concentrations as low as 10 ppm result in a clean spectrum of malathion with Metrohm Raman's P-SERS substrates.

Conclusion

SERS is a powerful technique for many applications from trace detection of narcotics to food additive and pesticide analysis. In combination with Mira DS, this technique has the potential to speed up forensic laboratory analysis and relieve crime lab testing backlogs. Field-based professionals will benefit from automated routines that improve ease and quality of data collection, as well as safety of the user. Metrohm Raman's innovative low cost and highly sensitive P-SERS substrates coupled with the Mira DS handheld analyzer will dramatically expand the reaches of Raman analysis in analytical and field-based applications.

References

E. P. Hoppmann, W. W. Yu, and I. M. White, «Highly sensitive and flexible inkjet printed SERS sensors on paper,» Methods, vol. 63, no. 3, pp. 219–224, 2013.

W. W. Yu and I. M. White, «Inkjet-printed paper-based SERS dipsticks and swabs for trace chemical detection,» Analyst, vol. 138, no. 4, pp. 1020–5, Feb. 2013.

W. W. Yu and I. M. White, «Chromatographic separation and detection of target analytes from complex samples using inkjet printed SERS substrates,» Analyst, vol. 138, no. 13, pp. 3679–86, Jul. 2013.

NIH Publication No. 04-446

FDA IA 99-30

EPA-HQ-OPP-2009-0317 Docket

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