International Journal of Pharmaceutics 417 (2011) 151–162

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Raman mapping of pharmaceuticals

Keith C. Gordon[a], Cushla M. McGoverin[b,*]

[a] MacDiarmid Institute for Advanced Materials and Nanotechnology, Chemistry Department, University of Otago, Dunedin 9054, New Zealand
[b] Department of Food Science, Stellenbosch University, Private Bag XI, Stellenbosch 7602, South Africa

ARTICLE INFO

Article history:
Received 30 September 2010
Received in revised form
21 December 2010
Accepted 21 December 2010
Available online 29 December 2010

Keywords:
Raman spectroscopy
Mapping
Imaging
Confocal
Chemometrics

ABSTRACT

Raman spectroscopy may be implemented through a microscope to provide fine scale axial and lateral chemical maps. The molecular structure of many drugs makes Raman spectroscopy particularly well suited to the investigation of pharmaceutical systems. Chemometric methods currently used to assess bulk Raman spectroscopic data are typically applied to Raman mapping data from pharmaceuticals; few reports exist where the spatial information inherent to a mapped dataset is used for the calculation of chemical maps. Both univariate and multivariate methods have been applied to Raman mapping data to determine the distribution of active pharmaceutical ingredients (APIs) in tablets, solid dispersions for increased solubility and controlled release devices. The ability to axially (depth) profile using Raman mapping has been used in studies of API penetration through membranes, cellular uptake of drug delivery liposomes, and initial API distribution and subsequent elution from coatings of medical devices. New instrumental developments will increase the efficiency of Raman mapping and lead to greater utilisation of Raman mapping for analyses of pharmaceutical systems.

1. Introduction

1.1. Raman spectroscopy

The Raman effect was first reported by Sir C.V. Raman in 1928 (Raman, 1928; Raman and Krishnan, 1928, 1929) and is the inelastic scattering of light. Incident photons lose or gain energy by interaction with the sample, the loss of incident photon energy is a much stronger effect and is termed Stokes scattering – the reverse process, in which the incident photons gain energy, is known as anti-Stokes scattering (Fig. 1). In the context of pharmaceutical samples almost all spectra are due to Stokes scattering, with sample-photon interactions primarily involving vibrational modes of the sample molecules (Smith and Dent, 2005). Raman scattering therefore reflects the vibrational energies of the molecules within samples; these in turn are related to the nature of the bonding within the compounds of interest (Long, 2005). Thus Raman spectroscopy probes the same range of energies as infrared spectroscopy, however, the selection rules for Raman transitions differ from the infrared. In the case of infrared transitions, the dipole moment associated with a molecular vibration determines the intensity of the transition. This is not the case for Raman scattering which relies upon: (1) the creation of an induced dipole (polarization) in the molecule; (2) the modification of this dipole by molecular vibration; (3) the subsequent scattering of a photon from this modified oscillating dipole (Long, 2002). Raman spectra are presented as the difference in energy (in wavenumbers) between incident and scattered light, therefore in a Raman spectrum the wavenumber will be the same for a specific mode in a specific molecule, regardless of the excitation wavelength (a stretch at 1600 cm^{-1} will occur whether the source is 1064 or 514 nm). As Raman transitions are due to inelastic scattering phenomena they are inherently rare events, hence Raman spectra are weak and low concentrations of analyte may be difficult to observe. Raman transitions are also much weaker than the elastic scattering of the excitation source – the Raleigh scattering. Optics that block Raleigh scattering, single stage spectrographs, and sensitive charge-coupled device (CCD) detectors have transitioned Raman spectroscopy beyond academic applications and into the industrial realm (McCreery, 2000).

The differing physical phenomena that underpin Raman and infrared spectroscopy mean that strong infrared bands need not be intense in Raman spectra and vice versa. The most striking example of this is for water, the infrared spectrum of water is intense causing a great deal of difficulty in using infrared in aqueous systems – however the Raman spectrum of water is weak and there is generally no difficulty in collecting Raman data from aqueous samples. The reasons for this are directly related to the electronic structure of water – it is a σ-bonded structure which has a strong dipole moment, hence the vibrations are infrared active, but the electrons are not easily polarized and thus dipole induction is difficult and Raman scattering is weak. In general molecules that possess π-electrons, such as many active pharmaceutical ingredi-

* Corresponding author. Tel.: +27 21 808 3578; fax: +27 21 808 3510.
E-mail address: cushla@sun.ac.za (C.M. McGoverin).

0378-5173/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2010.12.030

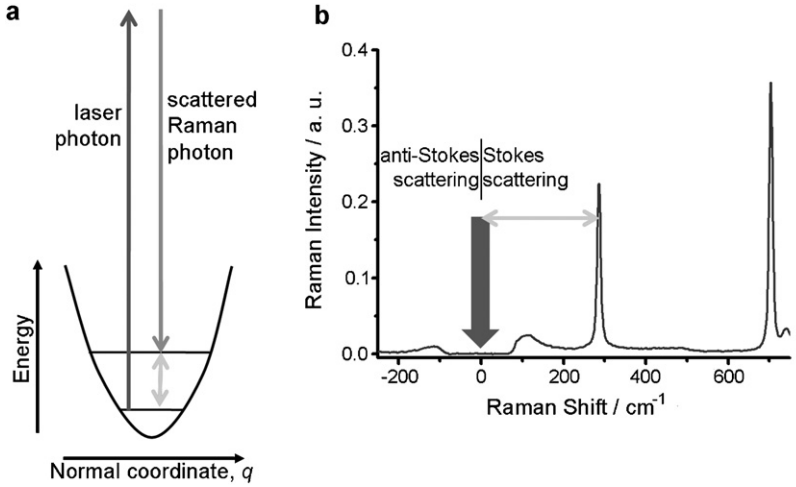

Fig. 1. (a) Raman scattering, the basis of Raman spectroscopy, is a coherent two photon event. Photons that have been Raman scattered have either lost or gained energy equivalent to that of a molecular vibration. Stokes scattering involves an energy loss and is the most common form of Raman scattering. (b) Raman spectra are depicted in terms of the energy difference between the incident and scattered photons, by convention Stokes scattering is positive. Each peak (band) in a Raman spectrum corresponds to a molecular vibration inherent to the analysed sample.

ents (APIs), show strong Raman scattering because they are easily polarized, conversely compounds which are primarily σ-bonded, such as excipients, may be very weak Raman scatterers.

1.2. Raman microscopy

Raman spectroscopic experiments may be carried out using microscope optics to guide the excitation laser and collect the resulting Raman scattering. The use of visible wavelength lasers means that when using a microscope spatial resolution and spot sizes down to a 1 μm diameter may be achieved. When using confocal microscopy a small well-defined volume of the sample is analysed. The dimensions of this volume are determined by the spot size (lateral resolution) of the irradiating laser and the depth of field (depth resolution) of the microscope. The lateral resolution of Raman microscopes is largely determined by the excitation wavelength and the numerical aperture of the objective. If one assumes the objective lens is filled by a beam of uniform intensity, the Airy disc approximation, then the focused spot diameter, d is given by Eq. (1):

$$d = \frac{1.22\lambda}{\mathrm{NA}} \tag{1}$$

in which λ is the laser wavelength and NA is the numerical aperture of the objective. For example 785 nm excitation and a 100× objective with NA = 0.9 gives a spot size of 1.06 μm. This deals with lateral resolution, at least to a first approximation, but the depth of the image from which Raman scattering is collected is not well controlled until a confocal configuration is applied to the experiment. A confocal microscope limits the depth of field by placing a pinhole aperture in the path between the objective and detector (Fig. 2). The confocal advantage may be applied to Raman microscopes such that a minor improvement to lateral resolution, and a major improvement to axial resolution, is made.

An element of caution is required when analysing confocal Raman data. Instrument manufacturers report axial resolution of Raman confocal microscopes as the full width at half maximum (FWHM) of the 520 cm^{-1} band of silicon as the focus is scanned through the air/silicon wafer interface. This a well defined test and certainly provides the axial resolution for *such an experiment*; however, when examining a buried structure or sample beneath a glass coverslip this axial resolution test underestimates the sampled depth of field.

In a series of studies Everall et al. (Everall, 2000a,b; Everall et al., 2007) have investigated the difference between the theoretical operation of a confocal microscope and the reality of performance with realistic rather than idealized samples. In principal the depth resolution (DR) is given by Eq. (2) (Juang, 1998):

$$\mathrm{DR} = \frac{2.2n\lambda}{\pi(\mathrm{NA})^2} \tag{2}$$

in which n is the refractive index of the material, λ is the laser wavelength and NA is the numerical aperture of the objective. This equation suggests that the DR may be improved simply by increasing NA. Indeed for a typical excitation source, such as 532 nm, and an objective with NA = 0.95 and material of n = 1.5 the DR = 600 nm. Everall examined a variety of substrates in which materials of differing refractive index were layered and found that the depth resolution was a complicated function related to the refractive

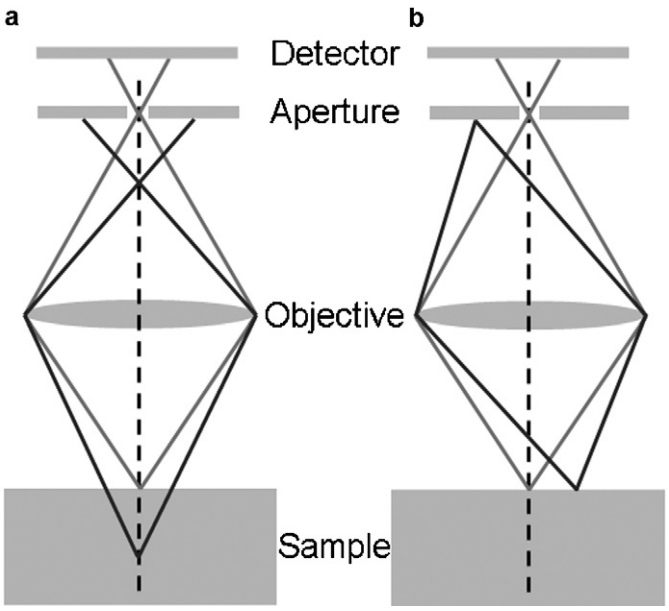

Fig. 2. A confocal aperture allows depth of field to be controlled and enables axial profiling. Only Raman scattering from the focal point (thin gray line) is focused through the confocal aperture. Scattering outside the focal point (thin black line), either axially (a) or laterally (b), is blocked.