Rapid non-invasive quantitative assessment of pharmaceutical capsules using transmission Raman spectroscopy

C. Eliasson, N. A. Macleod and P. Matousek
Central Laser Facility, STFC, Rutherford Appleton Laboratory, HSIC, Didcot, Oxon OX11 0QX, UK

L. C. Jayes, F. C. Clarke, S. V. Hammond and M. R. Smith.
Pfizer, Process Analytical Support Group (PASG), Pfizer Ltd, Ramsgate Road, Sandwich, Kent CT13 9NJ, UK

Contact | pavel.matousek@stfc.ac.uk

Introduction

In a number of analytical pharmaceutical applications there is a need for techniques capable of probing capsules or coated tablets *non-invasively* in order to quantitatively monitor the chemical composition in terms of both active pharmaceutical ingredients (API) and other constituents or impurities. For example, in quality control, information is needed rapidly and non-destructively to quantify the presence (or absence) of polymorphs or undesired salt forms of the active ingredient, and unwanted contaminants (including unreacted chemicals from the production and purification stages).

The need to obtain such information rapidly and non-invasively is dictated by the practical requirements of applications such as quality monitoring on production lines or when quantifying product shelf lifetime. Raman spectroscopy is of particular potential in this area due to its high chemical specificity, speed and compatibility with water containing samples. However its use has been limited to cases where the Raman signal and fluorescence emanating from the capsule shell does not interfere strongly with the Raman signal of the material held within the capsule. This problem stems from the backscattering geometry in which the vast majority of commercial systems operate.

This work builds on recent developments in the area of deep non-invasive probing of diffusely scattering samples using Raman techniques[1]. These techniques stem from a foundation provided via research access to the LSF's Ultrafast Spectroscopy Laboratory. In particular, this activity has revealed[2,3] that *transmission Raman* spectroscopy is particularly effective in probing the *bulk* content of non-absorbing or weakly absorbing pharmaceutical and biological samples at depths well beyond the reach of conventional approaches. This is

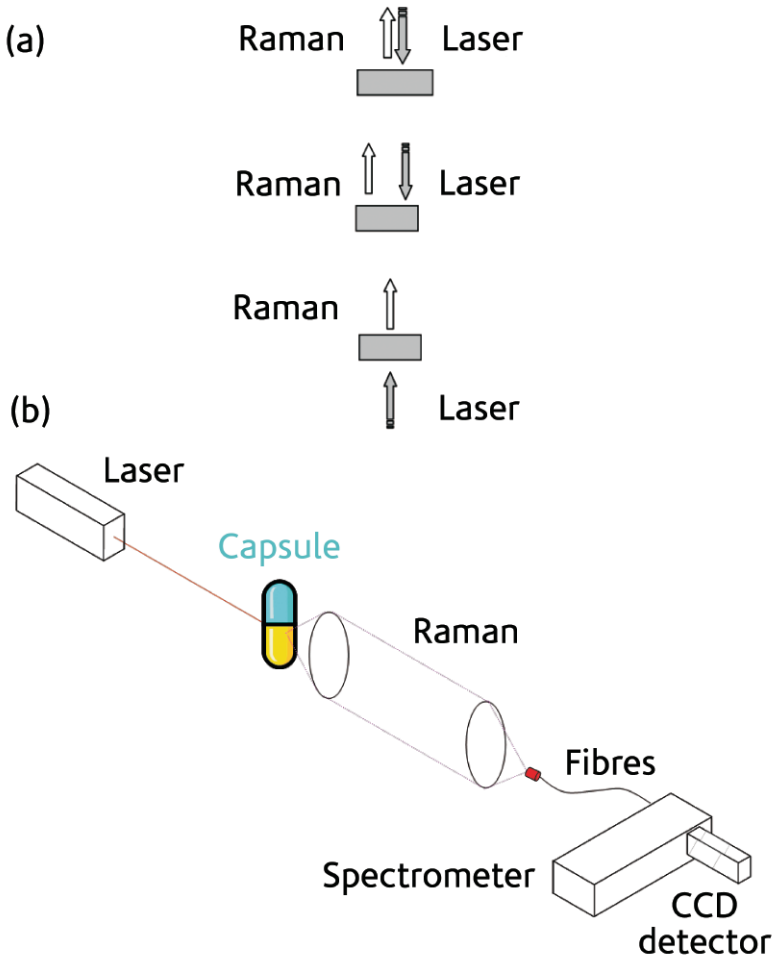

Figure 1. (a) Illustration of the conventional backscattering Raman, Spatially Offset Raman Spectroscopy (SORS) and Raman transmission geometries (from the top to the bottom). (b) Schematic diagram of the experimental layout employing transmission collection geometry.

achieved by removing the bias towards surface layers of the conventional Raman approach (sub-sampling)[2]. In the transmission Raman geometry, the laser beam is incident upon one side of the probed capsule and the Raman light is collected from the *opposite side* (see figure 1). Although

the transmission Raman technique was demonstrated in the early days of Raman spectroscopy[4], its benefits for the non-invasive probing of the bulk content of pharmaceutical samples had not been previously recognised or exploited. In particular, these include the removal of the so-called sub-sampling problem[2] (over-sensitivity to the surface layers of the probed medium), from which conventional Raman spectroscopy suffers[5], and the effective suppression of fluorescence components emanating from coating or capsule layers[3]. A more detailed account of this work is given in reference[6].

This work demonstrates the ability of the transmission Raman method to provide *quantitative information* on the internal composition of capsules where a strong interfering capsule shell Raman signal is present. The provision of this information is a key prerequisite for the deployment of this method in PAT applications. The general feasibility of the quantification of tablets and capsules using transmission Raman was recently demonstrated by Johansson *et al.*[7]. This study, using 20 test tablets and an unspecified number of capsules specifically formulated for the trial runs, achieved a relative root mean square error of prediction (for the concentration of the API) of 2.2% and 3.6%, respectively, with a 10 s acquisition time. In this work, we applied the transmission Raman technique to an existing, mass produced, pharmaceutical product. The formulations were prepared in a laboratory environment within the relevant production concentration range.

The capsules used possess strong interfering Raman signals which hamper or preclude the use of other PAT compatible optical spectroscopic methods (e.g. NIR absorption or conventional Raman) for the purposes of non-invasively quantifying the API present in the capsules. The entire sample set contained 150 capsules. The measured relative root mean square error of API prediction was superior (1.2 % and 1.8 % with 5 and 1 s acquisition times respectively) to that achieved before thus reaching production line relevant accuracy and measurement times. The study demonstrates quantitatively that the transmission geometry suppresses the interfering capsule shell Raman signature (TiO_2), by a factor of 33, leading to improved accuracy and stability of the prediction models.

Experimental section and methods

Instrumental

The Raman spectra were measured using a home built Transmission Raman apparatus[6]. The probe beam was generated using a frequency stabilised diode laser operating at 830 nm (Process Instruments, model PI-ECL-830-300-FS). The laser power at the sample was 250 mW and the laser spot diameter was ~3-4 mm.

Raman light was collected from the opposite side of the sample from the illumination site using a 50 mm diameter lens with a focal length of 60 mm. The scattered light was collimated and passed through a 50 mm diameter holographic notch filter (830 nm, Kaiser Optical Systems, Inc) to suppress the elastically scattered component of light. A second lens, identical to the first, was used to image, with magnification 1:1, the sample collection zone onto the front face of a fibre probe (CeramOptec Industries Inc., numerical aperture = 0.37) made of 22 active optical fibres. At the output end the fibres were arranged into a linear shape[8] oriented vertically and placed in the input image plane of a Kaiser Optical Technologies Holospec 1.8i NIR spectrograph. Raman spectra were collected using a NIR back-illuminated deep-depletion thermoelectrically cooled CCD camera.

Samples

The accuracy of the concentration of the two dominant constituents (API and lactose), depends on the accuracy of the weighing and blending process as reference potency data was not available for this sample set. For this feasibility study, the samples were laboratory prepared using a PK blender and encapsulated using a hand filler into gelatine capsules. The target weight (274 ± 15%) was based on a target blend weight of 225 mg and a capsule weight of 49 mg. Given the nature of their composition with only two dominant variable components (API and lactose) a strong concentration correlation was present between these two components.

Numerical analysis

Spectra were analysed using the MATLAB software package (Version 2006b, Mathworks Inc., Natick, USA) with the PLS toolbox (Version 4.0, Eigenvector Research Inc., Wenatchee, USA) with a combination of in-built and user written scripts.

The pre-processing routine comprised baseline subtraction (with a non-negative spectral constraint[9], normalisation to unit length (division by the sum of squares of all data-points in a spectrum) and mean-centering of the entire dataset. Additional multivariate analysis was performed using The Unscrambler® (Version 9.6, CAMO Software AS., Norway).

The multivariate techniques applied included principal components analysis (PCA) and partial least squares (PLS) regression[10]. The dataset comprised ~150 individual spectra representing 15 distinct chemical compositions ('blends') with ca. 10 capsules in each blend. Clear outliers (i.e. spectra not representative of the rest of the dataset) were identified visually in the PCA analysis and excluded from further analysis. For the calibration and regression methods, the remaining dataset (containing ~140 samples) was randomly split into a calibration set (two thirds) and a prediction set (one third); this division was repeated 10 times. A model was constructed from each of the calibration sets and applied to the prediction sets; quality of fit was estimated from the root mean square error of prediction (RMSEP, equation 1), where Y_{meas} is the reference concentration of the species of interest, Y_{pred} is the predicted concentration and n is the number of points in the prediction set.

$$RMSEP = \sqrt{\left(\Sigma\,(Y_{meas} - Y_{pred})^2 / n\right)} \qquad (1)$$

The number of latent variables used in the calibration (analogous to the number of principal components in PCA) was assessed by a cross-validation procedure using 10 subsets randomly selected across the calibration set; the optimum number of latent variables was defined as the

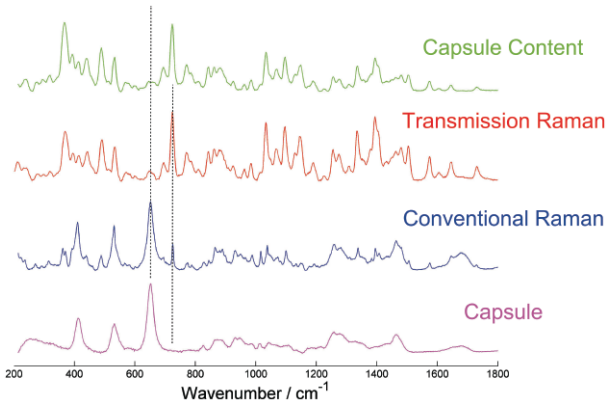

Figure 2. Non-invasive Raman spectra of capsules. The spectra were obtained using a standard commercial Raman system (Renishaw) in conventional backscattering geometry and our laboratory instrument used in the transmission Raman geometry. The Raman spectra of an empty capsule shell (lowest trace) and the capsule content itself (top trace) are also shown for comparison.

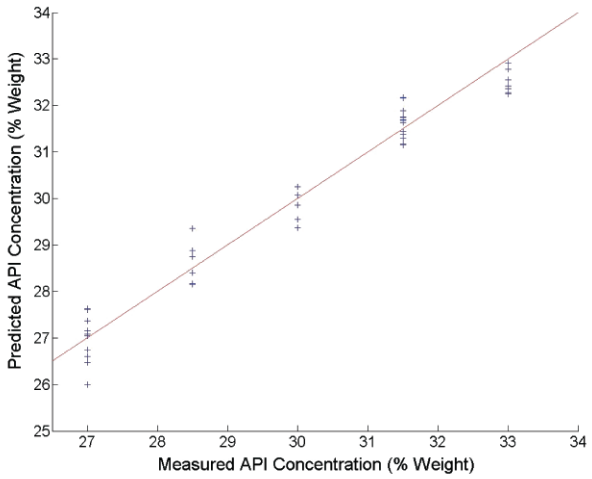

Figure 3. Partial least squares (PLS) calibration of the API dataset (5 s accumulation time). Spectra were baseline corrected, normalised to unit length and mean-centred. The dataset was randomly split into a calibration set (two thirds) and a prediction set (one third); obvious outliers from the PCA analysis were excluded from the analysis. Predicted versus measured API concentration of the prediction set, the straight line represents the 45° diagonal.

minimum of the root mean square error of cross validation (defined in a similar fashion as the RMSEP).

Results and discussion

Figure 2 shows non-invasive Raman spectra of one of the capsules measured using two different approaches: using a standard commercial Raman system (Renishaw inVia) in conventional backscattering geometry and our Transmission Raman instrument. The analysed sample represents the mid-point of the concentration range for both the API and the lactose excipient. The conventional Raman spectra is strongly contaminated with a Raman spectrum originating from the capsule shell as is evident from comparison with the pure spectrum of the empty capsule shell (figure 2). The capsule shell signal is dominated by the Raman spectrum of TiO_2 (anatase)[11] and contaminates the conventional Raman spectrum of the formulation with undesired noise, thus diminishing its prediction sensitivity and accuracy. Such layers are also known to present a considerable challenge to NIR absorption.

A dramatic reduction of the interfering Raman signal of the capsule shell relative to that of the capsule interior, by a factor of 33, was accomplished using the laboratory system operated in the *transmission Raman geometry* (figure 2). The absolute Raman signal of the capsule interior diminished *only* by a factor of 18 enabling relatively short acquisition times to be maintained.

Figures 3 shows the predicted versus measured concentration of the API collected with 5 s accumulation time; similar results were obtained using the 1 s dataset. The entire spectral range (200 cm^{-1} to 1800 cm^{-1}) was used in the calibration; typically 1-2 latent variables were required to model the API concentration while 2-3 were required for the lactose excipient. The analysis yielded a prediction error (RMSEP) for the API of 0.4% (absolute prediction error corresponding to 1.2% relative prediction error).

This feasibility study has demonstrated the potential of Raman spectroscopy, in transmission mode, for the quantitative analysis of pharmaceutical samples under the presence of strong interfering Raman signal of capsule shell. The overall accuracy of prediction (~1% of the measured concentrations) is comparable with those derived from other non-invasive techniques, i.e. NIR absorption, for which a relative prediction error of 1-2% would typically be considered acceptable in a routine manufacturing environment.

Conclusions

This study has demonstrated experimentally that the transmission geometry of Raman spectroscopy is ideally suited for the non-invasive probing of pharmaceutical capsules. Substantial reductions, relative to conventional backscattering Raman, in both Raman and fluorescence signals from the capsule shell allow the spectrum of the internal, bulk, components to be obtained. This property, in combination with the superb chemical specificity of Raman spectroscopy, rapid sample acquisition and ease of deployment makes the transmission Raman concept particularly well suited for the rapid analysis of pharmaceutical capsules and tablets.

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