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Raman imaging of drug delivery systems☆

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ABSTRACT

This review article includes an introduction to the principals of Raman spectroscopy, an outline of the experimental systems used for Raman imaging and the associated important considerations and limitations of this method. Common spectral analysis methods are briefly described and examples of interesting published studies which utilised Raman imaging of pharmaceutical and biomedical devices are discussed, along with summary tables of the literature at this point in time.

Contents

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1. Introduction

Raman spectroscopy is a technique in which scattered light is used to interrogate the nature of molecules within an irradiated volume. It is a technique that has developed significantly with advances in laser and detector technologies [1]. It is well suited to the analysis of materials with micrometre length scales, consequently Raman microscopes that are able to measure micron structures are now off-the-shelf instruments. In this review we explore the important aspects of Raman imaging with respect to biomedical devices, solid dispersions, percutaneous drug delivery, drug dissolution, intracellular drug distribution and microparticle composition. We include consideration of the important optical and physical properties associated with imaging as well as discuss the extensive use of chemometric techniques for image data analysis. We have focused on *spontaneous* Raman spectroscopy rather that the surfeit of interesting techniques that use non-linear optical responses, such as femtosecond stimulated Raman spectroscopy [2] and coherent anti-Stokes Raman spectroscopy (CARS) [3].

1.1. Principal of Raman spectroscopy

Raman scattering is the inelastic scattering of light from a sample. It was first described in 1928 by Raman and Krishnan [4] from which it gets it name. It occurs because the scattered photons lose or gain energy from the molecules in the irradiated sample. The pattern of Raman scattering (the energies of the scattered photons and the intensities of those transitions) informs on the molecules present in the irradiated volume. The scattering phenomenon is a rare event and the majority of scattered photons are *elastically* scattered; this is called Rayleigh scattering. Rayleigh scattering has a probability of about 10^{-5} in 1 m of air. Importantly this process is strongly dependent on wavelength (λ^{-4}) and it is Rayleigh scattering that makes the sky appear blue. The probability of Raman scattering occurring is $1{:}10^7$ scattering events. So the strength of signal is very small and although an individual Raman scattering event occurs in a femtosecond (10^{-15} s) [5] to get appreciable signal from a sample (which is many events) can take nanoseconds. This has some very important consequences for imaging in that there exists a fundamental difference between Raman scattering and absorption or emission techniques. The latter have transition probabilities that range from 1:10 (transition event:photon number), for emission, to $1{:}10^5$, for near-IR. This means that for the high probability methods the signal is likely to originate from the irradiated volume. However for Raman spectroscopy the intrinsic low probability of observing the signal means that the irradiated volume provides some of the signal but not all. Photon diffusion and scattering play a greater role in how the signal is observed. This problem becomes much more serious as attempts are made to collect data from deeper inside the sample [6]. This intrinsic problem is described in more detail in Section 1.5.

An additional point to appreciate is that the intensities of Raman transitions vary greatly depending on the analyte material. The mechanism whereby Raman scattering occurs is related to how a compound interacts with the electric field component of the photon. The more easily the compound can be polarised — the more responsive the electrons within it are to the driving electric field of the photons — the more intense the Raman signal will be. We can consider the ability to be polarised (polarisation, α) to be related to the elasticity of the electron cloud of the molecule. In an analogous fashion to infrared spectroscopy, in which the change in dipole with vibration determines absorption strength, it is the change of polarisability with vibration that determines Raman activity.

The scattering processes are described in Fig. 1. These show that scattering may be elastic (Rayleigh scattering) in which the vibrational state of the molecule is unchanged. Of the inelastic scattering processes, Stokes scattering red-shifts the laser photon by the vibrational energy ($v = 0 \rightarrow v = 1$) and the anti-Stokes blue-shifts it ($v = 1 \rightarrow v = 0$). In almost all normal Raman microscopes the Stokes scattering is that observed. The reason that the anti-Stokes lines are much weaker is that the population of molecules that are in the $v = 1$ state is lower thus these transitions occur less frequently. The population of the respective states is related to the energy between them (ΔE) by the Boltzmann distribution, Eq. (1).

$$\frac{N_{v=1}}{N_{v=0}} = \exp\left[\frac{-\Delta E}{kT}\right] \tag{1}$$

At room temperature, for transitions at about 100 cm^{-1} $\frac{N_{v=1}}{N_{v=0}} = 0.62$, thus the anti-Stokes and Stokes lines have comparable intensity; but for 1000 cm^{-1} this ratio drops to 0.008.

Molecules with electrons that are easy to polarise will give stronger Raman scattering than those held tightly. This has an important ramification for the study of pharmaceuticals in formulations and that is that most excipients are σ-bonded molecules like cellulose and starch and most APIs contain π-electrons. This means that APIs typically give stronger Raman signals than the excipients as shown in Fig. 2. This is exemplified by the data shown in Table 1 which shows the Raman cross sections $\left(\frac{\partial\sigma}{\partial\Omega}\right)$ [7] for different molecules and as a function of excitation wavelength. These data show that the Raman cross sections vary dramatically with molecule, for example the benzene Raman transition at 992 cm^{-1} is ten times more intense than the 758 cm^{-1} band of $CHCl_3$. Furthermore the Raman cross section varies with λ_{exc}. Notably it becomes intense when the laser wavelength is coincident or approaching an electronic absorption energy of the analyte; this is the resonance Raman effect — it can enhance signals by 10^6 as seen for β-carotene. This can be problematic if colouring agents are added to formulation as by their nature such agents are highly absorbing in the visible region.

1.2. Experimental systems — Raman microscopy

Raman microscopy is a term used to describe the fusion of Raman spectroscopy with optical microscopy. It can be performed using many of the various Raman spectroscopic techniques including dispersive Raman, FT-Raman, CARS and stimulated Raman scattering (SRS). Typically, the optical microscope is equipped with an excitation laser and spectrograph. The excitation laser beam is directed onto the sample through the objective lens and Raman scattering is produced. Scattered light is then collected using the same objective and directed to the spectrograph. Various different excitation laser wavelengths can be used depending on the sample and analytical question. Several other features