Raman Spectroscopy for Future Drug Discovery

a report by

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In recent years, Raman spectroscopy has enjoyed a strong resurgence in popularity due to advances in the technology, which gives higher sensitivity, ease of use and lower-cost instruments. In particular, it has been finding application as a technique for routine and non-routine analysis within a regulated pharmaceutical environment. Many different techniques are already utilised by the pharmaceutical industry, including infrared (IR) spectroscopy, nuclear magnetic resonance (NMR), X-ray diffraction (XRD), high-performance liquid chromatography (HPLC) and thermal methods. Indeed, many of these techniques have been developed in order to offer automated analyses. However, it is now evident that Raman spectroscopy is uniquely placed to support the growing demands of the pharmaceutical industry, especially in relation to high-throughput screening (HTS), rapid analyses, non-invasive analyses (through sample vials and packaging), no sample preparation and ease of use.

The 'Raman effect' involves inelastic scattering of light – the radiation scattered by molecules is shifted to a different frequency (energy) than the incident radiation. This inelastic scattering was first predicted in 1923 by A Smekal[1], however it wasn't until 1928 that Sir C V Raman carried out the first experiments[2], which confirmed the prediction and led to the award of his Nobel prize in 1930. It received considerable attention as a method of non-destructive chemical analysis in the years following its initial discovery, however this interest eventually waned due to advances in IR spectroscopy. The current renaissance in Raman has been driven by advances in the photonics sector, most notably by the introduction of lasers in the 1960s. More recently, multichannel detectors (initially photodiode arrays (PDAs), but now charge-coupled detectors (CCDs) designed specifically for spectroscopy), more advanced laser sources and high-performance optical filters and spectrographs are all allowing the development of ever more sensitive and reliable Raman instruments. This process of innovation and improvement has pushed backed the boundaries and allowed Raman spectroscopy to become a powerful analytical tool that can be accessed by specialists and non-specialists alike. The technique has always had the potential to find wide application in the pharmaceutical industry but it is only now that the instrumentation has evolved to the stage where its potential can be realised.

What is Raman?

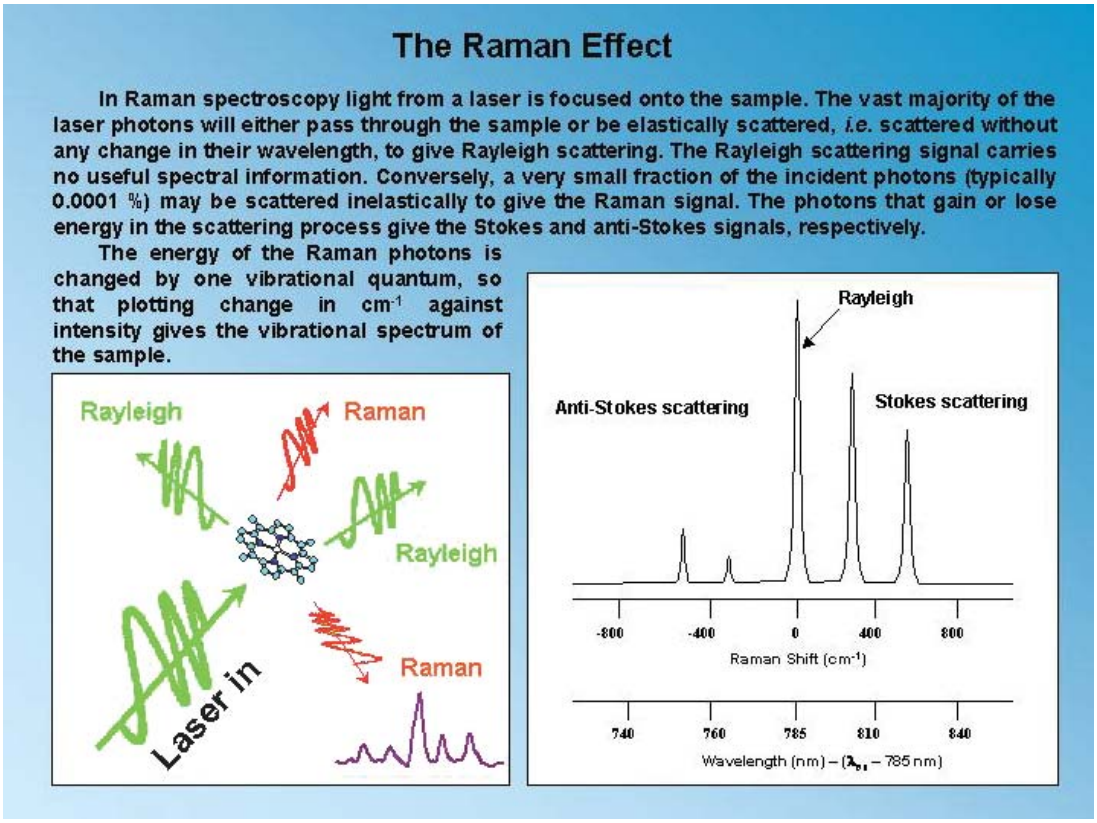
Raman spectroscopy is based on detection of light that has been scattered inelastically by a sample (the 'Raman effect'). This article will concentrate on the vibrational data these measurements can give; the overwhelming majority of studies and applications of the technique are vibrational Raman experiments.

In general, when light interacts with a substance it does so in three main ways: the light may be absorbed; it may be transmitted through the sample unchanged; or it may be scattered. *Figure 1* illustrates the general principle of the measurement, which is that the light from a powerful monochromatic light source (invariably a laser) is focused onto the sample of interest and as many as possible of the photons that scatter from the sample are collected and dispersed in a spectrometer. The photons that are scattered elastically (i.e. with no change in their wavelength) comprise the Rayleigh scattering, which is intense but carries no vibrational information and so is filtered out of the signal. In addition to the Rayleigh scattering, Raman scattering, in which the frequency of the incident photons changes due to interaction with the sample, can also be detected. Unfortunately, the Raman scattering comprises a very small fraction of the incident photons, typically 0.0001%, hence the need for an intense source and sensitive detector.

Not all Raman-scattered photons have the same change in energy. Some photons lose part of their energy to give Stokes scattering, while others gain energy and generate the anti-Stokes signal. To generate an anti-Stokes signal, photons must encounter a vibrationally excited molecule in the

1. *A Smekal,* Naturwissenschaften, *11, 1923, p. 873.*
2. *C V Raman & K S Krishnan,* Nature, *121, 1928, p. 501.*

Figure 1



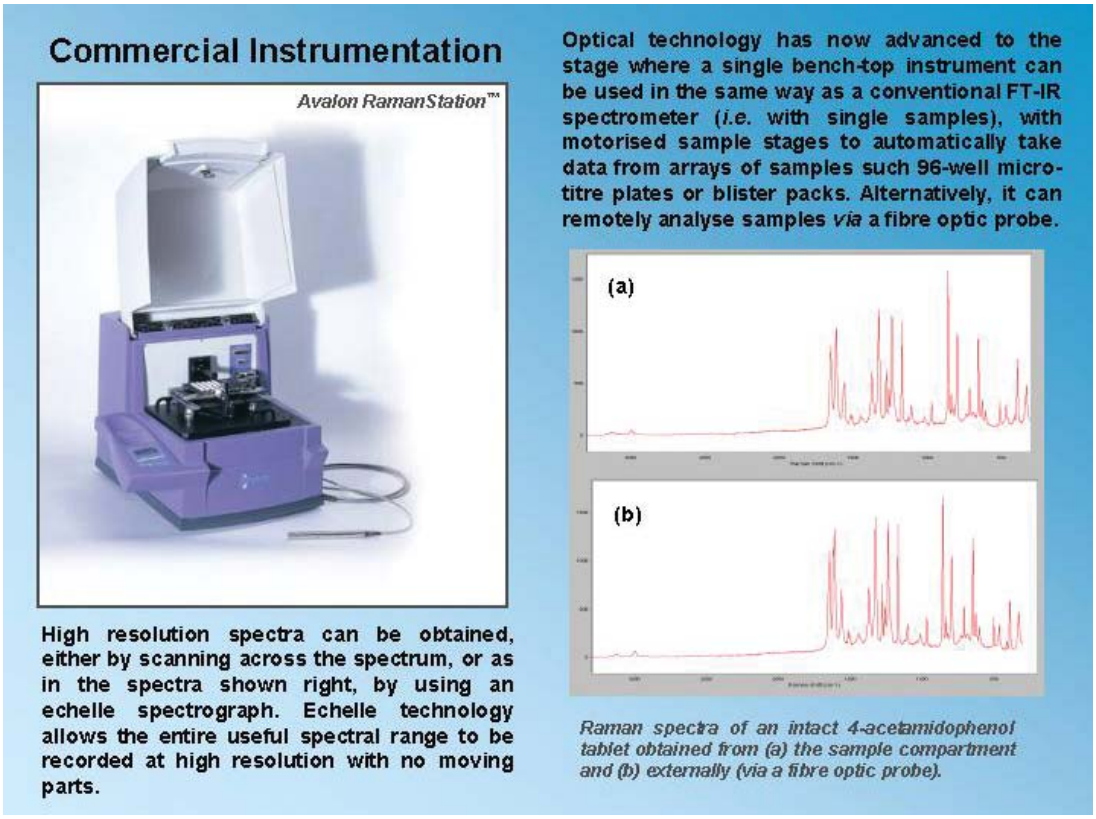
sample; Stokes scattering has no such requirement so Stokes signals tend to be stronger than anti-Stokes and spectra are normally recorded only on the Stokes (energy loss) side of the spectrum. Stokes photons lose energy by depositing it into vibrational excitation of the sample so that the pattern of energy loss reflects the vibrational levels within the sample. In practice, plotting the intensity of Raman-scattered photons against frequency difference between incident and scattered radiation maps the vibrational spectrum of the sample. Overall, Raman and IR signals measure the same vibrational levels (with subtly different selection rules), but the major difference is that the presence of a vibrational band is detected in IR measurements directly by the absorption of an IR photon, while in Raman experiments the same vibration causes the scattering of a photon, which retains most of its energy but shows a small loss corresponding to the same energy absorption by the sample as occurs in IR spectroscopy.

Every compound has its own unique Raman spectrum that can be used for both sample identification and quantification. Raman and IR spectroscopy can be used as complementary techniques because, due to differences in the spectroscopic selection rules, each is sensitive to different components of a given sample. For example, IR spectroscopy is generally more sensitive to polar bonds such as O-H stretches, whereas Raman is much more sensitive to vibrations of carbon backbone structures and symmetrical bonds, such as C = C groups. Using both techniques to characterise a particular substance can provide twice as much information on its chemical composition as that obtained by using either technique on its own.

Given that inexpensive Fourier-transform (FT) IR absorption spectrometers are widely available, the advantages of Raman spectroscopy would need to be significant to justify changeover. There are indeed many advantages, the most notable being:

- no sample preparation is required – Nujol or KBr matrices are not used; the laser is simply directed onto the sample;
- wet samples or even aqueous solutions can be analysed because water is a particularly poor Raman scatterer;
- *in situ* analysis is straightforward with no sample preparation required – Raman can even analyse samples through glass and plastic;
- fibre optics up to hundreds of metres in length can be used for remote analysis;
- Raman bands are narrower than those typically observed in mid-IR spectra and can be used more readily for quantitative analysis;
- the technique is suitable for use with both organic and inorganic materials;

Figure 2



- Raman spectroscopy can measure vibrations from symmetrical molecular modes, which are frequently very weak in IR spectra; and

- Raman bands can be related more easily to chemical structure due to the fact that fundamental modes are measured.

Most of the disadvantages of Raman methods arise directly from the fact that it is a weak effect, which leads to the need for intense laser excitation sources and sensitive detectors. In the past, this, in turn, led to relatively high costs for Raman instrumentation, which was one of the main obstacles to the widespread application of Raman spectroscopy for routine chemical and biological analyses. However, this situation is now changing, as laser and detector costs have fallen significantly, while their performance has improved steadily.

There are numerous variations on general experimental Raman methodology but the most commonly encountered variants are dispersive and FT Raman methods. In general, Raman instruments, whether interferometric (FT) or dispersive are constructed of three basic components: the laser excitation source; the spectrometer (or energy analyser); and the detector. FT-Raman typically employs a 1,064nm excitation laser, an inferometer (which encodes the unique frequencies of the Raman spectrum into a single scan) and a single-channel near-infrared (NIR) detector. In comparison, dispersive systems use visible or near-infrared excitation lasers (488, 532, 633 and ca. 785nm are employed most commonly), a grating for dispersion and a multichannel CCD detector. Overall, the dispersive systems are much more sensitive than those based on 1,064nm laser/inferometer combinations, primarily because of the exceptional quantum efficiency of modern CCD detectors and the enhancement in Raman signal that occurs at shorter wavelengths. This increased sensitivity allows data to be acquired over shorter time periods, which has significant implications for HTS applications. The dispersive instruments are also less expensive than FT instruments.

A significant factor in considering any Raman experiment on unpurified samples is the possibility that the sample may give a broad optical emission signal. In Raman spectroscopy, such background fluorescence is problematic because the Raman signal is relatively weak, so any other emission coming from the sample can drown it out. Problem levels of background fluorescence can arise not only from samples that contain known flurorophores, but also from adventitious fluorescent impurities that may be present at relatively low (sub-millimolar) concentrations. The problem of sample luminescence has been recognised for a considerable time and many different strategies have been employed, with reasonable success, to overcome it. These include quenching the luminescence by using surface-enhanced Raman spectroscopy (SERS) and

shifting the excitation wavelength to one that does not generate luminescence or gives luminescence that lies at a different wavelength range to the Raman signal[3]. Until the 1990s, the only widely available technique was FT Raman, which utilised long excitation sources and therefore did not excite the fluorescence that was observed when using visible excitation. Recently, dispersive instruments, such as the example given in *Figure 2*, with long wavelength (NIR) excitation have begun to find favour as a compromise, which reduces sample fluorescence in much the same way as FT instruments, but gives the sensitivity advantages inherent with multichannel CCD detection. Typically, these use ca. 785nm excitation and a classical spectrograph, although the advantages of using an echelle spectrograph, which allows the entire useful spectral range to be recorded at high resolution with no moving parts, are now being recognised and exploited.

The final major choice to be made in specifying a Raman spectrometer is whether to opt for a microscope-based system or use 'macroscopic' sampling. A wide range of commercial instruments based on both sampling protocols is available and the choice of a microscope-based system is easy if sampling of very small (<10μm diameter) is required. If this is not the case, the choice is much more complex as it is necessary to balance the increased cost and complexity of a microscope-based system (with its potential for sample damage from the highly focused laser source) against typical macroscopic systems, which are simpler, more rugged and less costly and allow sampling of regions ca. 100–200μm diameter. This macroscopic sampling is clearly a technique that has application for HTS, allowing the user to sample from a larger area and providing additional assurance, especially if the samples are inhomogeneous. Clearly, there is no single best option that would satisfy all users; individual requirements are likely to determine the optimum choice.

Raman Spectroscopy in the Pharmaceutical Industry

Raman spectroscopy has been applied successfully in the study of various drugs, including pharmaceuticals[4], narcotics[5] and drug delivery devices[6,12]. Historically, visible Raman spectroscopy did not find application within the pharmaceutical industry due to the problems associated with fluorescence and photodegradation. FT-Raman yielded spectra outside the fluorescent region, although this technique required high laser power, which often resulted in sample decomposition. The use of dispersive Raman did, however, offer an excellent compromise to these problems and has found increased utility in this area.

The implementation of swift and safe drug development processes is clearly a priority for the pharmaceutical industry. With current technology unable to match the pace of drug discovery and design, there is an obvious need to address this bottleneck and provide innovative new technology to improve and accelerate drug discovery. Already, the use of robotics, HTS and advanced software and computers has transformed the industry. However, there will always be a need to improve the efficiency of such systems and drive costs downwards. The development of analytical techniques that push back the boundaries is therefore critical and should be implemented to improve quality, offer additional assurance, increase efficiency and reduce cost.

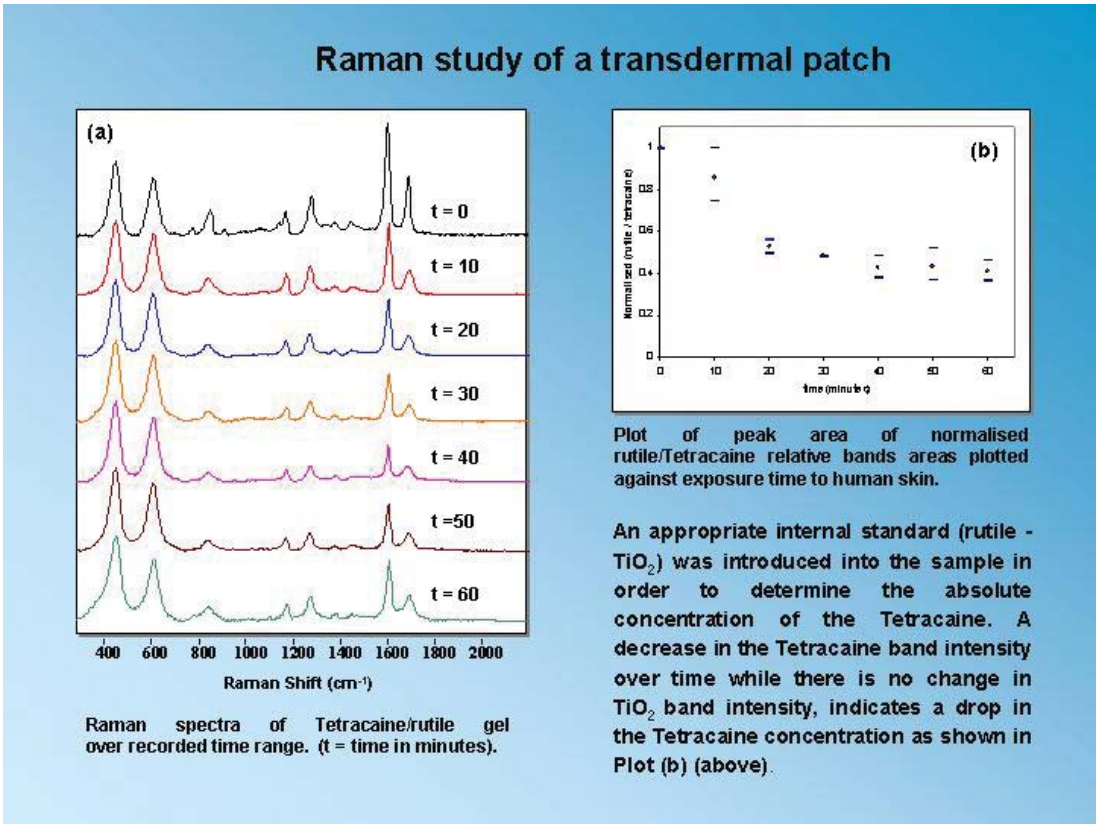
Raman spectroscopy is uniquely placed to support the growing demands of the pharmaceutical industry, especially in relation to HTS, rapid analyses (sometimes less than one second per sample) and non-invasive analyses (through sample vials and packaging) – all without the need for sample preparation. Many different techniques are already utilised by the pharmaceutical industry, including IR spectroscopy, NMR, XRD, high-performance liquid chromatography (HPLC) and thermal methods. Indeed, many of these techniques have been developed in order to offer automated analyses. However, Raman spectroscopy offers a range of significant advantages over these techniques and has been applied in the investigation of pharmaceutical samples, including:

- rapid identification of raw materials;
- analysis of drug mixtures, APIs and excipients;
- identification of contaminants in samples;
- characterisation of formulated materials; and
- process monitoring.

In particular, Raman has found application in the analysis of polymorphs (compounds with more than one crystalline form)[7]. Compounds often exist as more than one polymorph so the properties and,

3. *S E J Bell, E S O Bourguignon and A Dennis,* Analyst, *123, 1998, pp. 1,729–1734.*
4. *L S Taylor,* American Pharmaceutical Review, *4, 2001, pp. 60–67.*
5. *S E J Bell, D T Burns, A C Dennis and J S Speers,* Analyst, *125, 2000, pp. 541–544.*
6. *A C Dennis, J J McGarvey, D A Woolfson, A O'Grady and D F McCafferty,* Proc. of the XVIIth Int. Conf. on Raman Spec., Beijieng, *2000, pp. 1,078–1,079.*
7. *R Hilfiker, J Berghausen, C Marcolli, M Szelagiewicz and U Hofmeier,* European Pharmaceutical Review, *2, 2002, 2, pp. 37–43.*

Figures 3a and 3b



importantly, the stability and reactivity of the drug will be largely influenced by the existence of these polymorphs. If more than one polymorph exists, the Raman spectra will differ, usually quite significantly. In addition, Raman can also be used to distinguish between pseudo-polymorphs, where the degree of hydration differs. Not only does Raman provide a rapid method for the analysis of different polymorphs, it also allows the work to be carried out without additional sample preparation and is non-contact. IR spectroscopy usually requires grinding of the sample to form a mull or preparation of a KBr pellet, which may influence the crystalline form of the sample. Also, techniques such as XRD and DSC take much longer when compared with Raman analyses. Hence, Raman provides a unique platform to discriminate between different polymorphs, which is more rapid, flexible and robust than existing analytical techniques.

Tetracaine gels are another example of the efficacy of Raman spectroscopy for drug monitoring or analysis. Tetracaine, in an aqueous gel, provides an effective percutaneous anaesthesia of intact healthy skin and has found useful application in a bioadhesive transdermal patch[6]. Its effectiveness is due to a low melting point metastable hydrate in aqueous media. Drug delivery involves Tetracaine undergoing a phase change from a solid to an oily suspension at skin temperature[12]. The function of Tetracaine is to diffuse through the outer layers of the skin and desensitise the nociceptors within the epidermis. *Figure 3(a)* shows Raman spectra for Tetracaine after various periods of exposure to skin. An appropriate internal standard (rutile-TiO_2) was introduced into the sample in order to determine the absolute concentration of the Tetracaine. A decrease in the Tetracaine band intensity over time with no change in TiO_2 band intensity indicated a drop in the Tetracaine concentration as shown in *Figure 3(b)*. The clinical applications of Tetracaine include painless venepuncture and miscellaneous topical surgical procedures.

Despite the success of Raman methods in analysing bulk materials, investigations involving very low concentrations were prohibited by the low sensitivity of normal Raman methods. However, the development of SERS, which involves the analysis of samples that have been adsorbed to, or interact with, metal surfaces (typically a roughened metal electrode or a metal colloid) has allowed trace analysis by Raman methods to be carried out[8,9]. Under optimised conditions, SERS can provide spectral enhancements of 10^{14} or even 10^{15} compared with normal Raman scattering[10]. For

8. A C Dennis, J J McGarvey and S E J Bell, Proc. of the XVIIth Int. Conf. on Raman Spec., Beijieng, *2000, pp. 682–683.*
9. Y Ye, J Hu, L He and Y Zeng, Vib. Spec., *20, 1999, pp. 1–4.*
10. A M Michaels, J Jiang and L Brus, Jn. Phys. Chem., *B., 104, 2000, pp. 11,965–11,971.*

example, it can be used for HTS and detection of low concentrations of drug compounds[11].

These are only a few examples of how Raman spectroscopy can be exploited for the analysis of pharmaceuticals. However, what is clear from this work is that Raman has now become a very important and powerful analytical tool. Its utility within the pharmaceutical sector is now of particular significance, because it can provide selective, rapid HTS and highly sensitive methods of analysis, which are low cost, non-contact and do not require any time-consuming sample preparation of the type that exists with many of the current analytical techniques available. It has evolved in the last decade from a complex research tool to a fairly user-friendly technique that is for everyday use.

Raman spectroscopy has the potential to play a key role in the pharmaceutical industry and allow a more streamlined approach to drug development. Undoubtedly, it will have a positive impact in this area in the coming years, with the capacity to be used throughout the drug development life-cycle. ■

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11. S E J Bell and S J Spence, Analyst, *126, 2001, pp. 1–3.*
12. A D Woolfson and D F McCafferty, Int. J. Pharm., *94, 1993, pp. 75–80.*