Raman

IN-DEPTH FOCUS

Raman: a valuable tool in high-throughput screening

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High-throughput screening (HTS) in the pharmaceutical industry has traditionally been a drug discovery endeavour. In general, HTS involves testing collections of hundreds or thousands of samples and subsequent analysis, in order to quickly assay the biological or biochemical activity of a large number of drug-like compounds. It enables discovery of ligands for receptors, enzymes, or other pharmacological targets, and pharmacologically profiles a cellular or biochemical pathway of interest[1]. Nowadays, technological advancements have forced HTS to engulf more applications. Hence, today, HTS techniques are used to characterise polymers and medicines by being incorporated into manufacturing processes to provide real-time measurements. Due to the large number of samples, HTS requires a high degree of automatisation and small sample preparation. Raman spectroscopy, a non-contact and non-destructive technique, fills this need and is used extensively for automated HTS and assay measurements. In this review, Raman instrumentation and its HTS applications are described.

Instrumentation

The modern Raman systems use a combination of automated sample movement, autofocus devices, and automated data acquisition and analysis procedures to acquire spectra from hundreds of samples sequentially. HTS screening and automated measurements can even be integrated with full robot handling, removing the need for expertise and operator intervention.

Usually, typical HTS analyses involve the deposition of liquid or powder in a standard multiwell plate which is placed on an automated stage (**Figure 1**; page 23). As the stage moves, the laser beam focuses on each sample to obtain the spectrum. At this point it worth mentioning that one of the most challenging issues for HTS used to be the possible loss of focus of the laser beam. This has been resolved by using autofocus technology, whereby during the scan of the sample surface, the focal point microscope objective is moving up and down to focus exactly at the sample surface. Autofocus considers that at the maximum light reflection, the refractive intensity from the laser is also at a maximum. However, in practice, this may not be true and usually an offset must be set.

One disadvantage of the Raman technique is interference due to fluorescent samples. This has been resolved by incorporating excitation lasers of different wavelengths which can be changed to the near-infrared to reduce fluorescence of problematic samples.

Finally, the software must be developed to be sophisticated in order to control all the various parameters during the HTS process, such as the autofocus and the speed. Also, it must have the capabilities to take spectra in an array format, apply complex analytical tools, and represent the data in an understood format.

As well as the large modern Raman devices, handheld Raman spectrometers have been developed. These spectrometers are designed for non-specialists and provide easy, rapid measurements in a matter of minutes. These types of Raman allow you to measure through plastic and glass containers, and take *in situ* measurements of

liquids and powders. Moreover, the new models can offer even quantitative and chemometric analysis.

Applications

A typical application of Raman HTS is the identification of polymorphs and cocrystals of pharmaceutical products. Raman microscopy is a valuable technique for salt and cocrystal screening, since spectra obtained provides not only physical information of polymorphism but also chemical information of salt and cocrystal formation. Also, as it is described as follows, Raman HTS is currently used to detect counterfeit drugs, in genomics and as a process analytical technology (PAT) tool.

Cocrystals

Cocrystals have attracted the interest of the pharmaceutical industry since they offer unique possibilities not only to enhance solubility and dissolution properties of the active pharmaceutical ingredient (API), but also to provide better stability. Pharmaceutical cocrystals are solid components formed through noncovalent interactions between a drug molecule and a coformer. Raman microscopy, which could evaluate time-dependent transformation of crystals such as polymorphic conversion and cocrystal formation during the reaction in slurry, was once used for *in situ* analysis. Specifically, Kojima *et al* screened cocrystals of indomethacin. 46 cocrystal formers and potential cocrystals were prepared on a large scale and also, pharmaceutical cocrystals of nitrofurantoin were prepared and screened[2].

Normally, the first step to confirm the formation of cocrystal is by means of powder X-ray diffraction. However, by also comparing the data for the screening samples from spectroscopic tools, a more informed determination may be achieved. These tools can provide evidence of cocrystal formation by investigating the intermolecular interactions among the components. These intermolecular interactions can be attributed by peak shifts which would confirm the interactions of the components.

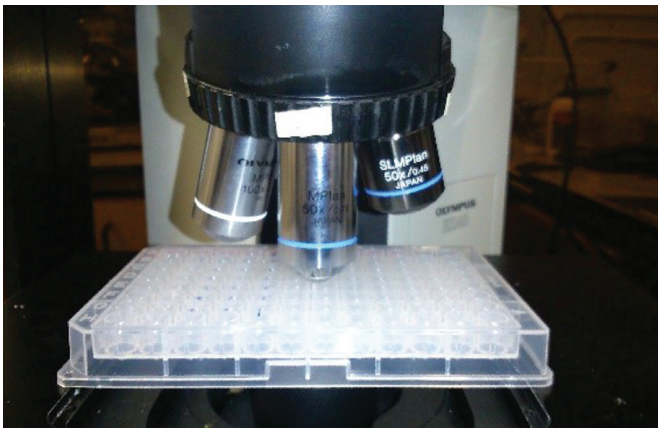

Figure 1: Photographic image of Jobin/Yvon LabRam 320 with a wellplate

Polymorphism

Polymorphism is the ability of a molecule to exist in more than one crystalline form. This is a very important aspect of pharmaceutical technology since the different forms exhibit different pharmacological properties such as varying bioavailability, solubility, manufacturability and processibility. Moreover, the conditions that can favour specific forms can be numerous, involving the crystallisation kinetics, temperature, solvents and the salts used[3]. Therefore, the development of methods that could screen quickly and accurately are of high importance. The most widely-used methods include X-ray

powder diffraction, microscopy and differential scanning calorimetry. Thermal gravimetric analyses are also widely used.

However, none of these methods are particularly rapid and they are therefore not particularly well-suited to HTS. Raman, on the other hand, has proved its suitability for the rapid analysis of polymorphs. A typical example involves the work of Pfund and Matzger whereby using just 0.2mg of material, different polymorphs of four drugs could be identified[4].

Counterfeiting

Another important issue within the pharmaceutical industry where Raman can help is the identification of counterfeit drugs. Both branded and generic products can be counterfeited, which may include those with the correct ingredients or with the wrong ingredients, products without any active ingredients, with insufficient active ingredients or with fake packaging. Sub-standard and falsified medicines are a great threat to public health. They may cause death due to the absence of active drug, as found in some vaccines and antibiotics, or because of the presence of a toxic material[5].

In the hope of combatting this illegitimate trade, the United States Food and Drug Administration has been being developing spectroscopic methods to assess the quality of drugs. The differences between the original product and the counterfeit ones can be related to differing polymorphic forms, divergent ingredients (excipients) and contrasting concentrations. As has already been mentioned, Raman can provide information about the presence of contaminants and polymorphs. Towards this direction, over the past few years, handheld Raman spectrometers have been used to screen pharmaceutical materials in a high-throughput manner. These have several key benefits, particularly for the analysis of incoming raw materials. For example, a major cost/time savings benefit is moving verification of raw materials from a central research laboratory to point-of-use analysis in the warehouse or loading dock. A typical example comes from Veij *et al* who employed a portable Raman spectrometer and by using characteristic peaks in the range 1150-700cm^{-1} detected different excipients in counterfeit Viagra® (sildenafil) formulations[6].

PAT tool

The simplicity and fast analysis of Raman spectroscopy has rendered it an excellent tool for in-line monitoring of relevant quality parameters in production processes by giving real-time information. Raman in combination with fiber optical probes has been applied in several pharmaceutical processes such as in granulation and mixing[7]. Specifically, the blending process has traditionally been monitored in order to determine the significant effects of two process variables – blending speed and loading of the blender – and of a formulation variable (concentration of API)[8]. The incorporation of Raman has not only allowed in-line and real-time monitoring of the blend homogeneity, but also helped to understand the process better in combination with experimental design.

Beer *et al.* has demonstrated how in-line monitoring of a Raman system has been applied in order to study the significance of process variables (mixing speed and height of the stirrer in the reactor) and of formulation variables (concentration of ibuprofen and the viscosity enhancer on the time required to homogenise an aqueous pharmaceutical model suspension as response variable for the production of aqueous pharmaceutical suspension)[7]. Apart from these examples, Raman as a PAT tool has the potential to be incorporated into monitoring other pharmaceutical situations including tabletting, where the real-time quantification of API takes place, and coating in order to analyse and quantify coating variations.

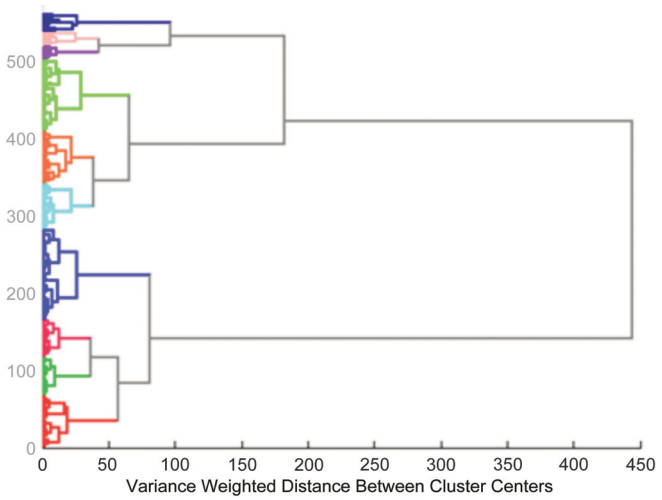

Figure 2: Example of dendrogram produced by HCA

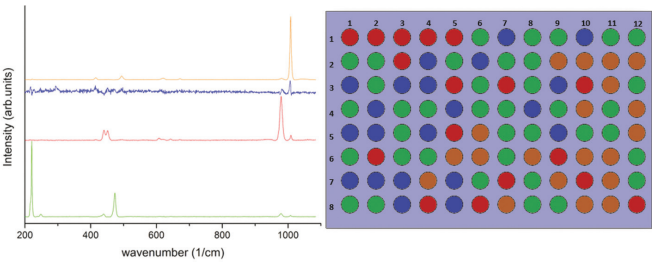

Figure 3: MCR analysis illustrating the distribution of each component

Genomics

One of the most recent applications in Raman HTS comes from the field of genomics. Specifically, surface-enhanced resonance Raman scattering (SERS) can identify the enzymatic activity and selectivity on specific enzymatic recognition sites by adding a dye to be released as a SERS-active species[9]. Moreover, the surface-enhanced Raman gene probes have been used to detect DNA biotargets (e.g., gene sequences, bacteria and viral DNA) via hybridisation to DNA sequences complementary to these probes[10]. It is necessary to apply SERS to biological samples due to the small concentration of analytes which makes their detection very difficult. In SERS, the molecules are conjugated with metallic surfaces in order to enhance the signals. The HTS capabilities of SERS could help the medical industry to develop diagnostics and biological imaging protocols.

Data analysis

Due to the large amount of the data produced by HTS, chemometric tools are applied. Firstly, each Raman spectrum, which represents the contents of an individual well at a given time, is filtered to remove background and to accentuate Raman peaks and shoulders where, subsequently, the proper chemometric tool is applied. These chemometric tools can be classification tools such as hierarchical clustering

and principal component analysis which can group the spectra that are close to similar (**Figure 2**; page 24).

In terms of quantitative analysis, both univariate and multivariate methods can be used for the development of quantitative models. Multivariate methods in this area are based mostly on regression analysis, which consists of multiple linear regression, principal component regression, and partial least square. According to these methods, a calibration data set is built based on the spectral data set. Hence, for instance, in order to predict the concentrations of compounds in a formulation, a calibration data set is constructed from a set of spectra of known concentrations, whereas a second known data set is used to validate the model[11].

Finally, for a clear visualisation of the results, the modern software offers colour-coding when wells are used, to indicate the group into which each falls. The analyst is then able to inspect the spectra from the individual wells or from the outliers and draw the final conclusions from the experiment. Thus the ability to perform automated data processing and data analysis does not eliminate the need for a trained person (**Figure 3**; page 24).

Conclusion

Raman spectrometers in combination with HTS accessories and chemometrics have revolutionised the automated screening of pharmaceutical and biological products. The speed, low sample preparation, its non-destructive capabilities and the plethora of information taken from a single spectrum render Raman a valuable tool in this area. Moreover, technological advances in Raman introduce it to new fields related to biologic analysis and PAT.

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Use of Raman in analysing polymorphism in pharmaceutical drugs

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Polymorphs are different structural forms of pharmaceutical solids, which are mostly organic. Their formation is driven by thermodynamic considerations, which can therefore lead to differences in chemical and biological properties of drug candidates that, in turn, impacts on their quality control, licensing and patent protection requirements[1]. This article will provide a brief overview of the use of Raman spectroscopy, particularly in the low frequency lattice mode region, to detect and understand polymorphs in pharmaceutical drugs. Examples on acetaminophen from earlier work and recent results on polymorphism with fenofibrate, including *in situ* temperature-dependent measurements, are discussed.

One interesting feature about polymorphs of organic crystals pointed out by Price[1] is that there is a tendency for the most thermodynamically stable forms to crystallise from the melt or amorphous phase last. One well-known example of polymorphism occurs in the HIV/AIDS drug ritonavir (Norvir®), which was first introduced in 1996 by Abbott as ordinary capsules in its bioavailable, water-soluble polymorphic form I. The more stable and less soluble polymorphic form II was discovered in 1998, leading to the withdrawal from the market of the water-soluble, metastable form I capsules because it turned out that even the presence of traces of polymorph II catalysed the conversion of the metastable bioavailable form I to form II. Initially, Abbott replaced the capsules with refrigerated gel caps to prevent the conversion of polymorph I to II. Abbott (now AbbieVie, Inc) was fortunately able to resolve this issue in 2000 with the introduction of Liponavir/Ritonavir® tablets, presumably in stabilised polymorph I form.

Polymorphism can be exemplified by the structures of two polymorphs of acetaminophen shown in **Figure 1** (page 27) – polymorph I is the more common stable active ingredient present in Tylenol® (acetaminophen) with the molecules in a puckered conformation, and polymorph II is the metastable form with molecules

flattened out to give a layered structure that transforms in a matter of days to the more stable polymorph I structure. For reasons similar to those for the HIV drug, polymorph II of acetaminophen cannot be used in pharmaceutical applications in spite of attractive features, such as higher plastic deformability under tabletting conditions and a higher water dissolution rate. Several methods have therefore been employed in attempts to stabilise polymorph II. For example, Łuczak *et al*[2] used milling to micronise coarse acetaminophen powder to increase its specific surface area, followed by rapid cooling to form polymorph II, which remains stable for nearly two years.

It was suggested that stabilisation is due to pinning of the molecules in the planar conformation through interactions with defects created by the milling process. Many types of defects exist in milled organic crystals due to varying grain sizes, edge dislocations, and effects due to grain boundaries. Crystalline defects of this type are higher energy, thermodynamically unstable sites that progressively lead to disorder during processing and the formation of an amorphous phase. Milling performed at low temperatures, referred to as cryomilling, enhances defect formation in the pharmaceutical drug griseofulvin (Gris-peg/Grifulvin V®) to give rise to disorder-induced inelastic light scattering[3]. Another approach to stabilising metastable polymorphs is by templating. This has been achieved for acetaminophen's polymorph II by direct compression in the presence of polymeric materials, such as polyvinylpyrollidone (PVP), gelatin and starch, resulting in stabilisation of polymorph II for an extended period of 11 months[4,5].

Traditionally, polymorphs have been discovered during drug development by thermal techniques, such as differential thermal calorimetry and X-ray diffraction. More recently, Raman spectroscopy has emerged for this use, driven by advances in miniaturisation and optical technologies, to provide highly efficient drug fingerprinting and polymorph detection using off-line, and potentially in-line monitoring methodologies. Another approach that complements solid state screening is computational crystal structure prediction of polymorphs in flexible organic molecules. This approach was shown to be attractive by the successful quantitative prediction of polymorph 1V of piracetam[6].

Low frequency Raman spectroscopy

Raman spectroscopy is used to detect vibrational modes of molecules in the gaseous, liquid and solid phases, as well as the rotational modes

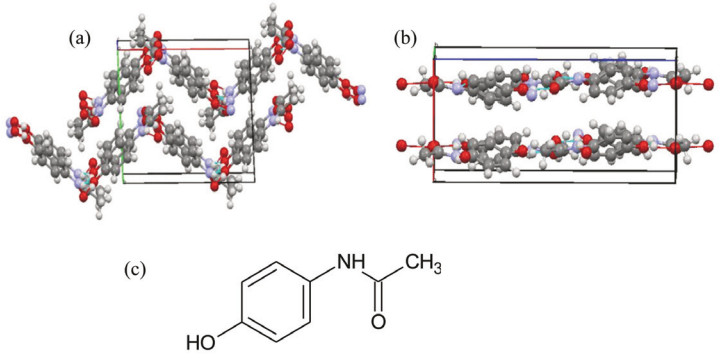

Figure 1: Crystal unit cells of acetaminophen polymorphs: **(a)** Stable polymorph I, **(b)** Metastable Polymorph II, and **(c)** Molecular structure of acetaminophen. In (a) and (b), oxygens are red, nitrogens are blue, hydrogens are light grey, and carbons are dark grey

in gases and vapours. In organic solids, it can detect four types of vibrational modes: internal or intramolecular modes, higher order internal modes due to combinations and overtones of the fundamentals, and external or lattice ones associated with intermolecular translations and hindered molecular rotations or librations, and their corresponding higher order combination and overtone modes.

Typical pharmaceutical and biomedical applications of Raman spectroscopy have largely involved investigating intra-molecular modes, showing Raman shifts that are typically greater than 200cm^{-1} from the excitation line. Lower frequency modes have not been investigated because of limitations of optical filters used to remove the intense, elastic Rayleigh scattering from the samples. However, with recent advances in optical technologies, low frequency Raman spectroscopy (LFRS) to well below 200cm^{-1} can now be rapidly performed with the same sensitivity and resolution as higher frequency spectroscopy to study intermolecular interactions in pharmaceutical and biomedical solids. LFRS of the lattice vibrations makes it possible to detect crystalline features in the early or later stages of crystallisation from the amorphous or the molten phase, and also to detect emerging stable or metastable crystalline polymorphs.

Raman spectroscopy in the low frequency region is particularly sensitive to structural changes. For example, with the onset of disorder leading to the formation of an amorphous phase, the external lattice modes broaden and essentially show a broad featureless background. In some organic solids, the amorphous phase retains short-range order as shown by the appearance of very broad

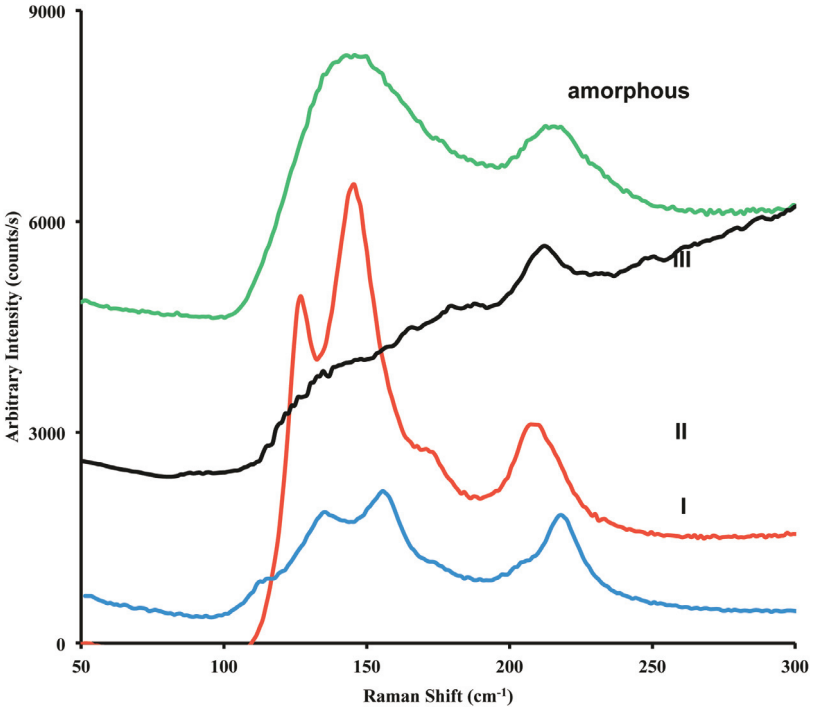

Figure 2: The low frequency Raman spectra of the amorphous and crystalline phases I, II and III polymorphs of acetaminophen

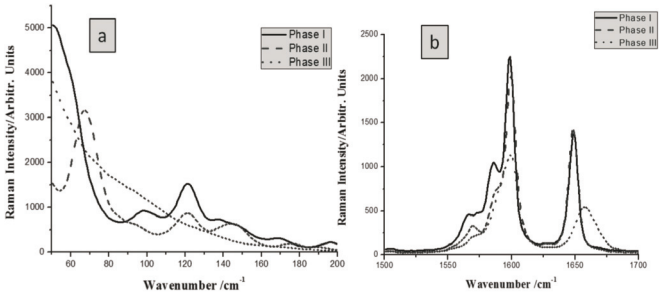

Figure 3: LFR spectra at 25°C of pure fenofibrate in the 50-200cm^{-1} (panel **a**) and high frequency Raman spectra in the 1500-1700cm^{-1}(panel **b**) spectral regions for its phase I and II polymorphs, and the amorphous phase III

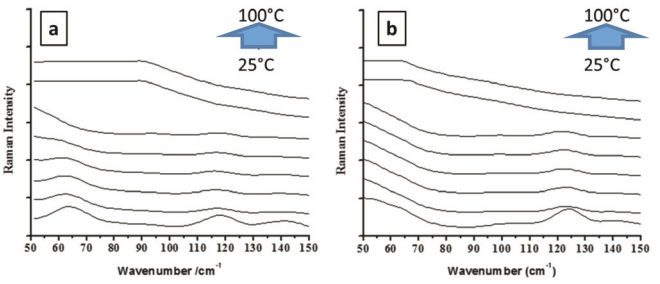

Figure 5: LFR spectra of the second heating ramps of: **(a)** fenofibrate-triblock copolymer PF/127 25%, and **(b)** fenofibrate-hydroxylpropyl methyl cellulose HPMC/E3 25%. 1g fenofibrate in 0.25g polymer is referred to as 25% polymer

Raman peaks. Intramolecular vibrations associated with symmetric and antisymmetric stretching motions, and molecular deformations coupled with rocking, wagging and breathing modes are also sensitive to changes in the local crystal field due to disorder and/or formation of a new crystalline structure or polymorph. In addition to line-broadening, defects and disorder can give rise to background scattering independent of excitation frequency, as observed for cryo-milled griseofulvin[3].

> "With recent advances in optical technologies, low frequency Raman spectroscopy (LFRS) to well below 200cm^{-1} can now be rapidly performed"

Polymorphism in acetaminophen and fenofibrate

The LFR spectra of acetaminophen in its amorphous and three polymorphic phases are shown in Figure 2 (page 28). A line near 210cm^{-1}, which can be assigned to a largely internal deformation mode of the acetaminophen molecule, is observed in all four phases. Below 200cm^{-1}, two strong lines between 100 and 175cm^{-1} are seen, which can be assigned to mixed translational-librational external modes in the crystal. The lines increase in intensity in the metastable polymorph II due to the greater contribution from the librational mode, which is typically more intense in molecular solids. In the increasingly metastable polymorph III, the low frequency lines are very broad. This indicates that polymorph III has substantial short-range, possibly dynamic disorder, although X-ray diffraction measurements show that the structure has long-range crystallinity. In the amorphous phase, the low frequency modes broaden to a single line peaking near 150cm^{-1} due to the presence of short-range order in the amorphous phase.

The LFR spectra of the anti-cholesterol drug, fenofibrate, in its stable polymorph I, metastable polymorph II and amorphous phases are displayed in Figure 3(a) together with the higher frequency internal mode fingerprint lines associated with C=O and C=O/C=C vibrations for the two crystalline polymorphs and the amorphous phase in Figure 3(b). The metastable polymorph II has a strong Raman line at 65cm^{-1} which can be attributed to an external librational mode of the crystal. The weaker lines between 90 and 180cm^{-1} are assigned to external translational modes of polymorphs I and II. In contrast to acetaminophen, the amorphous phase of fenofibrate shows no evidence of short-range order because only a featureless low frequency scattering is observed in this phase.

Raman spectroscopy also allows for a detailed investigation of fenofibrate polymorph crystallisation in the pure state and in the presence of stabilising polymers, such as triblock copolymer – PF/127 and hydroxylpropyl methyl cellulose – HPMC/E3[7]. This can be performed by *in-situ* temperature-dependent LFR spectroscopy. Two heating and cooling cycles for pure fenofibrate are shown in Figure 4 between 25°C and 100°C at intervals of 10°C. All three phase transformations are observed during the heating and cooling cycles shown in the figure. Formation of the amorphous phase occurs in the temperature range of 90°C to 100°C, just above the melting temperature during the first heating ramp in Figure 4(a). Crystallisation of polymorph II occurs at 25°C during the first cooling stage in Figure 4(b), as shown by the appearance of the strong line at 65cm^{-1} assigned, as discussed above, to a librational lattice mode. In the second heating ramp, the transformation from polymorph II to the stable polymorph I occurs at 80°C to 90°C followed by formation of the amorphous phase, as evident

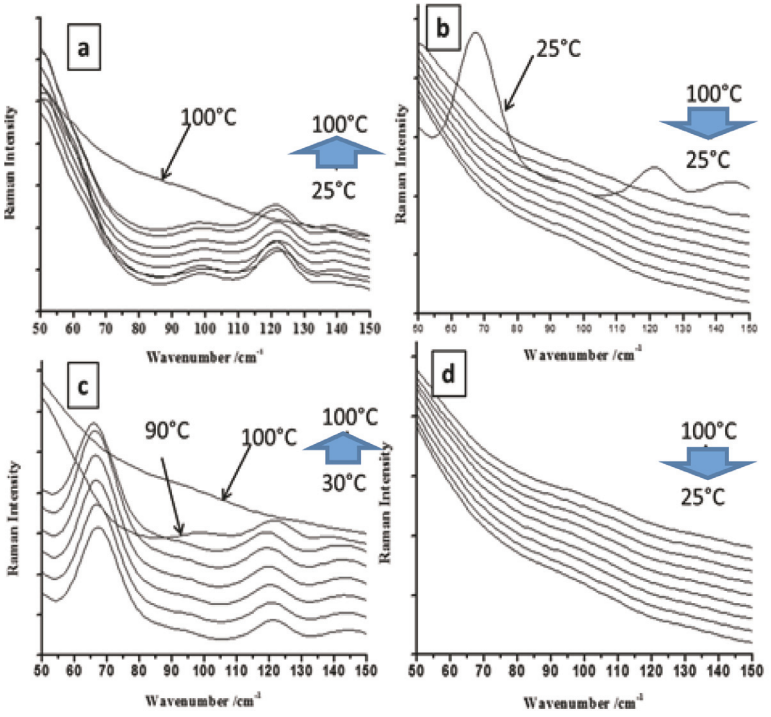

Figure 4: LFR spectra between 50 and 150cm^{-1} of pure fenofibrate as a function of temperature in the 25°C-100°C range during: **(a)** 1st heating, **(b)** 1st cooling, **(c)** 2nd heating, and **(d)** 2nd cooling

from **Figure 4(c)** (page 29). **Figure 4(d)** shows that the amorphous phase does not transform to a crystalline form through the second cooling stage due to hysteresis.

Temperature-dependent LFR spectra during the second heating ramps of fenofibrate mixed with PF/127 and hydroxylpropyl methyl cellulose HPMC/E3 are shown in **Figure 5** (page 29). The internal mode Raman spectra in the higher frequency range for fenofibrate in the presence of different amounts of the stabilising polymers do not change, indicating that hydrogen and chemical bonds are not formed. With the increase in temperature of the second heating ramp shown in **Figure 5(a)**, polymorph II crystallises and then transforms to polymorph I prior to forming the amorphous phase with some short-range order. The crystallisation behaviour of fenofibrate in the presence of the block copolymer PF/127 is therefore similar to that for pure fenofibrate. As for fenofibrate with HPMC/E3 shown in **Figure 5(b)**, metastable polymorph II is not formed, indicating strong non-bonding interaction with the polymer.

Acknowledgement

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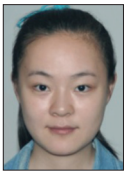

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Upcoming Raman calibration standards

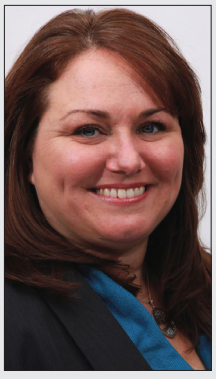
The European Pharmacopeia published Supplement 8.7 in October 2015, which included a revision to Chapter 2.2.48 – the section covering Raman spectroscopy. The chapter includes new acceptable tolerances for benchtop and handheld Raman instruments for polystyrene, paracetamol and cyclohexane, which all Raman instrument manufacturers must comply with from 1st April 2016. The specifications are shown in **Table 1** below.

Table 1: Wavenumber shifts (and acceptable tolerances) of polystyrene, paracetamol and cyclohexane

	Wavenumber shifts[A] [cm^{-1}]	Tolerances	
		Benchtop [cm^{-1}]	Handheld [cm^{-1}]
Polystyrene[B]	620.9	± 1.5	± 2.5
	1001.4	± 1.5	± 2.0
	1031.8	± 1.5	± 2.0
	1602.3	± 1.5	± 3.0
	3054.3	± 3.0	NA[E]
Paracetamol[C]	797.2	± 1.5	± 2.5
	857.9	± 1.5	± 2.0
	1168.5	± 1.5	± 2.0
	1236.8	± 1.5	± 2.0
	1323.9	± 1.5	± 2.5
	1648.4	± 1.5	± 3.0
	2931.1	± 2.0	NA[E]
Cyclohexane[D]	801.3	± 1.5	± 2.5
	1028.3	± 1.0	± 2.0
	1266.4	± 1.0	± 2.0
	1444.4	± 1.0	± 2.5
	2852.9	± 2.0	± 3.0

A: Standard guide for Raman shift standards of spectrometer calibration (American Society for Testing and Materials ASTM E 1840); B: Polystyrene film (e.g. 76 µm), pellets (e.g. NIST 706a) or rod; C: Paracetamol for equipment qualification CRS (which represents monoclinic form I); D: Cyclohexane R; E: NA: beyond detector range.

Kerstin Barr, Product Manager for handheld Raman instruments at Thermo Fisher Scientific, comments on the revised tolerances



"The pharmaceutical industry has rapidly adopted the use of handheld Raman for the identification of raw materials over the past five years. But now, for the first time, the European Pharmacopoeia has set specific wave number accuracy requirements for these handheld spectrometers. These new specifications define the minimum requirements for wavenumber accuracy for users of handheld Raman spectrometers in GMP test environments."

Kerstin believes the change to provide industry specifications that can help ensure reliable identification and the safe manufacture of medicines is "largely overdue".

"The new specifications provide pharmaceutical manufacturers clear guidance on the requirements for use in GMP environments," she says. She goes on to explain that this clarity gives manufacturers across the industry well-defined parameters without the need to justify analytical performance individually. "Overall, these changes should streamline the deployment of handheld solutions for the release of raw materials."

So how do the changes to Supplement 8.7 affect users of Thermo's TruScan RM analysers? Kerstin explains: "Thermo Scientific TruScan users can have confidence that their equipment will be compliant with the new regulatory standards. Qualification and recertification documents are being updated in advance of the 1st April implementation date."

We also ask Kerstin how Thermo Fisher plans to address any forthcoming challenges: "Due to the inherent laser and spectrometer stability in our TruScan instruments, there are no changes required for hardware or manufacturing operations to comply with the new specifications. We will closely monitor the regulatory expectations and work with our customers to help meet their requirements."

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Kaiser Optical Systems, Inc.

Mark Cabell
Global Market Manager,
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European Sales Director
for Pharma, B&W Tek

Moderator
Zafar Iqbal, New Jersey Institute of Technology

How can Raman spectroscopy be used for pharma drug concentration quantification in solid dosage forms in use today?

Esmonde-White: The sampling versatility and unambiguous materials identification provided by Raman spectroscopy renders it a valuable tool in measuring solid dosages from the research laboratory to the manufacturing environment. Raman spectroscopy has a strong advantage over other techniques for real-time in-process identification of vanishingly small amounts of material, as demonstrated in the PhAT System™ Raman technology. This important feature increases the efficiency of manufacturing low dosage formulations, or identifying small amounts of impurities or polymorphs, by enabling real-time process corrections. Transmission Raman has been demonstrated in a release testing context, after the dosage has been formulated.

Cabell: Outside of the traditional lab, handheld Raman could be used to quickly screen for falsified and substandard medicines to help protect patient safety and the efficacy of pharmaceutical products. Handheld Raman tools allow for quick screening in warehouses, ports and borders without the inherent time delays with current sampling/laboratory analysis workflows.

Fischer: In Raman spectroscopy, quantification requires precise calibration in order to link the measured relative spectral intensities with the calibrated data. It is necessary to maintain the same instrumental and experimental conditions. An absolute determination and adjustment of the laser power with excellent reproducibility is essential for such a setup. It is also important to define the spot size depending on the application: a global illumination provides general information whereas a high-resolution imaging setup results in a more detailed analysis.

Diz: The intensity of a spectrum in Raman spectroscopy is directly proportional to the concentration of the components. Since Raman is not a direct technique for quantitative measurements, correlation models or calibration curves are required. Raman is advantageous

because of the intrinsic fingerprint nature of the technology. Also, physical aspects of chemicals (e.g., granulation) do not affect the quantification and an accurate measure can be achieved through the coating elements of the solid form of the pharmaceutical drug. The technique allows the identification and quantification of complex mixtures, ratio of polymorphic forms, and the content homogeneity of the components.

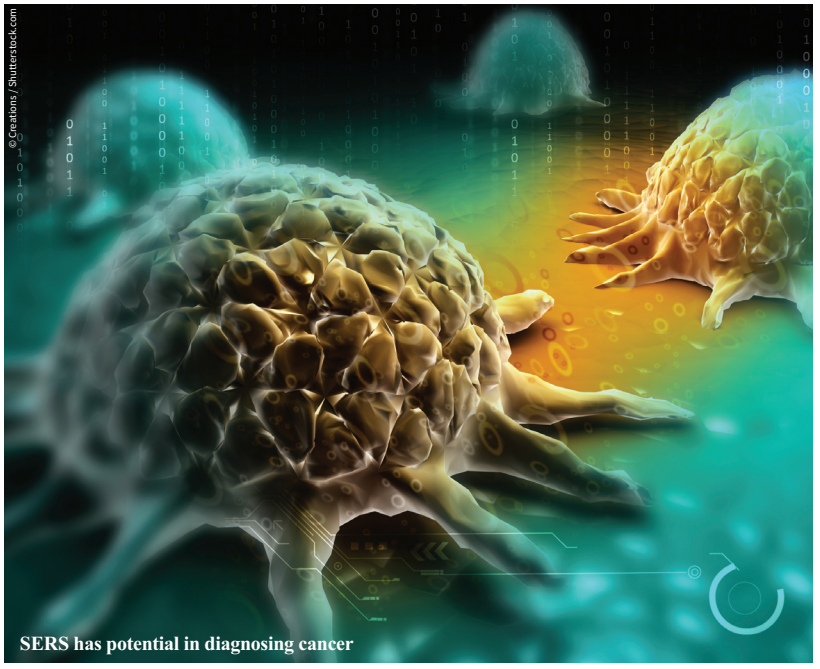
Surface-enhanced Raman has the potential for single molecule detection. How do you see its implementation in the pharma industry?

Cabell: Surface-enhanced Raman spectroscopy (SERS) has tremendous potential in new applications where trace levels of analytes need to be evaluated. Challenges exist today in the reproducibility of quantitative results and overall SERS substrate reproducibility. Research in specific applications will require matching sample prep (swabbing/pre-concentration steps) with the appropriate SERS substrate for optimal analytical performance.

Diz: There are now SERS substrates on the market that offer specific, consistent and reliable results, and it is only a matter of time before the pharma industry starts introducing the technology into the routine operations. Today, Raman technology is already available in compact and fully portable devices that offer fast and reliable results in diagnostic areas. New applications are continually being developed as we speak. The detection of contamination, impurities, reactors cleaning process assurance, etc. are examples of where there is the greatest interest, mostly because of the fast and reliable response of the SERS.

> "For single molecule detection with SERS, sample preparation is critical in achieving good results"
>
> *Harald Fischer*

Fischer: For single molecule detection with SERS, sample preparation is critical in achieving good results. It requires that the drug molecules become attached to the SERS-active substrate prior to measurement. This allows the detection limit to be significantly reduced and could facilitate the screening of low concentration drug-formulations. Due to the sample preparation this technique is quantitatively challenging and ceases to be non-invasive.



SERS has potential in diagnosing cancer

Esmonde-White: SERS is a vibrant academic research technique with exciting potential for ultra-trace Raman analyses. There are major materials manufacturing and application concerns that need to be addressed if the technology becomes implemented in a pharmaceutical industry setting. Validating non-specific binding or biological toxicity of SERS particles, with or without attached labels, in high throughput screening (HTS) or discovery applications is a major concern. Representative sampling and process compatibility (for example, vessel fouling or incompatibility with cleaning protocols) are concerns for using SERS in any regulatory environment.

What would be the best way to use Raman spectroscopy for the detection of polymorphs (including the amorphous phase) of pharmaceutical drugs?

Fischer: A Raman spectrometer in combination with a high-performance confocal microscope for three dimensional-Raman imaging is ideally suited for the detection of polymorphs within an amorphous phase. The smallest conglomerates of polymorphs can be distinguished and imaged by analysing, for example, the peak widths of the spectral data. Using a state-of-the-art Raman imaging system, sample volumes can be as low as approximately 230nm x 230nm x 500nm while attaining the highest spectral resolution. This setup can be used for the various pharmaceutic formulations such as emulsions, solids, core-shell particles or drug-delivery coatings.

Cabell: Raman spectroscopy has long been used to discriminate polymorphic active pharmaceutical ingredients in multiple workflows in the pharmaceutical industry. Research into complex polymorphic systems has been aided by advances in low frequency Raman spectroscopy which allows deeper analysis of the fingerprint region for enhanced discrimination in challenging systems.

Diz: Raman has enormous advantages over other spectroscopy technologies because it is capable of providing information on compound crystallinity as well as functional groups. Typically, a crystalline material produces very sharp peaks on a Raman spectrum, while amorphous materials disperse the light in a way that the recorded peaks are broader. The bands associated with the crystal structure are generally below 100cm^{-1} Raman shift. Raman instruments that produce good resolution and extended range close to the laser emission line (50-100cm^{-1} Raman shift) or lower are important to see and analyse these critical bands to differentiate polymorphs.

Esmonde-White: The wealth of literature and industrial examples establish Raman spectroscopy as a universal

tool for understanding, identifying and controlling polymorphism. The best way to leverage the power of Raman spectroscopy is to incorporate it throughout discovery and development. Raman spectroscopy provides valuable information on polymorphism including unambiguous crystal form discrimination and insight into the affected intermolecular forces. Method transferability is an excellent feature of Raman that enables a Raman method to be used from HTS to manufacturing. As a PAT tool, in-process Raman measurements improve process efficiency by enabling process corrections in real-time.

What was the most innovative application of Raman spectroscopy in the aerospace industry?

Esmonde-White: Technological advances in detectors, optics and lasers have enabled Raman spectroscopy for standoff and planetary applications. Two exciting applications come to mind. The combination of Raman spectroscopy with Light Detection and Ranging (LIDAR) has improved remote sensing for atmospheric water vapour on Earth. Feasibility of standoff and planetary Raman measurements have been demonstrated in extreme Earth environments with an eye toward space missions. Raman instruments aboard the EXOMARS 2018 mission and NASA 2020 rover missions will provide mineral identification with the exciting possibility of identifying biosignatures of life, such as bacterial pigments, on Mars.

> “As the aerospace industry moves to lighter composite materials, new tools to assess the structure and performance are critical”
>
> *Mark Cabell*

Cabell: As the aerospace industry moves to lighter composite materials, new tools to assess the structure and performance are critical. Raman microscopy offers a unique window into the structure and chemical composition of these new nano-materials and composites leading to a greater understanding of performance characteristics.

Diz: Raman can be used in the correlation of physical properties such as strength or breaking point in polymers or correlation of composition with glass transitions. The aerospace industry is strongly associated with these properties and characterisation of these materials while in use. Raman allows for non-destructive verification of the material properties that are present by simply analysing the spectroscopic material signature, unlike many previous tests that were destructive and thus were inappropriate for the industry. The excellent correlation existing between Raman molecular spectroscopy and macroscopic properties are in our opinion one of the most innovative associations.

Raman will be used to analyse minerals on Mars

What could be the most innovative use of Raman spectroscopy in the pharma industry?

Diz: Raman spectroscopy as a routine tool in drug development as well as in product testing – from production to final distribution to end customers – are areas of innovative use of Raman in the pharmaceutical industry. With the miniaturisation of Raman spectrometers, and development of intuitive user interfaces for both portable and handheld Raman spectrometers, this sensitive and selective analytical tool is being widely adopted at all stages of drug development. With early development including API synthesis, polymorph screening, and crystallisation studies, the portability of Raman used with fiber-optic probes allows for monitoring of even small-scale processes.

Fischer: We regard truly confocal microscopy, which provides spatial resolution down to the diffraction limit while simultaneously maintaining the highest spectral sensitivity, as the most significant innovation for the analysis of drug delivery systems in the pharmaceutical industry. Combining this with confocal large-area imaging capabilities as realised in TrueSuface microscope extends the range of applications to the macroscopic level.

A great potential for the application of Raman innovations in the pharmaceutical industry might be the direct monitoring of the drug delivery and distribution levels down to the subcellular levels or the rapid bacteria identification securing a more specific antibiotics treatment.

Esmonde-White: Raman applications in the pharmaceutical industry have brought improved scientific understanding and increased process efficiency, resulting in safer medicines. Extension of these principles to biopharmaceuticals is innovative and is already yielding cost savings in upstream bioprocesses. The high molecular specificity of Raman enables simultaneous measurements of multiple bioprocess parameters *in situ*. Raman enables optimised feed strategies and provides scalable analytical models. Raman bIO PRO technology is compatible with universal platforms which are independent of cell or media type and process conditions. Raman technology enables yield enhancements while ensuring quality product. We also anticipate exciting Raman applications in downstream bioprocessing.

Cabell: SERS could play an important role in the future of diagnosing infections, cancers and other precursors of serious medical conditions. In addition, SERS could lead to a revolution in personalised medicine whereby patients could easily monitor pharmaceutical treatment performance to minimise side effects while maximising clinical results.