New Developments in SERS for the Pharmaceutical Industry

With advances in surface enhanced Raman scattering (SERS) technology, the accepted advantages of Raman analysis – specificity, speed of measurement, application to aqueous systems and ease of sampling – can now be transferred to a range of pharmaceutical applications that were previously the domain of HPLC and GCMS techniques.

Raman spectroscopy has now gained market acceptance as a robust, rapid and specific materials identification tool. The last decade has seen the release of a new generation of portable instruments that take the technique out of the laboratory and into the field or production site. However, a major limitation of conventional Raman spectroscopy is a lack of sensitivity for the identification of analytes in solution, preventing the application of this technique in addressing some important problems of relevance to the pharmaceutical industry. This lack of sensitivity can be overcome through the use of surface enhanced Raman scattering (SERS); with this technique, interactions between the analyte and an appropriate SERS substrate cause the weak Raman signal to be amplified by many orders of magnitude, allowing detection levels as low as parts-per-billion (ppb). Sample preparation is rapid and easy to perform, but quantitative procedures have been difficult to develop due to a lack of reproducibility in substrate manufacturing. Now that this problem has been overcome, there is considerable scope to develop new applications.

RAMAN SPECTROSCOPY

Raman spectroscopy – in its most basic form – consists of irradiating a sample with a laser and measuring the scattering through a spectrometer. The Raman scattering arises when light interacts with a molecule and there is energy transfer from the light to the nuclei starting a vibration. This reduces the frequency of the scattered light and gives a series of bands, each one arising from a specific vibration within the molecule. The advantages of the technique include specificity, speed of measurement, ease of sampling, stand-off detection and ease of use in aqueous systems. The last decade has seen a mini-revolution in instrument design and software. This has won Raman spectroscopy new-found recognition as a standard technique for materials analysis in a variety of applications and industries. However, a major factor that limits the applications of the technique is a lack of sensitivity. Very small quantities can be detected using a Raman microscope, but the low scattering efficiency means that analytes dissolved in solution or in the gas phase are difficult to detect except in relatively high concentrations, and any fluorescing material present can act as an interferent. Clearly, to apply robust material identification techniques in trace analytical regimes, a method for amplifying Raman signals is required.

SURFACE ENHANCED RAMAN SCATTERING

SERS can increase the sensitivity of measurement by up to 10^6 times, extending the range of Raman measurements to as low as ppb levels. Surface enhanced resonance Raman scattering (SERRS) is the Raman scattering observed when the analyte contains a chromophore, either naturally or by attachment, that is resonant at the laser wavelength being used. The observed signal enhancement with SERRS can be up to 10^14 times, which is much stronger than with SERS. An additional benefit to both techniques is that any fluorescence from an analyte or impurity attached to the surface is quenched (1,2).

Since signal intensity is intrinsically linked to analyte concentration, SERS allows for quantification protocols based on suitable calibration curves. Through building a calibration data-set based on known concentrations, the concentration of an unknown sample can be quickly and reliably determined. In any spectroscopy-based approach, data collection times are typically very short, and this is also true for SERS. Spectra are often collected in a matter of seconds, and if spectral processing and analysis is automated, the identity of unknown samples and their concentration can be determined extremely quickly. The variable nature of substrate manufacturing is an underlying problem that has prevented SERS from gaining acceptance as a robust analytical technique. Typically, the level of signal enhancement is quoted through the term ‘enhancement factor’. A robust quantification protocol is only possible if every
measurement made during the calibration stage uses substrates which have a very similar enhancement factor. A very wide range of SERS substrates have been synthesised by groups around the world. However, the predominant substrates used today are either surfaces for which roughness has been engineered on a nanoscale, or colloidal suspensions. The former can be made in many ways, with just two examples being the fabrication of nanostructures by electron beam lithography, or templating surfaces with materials such as polystyrene spheres. Substrates are usually then coated with SERS active metals such as silver or gold. Colloidal suspensions are very effective, but particle size control to regularise the SERS effect is essential, and particle size and size distribution must be measured to ensure the degree of control required. In addition, aggregation of the colloidal particles to larger clusters is usually necessary and this requires control of the chemistry.

For a solid substrate to be effective in routine analysis, it is necessary that the signal enhancement is even across the whole surface, and that batch-to-batch variation is small. At Renishaw Diagnostics we have developed a proprietary SERS substrate (Klarite®) that is fabricated on a batch manufacturing scale using techniques well established in semiconductor manufacturing. Klarite consists of photolithography-etched silicon, which is gold-coated using a highly specific evaporation technique (see Figure 1). By manufacturing on the semiconductor wafer scale, thousands of chips can be fabricated that exhibit consistent enhancement factors, allowing robust quantification protocols to be established. This, combined with the ease with which measurements can be made using a solid state substrate in contrast to a colloidal suspension, results in the most commercially attractive solution for applications where ease-of-use and experimental simplicity are important, especially when the technique is being carried out by non-specialists.

**APPLICATIONS FOCUS**

As a rapid, molecularly-specific and sensitive technique, SERS can be utilised in a variety of pharmaceutical applications. In analytical chemistry, it represents an attractive alternative to HPLC or IR spectroscopy. Typically, volumes required for analysis are on the scale of 1-40µl, massively reducing solvent or other consumable waste. In addition, water is only a very weak Raman scatterer, so aqueous solutions represent ideal samples – unlike in IR spectroscopy where the water absorption bands may dominate and obscure a spectrum. Thus SERS has advantages in fields such as material analysis within drug discovery, quality control and cleaning verification. When incorporated into a portable instrument, the detection equipment can be moved easily to various positions within a production site or laboratory. A wide variety of APIs have been investigated and the concentration dependence of these spectra and potential sample deposition routes (including practical swabbing methods) are well understood. An example of this concentration dependence on an API is shown for ibuprofen in Figure 2.

SERS can also be used to identify trace level contaminants in additional matrices such as pill formulations or foodstuffs – a topical issue given the relatively sharp increase in counterfeit pharmaceuticals and contaminated foodstuffs from developing countries. SERS and Klarite have been found to provide a quantifiable test for melamine (in its pure form), with a limit of detection (LOD) of 2.6 x 10⁻⁷M (~33ppb). Calibration models have been built using both univariate and partial least square analysis. Both

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**Figure 1:** The structure of Klarite® is a series of microscopic pits (A) designed to shape the surface plasmon into localised structures that will be in resonance with the laser frequency chosen. The surface of each pit is coated with gold to a specified roughness (A). It is then mounted in various ways (B) so that it can be inserted into the sample compartment of standard Raman spectrometers.

**Figure 2:** Concentration dependence of SERS signals from an API (in this case ibuprofen) obtained by dropping a spot of solution containing the API onto Klarite.
methods gave excellent prediction models. In a detailed comparison of SERS with HPLC, the time-scales of SERS measurements were found to be significantly shorter than the equivalent HPLC method (a total detection time for eight samples of 30 minutes for SERS versus three hours for HPLC). The LOD of SERS (33ppb) is much lower than that of HPLC (1ppm) when measuring melamine in solution. SERS has a further advantage of requiring minimum sample preparation because no centrifugation, filtration or clean-up steps are needed (3).

SERS uses a dye label and is of interest for the development of tests for drug discovery or quality control of processes using biological molecules such as DNA and antibodies. The huge difference in enhancement between SERS and SERRS means that normally only signals from the label are detected, reducing interference and simplifying the spectrum. The detection sensitivity is very high and has been shown to be greater than that of equivalent fluorescence techniques in one comparison using practical equipment (4). As shown in Figure 3, the sharp peaks in the molecularly specific SERRS spectrum make positive, in situ identification of an analyte relatively easy, even in the presence of several analytes.

CONCLUSION
The development of a reproducible SERS substrate using large-scale manufacturing methods has led, for the first time, to the possibility of extending Raman analytical methods into trace detection applications within the pharmaceutical arena. With advances in instrumentation, the accepted advantages of Raman analysis – specificity, speed of measurement, application to aqueous systems and ease of sampling – can now be transferred to a range of applications previously the domain of HPLC and GCMS techniques. In addition, the ongoing development of Raman analytical technology has led to the exciting possibility of portable, at-source analysis using SERS methods, with the potential to greatly improve process efficiency through time and cost reductions.

References