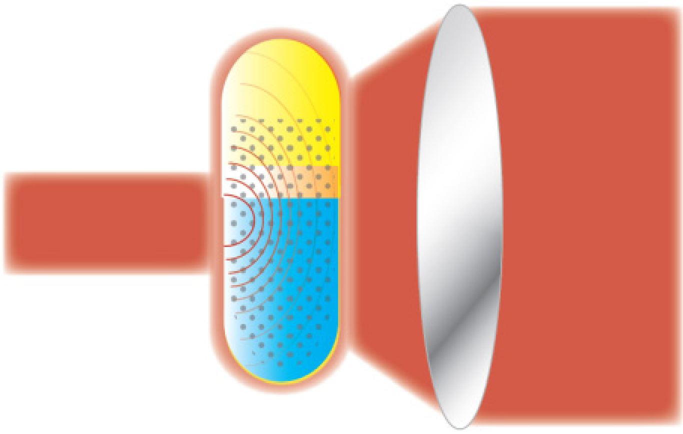
Technical Note

Transmission Raman Spectroscopy



Transmission Raman spectroscopy is a powerful analytical tool that allows bulk averaging of large opaque samples such as tablets and powders. Transmission Raman fits pharmaceutical analysis very well, particularly for drugs in capsules, but is generally useful for rapid bulk-characterisation of chemical mixtures or biological systems.

Transmission Raman spectroscopy (TRS) is an emerging technique with a number of unique analytical properties. TRS preserves the high chemical specificity of conventional Raman spectroscopy, surpassing that of near infra-red (NIR) absorption. NIR methods are commonly used in pharmaceutical analysis, typically in reflectance mode, but have limitations such as complexity in data modelling.

TRS removes some key limitations that have previously hampered the analytical applicability of Raman spectroscopy. The benefits of TRS include:

- Removal of the sub-sampling problem – an overly high sensitivity to surface sample layers
- An ability to provide bulk averaged information on the composition of dosage forms
- Penetration through coatings and capsule shells

These new characteristics represent a step change in Raman analytical capability in areas such as non-invasive pharmaceutical concentration measurements and *in vivo* disease diagnosis.

Unlike conventional Raman spectroscopy, where the signal is collected back-scattered from the sample illumination zone, a TRS signal is collected from the opposite side of the sample (see figure at top of page). Although the TRS approach was demonstrated in the early days of Raman spectroscopy, its benefits to non-invasive probing of the bulk content of turbid samples, including the elimination of the sub-sampling problem and the effective suppression of surface fluorescence, have not been recognised and exploited until very recently.

Bulk Probing Capability

Bulk probing is required in a number of analytical applications and has been demonstrated both theoretically and experimentally. Figure 1 illustrates the comparative volumetric sampling capability of TRS in the measurement of a two-layer sample in two different orientations between conventional Raman and TRS. In these measurements conventional Raman spectroscopy is limited to sensing the top layer of the probed sample and nothing beneath. In sharp contrast, TRS yields a chemical signature of both layers irrespective of the sample orientation, as expected for a technique with volumetric sensing capability.

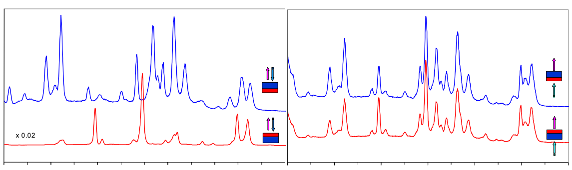


Figure 1: Raman spectra of a stacked 4mm acetaminophen tablet and 2mm of *trans*-stilbene powder measured using conventional Raman (left) and TRS (right).

Suppression of Fluorescence and Raman Signals from Surface Layers

An additional benefit of TRS is the ability to suppress interfering fluorescence and Raman signals from surface or near-surface layers of turbid samples – in contrast with conventional Raman spectroscopy. Such interference often generates excessive photon shot noise that can overwhelm much weaker Raman signals from deeper layers, precluding their effective measurement. Beneficial areas include the probing of pharmaceutical capsules or coated tablets or human tissue through fluorescing melanin layers. This feature is illustrated in Fig 2 depicting the measurement of a highly fluorescing green capsule containing a acetaminophen formulation. In the conventional Raman measurement the Raman signal of the formulation was completely obscured by a high level of fluorescence from the capsule shell. In contrast, the TRS measurement was straightforward with fluorescence levels being dramatically reduced.

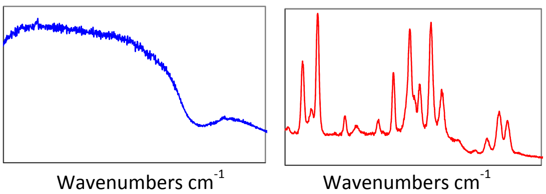


Figure 2: Signal from an acetaminophen formulation in a green capsule: conventional Raman is fluorescence limited (left) but TRS is not (right).

Non-invasive Quantification of Pharmaceutical Tablets and Capsules

Raman signal from the surface reduces accuracy when quantifying the composition of the formulation within the capsule. This effect is dramatically reduced with the TRS method and enables the quantification of the API within the capsules using the Partial Least Squares (PLS) method to an accuracy of around 1%[iv] (see Fig 3).

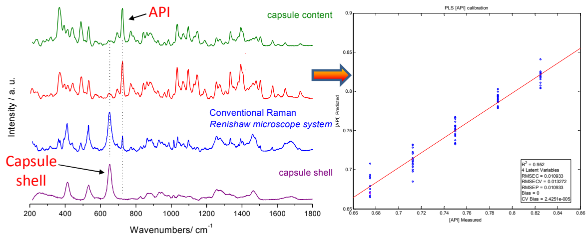


Figure 3: Turning Raman spectra into quantification of API in capsules using TRS and PLS.

In pharmaceutical applications one also benefits from a high robustness of data models to processing changes. Since the data is largely unaffected by particle characteristics the analysis does not need to take these into account. This leads to leaner training sets compared to NIR absorption spectroscopy. These benefits were experimentally explored and discussed by Johansson *et al.*[v]

Other applications currently under development include the diagnosis of breast cancer using TRS. Penetration depths of 25mm into living tissue were successfully demonstrated when looking for calcifications within the tissue.[vi]

Conclusions

The advent of TRS has stimulated the development of new analytical methods for probing tissues and powders at previously inaccessible depths. Many exciting applications are emerging, including accurate quality control of pharmaceutical products, disease diagnosis and process analysis.

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