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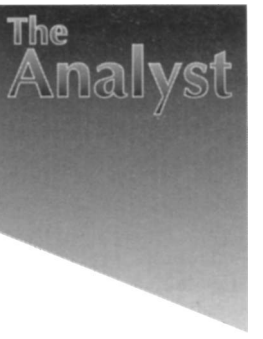

Tutorial Review

Time-resolved Resonance Raman Spectroscopy

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Vibrational Raman spectroscopy is now widely recognized as a useful technique for chemical analysis. It has become increasingly popular for the characterization of stable species since the technology which underpins Raman measurements has matured. Time-resolved Raman spectroscopy has also become established as an excellent method for the characterization of transient chemical species but it is not so widely applied. However, the technical advances which have reduced the cost and increased the reliability of conventional Raman systems can also be exploited in studies of transient species. In some cases it is just as straightforward to record the Raman spectra of a short-lived transient species as it is to monitor a more stable sample. This raises the possibility of routinely adding time-domain Raman measurements to more conventional Raman techniques, increasing the selectivity of the analysis while retaining its ability to provide spectral information which is characteristic of the species under investigation.

Keywords: *Vibrational spectroscopy; Raman spectroscopy; resonance Raman spectroscopy; time-resolved Raman spectroscopy; transient Raman spectroscopy; excited-state spectroscopy; reaction kinetics*

Introduction

Vibrational spectroscopy has long been recognized as a useful and widely applicable method for the characterization of a wide range of chemical species. For many years IR absorption spectroscopy dominated the field but the development of intense monochromatic laser sources in the late 1960s, and latterly multi-channel detectors, has led to a renaissance in Raman spectroscopy. The term 'Raman spectroscopy' is generally used to encompass a whole family of related techniques, each with their own inherent advantages and problems.[1] Normal, spontaneous Raman scattering is an inherently weak effect but Raman scattering probabilities, and therefore the signals themselves, can be enhanced dramatically through surface enhancement, resonance enhancement or a combination of both.[2] The increased sensitivity which these enhanced scattering mechanisms can provide has already led to analytical applications.[3–5] Both resonance and surface-enhanced Raman spectroscopy (SERS) are most widely applied to stable species in the form of pure compounds or in mixtures, solutions, *etc*. However, it is a relatively straightforward step to expand Raman methods to the study of transient species, *i.e*., to use time-resolved Raman (TR^2) spectroscopy.

TR^2 spectroscopy can provide information on both the structure and dynamics of transient species so that it allows the time evolution of the composition of a changing sample to be recorded. Up to this point, most time-resolved studies have been aimed at obtaining a detailed understanding of the changes in chemical structure which occur within a sample after a reaction had been initiated. In these types of studies the experiments are very carefully designed around the species of interest so that it is not possible to outline a single set of experimental conditions which constitute a general analytical technique.

The approach in this review will be to outline the general principles of the most commonly used time-resolved methods, discussing the experimental protocols which may be implemented for different types of sample and/or chemical process (in the interest of brevity, time-resolved coherent anti-Stokes Raman methods have been omitted from this tutorial review; an excellent review of the topic was given by Kamalov *et al*.[6]). Illustrative examples will be used alongside the descriptions of the methods wherever possible. The coverage is biased in favour of techniques based on pulsed lasers (particularly those with nanosecond pulse durations), since this reflects the balance within the literature and because such systems are now based on a mature technology and are both reliable and easy to operate. As such they probably constitute the easiest entry point for new TR^2 users, particularly since, in favourable cases, they allow time-resolved (or transient) Raman spectra to be recorded with little more difficulty than those of stable species. Indeed, the experimental complexity of some TR^2 methods is not much higher than that of other optical analysis techniques such as time-domain fluorescence measurements. Moreover, the apparatus required for such experiments, or at least some of the major components, such as pulsed laser systems and sensitive multi-channel light detectors, has become much more commonplace so that the cost of adapting existing optical systems to transient Raman techniques is much lower than might be expected. *It is hoped that, by demonstrating just how straightforward some TR^2 experiments can be, this review will stimulate new applications of the technique*. However, even in favourable cases, careful design of the experiment is needed to obtain spectra of useful quality. The experimental section is intended to provide enough information to allow potential users to judge the feasibility of carrying out such measurements on their own samples and to give some idea of the type of information which they might obtain. The difficulties that may be encountered, as well as the advantages, are discussed.

Although time-resolved Raman studies are still not as commonplace as those of stable species, there is still a very extensive literature on the subject.[7–10] A single review could not possibly cover in detail all the studies which have been carried out since the first reports of time-resolved experiments in 1976.[11] Although the literature cited in this review has been taken primarily from work published since 1989, limiting the coverage to the last 6 years does not reduce this body of material significantly, since much of the work in the area builds on earlier studies which need to be discussed in order to put more recent work in context. Fortunately, much of the research can be divided into groups involving similar broad categories of

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compounds, such as haem enzymes, and recent comprehensive reviews of the TR[2] of many of these broad categories are available. While it is desirable to discuss the types of problems which can be studied alongside descriptions of the experimental protocols, the number and range of experiments carried out with pulsed laser techniques makes it difficult to provide reasonable coverage of even the general classes of transient species which have been studied with this technique alongside the descriptions of the experimental procedures. For this reason, in the discussion of pulsed laser experiments a single class of compounds, the excited states of metalloporphyrins, is used to provide the illustrative examples. A broad overview of the application of pulsed laser Raman techniques to other classes of compounds is provided as a separate section.

Raman Spectroscopy—General Considerations

In order to keep this brief review as self-contained as possible, it is useful to summarize some of the general features of Raman scattering experiments before discussing those features which are most important to transient Raman measurements. Conceptually, the experiments are very straightforward: a sample is irradiated by intense monochromatic radiation and the scattered photons are then collected, dispersed by a mono- or polychromator and detected. The resultant spectrum is plotted as intensity *versus* wavenumber shift (cm^{-1}) from the excitation wavelength, as shown in Fig. 1. The frequency shift from the excitation line gives the frequency of a vibrational mode within the sample. Scattered photons appear at both higher and lower wavenumbers than the incident radiation; those which appear at lower wavenumber, the Stokes lines, are normally stronger than the anti-Stokes scattering and spectrometers are normally set to collect photons from just the lower wavenumber side.[1]

In modern spectrometers, a laser is used as the light source. It may be any one of a variety of commonly available types, the main requirements being that it is sufficiently powerful to produce detectable signals (typically milliwatt output powers are used) and has a narrow linewidth (typically $< 1\ cm^{-1}$). The sample may be a solid, liquid or gas and the detector is either a photomultiplier (in which case the spectrum is scanned point by point) or a multichannel silicon detector (a diode array or CCD), in which case the signals from a large spectral region can be detected simultaneously. Multichannel detectors have been used in most transient experiments.

The main experimental difficulties arise because of the inherent weakness of the Raman effect, which means that high input laser irradiances (input power per unit area, W cm^{-2}) are needed to produce detectable numbers of Raman scattered photons. This can be a serious problem in that the probe light can cause photochemical sample decomposition or the heating it creates can cause thermal decomposition. In addition, the scattered radiation primarily consists of reflected or elastically scattered (Rayleigh) photons, which are at the same frequency as the incident radiation, and a small number of Raman scattered photons, which lie close to them in wavelength. It is necessary to separate the weak Raman scattering from the overwhelmingly intense Rayleigh line. Until recently, this was achieved with large (and expensive) double- or triple-stage polychromators whose throughput was low. These can now be replaced with comparatively low-cost optical filters, such as holographic notch filters, which have very high optical densities (> 6) over a very narrow spectral window and have high transmittance over the rest of the required wavelength range.[12] These filters effectively reject the strong Rayleigh line and so can be used with compact and inexpensive single-stage polychromators to transmit the Raman scattered photons without stray light interference, as shown in Fig. 1.

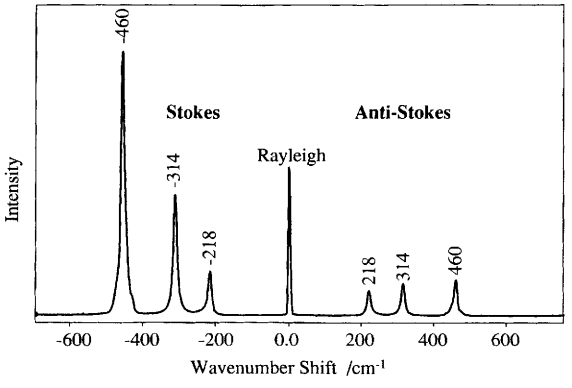

Fig. 1 Raman spectrum of CCl_4, obtained using a single-grating polychromator and a CCD detector. Raman bands to both the high- and low-wavenumber sides of the incident light (anti-Stokes and Stokes scattered photons, respectively) are of comparable magnitude to the small fraction of the elastically scattered light which is not blocked by the filter and appears at 0 cm^{-1}.

Raman experiments on transient species normally involve attempts to record spectra of relatively low-concentration solutions (typically 10^{-5}–10^{-3} mol dm^{-3}). In such cases, some enhancement mechanism is needed to raise signal levels to detectable values. The most common approach is to use resonance enhancement of the transient signal, *i.e.*, time-resolved resonance Raman (TR^3) spectroscopy. Resonance Raman spectroscopy (which can be applied to either stable or transient species) takes advantage of the increase in Raman scattering probability which is observed when the wavelength of the incident radiation is chosen so that it falls within a strong electronic absorption band of the species of interest. Enhancement factors are difficult to calculate theoretically but are typically of the order 10^3–10^5. The only vibrations which are enhanced are those of the chromophore involved in the particular electronic transition which is in resonance; this gives both increased selectivity and sensitivity.[1] The selectivity can be used to good effect in studies on complex sample mixtures, *e.g.*, biological samples, where small regions of very large molecular assemblies can be selectively probed.[13] On a more prosaic level it also allows vibrational spectra of dilute coloured samples within a non-absorbing solvent to be recorded since the solute Raman bands are enhanced over those of the solvent.

One disadvantage of tuning the excitation source into an electronic absorption band is that absorption of incident radiation reduces its absolute intensity in regions deeper within the sample. This is compounded by the fact that the Raman scattered photons emanating from the sample will be at wavelengths similar to the incident radiation and can also be absorbed before emerging from the sample and reaching the detector. The result of these self-absorption effects is that although the relative magnitudes of solute *versus* solvent bands can be dramatically increased by resonance effects, the total Raman signal will actually fall under resonance conditions, as shown in Fig. 2.

Experimental Methods for Time-resolved Raman Spectroscopy

Time-resolved Raman methods have been used to record the vibrational spectra of a large number of different transient species. This diversity in sample types has led to the development of many different initiation and Raman monitoring protocols, some of which are illustrated in Figs. 3 and 4. These experimental techniques can be divided into two broad categories, those which use laser photolysis (or pulse radiolysis) for initiation of the reaction and those in which the process is initiated chemically or electrochemically. The ultimate time resolution of the pulsed methods is extremely fast,[14–16] but the

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methods are limited to studies of processes which can be initiated by light or pulse radiolysis.

For our purposes, it is logical to discuss these broad categories separately, in the order shown below, beginning with the most generally applicable chemical methods before dealing with the various photochemical experiments in increasing order of technical sophistication:

(*i*) Chemically initiated processes.
(*ii*) Photochemically generated transient species.
(*iii*) General considerations for photochemical initiation.
(*iv*) Single-colour methods.
(*v*) Two-colour experiments.

Chemically Initiated Processes

The simplest form of time-resolved Raman experiments can involve the monitoring of relatively slow changes in chemical composition with time. If the time-scale for the process is hours then modern spectrometer systems, which can record spectra within a few minutes, can be used to record the spectral changes in much the same way as for stable samples. The general protocol is illustrated in Fig. 3(a). The limiting time-scale for the measurements is the time required to acquire a spectrum of appropriate signal-to-noise ratio [t_a in Fig. 3(a)]. This accumulation time can be reduced if the signal level can be increased, through either resonance or surface enhancement mechanisms. Many of the studies in this area have used surface enhancement,[17–24] since this can be particularly effective at increasing signal levels, but there is no fundamental reason why real-time TR^3 measurements should not be used instead, provided that the signal levels are adequate.[25] Recent reports of real-time or time-resolved SERS (TRSERS) measurements have shown that the total signal accumulation time can be reduced to a few seconds or less, which has allowed the dynamics of species localized on SERS-active substrates to be monitored on the seconds time-scale. Similarly, TR^3 measurements have been made on smooth electrode surfaces which are not SERS active.[25] The combination of surface and resonance enhancement mechanisms (surface-enhanced resonance Raman spectroscopy, SERRS) should allow even greater enhancement factors with correspondingly higher time resolution. Although SERS is normally carried out on Ag or Au surfaces, it has been possible to monitor the formation of surface species created from adsorbed gaseous molecules on other metal surfaces by overcoating an Au surface with ultra-thin layers of transition metals, such as Rh, and then monitoring reaction dynamics on the time-scale of seconds.[17,18]

The short signal accumulation time required for strongly scattering SERS-active species has prompted the use of time-dependent Raman signals as a chromatographic detection method. Since the eluent, whose composition changes with time, must flow past the detector, the SERS-active medium must either be continuously mixed with the sample upstream from the detector[22,23] or must be fixed in the detection zone and regularly regenerated, *e.g.*, by electrochemical oxidation–reduction cycles.[24] A most striking example has been in the use of SERS to detect purine bases eluted from an HPLC column, where the differences between the Raman signatures of the bases has allowed them to be readily identified as they elute from the column.[23]

Because SERS and resonance Raman measurements can be carried out on electrode surfaces within electrochemical cells, they can be used to monitor processes which are themselves initiated electrochemically, rather than through external chemical methods. For example, by applying a step potential and then recording a series of spectra after this initiation step [Fig. 3(a)], the intermediates formed on reduction of *p*-nitrobenzoic acid

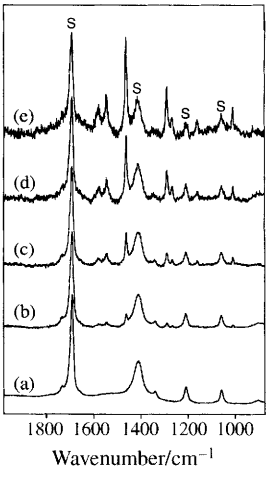

Fig. 2 Effect of increasing concentration on the resonance Raman spectra (λ_{ex} = 457.9 nm) of a metal–polypyridyl complex, $Cu(Bpy)_2^+$ (Bpy = 2,2′-bipyridyl), in acetone solution. At the lowest concentration the spectrum is almost that of the pure solvent; at the highest concentration the Raman bands due to the complex solute have increased with respect to those of the solvent, but this has been achieved at the cost of a 10-fold decrease in the absolute Raman signal. The decrease in the absolute signal intensity is clearly apparent from the increased apparent noise level at the higher concentrations. All spectra were accumulated for only 10 s to emphasize the effect; superior signals could readily be generated using longer accumulation times. Concentrations: (a) acetone only; (b) 3.0×10^{-5}; (c) 1.0×10^{-4}; (d) 2.5×10^{-4}; and (e) 4×10^{-4} mol dm^{-3}.

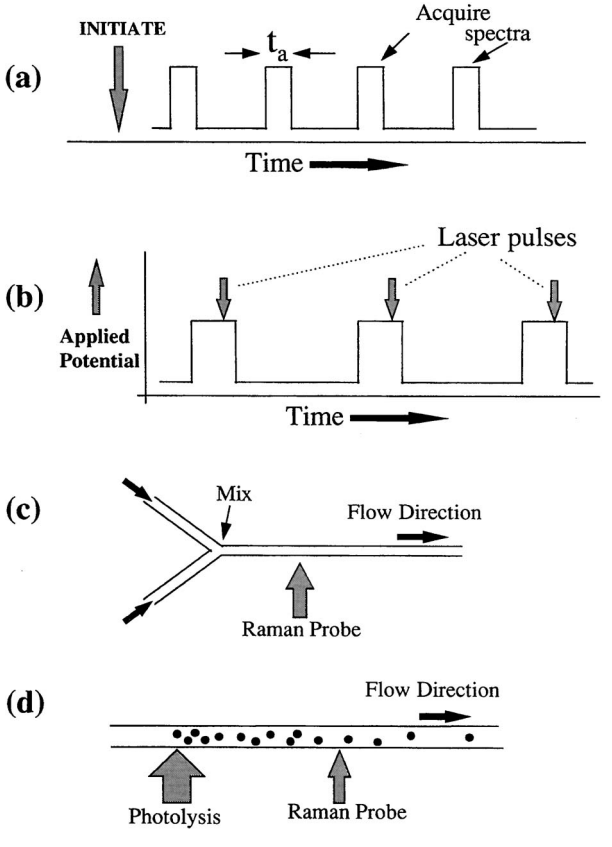

Fig. 3 Schematic diagrams of a range of protocols which have been used to obtain TR^3 spectra. (a) Initiation, either chemical or electrochemical, followed by a sequence of spectral acquisitions. (b) Synchronization of an extended series of probe laser pulses with a repetitively cycled electrochemical potential step. (c) Rapid mixing followed by downstream Raman probing with a CW laser. (d) Continuous photochemical initiation followed by downstream monitoring.

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were recorded at 10 ms time intervals.[19,20] Similarly, the formation of surface metal oxides and hydroxides on electro-oxidation of Pt, Au, Rh and Ru overlayers on roughened Au substrates have been recorded at 8 s intervals during the potential sweep.[21] It is possible to increase the time resolution of such experiments if a pulsed laser is used as the monitoring source, but a different protocol is required since the signal obtained from a single laser pulse (or even a short sequence of several laser pulses) will be very weak. The solution to this problem is to take advantage of the fact that the experiment can be repetitively cycled by synchronizing the laser pulse to fall on the sample at the same point in the electrochemical cycle at each repetition, as shown in Fig. 3(b). The signal can then be accumulated for as long as is necessary (typically thousands of laser pulses) before the time delay is changed. This strategy has been used to study the first steps in the reduction of heptylviologen films to radical cations with sub-millisecond time resolution, using both TR^3 and TRSERS measurements.[25]

In the case of chemically generated species, fast mixing techniques, which generate a continuous flow of reacting species downstream from the mixing chamber, have been widely used, particularly for studies of biochemical processes which cannot be initiated photochemically. In these experiments [illustrated in Fig. 3(c)], the sample composition at a given point downstream from the mixing chamber remains constant, so that the Raman experiments can be carried out in much the same way as normal Raman experiments on stable species. In such experiments, the time resolution is limited by the sample mixing time. Considerable ingenuity has been expended in reducing these mixing times, with the development of turbulent mixing systems with dead times as low as hundreds of microseconds.[26] Even more sophisticated systems, based on collisions between streams of droplets of approximately 100 μm diameter, have been developed. With these systems, mixing times of hundreds of microseconds have been demonstrated for the reaction of Fe^{II} with 1,10-phenanthroline.[27,28]

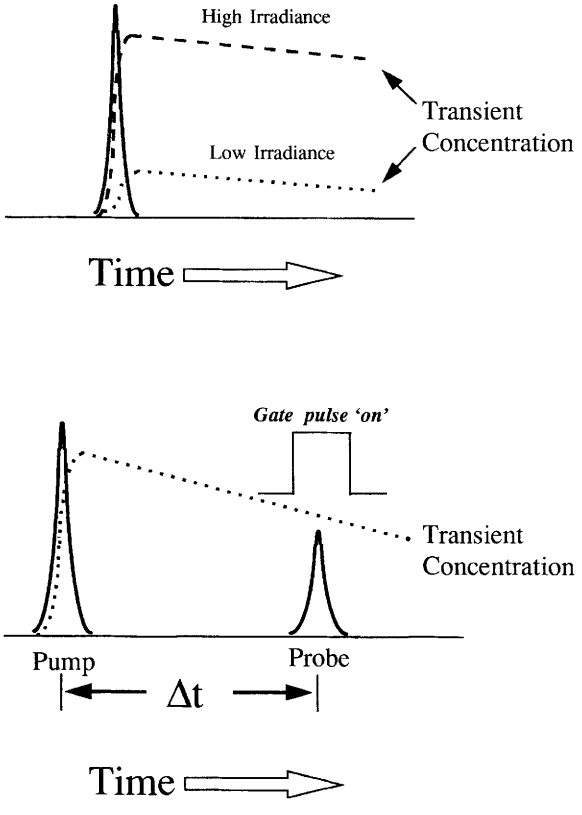

Fig. 4 Schematic diagrams of the processes involved in single-colour transient resonance Raman experiments (upper diagram) and two-colour TR^3 experiments (lower diagram). In both cases the transient population rises during the excitation pulse. In the single-colour technique this pulse also acts as the Raman probe and in the two-colour technique a second (time-delayed) laser pulse is used as the Raman probe.

Photochemically Generated Transient Species

General considerations

Photochemical generation of transient species is a clean and convenient method of making large concentrations of a transient of interest within a very short time and in an easily accessible form, typically in the body of the spectrometer itself. It is not surprising that the majority of transient species studied have been generated photochemically, rather than chemically. Although the method cannot be completely general, in that it depends exclusively on light initiation, it is not confined exclusively to excited-state studies since the photolysis can be used to generate ground-state species, *i.e.*, photoinitiation can mimic the effects of thermal processes such as deligation or radical formation.

For most photochemical TR^3 studies, the initiation is by a pulsed laser source but for very photolabile compounds pulsed laser irradiation rapidly leads to decomposition so, as an alternative method, CW irradiation can be coupled with flow systems as shown in Fig. 3(d).[29,30] In the most straightforward variant of this approach, the same laser is used as the photolysis source and Raman probe. As the sample enters the irradiated volume it is photolysed and for the remainder of the time it remains within the beam and the photoproducts thus generated can Raman scatter. A second variant of this techniques uses two CW lasers, one positioned upstream and used to generate a photoproduct continuously and the other monitoring the photoproduct as it passes downstream. The time resolution is set by the length of the stream which is irradiated and the flow rate. If the photoreaction is reversible, the sample can be recirculated or both lasers can be focused at different points of the circumference of a rotating cell where again the sample passes first through a photolysing and then a monitoring region. This approach has been extensively used in studies of visual pigments (see below).

The most popular experimental technique for Raman studies of photochemically generated transient species involves pulsed lasers, which are used to both initiate the chemical reaction and to give the Raman probe radiation. There are two main variants: single-pulse techniques, where the same laser pulse is used for initiation and probing, and two-colour experiments, where pairs of laser pulses are employed. In the latter, one pulse initiates the process and the second is used as the Raman probe, as illustrated in Fig. 4. The same general protocols are used irrespective of whether nanosecond or picosecond pulses are used but, for the purposes of illustration, discussion in this review will assume that nanosecond duration pulses are used. Typically, the transient species are generated at low concentration so that the experiments are carried under resonance conditions, *i.e.*, the wavelength of the probe laser is tuned to fall within a strong electronic absorption band of the transient of interest. The photolysis pulse must, of course, also fall within an electronic absorption band to allow optical pumping to take place. Alternatively, a pulse of high-energy electrons (10 ns, 2 MeV) can initiate the reaction.[10]

The use of pulsed lasers in either single- or two-colour experiments, coupled with absorbing samples, exacerbates the problem of sample decomposition, since very high peak powers and irradiances can be generated. Indeed, it is possible to generate non-linear effects within samples even using the output

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of small pulsed lasers. For example, a 10 mJ pulse with a half-width of 10 ns (assumed square) has a peak power of

$$(10 \times 10^{-3}\ \text{J})/(10 \times 10^{-9}\ \text{s}) = 10^{6}\ \text{W}$$

If this pulse is focused into a sample with a beam waist of 50 μm, the peak irradiance will be

$$10^{6}\ \text{W}/\pi(0.0025)^{2}\ \text{cm}^{2} = 5 \times 10^{11}\ \text{W cm}^{-2}$$

Under such conditions, it is hardly surprising that two-photon effects and sample decomposition can occur. The obvious solution to these problems is to reduce the input laser pulse energy but, since the Raman signal generated in a given time is directly proportional to the total number of input photons (*i.e.*, the average incident power), this reduces the signal which can be accumulated within a given time. A second option is to decrease the irradiance at the sample by defocusing the input beam and, since the irradiance is proportional to 1/(diameter)^2, this readily decreases the irradiance. However, defocusing the beam but retaining the energy per pulse will not give the same detected Raman scattering intensity, since the image of the sample which falls on the spectrometer slit will also grow larger and thus a smaller fraction of the scattered photons will actually pass through the slit and enter the detector. This is the reason for the common practice, when using a back-scattering geometry, of using a cylindrical lens to focus the input laser to a line on the sample, rather than focusing to a point. By defocusing to a line the irradiance is decreased compared with the point focus, but the Raman scattered photons may still be collected efficiently since the line image will still pass through the entrance slit to the monochromator. In practice, experiments are typically carried out near the sample damage threshold to maximize the signal which can be obtained.

The best way of maximizing the average power incident on the sample (and therefore the Raman signal) but keeping the peak power generated within the laser pulses low (and so reducing photolysis and sample damage problems) is to use pulsed laser systems with high repetition rates. This approach is well established for picosecond TR^3 experiments, where mode-locked lasers can be used to produce either relatively low-energy (nJ) pulses at MHz repetition rates or amplified kHz pulse streams with higher energies (μJ) by a range of different methods.[14,15,31] In picosecond measurements the short duration of the pulses means that peak powers in the non-linear regime are reached at much lower pulse energies than with nanosecond pulsed systems, so that higher repetition rates are necessary to reduce peak powers to usable levels while maintaining the average power needed to record Raman spectra. With the nanosecond pulse duration, kHz repetition rate Nd : YAG lasers which have recently become available, this high repetition rate approach should become much more common for transient studies using nanosecond pulses.[32] These lasers have the potential to increase dramatically S/N and/or reduce the signal accumulation times compared with more conventional systems operating at < 100 Hz.

A major problem with many samples which have been photolysed by pulsed lasers is that they may luminesce strongly in the same spectral region as the Raman scattered photons. Since this luminescence creates a broad background upon which the Raman bands are superimposed, even moderate luminescence can degrade signal quality and at higher levels can completely prevent observation of the bands. The problem is not merely that the Raman bands are concealed by the background. If this were the case then subtraction of a smooth background correction function could eliminate it. Rather, it is that the random photon shot noise associated with the background can be larger than the Raman signals themselves. This noise cannot be digitally subtracted away. Many different approaches have been taken to reduce background luminescence, which is a problem in both normal and transient Raman experiments. Methods which work by quenching the electronically excited states responsible for the luminescence, *e.g.*, by adsorbing the sample on a metal surface (*i.e.*, SERS and SERRS), are inappropriate if it is these states which are the target of the TR^3 study. In some circumstances it has been shown that photoreactions can be induced and monitored on SERS-active surfaces, but these are very much the exception.[33] Another approach is to use any differences in the duration of the Raman signal (scattering occurs only within the duration of the probe pulse) and luminescence background. The most widely used strategy is to use a 'gated' multichannel detector in which incident photons are amplified by an intensification stage before falling on the array of light sensitive elements in the diode array or CCD. The accelerating voltage on the intensifier can be turned on and off in less than 10 ns so that the detector can be made active only during the time period when the laser pulse is falling on the sample and the Raman scattering is generated. This means that other background radiation, either luminescence from an electronically excited sample or broad background radiation caused by the photolysis pulse, is rejected.

It is useful to separate the discussion of single- and two-colour experiments but to illustrate the points made using examples of experiments made on a single class of compounds. There are several classes of compound for which sufficient literature data are available, such as haem enzymes, but recent reviews of these systems are already available.[34–36] One major class of compounds which has been extensively studied in the past 5 years and which provides useful illustrative examples is the porphyrins. No extensive review of the TR^3 of excited states of porphyrins has yet been published but they do show a remarkable variety in their excited-state properties, due to alterations in the nature of their lowest excited states with changing central metal ion, exogenous ligands and porphyrin structure.[37] Many of these excited states have now been characterized by transient Raman methods. Some experimental data on these systems are presented in the following section (the nomenclature used is shown in Fig. 5) and a fuller discussion is given along with those of other well-characterized systems in a later section.

Single-colour experiments

The simplest pulsed laser experiments are so-called single-colour experiments where high irradiance laser pulses are both used to initiate the photoreaction and then to Raman probe the transient species created. At low irradiance the total number of incident photons per pulse will be lower than the number of

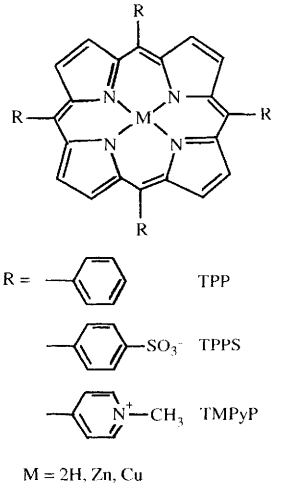

Fig. 5 Nomenclature used for the *meso*-substituted porphyrins used as examples herein.

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absorbing molecules present in the sample and the Raman scattering will necessarily be dominated by scattering from the unphotolysed sample. As the irradiance is increased, either by increasing the energy per pulse or focusing the beam more tightly, the leading edge of the pulse will contain sufficient photons to initiate the photoreaction. The trailing edge of the pulse will therefore encounter a photolysed sample and the total Raman signal will contain contributions from both the photolysed and unphotolysed sample (see Figs. 4 and 6). At very high irradiance, most of the incident photons will encounter photolysed sample and the signal is dominated by the transient species which are created. The irradiance level at which this occurs will depend on a number of factors:

(*i*) the sample concentration;
(*ii*) the absorption coefficient of the unphotolysed sample at the laser wavelength;
(*iii*) the lifetime of the transient species; and
(*iv*) the relative Raman scattering probabilities (cross-sections) of the starting material and the transient.

The first two factors are interrelated; a sample with a lower absorption coefficient at the laser wavelength will typically be used at higher concentration to achieve a reasonable absorbance. This will mean that the number of photons required to achieve, for example, a two-fold excess of incident photons over sample molecules will increase proportionately, *e.g.*, the sample will be more difficult to pump optically.

The lifetime of the transient species is an important variable only if it is shorter than the laser pulse duration. If it is longer, then the average concentration of the transient species which is encountered by the laser pulse will be the same irrespective of whether its ultimate decay takes place shortly after the pulse has entered the sample or at a time delay hundreds of times that of the laser pulse width. For this reason, the term transient Raman (or resonance Raman) spectroscopy should be preferred over time-resolved resonance Raman (TR^3) spectroscopy, since these single-pulse techniques give only limited dynamic information in comparison with the two-colour pulsed technique described below.

The final factor that controls the relative contributions of the transient and unphotolysed molecules to the Raman spectrum is their relative Raman scattering cross-sections. The most convenient conditions are those where the Raman scattering cross-sections of ground-state and transitory species are of similar magnitude and so follow the relative concentrations of material present in the sample. These conditions can often be realized relatively easily in practice since the experimental wavelength is chosen so that both ground-state and transient species are strongly absorbing. If the imbalance is too great then it can be difficult to record spectra of both transient and unphotolysed species using laser pulses of the same duration and wavelength. For example, the contribution from a weakly scattering transient species may be drowned by residual unphotolysed material, while a very strongly scattering transient will dominate spectra even under conditions where only a few per cent. of the sample has been photolysed. This second problem is particularly difficult to solve since the only way to reduce the proportion of transient present is either to decrease the incident irradiance or increase the sample concentration. Unfortunately, decreasing the laser irradiance also reduces the Raman signal, whereas increasing sample concentration will also decrease the absolute intensity of the Raman signal, owing to self-absorption, and may be precluded by low solubility. For example, whereas the MLCT state of $Ru(Bpy)_3^{2+}$ (whose lifetime is approximately 600 ns) can readily be observed using 10 ns duration laser pulses,[38] it is extremely difficult to reduce the input laser irradiance to sufficiently low levels to observe a spectrum of the ground electronic state with the same pulses. Indeed, the transient is sufficiently strongly scattering that it can be observed even using a tightly focused CW laser, whose power is of the order of 100 mW, as opposed to the MW peak powers of nanosecond pulsed lasers.[39]

The one great advantage of the single-colour technique, apart from its experimental simplicity, is that it can be used to record the Raman spectra of transient species which have

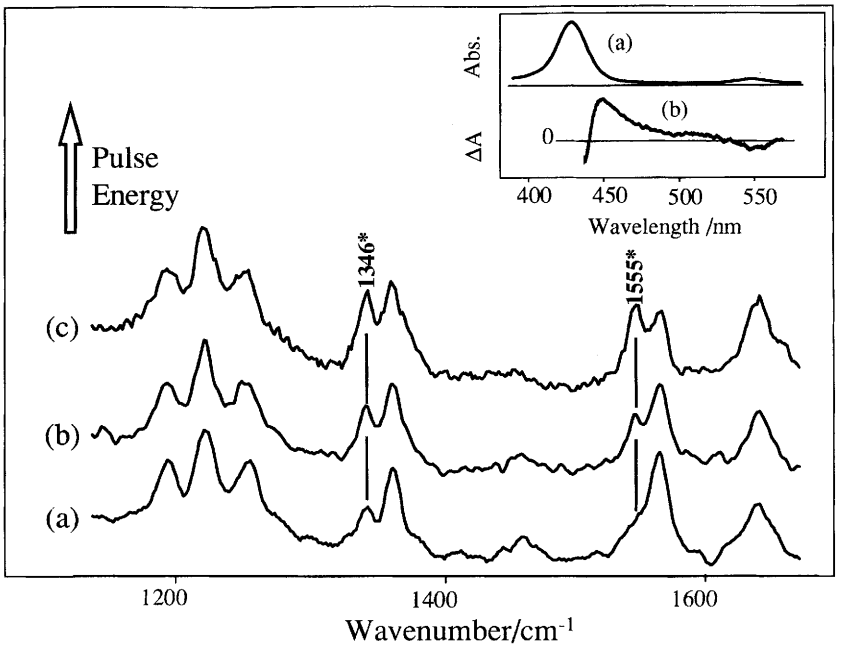

Fig. 6 Example of single-colour Raman data showing the effect of increasing laser pulse energy (λ_{ex} = 435 nm) on the resonance Raman spectrum of Cu(TMPyP) in aqueous solution with excess 5′-deoxythymidine monophosphate (5′dTMP). Pulse energies: (a) 0.1, (b) 1.5 and (c) 4.0 mJ. The two strongest transient bands (marked with asterisks) increase with respect to those of the unphotolysed complex at higher pulse energies. Inset: (a) UV/VIS absorption spectrum of a similar sample and (b) its transient absorbance difference spectrum. Note that both ground-state and transient species have significant absorbance at 435 nm.

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lifetimes significantly shorter than the laser pulse duration. This is because, as discussed above, extremely high photon fluxes can be created under what would, at first sight, appear to be relatively mild conditions. For example, a single pulse at 500 nm with an energy of 1 mJ contains 2.5×10^{15} photons, and if this pulse is incident on a sample which is 100 μm in diameter, 1 mm deep and has a concentration of 10^{-4} mol dm^{-3}, the number of solute molecules it encounters is

$$(50 \times 10^{-6}\ \text{m})^2 \times 3.14 \times (10^{-3}\ \text{m}) \times (10^{-4} \times 10^{3}\ \text{mol m}^{-3}) \times (6 \times 10^{23}) = 4.7 \times 10^{11}.$$

This is approximately a 5000 : 1 ratio, so that within the duration of a 10 ns laser pulse each molecule would have available 5000 photons, *i.e.*, one photon per 2 ps. Of course, only a fraction of these photons would be absorbed, but clearly even if only 1% are absorbed the pumping rate is sufficiently high that a transient with a lifetime as short as 200 ps will have a significant pseudo-steady-state population throughout the laser pulse. The limiting factor in such experiments is normally not how to generate these high photon densities but whether the sample is sufficiently robust to withstand them without photodecomposition. However, even in cases where lower irradiances are used (to prevent decomposition), the excess can remain so large that the phototransient is observed at all but the very lowest incident power levels. For example, the transient species whose spectrum can be seen to increase with increasing irradiance in Fig. 6 has a lifetime of < 1 ns[40] but it is observed (albeit only as shoulders on the main bands) even when the input pulse energy is reduced to one fortieth of its highest value.

Two-colour TR³ spectroscopy

In two-colour TR³ spectroscopy, pairs of laser pulses of different wavelength are used to photolyse (optically 'pump') and then to Raman probe the transient of interest. The spectral window of the detector is set so that it corresponds to the frequency range of the Raman scattering from the probe laser and the time evolution of the transient signal is monitored by recording a series of spectra at different delays after the photolysis event.[1,7–10] The most flexible arrangement is to use two synchronized lasers as the sources of pump and probe light, but both pulses can be taken from the same laser source if it is coupled with a wavelength-shifting device and a means of generating an optical time delay between the shifted and unshifted pulses. With picosecond pulsed lasers this second method is the norm,[14,15] optical time delays between pulses up to a few nanoseconds being readily generated by moving optical stages. For experiments on the nanosecond time-scale, it is still feasible to introduce optical delay paths of perhaps 30 m (approximately 100 ns), but generating delays of hundreds of nanoseconds using even longer delay paths becomes impractical.

A schematic diagram of the system used in the author's laboratory, which is a standard configuration for these types of experiments, is shown in Fig. 7. The basic design of these systems has not changed significantly for several years.[41] Higher repetition rate lasers, holographic notch filters and CCD detectors can be incorporated into this design and will probably become the norm over the next few years. Much more rapid technological progress has been made in the design of systems used for measurements on the picosecond time-scale. The specific design criteria for such systems and the extraordinary progress which has been made in meeting these criteria have recently been reviewed.[14]

The essential components of a nanosecond TR³ system are shown in Fig. 7. The core of the system is the pulsed lasers, which can be any one of a number of types, the main requirements being:

(*i*) sufficient energy in the photolysis pulses to produce a detectable concentration of transient;

(*ii*) average power in the probe pulse stream high enough to give detectable Raman scattering;

(*iii*) narrow linewidth in the probe pulses, preferably < 1 cm^{-1}; and

(*iv*) pump and probe pulses both at appropriate wavelengths for the sample.

In the system shown in Fig. 7, two Nd : YAG lasers produce 1064, 532, 355 and 266 nm pulses with a half-width of approximately 8 ns at a repetition rate of 10 Hz. Firing of both lasers is synchronized *via* a digital pulse generator, the timing jitter between the pulses being < 2 ns. The intensified diode-array detector is gated and also triggered from the pulse generator so that it is active for about 10 ns when the Raman photons from the probe laser fall on the detector but inactive for the remainder of the experimental cycle. This gating therefore rejects the scattered light from the high intensity pump pulse and also acts to remove luminescence. To give more wavelength tunability than is provided by the Nd : YAG lasers, the output pulses are either used to pump a dye laser or are Raman shifted with simple gases (H_2, D_2 or CH_4). Raman shifting is a much less expensive means of generating a range of output wavelengths than is a dye laser; both the initial cost of the equipment required and the running costs are lower, but it does not provide a continuously tunable wavelength range. However, a large number of output wavelengths can be generated using this system (the wavelengths generated by Raman shifting the fundamental and frequency doubled and tripled outputs of Nd : YAG lasers are given in ref. 42) and for most purposes it is not essential to be exactly on-resonance with a UV/VIS absorption band of the sample. Both pump and probe pulses must be focused into the same region of the sample. The simplest method of achieving this is to combine the beams and then to bring them into the sample colinearly. Normally the diameter of the pump beam at the sample is set slightly larger than that of the probe to ensure that only photolysed sample is Raman probed. The sample can be contained in a variety of ways, but the most usual methods for ensuring that the probed volume is constantly being regenerated are to flow the sample as a jet (or within a capillary) or to put the solution into a spinning cylindrical cell, such as an NMR tube.

In an ideal experiment, the sample is optically pumped at a wavelength where it is strongly absorbing but is then probed at a different wavelength, where the transient species of interest is strongly absorbing but the unphotolysed sample is not. Until recently, experiments were limited to transient species which absorbed in the visible region, but methods for TR³ spectroscopy with probe wavelengths in the UV region have now been developed and applied to both biological and simple chemical transients.[43–48] Under ideal absorption conditions, a high

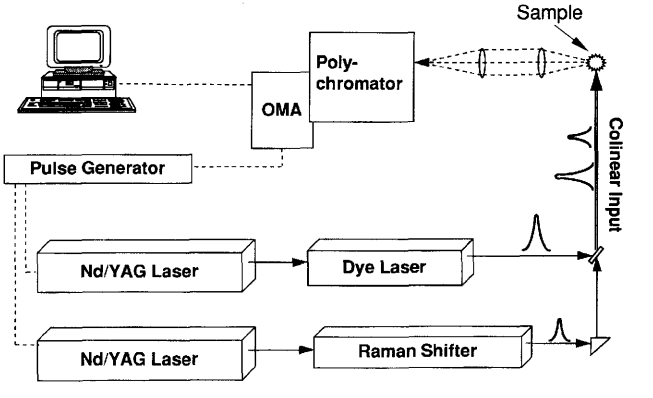

Fig. 7 Laboratory layout of a typical TR³ experimental system operating in the nanosecond time regime.

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proportion of the transient can be generated and its Raman signal will be strongly resonance enhanced. Indeed, the only signal, apart from solvent, which will be detected will be due to the transient, since the Raman signal of unphotolysed starting material will be vanishingly small. The best chance of achieving these conditions will be when pump and probe wavelengths are well separated, *e.g.*, in stilbene, where the λ_{max} of the ground state is around 300 nm but that of the lowest excited singlet state is at 600 nm.[14] In the absence of a photolysing pulse, a dilute stilbene sample has no absorbance at 600 nm and the Raman signal is merely that of the solvent. The transient signal only appears upon photolysis and is therefore easy to distinguish.[49] However, for many samples the choice of pump and probe wavelengths is less clear because there is no wavelength at which the transient absorbs but the ground state does not. This gives the minor problem that the unphotolysed sample may give strong Raman signals, which will interfere with those of the transient unless the photolysis pulse is 100% effective in depopulating the starting material. More significantly, if the sample absorbs at the probe wavelength, then high irradiance probe pulses may generate a transient species, irrespective of whether the sample has been photolysed or not, and indeed this is the essence of the single-colour technique, but in TR^3 experiments it has the effect of eliminating the dynamic information. To circumvent this secondary photolysis problem, it is necessary to reduce the irradiance of the probe pulse at the sample, with the associated loss in overall signal levels. Indeed, one of the reasons why the single-colour technique is experimentally simpler than two-colour methods is that in the former the transient signal is improved by increasing the probe laser irradiance (subject to sample damage limitations) whereas in the latter it is necessary to reduce the probe laser irradiance at the sample until no transient signal is present. In effect, the lowest irradiance level used in single-colour experiments is the upper limit in two-colour TR^3 experiments.

The major advantage which the TR^3 approach provides over single-colour transient Raman experiments is the ability to monitor the dynamics of the transient species created in photolysis by monitoring the time evolution of the signal. In many cases the dynamics can be measured much more easily using conventional flash photolysis, so it might appear that little additional information is given by TR^3, as opposed to a combination of single-colour Raman and conventional flash photolysis measurements. However, given the ease with which single laser pulses can generate even short-lived transients, it is important to confirm that the species probed in a single-colour experiment is indeed the transient of interest, rather than a species whose lifetime is similar to that of the laser pulse. Such a transient can dominate the spectrum recorded in a single-colour Raman experiment but can be very difficult to discern using flash photolysis. TR^3 data can show that the dynamics of processes measured using flash photolysis and Raman scattering techniques are similar. For example, in the TR^3 spectra of ZnTPPS,[50] shown in Fig. 8, a transient species with a strong band at approximately 1600 cm^{-1}, is present 30 ns after photolysis and decays away within 10 μs; the species probed is the T_1 state since its lifetime matches that recorded using flash photolysis measurements.

Of course, the experimental conditions needed to record transient Raman data are critically dependent on the properties of the transient, so that the most efficient approach is to obtain as much information on the transient species as possible (*e.g.*, using conventional flash photolysis) before embarking on Raman experiments. For example, in the ZnTTPS case discussed above, the lifetimes of the excited singlet and triplet states (approximately 2 ns and >5 μs) and their absorption spectra (both absorbing strongly at approximately 430 nm) were determined in advance. On the basis of this information, it was clear that the optimum laser probe wavelength should be approximately 430 nm but that single-colour experiments with 10 ns pulses could give transient spectra with at least some excited singlet-state character in addition to the triplet. However, using the same probe laser wavelength (with low-energy pulses) but optically pumping at 532 nm, where the ground state absorbs, ensures that only the longest lived transient is observed. As expected, the spectra show the largest population of transient at the shortest delays and a simple regeneration of ground-state bands as relaxation proceeds.

Another illustration of the advantages of TR^3 over single-colour experiments is given by the studies of the transient species formed by excitation CuTMPyP in the presence of a high molar excess of a mononucleotide (5′dTMP). The single-colour data on this system (shown in Fig. 6) provided spectra of the transient, which was generated within the duration of a single 8 ns laser pulse, but gave little further information on the dynamics of its formation and subsequent decay. By moving to two-colour TR^3 experiments with shorter pulses, it is possible to monitor both the formation of the transient (approximately 50 ps) and its subsequent decay (Fig. 9). In fact, for this system both processes are complete in about 1 ns so that within the 8 ns pulse duration used for the single-colour experiments several excitation–decay cycles can take place.

The time resolution of TR^3 experiments is set by the laser pulse duration and can be as fast as a few picoseconds or less,[16] the ultimate limit set by the Heisenberg uncertainty principle.[14] With Q-switched Nd:YAG lasers the resolution is typically tens of nanoseconds, but a recent report demonstrated that by using pairs of about 5 ns pulses from the same laser source it was possible to record the dynamics of a transient species whose lifetime was of the same order as the cross-correlation time of the laser pulses (approximately 100 ps).[51] Sub-nanosecond events have also been characterized by 'optical depletion timing' using pulses of similar duration.[52]

Data Analysis

Most of the applications of TR^3 listed in the following section are concerned with following structural changes in key components in the sample following excitation, but this is a

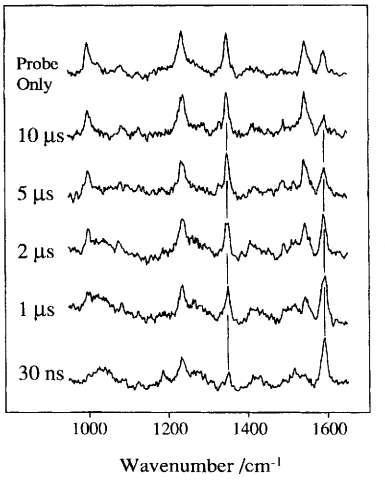

Fig. 8 TR^3 spectra of Zn(TPPS) in aqueous solution, obtained at the pump–probe time delays marked. The spectrum of the triplet state (Δt = 30 ns) is dominated by a strong band at approximately 1600 cm^{-1}; recovery of the ground state is accompanied by loss of this band and growth of the strong feature at approximately 1350 cm^{-1}. The similarity of the spectra obtained at 10 μs and and with the probe laser only shows that relaxation to the ground state is complete at this time delay. λ_{pump} = 532 nm, 8 mJ; λ_{probe} = 447 nm, 1.5 mJ.

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reflection of the interests of those who carried out the studies rather than a property of the method itself. *TR³ has the potential to be used as a direct and highly selective quantitative analytical method since, at the simplest level, for analytical applications it is not strictly necessary to assign the features characteristic of the species of interest to particular vibrational modes provided that some of the bands are indeed characteristic of the species of interest and distinct from those of possible interfering molecules.* The highly structured form of the spectra should allow identification of species of interest, and the method can be made highly selective by a combination of resonance effects (selection of the probe wavelength to match the absorption spectrum of the species of interest) and the use of the time domain to select, for example, only long-lived transient species from a complex mixture. Fig. 10 shows the ground-state and transient absorbance difference (ΔA) spectra of H_2TPP along with the Raman excitation profile of the ground and T_1 states.[53] It is clear that strong Raman scattering by the transient is only observed over a relatively narrow probe laser wavelength range. By selecting a probe wavelength outside this range or monitoring at longer delay times, it is easy to discriminate against the signal for this species.

The most straightforward quantitative analysis used for TR³ data, and the one most commonly employed, is simply to measure the intensity of transient bands with respect to an internal standard, such as a solvent band. However, much more sophisticated multi-dimensional least-squares data analysis of excited state Raman spectra has been demonstrated for model systems.[54,55] Such treatments are valuable in situations where saturation of the transient species is important, *i.e.*, where the transient signal, even after correction for self-absorption effects, does not increase as the square of the incident irradiance. The single-colour technique clearly has potential as an analytical tool which has not been widely exploited, presumably because of the technical difficulties which were undeniably present until even a few years ago.

Up to this time, the main objective of most transient Raman studies has not been direct quantitative analysis but rather structural determination of the transient species, and for this reason the simple fingerprint analysis has been supplemented by a range of vibrational mode assignments of varying complexity and rigour.

At the simplest level, the vibrational features can be compared with model, chemically stable species. A clear example of this is in studies of $Ru(Bpy)_3^{2+}$,[38,39] whose excited-state spectrum contains two sets of features, one set in positions very close to those of the ground-state complex and a second set which are moved to a lower wavenumber and closely resemble those of chemically reduced Bpy in Li^+Bpy^-. The structure of the excited-state species can therefore be formulated as $Ru^{III}Bpy_2(Bpy^-)$, the shifted bands arising from an excited state where the optically excited electron is localized on just one of the three available Bpy ligands.

The next step upwards in sophistication in the analysis of TR³ data is to carry out some form of normal-mode analysis of the transient species investigated. This tends to be a much more difficult task but it has been completed for some systems such as the charge-transfer excited state of $Ru(Bpy)_3^{2+}$ referred to above.[56] Even in the ground state a full normal-mode analysis requires considerable experimental effort, typically involving studies of isotopically substituted compounds, use of a series of different excitation wavelengths, if resonance Raman spectra are required, and IR absorption spectra to find Raman-silent modes.[57] With transient species the data sets are normally much more limited, so that even if series of isotopomers are available it is not normally possible to record spectra at a series of probe wavelengths. Similarly, although there have been major steps forward in time-resolved IR spectroscopy,[58] transient IR spectra are not normally available. However, it may be possible to assign bands in transient spectra by analogy to ground-state counterparts whose origin is known. For example, Fig. 11 shows the ground- and T_1-state spectra of H_2TPP. (The spectra were obtained in a manner very similar to that used for the ZnTPPS spectra discussed above.) A strong band is clearly visible at about 1235 cm^{-1} in both the ground- and triplet-state spectra of the complex. It is known that this band in the ground-state spectrum is ν_1, the stretching vibration of the bond connecting the phenyl ring to the porphyrin[57] but the question to be addressed is whether the band which appears in a similar position in the T_1 spectrum has a similar origin. By recording spectra of a second isotopomer in which the aryl ring substituents have been deuterated (D_{20}), it is possible to monitor the effect of phenyl ring deuteration on the position of this band in the ground and triplet states.[59] As expected, the band is sensitive to this isotopic substitution, moving to about 1186 cm^{-1} in the ground state. Moreover, the same frequency shift is also observed for the unidentified triplet state band, confirming that it has a similar composition to the well characterized ground-state band. In extreme cases, the band positions associated with particular modes can move by very large

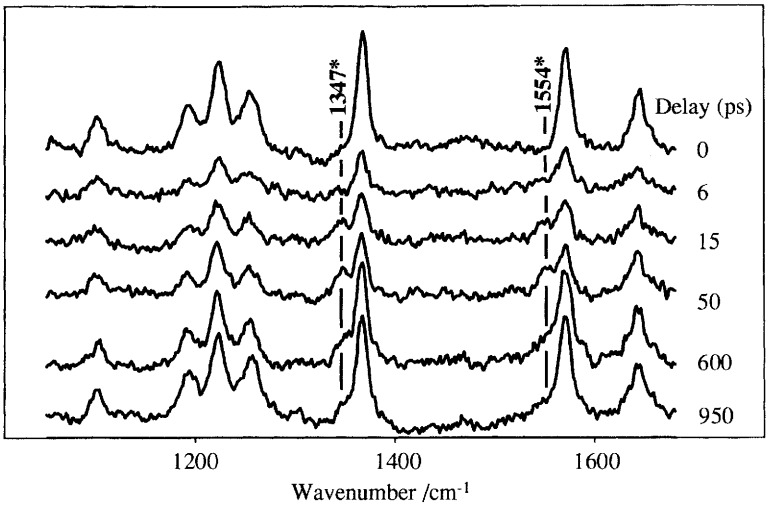

Fig. 9 TR³ spectra of Cu(TMPyP) in aqueous solution with excess 5′dTMP. Pump–probe time delays as shown. Bands due to the transient species (marked with asterisks, as in Fig. 6) build to a maximum at 50 ps before decaying, at 950 ps, to the same level as is observed at 0 ps (where the probe pulse just precedes the pump pulse). λ_{pump} = 540 nm, 15 μJ; λ_{probe} = 418 nm, 0.5 μJ.

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amounts. For example, in ground-state benzophenone the carbonyl C–O stretching vibration is at 1665 cm^{-1}, the lowest lying triplet state is n–π* in character and in its resonance Raman spectrum the band moves to 1222 cm^{-1}.[60] Many studies of the structural changes which occur following excitation have followed the approach of comparing changes in vibrational mode frequencies with calculated changes in structure and, as computational methods of reasonable sophistication become more accessible, this approach is likely to become even more widespread.

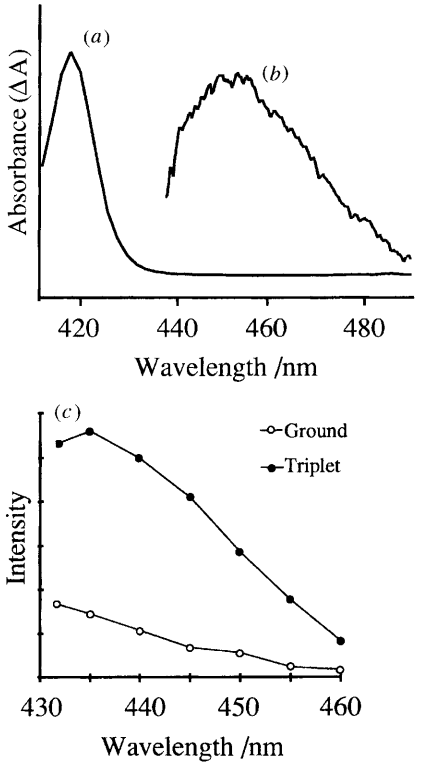

Fig. 10 (*a*) Ground-state UV/VIS absorption spectrum and (*b*) transient absorbance difference spectrum (Δt = 30 ns) of H_2TPP. (*c*) Resonance Raman excitation profiles of the ground and the triplet states of H_2TPP.

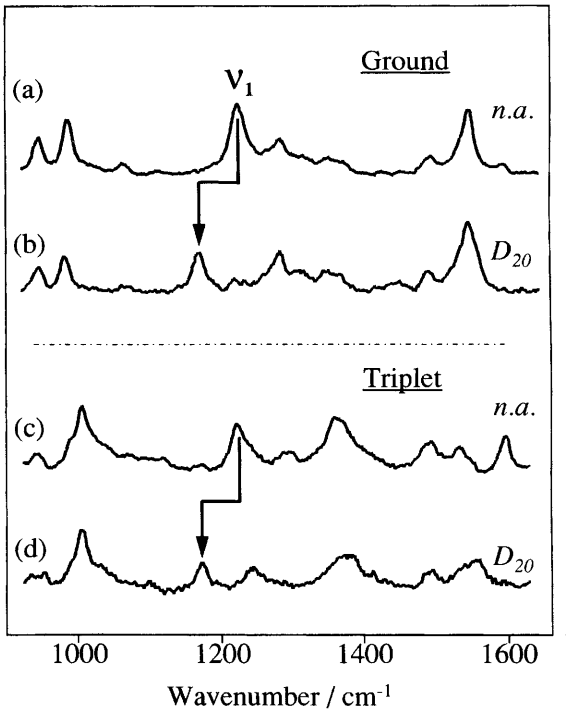

Fig. 11 Resonance Raman spectra of the ground and triplet states of natural isotopic abundance (n.a.) H_2TPP and its D_{20} isotopomer. Note this similarity in the isotope shifts of the ν_1 band (marked) in both the ground [(a) and (b)] and triplet [(c) and (d)] states.

Examples of Compounds Studied With Pulsed Laser Techniques

Because the use of pulsed laser photolysis has been so very widely adopted as the best method of characterizing the transient vibrational spectra of so many different compounds, it is not possible to discuss all examples in sufficient depth to be meaningful or helpful. This is partly because the number of vibrational studies is large, but mostly because the vibrational studies are not carried out in isolation and a great deal of background information needs to be included to place the data from Raman studies within their wider context. In addition, some comprehensive reviews, which cover in useful detail all the relevant work carried out on particular classes of compounds in recent years, are available. For these reasons, detailed descriptions and comprehensive lists of references have been replaced in this review by Fig. 12 and the text below, which is an attempt to illustrate some of the major classes of compounds that have been studied using pulsed laser techniques in the past 5 years, along with a very brief description of the type of information obtained, and recent references to work carried out by groups interested in these particular systems. It is hoped that this method of presentation will both illustrate the breadth of work available in the literature and demonstrate that TR^3 methods need not be confined to a small set of particularly favourable compounds.

Porphyrins and Metalloporphyrins

The properties of the excited states of metalloporphyrins are extremely diverse but are dominated by the nature of the central metal ion.[37] Their lifetimes vary by several orders of magnitude so that a range of transient Raman techniques have been used to characterize them. In broad terms, the photochemistry of the free-base (metal-free) and closed-shell metal complexes is dominated by porphyrin π–π* singlet and triplet excited states, with lifetimes of the order of a few nanoseconds and microseconds to milliseconds, respectively. TR^3 studies of several free base,[50,59,61–66] Mg[67] and Zn[50,68–72] porphyrins have been reported, as have spectra of closely related chlorins[73] and even bacteriochlorophylls.[74–77] The specific vibrational signature of the T_1 states of tetraphenylporphyrins has been used to monitor intramolecular electron transfer to a covalently attached quinone moiety.[72] With open-shell metal ions other low-lying *d–d*, metal-to-ligand and ligand-to-metal charge-transfer (MLCT and LMCT) states can be populated. Moreover, the energy differences between the various excited states is relatively small so that it is possible to switch the nature of the lowest excited state not only by changing central metal ion but also by altering porphyrin substituents or even the solvent. TR^3 studies of Ni porphyrins, in which the *d–d* excited state lies lowest in energy[65,78] and the MLCT state of $Ru^{II}TPP$ have been reported.[79] For iron porphyrins the primary effect of photoexcitation is either photoreduction[80] or dissociation of axial ligands[81] (used extensively in studies of haem enzymes, shown below). Copper(II) porphyrins show particularly complex behaviour which has been the subject of several publications. The Raman signature of the excited states change in solvents of

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different polarity and when water-soluble derivatives interact with DNA or DNA model systems.[82–90]

Haem Enzymes

Haem enzymes carry out a range of functions in living systems, from O_2 transport and storage by myoglobin and haemoglobin, to electron transfer, by cytochromes *b* and *c*, and transformation of substrates, such as O_2 to H_2O in cytochrome *c* oxidase. Although the chemistry of most haem enzymes is not photochemically driven, extensive TR^3 studies of haemoglobin and cytochrome *c* oxidase have been carried out by initiating their chemical reactions photochemically, through photodeligation of CO from CO adducts of their Fe^{II} haem groups. The subsequent reaction pathway depends on the enzyme involved. With haemoglobin the primary interest has focused on the relaxation of the protein structure around the haem group, following either the evolution of the haem vibrational modes from the first picosecond until the structure has fully relaxed, or using UV probe wavelengths to monitor changes in the protein. For cytochrome *c* oxidase, TR^3 methods have been used to follow the complex series of electron and proton transfer steps which follow O_2 binding. Recent comprehensive reviews of the application of TR^3 methods (using both pulsed and CW photoinitiation) to haem enzyme reaction dynamics are available.[34–36]

Small Organic Molecules—Excited States and Radicals

Studies of excited states of stilbene and its derivatives are inextricably linked to the development of picosecond optical spectroscopy and the molecule continues to provide a valuable testing ground for many of the phenomena associated with ultra-fast isomerization processes.[14,48,49,51,91–97] TR^3 spectra, with extremely high S/N, of the first few picoseconds following excitation are now available.[14,49,91] Although stilbene and its derivatives have been thoroughly examined by TR^3 methods, it is by no means the only compound probed by picosecond or nanosecond methods. Indeed, the range of questions in organic photochemistry addressed by TR^3 methods is almost as large as the field itself.[91–110] The particular strength of the method is that it allows structural characterization of excited states and therefore the investigation of links between structural changes upon photoexcitation and photochemical reactivity. Studies have ranged from investigation of the geometry (twisted or planar)[97] of the singlet and triplet excited states of biphenyl to the structural changes which accompany excitation of photochromic compounds.[98] Radical formation is a common outcome of photoexcitation and free-radical reactions (or their suppression) are physiologically important so that much interest has focused on this area, where the fact that technique can readily distinguish between excited states of different multiplicities and protonated and unprotonated radicals makes it particularly valuable. The spectra of large numbers of radical species generated either photochemically or by pulse radiolysis have

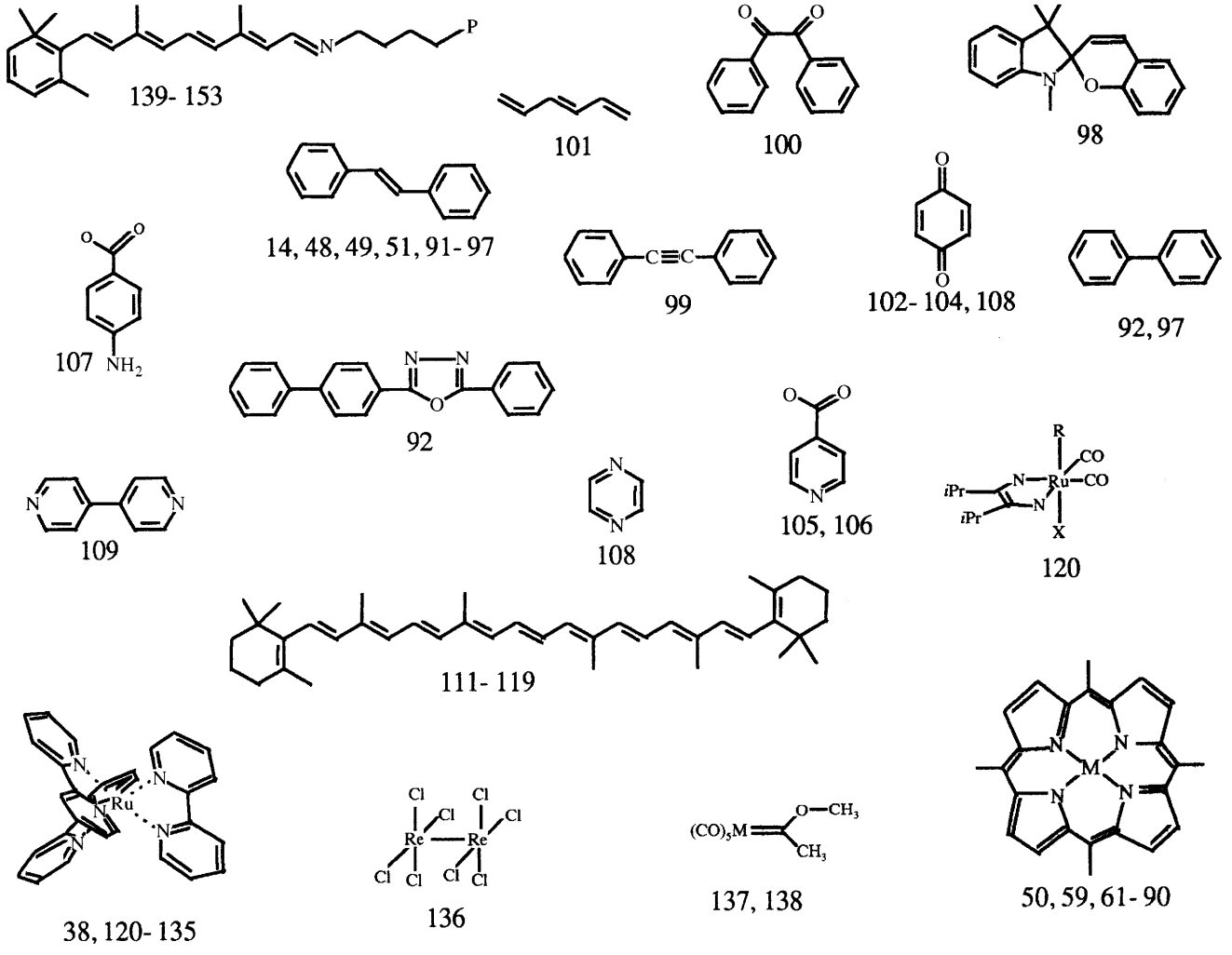

Fig. 12 Structures of some of the compounds whose excited states or radical products have been studied by resonance Raman spectroscopy. Only a single representative is shown for each general compound type. References cited under the structures cover both the compound shown and related compounds.

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been obtained[102–110] and in some cases the link between their electronic structure and chemical reactivity established. The structures of a representative range of compounds (not a comprehensive listing) are given in Fig. 12.

Photosynthetic Reaction Centres, Carotenoids and Bacteriochlorophyll

TR^3 studies aimed at understanding photosynthetic systems have focused on both the bacteriochlorophyll, whose excited-state Raman spectra have been recorded,[74–77] and on the photochemistry of carotenoids. The latter has been extensively investigated for many years[111] but new insights continue to stem from the intensive scrutiny to which they have been subjected. In recent years, TR^3 experiments have been carried out on a large number of carotenoids and their substituted derivatives in solution,[112–114] micelles[115,116] and *in vivo*.[117–119] The structural changes accompanying population of the lowest lying singlet and triplet excited states and radical cation formation have been determined.

Metal Complexes

The first resonance Raman studies of the lowest excited triplet metal-to-ligand charge-transfer excited states of $Ru(Bpy)_3^{2+}$ by Bradley *et al.*,[38] which showed that the optical electron was localized on a single Bpy ligand, firmly established transient Raman methods at the centre of studies of metal polypyridyl complexes.[8] The technique has now been extended to studies of a wide range of complexes with different metal centres,[120–126] and coordinated ligands.[127–135] TR^3 has now been used not only to find which ligand is reduced on excitation of heteroleptic complexes[127] but also to find in which region of asymmetric ligands the optically excited electron resides,[128,129] the effect of the environment on the excited state structure (including the changes brought about by interaction with DNA),[130–132] the nature of the excited states of polychromophoric complexes[133,134] and even the processes which occur during intramolecular electron transfer.[135]

While most transient and time-resolved Raman studies of metal complexes have involved charge-transfer excited states, studies on the δ–δ* excited states of $Re_2Cl_8^-$ and $Re_2Br_8^-$,[136] photoisomerization of metal carbene complexes[137,138] and investigations of the nature (MLCT or intra-ligand) of the lowest excited state of Ru(halide)$(CH_3,\ C_2H_5)(CO)_2$(a-diimine),[120] have also been carried out. The latter study compared both time-resolved IR absorption and Raman measurements on the same transient species.

Bacteriorhodopsin and Visual Pigments

The chemistry of vision is a natural area for exploration by photochemical techniques. TR^3 methods have been extensively used to follow the sequence of reactions which follow initiation of the process through light absorption in the chromophore of bacteriorhodopsin and related molecules.[139] The photochemical cycle involves several steps which occur on a wide range of time-scales but which ultimately return the chromophore to its original state within milliseconds.[139,140] In recent years, several groups have been involved in carrying out the wide range of experiments (using both pulsed and CW lasers) needed to characterize the complex reaction sequence and in studying pigments related to the native protein.[141–153]

Conclusions

Time-resolved Raman methods can be applied to a very large range of compounds, the exact experimental protocol depending on the time-scale of the measurement required and whether the process of interest must be initiated chemically, electrochemically or photochemically. A range of protocols has been developed for each of these types of reaction. Technical advances have dramatically improved the signals obtainable from picosecond laser systems, but these systems are extremely expensive. Transient experiments based on CW and nanosecond pulsed lasers are considerably less expensive and based on a mature and established technology, so that for routine measurements they are more attractive.

The technique offers the opportunity to monitor structural changes within samples undergoing time-dependent processes or the possibility of using the time domain simply as an additional variable with which to separate one component from a complex sample mixture. Sample concentrations are not excessively high, typically of the order 10^{-3}–10^{-4} mol dm^{-3}.

The major disadvantages of the technique arise from the inherent weakness of the Raman effect, which demands sensitive optical detectors and high input light irradiances (with associated sample decomposition problems). In addition, strong luminescence from samples may prevent studies being carried out. However, as the examples listed above illustrate, many chemical systems are amenable to TR^3 methods, which can provide extraordinarily detailed information on species with even the most fleeting existence.

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