JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 179–192

Received May 12, 2006
Accepted July 28, 2006
DOI 10.1211/jpp.59.2.0005
ISSN 0022-3573

Raman spectroscopy for quantitative analysis of pharmaceutical solids

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Abstract

Raman spectroscopy is experiencing a surge in interest in solid-state pharmaceutical applications. It is rapid, non-destructive, no sample preparation is required and measurements can be made in aqueous environments. It can be used for not only qualitative, but also quantitative, analysis. In this paper, the use of Raman spectroscopy for quantitative analysis of pharmaceutical solids is reviewed. The technique has been used for chemical and physical form analysis. Physical form analysis has involved quantification of polymorphism, hydrates, the amorphous form and, recently, protein conformation. Initially, simple powder systems were quantified, although this has since extended to complex pharmaceutical formulations, including tablets, capsules, microspheres and suspensions. Formulations have also been analysed through packaging. The characteristics of the technique make it ideal for process monitoring and it has been used to quantify changes in-situ during processes such as wet granulation and batch crystallisation. The theoretical basis of quantitative Raman spectroscopy, common data analysis approaches, including multivariate analysis, and sources of error in quantitative analysis are also discussed.

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Funding: JR and CS are grateful to the Academy of Finland for funding (decision number 109343).

Introduction

Raman spectroscopy has experienced a surge in interest from the pharmaceutical sector in the last two decades. A search under "Raman" and "pharmaceutic" in SciFinder Scholar (American Chemical Society) resulted in 177 hits before 1990, 489 hits from 1990 to 1999 and already 882 hits in the present decade. Of these, a significant proportion covers analysis in the solid state. Why has Raman spectroscopy become so popular for solid-state pharmaceutical analysis? This can be answered by introducing the theory and practical features of the technique.

Raman spectroscopy probes vibrational transitions in molecules and is complementary to infrared (IR) (both mid- and near-IR) spectroscopy. However Raman spectroscopy is based on light scattering while IR spectroscopy is based on absorption. In Raman spectroscopy samples are irradiated by monochromatic (visible or near-IR) laser light. Most of the light is scattered back at the same wavenumber (Rayleigh scattering). However, in a very small proportion of cases the light is scattered at slightly higher wavenumbers (anti-Stokes scattering) or, less often, lower wavenumbers (Stokes scattering), and this is collectively known as Raman scattering, although it is common to report the Stokes scattering as the anti-Stokes is much weaker. The wavenumber difference between the incident and scattered radiation corresponds to vibrational energy transitions, and it is these differences that are detected in Raman spectroscopy (Colthup et al 1990).

For Raman scattering to occur, there must be a change in the electronic polarisability of the molecule with the vibrational mode. One can think of polarisabilty as the elasticity of the electron cloud (i.e., the susceptibility of the electron charge to have a dipole induced within it). For this reason large polarisability changes, and hence strong Raman scattering, are associated with molecules that have delocalised electron systems (e.g., aromatic conjugated systems). In contrast, IR absorption is strongest with polar molecules and thus IR and Raman spectroscopy provide complementary information (Colthup et al 1990).

Before the mid 1980s, Raman spectroscopy on pharmaceutical materials was usually impossible because the Raman response was masked by sample fluorescence induced by the visible lasers. However, the advent of new experimental methods, including Fourier transform (FT) spectrometers and charge coupled device (CCD)-based experiments, provided a strategy to avoid fluorescence. Both of these new types of experiment allow one to

use far-red or near-IR lasers, thus the resulting Raman photons that are detected are spectrally red-shifted with respect to normal fluorescence (which dominates in the visible region, 450–680 nm). This opened up mainstream pharmaceutical analysis to Raman spectroscopy.

Raman spectroscopy has several practical features that make it attractive for pharmaceutical solid-state analysis. Most active pharmaceutical ingredients (APIs) contain aromatic or conjugated domains and are therefore strong Raman scatterers. Conversely, many excipients are aliphatic and exhibit much weaker Raman spectra. Thus Raman spectroscopy may be better than mid- and near-IR spectroscopy for studying APIs in formulations, especially when the drug concentration is low. Water, which is a prevalent excipient and is also present during manufacturing and drug dissolution, is a very poor Raman scatterer. While mid- and near-IR spectroscopic analyses may be very challenging in aqueous environments, Raman spectroscopy is ideal for solid-state analysis in the presence of water.

Like near-IR spectroscopy, Raman spectroscopy requires only a few milligrams (or in some cases less) of sample and no sample preparation. The technique is non-destructive and with current spectrometers (using FT spectrometers or CCD cameras), the method is rapid (within seconds or less). As with near-IR (but not mid-IR) spectrometers, Raman spectrometers can also be interfaced easily with a probe to improve sample access, which is especially useful for process analytical technology applications.

These theoretical and practical features make Raman spectroscopy an attractive technique for analysis of pharmaceutical solids. What is the potential of Raman spectroscopy for quantitative analysis of pharmaceutical solids? This article will attempt to answer this question by first introducing the basis and approaches for quantitative analysis, and then reviewing quantitative applications in pharmaceutical solid-state analysis.

Quantitative analysis

Basis for quantitative analysis

Raman scattering is proportional to the concentration of the substance, and this is the basis for quantitative Raman spectroscopy. The intensity of spontaneous Raman scattering in an ideal (non-absorbing) system during a particular vibration can be defined as:

$$L = P_D \beta D K \quad (1)$$

where L is the specific intensity of the Raman scattering (photons $sr^{-1}\,cm^{-1}\,s^{-1}$), P_D is the power density of the incident laser light (photons $cm^{-2}\,s^{-1}$), β is the differential Raman cross section ($cm^2\,mol^{-1}\,sr^{-1}$), D is the number of molecules per cm^3 and K is a geometric factor that depends on the observation geometry (incident and detection angle). A detailed analysis of Equation 1 is provided by McCreery (2000). The most important feature of Equation 1 in this context is that the intensity of Raman scattering of a particular mode is directly proportional to the vibrating species concentration (described by D). Although the Raman scattering is over the bandwidth of the mode and hence a frequency range, proportionality is maintained at a single Raman shift (McCreery 2000).

This relationship provides the basis for quantitative Raman spectroscopy.

Quantitative approaches

Quantitative analysis using Raman spectroscopy can be broadly defined into two categories, uni- (and bi-variate) and multivariate analysis. These two approaches differ principally in the number of variables or frequencies used for quantitative analysis.

Uni- and bi-variate analysis Uni- or bi-variate analysis uses one or two variables to extract information from a system (e.g. peak area, peak height or the ratio of two peak heights). Such analysis has been successfully and extensively used in the pharmaceutical setting. Its advantages include its intuitive sense and ease of calculation. However, this approach can be difficult to use when many or all of the peaks overlap, and the approach is vulnerable to undetected interferents. A popular method to overcome these problems is multivariate analysis.

Multivariate analysis Multivariate analysis differs from univariate analysis in that it uses many (up to thousands) of variables to obtain quantitative data. It offers several advantages over univariate analysis. Very small, but significant, spectral changes may yield excellent quantitative analyses so there is no difficulty with overlapping peaks. Several components in a sample can be analysed simultaneously, the precision is improved through multiple correlated measurements and interferents may be detected during component quantification in new samples. In addition, if interferents have been incorporated into a model, prediction of components may be possible even in the presence of interferents (Beebe et al 1998). The main disadvantage of multivariate analysis is that it is complex and not as intuitive as univariate analysis, and therefore misinterpretation of the data is more likely.

The most commonly used multivariate methods in pharmaceutical spectroscopic analysis can broadly be divided into two categories: classical least squares analysis (CLS) and inverse least squares analysis (ILS). Since these methods are so commonly used in quantitative analysis, their theory will be briefly introduced. Beebe et al (1998) provided a decision tree to assist with selecting an appropriate method based on CLS and ILS, and an adaptation is presented in Figure 1. Other multivariate analysis methods (e.g., neural networks analysis) have been much less used and therefore will not be further discussed in this section.

The CLS method is analogous to the Beer-Lambert law, and can be defined as:

$$X = yS \quad (2)$$

where X is the matrix of responses (spectra) for the samples, y is a vector of the analyte concentrations and S is a matrix of the sensitivities for each analyte at the different wavenumbers. Direct CLS uses pure components for calibration, whereas indirect CLS uses mixtures (with all components and concentrations known). The technique is straightforward and comparatively few samples are required to create a quantitative model. However for a CLS model all the analytes and their concentrations must be known and there should be no

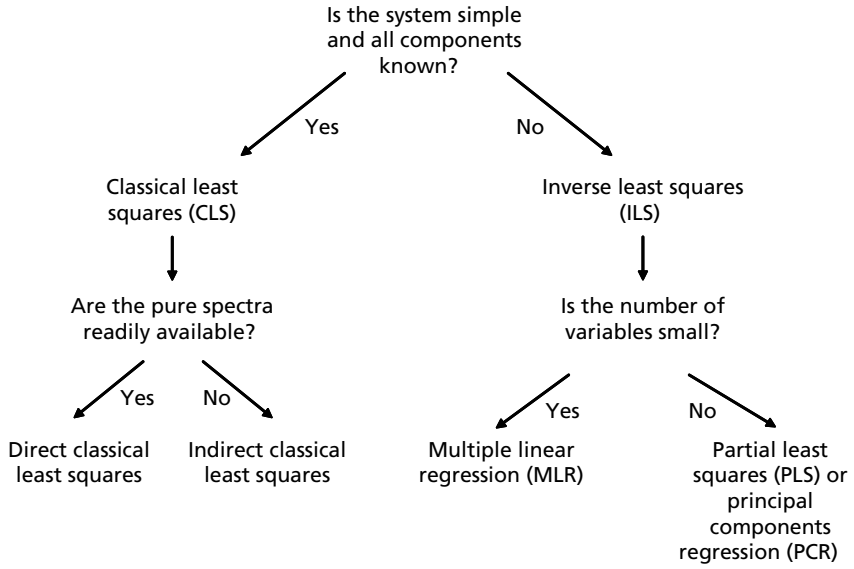


Figure 1 Decision tree to assist choice of appropriate multivariate analysis method, adapted from Beebe et al (1998).

interferents in either the calibration or the test samples (Beebe et al 1998).

ILS uses weighted amounts of each variable (wavenumber) to calculate the analyte concentration:

$$Y = Xb \tag{3}$$

where b is a vector denoting the regression coefficients (multiple regression). The coefficients render some variables more important than others in determining the analyte concentration. To set up a calibration model, samples with known concentrations, y, are measured using spectroscopy to obtain X. To correlate the spectral measurements to the analyte concentration, the regression coefficients, b, are estimated (Beebe et al 1998).

The three most common ILS methods used to estimate b are multiple linear regression (MLR), principal components regression (PCR) and partial least squares (PLS). MLR uses spectral intensities at a few selected wavenumbers. For MLR, the number of wavenumbers chosen must be less than the number of samples measured, thus restricting the ability to gain useful spectral information. In addition, if the wavenumbers chosen are correlated (i.e., they represent some of the same information), concentration estimation in unknown samples becomes unreliable.

PCR and PLS allow most of the meaningful spectral variation across the whole spectrum to be captured while avoiding correlated data and reducing the influence of noise. In these methods, the spectral data in X is first decomposed to obtain new dimensions that represent combinations of many wavenumbers. Most of the spectral variation is represented in few dimensions that are then used to estimate b. At the same time spectral noise that is represented in other dimensions is discarded. PCR and PLS differ in the way the new dimensions are determined: in PCR only the maximum spectral variation is considered, whereas in PLS the covariance between the spectral data and species concentration is maximised (Beebe et al 1998).

An advantage of using ILS over CLS is that not all components and their concentrations must be known to build a quantitative model. As long as the variation due to redundant variables has been present during model construction, then variation in these redundant components will not interfere in the prediction of the components of interest in future samples. This is especially useful for complex mixtures, such as those in drug formulations. Disadvantages of ILS include the more complex calculations and the requirement for more samples to make a reliable model.

Sources of error

Like with any method, quantitative analysis with Raman spectroscopy requires the quality of quantitative models to be assessed. The gold standard and industrial requirement in the pharmaceutical setting is a formal validation, the guidelines of which have been developed at the International Conference on Harmonisation (ICH Expert Working Group 1994).

In Raman spectroscopy, sources of error can be separated into sample, spectrometer and operator effects. A detailed description of these is provided elsewhere (Pelletier 2003). Some of the causes of error in solid-state quantitative Raman spectroscopy, mainly sample effects, have been analysed in pharmaceutical applications. In a quantitative crystallinity study, Taylor & Zografi (1998) investigated the effects of laser power, instrument variability, sample homogeneity and overall method error on the quantitative analysis. Laser power and instrument variability were small sources of error; the largest contributor to error was mixture subsampling. This was due to the small laser spot size (and hence sampling volume). In a similar but more extensive study, Campbell Roberts et al (2002) investigated instrument and day-to-day reproducibility, intraday reproducibility, sample mixing, sample positioning, laser power and particle size on quantitative model construction. Again, the largest source of error was sample inhomogeneity and small sampling area, which was minimised in this study by reducing and controlling the particle size range.

Langkilde et al (1997) described a novel sample setup where a powdered sample was placed in a nuclear magnetic resonance spectroscopy tube, which was rotated and moved up and down. This set-up led to a much larger sampling volume, and hence reduced errors due to inhomogeneous mixtures. Rantanen et al (2005) also found that sampling volume strongly affected quantitative analysis of mixtures; a larger spot size and dynamic mixing decreased sample inhomogeneity-related errors and improved the models.

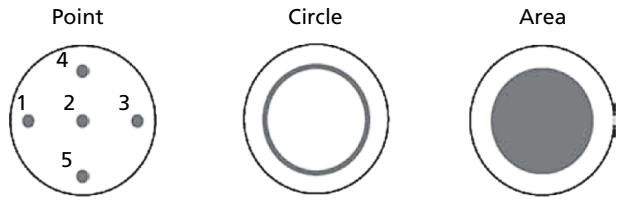
Johansson et al (2005) investigated the effect of different irradiance patterns on quantitative measurements of intact immediate release tablets. Various methods of spectral normalisation and a variety of univariate and PLS analysis were performed. Point irradiation resulted in the largest errors, followed in order by small circle, large circle and area irradiation (Figure 2). PLS analysis resulted in smaller root mean squared error of prediction (RMSEP) than univariate analysis. The authors also investigated the effect of tablet compression on concentration measurements, and found that with appropriate spectral pre-processing, measurements were insensitive to tablet compression forces between 5 and 20 kN (Johansson et al 2005).

In a study that avoided sample rotation, Bell et al (2004) proposed a grid irradiation approach, where the effect of spot size and number on the standard deviation of measurements of powder mixtures was investigated. It was shown that a Raman microscope (spot size 3 μm) led to much larger variation than a macro-Raman set-up with a probe (spot sizes of 75 and 150 μm) for the same number of sampling spots (particle size 40 μm). The authors concluded that to reduce the standard deviation of the measurements, a spot size larger than the particle size should be used for bulk quantitative measurements and sufficient sampling spots used. The authors also pointed out that Raman microscopy, while very good for defining local variations in crystallinity, is not suited to quantifying bulk components in mixtures, even when a large number of sampling spots is used.

Sources of error that have been less published in the pharmaceutical literature include fluorescence, absorption, and diffuse reflectance. Fluorescence generally looks like a gently curving spectral baseline that partially or completely masks the Raman response (Pelletier 2003). As previously mentioned, this is very common with visible excitation, but can usually be circumvented by using near-IR lasers. However, some molecules with very large π-electron systems may still emit, even with near-IR excitation, probably as a consequence of sample heating. The systems that display these problems are often very highly coloured. In this case, Raman analysis is usually not possible.

Absorption of the laser light and Raman scattered light, known as self absorption, may occur. With near-IR excitation this is due to overtone and combination vibrations (Schrader et al 1991) and attenuates some spectral peaks more than others. Factors that affect self absorption include sample concentration, sample thickness and sample distance from the laser focus (Everall 1994). Thus, if specific peaks that are attenuated due to self absorption are selected for quantitative analysis, a nonlinear response will be observed as a function of concentration. Approaches to overcome this problem include selection of bands that are not affected and multivariate analysis methods. Furthermore, sampling parameters should be kept as constant as possible. Unfortunately, there is minimal, if any, literature on self absorption in pharmaceutical analysis.

The effects of laser light diffuse reflectance by solids are widespread, with large influences for quantitative solid-state pharmaceutical analysis. According to Kubelka-Munk theory, diffuse reflectance by particles (much larger than the wavelength of the light) increases as particle size decreases. Therefore, overall Raman scattering decreases because the sample volume irradiated decreases (D'orazio & Schrader 1976; Schrader et al 1991). However, in contrast to this theory and early work, more recent studies observed increased peak intensities of powdered inorganic salts as particle size decreased (Pellow-Jarman et al 1996; Wang et al 2002). This discrepancy is probably due to sampling geometry effects. The Kubelka-Munk model assumes confocal excitation and collection, although this is not the case in many Raman set-ups, including those with probes. In these situations, increased diffuse reflectance improves the overlap between the excitation and collection beams, overriding the decrease in sampling volume, thus leading to an overall increase in the detected Raman response (Wang et al 2002). Wang et al (2002) also found that sample thickness, the surrounding medium (e.g., air or water) and sample compression also affected the spectra through diffuse reflectance and sampling geometry.



	Point	Circle	Area
Irradiated area (mm^2):	0.2	3.9 (small) 7.9 (large)	19.6
% of tablet front surface:	0.4	8 (small) 16 (large)	39

Figure 2 Different laser irradiance patterns for Raman assessment of tablets (Johansson et al 2005; reprinted with permission).

As with self absorption, diffuse reflectance sources of error should be minimised by using consistent sample preparation and sampling geometry. Despite the importance of self absorbance and diffuse reflectance for quantitative analysis, the literature of these effects on pharmaceutical analysis is minimal and should receive more investigation.

In addition to previously mentioned sample-related effects, other sources of error include laser intensity variation, sample alignment changes and, possibly, build up of the sample on the Raman–sample interface. In quantitative studies, this should always be considered and overcome as much as possible by normalising the spectra. There are many possible normalisation methods and an excellent overview of these has been provided by Pelletier (2003). In the papers reviewed in this article, the following normalisation methods have been used successfully: internal standard, peak ratios, baseline correction, background subtraction, mean centering, vector normalisation, multiplicative scatter correction, differentiation, standard normal variate (SNV) transformation and orthogonal signal correction.

Applications

Quantitative Raman spectroscopy has been used in diverse applications in solid-state pharmaceutical analysis. These can be divided into chemical and physical analysis applications, and the following section is divided accordingly. Additional information about the applications reviewed in this paper is provided in Table 1. Although a few quantitative surface-enhanced Raman spectroscopy (SERS) applications have been documented (Pal et al 1998; Wang et al 2003), they have not been included because the samples are dissolved before analysis.

Chemical analysis

One of the earliest reported quantitative applications of Raman spectroscopy for pharmaceutical analysis was the simultaneous analysis of phenylpropanolamine hydrochloride and paracetamol (acetaminophen) (King et al 1985). The set-up involved a monochromator, with step sizes of 1 cm^{-1} and one-second integration time. This would have resulted in considerable data collection times. Nevertheless, quantification from the powder mixtures was performed both in the solid state and in solution. Quantification first involved a univariate approach with peak intensity, and subsequently multivariate analysis using cross correlation and least squares approaches. The best results were obtained with three component cross correlation, though errors for all methods were typically less than 1% and, interestingly, the results from the solid state were better than those from solution. The authors concluded that the Raman method "has great potential for simultaneous multicomponent determinations on solid state samples with minimal sample pre-treatment . . . Thus Raman spectroscopy should receive greater attention in pharmaceutical analysis, for both qualitative and quantitative measurements". It was several years before this advice was followed.

In 1988, after FT-Raman spectroscopy was commercialised, Lewis et al (1988) quantified powdered polyene antifungal medicines. Overlapping bands were resolved using Lorentzian fitting, and peak areas were normalised against instrument response to quantify impurities in commercial samples of the drugs. The drugs fluoresce when irradiated with UV or visible radiation; however, this problem was avoided using the near-IR radiation associated with FT-Raman spectroscopy (Lewis et al 1988).

Quantitative analysis of active pharmaceutical ingredients using Raman spectroscopy has been widely published from the 1990s. Davies et al (1990) quantified diclofenac sodium in sodium alginate matrices in tablet form using FT-Raman spectroscopy (Figure 3). Using the areas of two peaks specific to the drug, a linear relationship was obtained between 5 and 60% drug in the matrix. The authors point out that many APIs contain aromatic groups and are therefore strong Raman scatterers (due to the large polarisability of such groups). In contrast, many excipients, including polymers such as sodium alginate, are not aromatic and heavily hydroxylated, which makes them weak Raman scatterers (Figure 3). This makes Raman spectroscopy particularly sensitive to APIs in solid-state formulations. Other studies on formulations based on univariate analysis include the quantification of calcium carbonate and glycine in antacid tablets using peak intensity ratios, and dipyrone in tablets using FT spectroscopy and peak intensity and area (Kontoyannis 1995; Izolani et al 2003).

In 1999, Ryder et al published a forensic application of Raman spectroscopy where cocaine was quantified in binary mixtures with glucose using PLS (Ryder et al 1999). In a follow-up study (Ryder et al 2000), cocaine was quantified in ternary mixtures containing caffeine and glucose, as typical diluents. Cocaine was quantified with a RMSEP of 4.1%. However, the authors acknowledged that illicit drug products contain enormously varying diluents; for a robust workable model based on ILS, all these excipients would need to be incorporated into a quantitative model. In a regulated pharmaceutical manufacturing environment, all excipients are known and controlled, making quantitative model construction and use more straightforward.

Many examples exist where the API has been quantified in tablets. Skoulika & Georgiou (2001) used univariate (peak intensity and area) and multiple linear regression to quantify ciprofloxacin in tablets. They showed that Raman spectroscopy could be used as a much faster and cheaper alternative to the HPLC method described in the United States Pharmacopeia. In a thorough study, Szostak & Mazurek (2002) quantified acetylsalicylic acid and paracetamol in powder and tablet form using the peak intensity, PCR and PLS. Quantification of the active ingredients was performed on various formulations including commercial tablets. The best results were obtained using PLS analysis, with the approach proving to be a rapid and reliable method for quantifying acetylsalicylic acid and paracetamol in commercial tablets. Raman spectroscopy has also been shown to be suitable for quantifying components through packaging in finished products. Skoulika & Georgiou (2003) quantified aciclovir in tablets through poly(vinyl chloride) blister packaging. The results compared well with those performed by pharmacopoeial methods and demonstrate the possibility of using Raman spectroscopy for quantitative analysis on pharmaceutical production lines, including on completed products. The study also shows the potential for long-term storage studies on the same samples

Table 1 Applications and methods of quantitative analysis in solid-state pharmaceutics using Raman spectroscopy

Application	Formulation	Set-up	Approach	Method	Normalisation	Comments	Reference
Chemical analysis							
Phenylpropanolamine HCl and paracetamol	Powder	514.5 nm, monochromator	Univariate and multivariate	PI and cross correlation	Internal standard		King et al (1985)
Impurities in polyene antibiotics	Powder	FT	Univariate	Lorentzian curve fitting and PA	Instrument response function		Lewis et al (1988)
Diclofenac in alginate matrix	Tablets	FT	Bivariate	PA	None		Davies et al (1990)
Sulfasalazine in polymeric microspheres	Powder	FT	Univariate	PA	Ratio (and logarithmic function)		Watts et al (1991)
Calcium carbonate and glycine antacid tablets	Tablets	488 nm, monochromator	Univariate	PI	Ratio		Kontoyannis (1995)
Bucindolol	Gel capsules	785 nm, CCD, probe	Multivariate	PLS	Numerous (linear baseline correction, 2nd derivative, MSC)	Samples emptied from capsule before measurement; Savitsky-Golay smoothing improved model	Niemczyk et al (1998a)
Bucindolol	Gel capsules	785 nm, CCD, probe	Multivariate	PLS	Linear baseline correction	Measurements on intact gel capsules and intact capsules through blister packaging	Niemczyk et al (1998b)
Radiosensitiser, 5-iodo-2′-deoxyuridine polymeric microspheres	Powder	FT	Univariate	Region areas	Ratio		Geze et al (1999)
Cocaine and glucose	Powder	785 nm, CCD	Multivariate	PLS	Mean centering and MSC		Ryder et al (1999)
Cocaine, caffeine and glucose	Powder	785 nm, CCD	Multivariate	PLS	MSC		Ryder et al (2000)
Ciprofloxacin	Tablets (ground before measurement)	FT	Univariate and multivariate	PI, PA and MLR	Baseline correction with/without internal standard		Skoulika & Giorgiou (2001)
Vitamin C	Powder	FT	Multivariate	PLS	MSC	Comparison with NIR and mid-IR spectroscopy	Yang & Irudayaraj (2002)
Diltiazem hydrochloride	Tablets	FT	Univariate	PA	Baseline correction and vector normalisation	Spot size identified as limiting factor	Vergote et al (2002)
Acetylsalicylic acid and paracetamol	Powder and tablets	FT	Univariate and multivariate	PI, PCR and PLS	Ratio or mean centering with/without added internal standard		Szostak & Mazurek (2002)
Dipyrone	Tablets	FT	Univariate	PI and PA	None		Izolani et al (2003)

Corn starch and lactose monohydrate	Powder and tablets	532 nm, CCD, microscope coupled with probe	Univariate	PI	Baseline correction and ratio	Probe coupled with Raman microscope for bulk measurements	Szép et al (2003)
Aciclovir	Tablets (in blister packaging)	FT	Univariate and multivariate	PI, PA and MLR	Baseline correction		Skoulika & Giorgiou (2003)
Ambroxol	Tablets	FT	Multivariate	PLS	Mean centering with/without added internal standard		Szostak & Mazurek (2004)
N-methyl-3,4-methylenedioxy-amphetamine (ecstasy)	Tablets	785 nm, CCD, probe	Multivariate	PLS	Internal standard (sorbitol, already in sample), mean centering and 1st derivative	Effect of spot size and number on sampling errors	Bell et al (2004)
Medroxyprogesterone acetate	Suspension	FT	Univariate	PI	Vector normalisation		De Beer et al (2004)
Active pharmaceutical ingredient tablet	Immediate-release	FT	Univariate and multivariate	PI, PA and PLS	Numerous for univariate and multivariate	Effect of sampling area and tablet density	Johansson et al (2005)
Polymorphism							
Chlorpropamide	Powder	FT	Multivariate	PCR	Normalised for sample weight and black body radiation, method not specified		Tudor et al (1993)
API (not stated) forms A and B	Powder	FT	Univariate	Intensity	Ratio	Rotating sample decreased variability. FTIR, DSC and XRPD also used	Langkilde et al (1997)
Ranitidine forms I and II	Powder and tablets (ground)	459.7 nm, CCD	Univariate and multivariate	PI, PA and PCR	None	PI and PA calibration curves nonlinear	Pratiwi et al (2002)
Paracetamol forms I and II	Powder	FT	Univariate	Peak fitting (deconvolution) together with PA	Ratio	Comparison with FTIR spectroscopy	Al-Zoubi et al (2002)
Mannitol forms β and δ	Powder	FT	Univariate	PI	Ratio	Effect of instrument, intra -and inter-day variability, mixing, packing, sample positioning, laser power and particle size on error reported	Campbell Roberts et al (2002)
Carbamazepine forms I and III	Powder	FT	Multivariate	PCR	None and baseline correction		Strachan et al (2004)

CCD, charge coupled device; FT, Fourier transform; MSC, multiplicative scatter correction; MLR, multiple linear regression; MIR, mid-infrared; NIR, near-infrared; PA, peak area; PI, peak intensity; PCR, principal components regression; PLS, partial least squares.

(cont)

Table 1 Applications and methods of quantitative analysis in solid-state pharmaceutics using Raman spectroscopy. *(cont)*

Application	Formulation	Set-up	Approach	Method	Normalisation	Comments	Reference
Benzimidazole forms A, B and C	Powder premix and suspension-emulsion	FT	Multivariate	Stepwise MLR	Vector normalisation	Drug 5% (m/m) in formulations	De Spiegeleer et al (2005)
Mannitol forms I and III	Powder and tablets	FT	Multivariate	PLS-1, cross validation	Mean centering and MSC	Powder mixtures used for tablet quantification	Auer et al (2003)
L-Glutamic acid forms α and β	Powder and suspension	785 nm, CCD	Univariate	PI and PA	Ratio	Kinetics and optimisation of polymorph formation during batch crystallisation	Ono et al (2004)
Compound A forms α and β	Powder	FT	Multivariate	PLS	2nd derivative	From abstract and figures only (in Japanese)	Ueno et al (2005)
Amorphous form							
Indometacin amorphous and γ-form	Powder	FT	Univariate	PI	Ratio	Sources of error Investigated	Taylor & Zografi (1998)
Compound A free amorphous base and crystalline hydrochloride	Granules and tablets	FT	Univariate	PI	Ratio	Effects of moisture during processing and storage on drug form investigated	Williams et al (2004)
Indometacin amorphous and γ-form	Powder and tablets	FT	Univariate	PI	Ratio	Effects of excipients on crystallisation of indometacin in tablets	Schmidt et al (2003)
Indometacin amorphous and γ-form	Powder and tablets	FT	Univariate	PI	Ratio	Effects of excipients, including polyethylene oxides, on crystallisation of indometacin in tablets	Schmidt et al (2004)
Compound B amorphous and form I	Powder	FT	Multivariate	PLS	1st derivative	From abstract and figures only (in Japanese)	Ueno et al (2005)
Mannitol amorphous and α, β, and γ forms	Powder	670 nm, CCD	Multivariate	Deconvolution and CLS	Background subtraction, internal standard (already within sample)	Spray dried inhalable powder with calcitonin. Instrumental sources of error investigated.	Vehring (2005)
Indometacin	Powder and tablets	FT	Multivariate	PLS	MSC and 2nd derivative	Compared with XRPD, alone and in mannitol; compression effects investigated	Okumura & Otsuka (2005)

Solvates							
Theophylline monohydrate and anhydrate	Powder and tablets	FT	Univariate	PI	Ratio	Effects of excipients on dehydration of theophylline monohydrate in tablets	Schmidt et al (2003)
Theophylline monohydrate and anhydrate	Powder and tablets	FT	Univariate	PI	Ratio	Effects of excipients including polyethylene oxides on dehydration of theophylline monohydrate in tablets	Schmidt et al (2004)
Theophylline	Powder and granules	784.8 nm, CCD	Univariate	PI	Ratio	In-line monitoring of hydrate formation during wet granulation with excipients	Wikström et al (2005)
Nitrofurantoin, theophylline, caffeine, and carbamazepine anhydrate and hydrate	Powder	784.8 nm, CCD	Univariate and multivariate	PI and PLS	Numerous for univariate and multivariate	Hydrates and anhydrous forms quantified	Rantanen et al (2005)
Carbamazepine forms I, II and III and dihydrate	Suspension	FT	Multivariate	PLS	Mean centering, MSC with/without 1st derivative	Kinetics of conversion of forms I, II and III to dihydrate studied with binary and ternary mixtures. Particle morphology large influence on conversion.	Tian et al (2006)
Peptides and proteins							
Monoclonal antibody	Powder (and solution)	FT	Univariate	Not stated (referenced)	Not stated (referenced)	Effects of lyophilisation and spray drying on secondary structure investigated	Sane et al (2004)

CCD, charge coupled device; FT, Fourier transform; MSC, multiplicative scatter correction; MLR, multiple linear regression; MIR, mid-infrared; NIR, near-infrared; PA, peak area; PI, peak intensity; PCR, principal components regression; PLS, partial least squares.

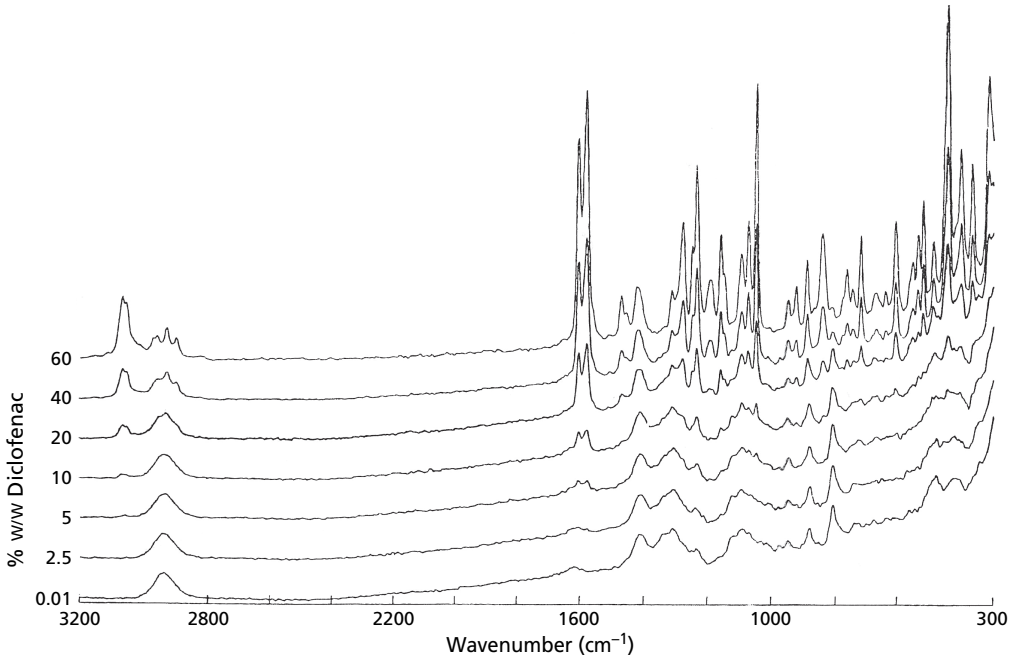


Figure 3 FT-Raman spectra of diclofenac (0.01–60% (w/w)) in sodium alginate (Davies et al 1990; reprinted with permission).

without disturbing the sample environment. Other studies where APIs have been quantified in tablets include those on diltiazem hydrochloride (Vergote et al 2002) and ambroxol (Szostak & Mazurek 2004).

An active ingredient, bucindolol, has been quantified in a gel capsule formulation using PLS. In a first publication, the contents of the capsule were removed and quantified using Raman spectroscopy and the results were compared with IR spectroscopy. With appropriate spectral pre-treatment, both methods resulted in a standard error of prediction (SEP) of below 2%. Raman spectroscopy, however, involved less sample preparation and therefore spectroscopic data collection was faster, making it the preferred technique (Niemczyk et al 1998a). In a subsequent study (Niemczyk et al 1998b), the same group quantified bucindolol in intact gel capsules with a probe set-up, and the SEP only increased to 2.37%. The capsule contents were also quantified using the intact gel capsule though blister packaging, with the SEP increasing to 3.22%.

Raman spectroscopy has also been used to quantify APIs in microspheres. Watts et al (1991) used FT spectroscopy to quantify the highly Raman-active sulfasalazine in the less-active Eudragit RS microspheres. The ratio of two peaks, one due to each of the compounds, was used to calculate drug concentration and encapsulation efficiency. Geze et al (1999) used spectral region ratios to quantify the content of a radiosensitiser, 5-iodo-2′-deoxyuridine, in microspheres with a precision of 1%. Although the calibration curve obtained was only quasilinear and the reason for this was not investigated, the model was used to show that sterilisation of the microspheres using γ-irradiation did not lead to drug degradation.

Since water is a very weak Raman scatterer, Raman spectroscopy is ideal for investigating solid-state systems in an aqueous environment. The technique has been used to quantify solid-state components in suspension. De Beer et al (2004) quantified medroxyprogesterone in suspension with no sample preparation. Using the normalised intensity of a peak unique to medroxyprogesterone, a linear calibration curve was obtained and validated. Sample uniformity was ensured by continual stirring. Commercial samples were tested using the constructed calibration model, and the measured and stated concentrations were in good agreement. Furthermore, the same samples were used to construct and test a calibration model using HPLC (European Pharmacopoeia IV). The calibration curve from the HPLC method contained a systematic offset, presumably due to the undefined sample density. This study shows that Raman spectroscopy is better than HPLC for quantitative chemical analysis of suspensions because the calibration model is more reliable, there is no sample preparation, the technique is non-destructive and measurement times are shorter (De Beer et al 2004). The potential of Raman spectroscopy to quantify chemical components quickly and reliably in suspension clearly should be further explored.

Solid-state form analysis

Vibrational modes are sensitive to changes in solid-state structure. Conformation and intermolecular bonding differences may alter Raman scattering frequency and intensity. These changes can be used to quantify different solid-state forms of the same chemical entity.

Polymorphism Tudor et al (1993) used near-IR FT-Raman spectroscopy to investigate and quantify mixtures of two chlorpropamide polymorphs in powder form. Not only was this study the first where Raman spectroscopy was used to

quantify solid-state forms, but ILS was used (the first example for chemical or physical analysis). Using PCR and two spectral regions where spectral differences between polymorphs A and B were evident (but the peaks still overlapped), a linear calibration curve was obtained. Instrument and sample reproducibility were investigated; sample variability affected the model more than instrument variability.

Langkilde et al (1997) quantified powder mixtures of two polymorphic forms of an unspecified API using the peak intensity ratio of two resolved peaks, one specific to each form. A linear calibration curve was obtained over the entire concentration range (1.8–15.4% (w/w) form A in B). Quantification by FTIR spectroscopy, differential scanning calorimetry (DSC) and X-ray powder diffraction (XRPD) was also discussed. FT-Raman spectroscopy was considered the method of choice. Spectral features are broader and overlap more in FTIR spectra (a difficulty with univariate calibration) and some sample preparation is necessary, including ensuring small particle size. In this case conventional DSC was complicated by concomitant transition to form B during melting of form A. Thus the enthalpy of melting of form A could not be determined unequivocally (Langkilde et al 1997).

FT-Raman and FTIR spectroscopy were also compared when Al-Zoubi et al (2002) quantified powder mixtures of the orthorhombic and monoclinic forms of paracetamol. Using intensity and area ratios, both techniques yielded linear calibration curves with a limit of detection (LOD) of 1.2% (mol/mol). Campbell Roberts et al (2002) quantified the α and δ forms of mannitol using peak intensity ratios, and quantified as low as 2% of the β form. Pratiwi et al (2002) quantified ranitidine hydrochloride polymorphic forms I and II in both powders and tablets. In this study, quantitative attempts using univariate analysis led to a nonlinear calibration curve because of overlapping peaks of the two forms. With PCR, however, the colinearity was overcome, and an LOD for both binary mixtures and tablets as low as 0.6% was obtained. This is lower than the LOD of 1.5% that had previously been obtained using diffuse reflectance FTIR spectroscopy and XRPD combined with artificial neural networks analysis (Agatonovic-Kustrin et al 2001). Multivariate analysis has also been used to quantify powder mixtures of carbamazepine forms I and III (using PCR) (Strachan et al 2004) and mannitol polymorphs I and III (using PLS) (Auer et al 2003).

The solid-state form of compounds has been studied in suspension. De Spiegeleer et al (2005) quantified three crystal forms of benzimidazole in premixes containing 5% of the drug, and a limit of quantification (LOQ) of 5–10% for each polymorph was estimated. Using the same quantitative approach, it was found that no polymorph transformation had occurred in constituted suspension-emulsions after twelve months at 25°C; the samples contained 100% form B (De Spiegeleer et al 2005). Ono et al (2004) quantified the polymorphic composition of L-glutamic acid during batch-cooling crystallisation in-situ by Raman spectroscopy. A calibration curve of α- and β-form mixtures in dry powder was constructed by taking the ratio of peak heights due to each form. The model was also found to apply to the suspension samples. At 25°C in aqueous solution, the α form dominated. However, a subsequent solvent-mediated transformation to the β form occurred, especially at higher temperatures. The in-situ quantification could be used to optimise crystallisation kinetics and polymorphic purity (Ono et al 2004).

Amorphous form Taylor & Zografi (1998) quantified the crystallinity of the much-studied indometacin using FT-Raman spectroscopy. Binary mixtures of the γ-crystalline and amorphous forms were quantified using peak height ratios. A linear correlation curve between 0 and 100% crystallinity and LOD of 1% for either the amorphous or crystal form were obtained (Taylor & Zografi 1998). Indometacin crystallinity was also the subject of a study by Okumura & Otsuka (2005). Using PLS, pure indometacin in binary mixtures of the γ-crystalline and amorphous forms, as well as the same binary mixtures in a model tablet containing 10% (w/w) indometacin in mannitol, were investigated. In the tablet formulation, an impressive 2% crystallinity (0.2% total mass of tablet) was detected. The authors also investigated the pressure-induced amorphous form formation from the γ-form, and found that increased pressures decreased the crystallinity, especially at the edge of the tablet (81% crystalline after 196 MPa compression). Schmidt et al (2003) used FT-Raman spectroscopy to quantify the crystallisation of indometacin during storage after compression into tablets with various excipients. Using a peak intensity ratio, it was shown that excipients with a high elasticity on compression could partially retard the recrystallisation of amorphous indometacin after compression (Schmidt et al 2003). In a follow-up study (Schmidt et al 2004), the same authors revealed that polyethylene oxides could dramatically reduce recrystallisation of compressed indometacin, which was attributed to a chemical interaction. Other studies have investigated the effect of processing conditions on moisture induced crystallinity (Williams et al 2004) and quantification of amorphous mannitol in the presence of multiple polymorphic forms (Vehring 2005).

Solvates Hydrates, the most common solvate in pharmaceutics, have been subject to several quantitative analyses. Hydrate/anhydrate mixtures of nitrofurantoin, theophylline, caffeine and carbamazepine have been quantified by Raman and near-IR (NIR) spectroscopy (Rantanen et al 2005). Raman spectroscopy, univariate (PI) and multivariate (PLS) analysis revealed that either approach could result in low detection limits. In addition, several spectral pre-processing methods were investigated with the PLS analysis, and SNV transformation generally resulted in the best models. Wikström et al (2005) interfaced Raman spectroscopy with a high-shear wet granulation process to monitor solvent-mediated transformation from anhydrous theophylline to the monohydrate form. The effect of several pre-processing conditions on conversion was investigated; mixing speed was found to have the greatest effect. It was not possible to monitor the process using NIR spectroscopy because the water masked useful spectral information. This study shows the potential of using Raman spectroscopy to monitor and quantify processes in-line where water is present, which hinders the use of many other spectroscopic approaches (Wikström et al 2005). In a study by Schmidt et al (2003), the influence of excipients

on the dehydration of theophylline monohydrate during tabletting was quantified.

Tian et al (2006) used FT-Raman spectroscopy to investigate the kinetics of transformation of different polymorphic forms of carbamazepine to the dihydrate form in suspension. Binary models of forms I, II or III with the dihydrate were used to construct calibration curves using PLS (an assumption was made that each form converts directly to the dihydrate). Using a ternary mixture with forms I, III and the dihydrate, no influence of one form on the conversion of the other form was found. Each form was found to convert to the dihydrate by first-order kinetics. However, particle morphology affected conversion kinetics more than polymorphic form, with rods converting to the dihydrate faster than prisms (Tian et al 2006).

Other applications Raman spectroscopy has been used to quantify secondary protein structure in solution and solid state. In an impressive application, the secondary structure changes of a therapeutic monoclonal antibody (rhuMAb) during freeze- and spray-drying have been quantified using FT-Raman spectroscopy. The amide I band region was used to estimate the proportions of different secondary structure types of the antibody. The secondary structure types, α-helix, unordered helix plus sheet, turn and unordered sheet, have distinct amide I band regions. Reference spectra for pure secondary structure types were calculated based on the Raman spectra of well-characterised crystalline proteins. The proportions of different secondary structure types in the antibodies were calculated as linear combinations of the pure reference spectra. The authors showed that in the absence of sugar, large changes in the antibody secondary structure were observed during lyophilisation and spray-drying. However, with increasing sucrose or trehalose content as structural stabilisers during drying, the structural perturbation was decreased and at sufficient sugar content concentrations structural changes were minimal. The authors also used Raman spectroscopy to demonstrate that increased structural changes during drying were correlated with increased levels of protein aggregation during long-term storage (Sane et al 2004).

Quantitative analysis of macromolecules, including proteins, remains relatively untouched by Raman spectroscopists. Given the increasing importance of proteins as active pharmaceutical ingredients, quantitative Raman spectroscopy of proteins both in the solid state and otherwise should be the subject of much more research.

Conclusions

It is clear from this literature review that in the last two decades Raman spectroscopy has become a popular method to quantify pharmaceutical materials in the solid state. Modern Raman spectroscopy has many strengths and advantages over other techniques, both spectroscopic and otherwise. It is rapid, requires no sample preparation and is non-destructive. The technique can be used to measure systems in aqueous environments and can be interfaced with a probe. Quantification problems due to poor peak resolution in spectra of mixtures have been overcome by using various peak ratio methods or, increasingly, multivariate analysis.

Raman spectroscopy has been used for chemical and physical quantification. Chemical applications have mainly involved quantifying APIs, while physical characterisation has covered polymorphism, hydrate formation and crystallinity. Initially, simple binary powder mixtures were quantified but this has since extended to complex formulations, including tablets, capsules, microspheres and suspensions. Sample preparation has varied from grinding the sample and sieving to recent publications of measurements of intact formulations through packaging materials. In an exciting development, Raman spectroscopy has recently been used to quantify structural changes in proteins. The technique is ideal for real-time quantification of pharmaceutical processes, and has been used to monitor processes such as wet granulation and batch crystallisation.

These advantages, combined with increased emphasis on process monitoring, will inevitably mean that quantitative analysis in the solid state by Raman spectroscopy will increase. It is hoped that not only will the use of quantitative Raman spectroscopy grow in the areas reported in this paper, but also that new applications will be investigated.

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