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TIME-RESOLVED RAMAN SPECTROSCOPY

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Introduction - Fast Reaction Techniques and Vibrational Spectra

Spectroscopy has long been known as an excellent tool for monitoring the course of chemical reactions, thereby providing valuable information on their kinetics. Over the years various techniques involving spectroscopy have evolved into commercial instruments. For example, many companies manufacture equipment for the rapid mixing of two (or more) solutions in flow systems, spectroscopic measurements on which may be made continuously or after the flow is stopped (stopped-flow apparatus). Others produce equipment for the photochemical initiation of reactions (flash photolysis) and yet others supply equipment for the study of fast reactions induced by temperature or pressure change (T-jump and P-jump). The time domains appropriate to each technique vary from milliseconds for the flow methods to picoseconds (10^{-12} s) for the best pulsed-laser flash-photolysis gear. A host of mechanisms in chemistry and in biochemistry have been characterized by exploiting these various fast reaction techniques. Although variations in a physical property such as the electrical conductance have occasionally proved helpful, most commonly optical absorption or fluorescence has prevailed as the preferred technique for following the course of such reactions.

The problem with optical spectroscopy in the visible and near-uv regions (200-800 nm) is that the spectra from molecular species in solution usually consist of only a few broad bands which yield little or no information on molecular architecture or the nature of chemical bonding. Such information is, however, vital to any deep understanding of reaction mechanisms, particularly when the reaction pathway involves one or more transient intermediate species. Moreover, when electronic spectra are measured for systems involving several different species with similar and overlapping spectra, results are often frustratingly ambiguous.

These shortcomings of uv-vis spectra prevail because of the jumble of molecular electronic transitions from which the spectra originate. To obtain sharp, narrow-banded, richly detailed spectra which can serve well for "fingerprinting" molecular species, the researcher usually prefers to focus on vibrational transitions. These may be observed either directly in infrared absorption or through the form of inelastic light scattering known as Raman spectroscopy. In principle, either of these techniques of vibrational spectroscopy is applicable in place of the less informative uv-vis electronic spectroscopy. However, to date only the Raman technique has been developed to the stage where it provides the means for identifying transient intermediates in fast reactions, analyzing their structures and bonding and also monitoring their kinetics of formation and decay. The technique is known as time-resolved Raman spectroscopy (TRRS).

So why hasn't everyone been stampeding into Raman rather than persevering with optical absorption or fluorescence spectroscopy? The reasons are manifold. Perhaps the most important is that the sensitivity of the Raman method is intrinsically much poorer than that of the others. Accordingly, Raman spectra are much more difficult to generate with interpretable signal-to-noise (S/N) ratios. Furthermore, Raman equipment tends to be more complex; several of the components are the products of quite recent technological development and thus quite expensive. Finally, real-world samples often fluoresce at intensity levels high enough to swamp the Raman signals.

To circumvent some of these problems, special non-linear techniques have been devised. Coherent Anti-Stokes Raman Spectroscopy (CARS) is an example. By generating Raman spectra at a higher frequency than the exciting source, it neatly avoids fluorescence. Generally speaking, its background noise is high, however, as is the expense of the required two lasers. Raman Induced Kerr Effect Spectroscopy (RIKES) is still another non-linear method. Like CARS, it requires two expensive lasers. Experimentally, it is simpler and background is generally weaker.

Unlike non-linear techniques, resonance Raman spectroscopy has been found to have considerable merit. By choosing an excitation frequency matching that of a strong absorption band, ordinary Raman intensities may be boosted by up to six orders of magnitude. And, by coupling resonance Raman with a pulsed laser, nanosecond time resolution may be attained. Finally, by lengthy signal averaging of many pulses, acceptable S/N ratios are attained. Through this combination, TRRS is rapidly gaining popularity as a probe of reaction kinetics.

Resonance Raman is of particular significance because of its selectivity; Raman spectra of each substance in a mixture can be enhanced simply by tuning the excitation wavelength to an appropriate absorption band of that substance, one showing little or no overlap with others in the mixture. By so doing a Raman spectrum of a transient reaction intermediate can be generated at a signal level far stronger than that of the reactants or the solvent, either of which may be present at much greater concentration than the transient species under study. It is also important to note that no sharpness or fine structural detail is lost in resonance Raman spectra. It is this rich structural detail that distinguishes resonance Raman most obviously from fluorescence. In general, not all Raman bands are affected equally by a resonance effect and band enhancement usually is confined to those vibrational modes associated with the chromophoric part of a molecule.

Chemical and biochemical reactions of virtually all types may be studied by TRRS methods. Although this is a relatively new field, many interesting examples have already appeared in the literature, examples drawn from areas as

diverse as solar energy conversion and the ligation (with O_2, CO, etc.) of hemoglobin. The experimental techniques which have been devised in conjunction with Raman spectroscopy for the generation and study of transient reaction intermediates are almost equally diverse, as is shown by the following illustrative list:

- Continuous and stopped flow.
- Matrix isolation. (Reaction intermediates may be trapped from the gas phase or quench-cooled and stabilized in matrices or may be generated *in situ* by photolysis)
- T-jump and P-jump. (Temperature- or pressure-sensitive reaction equilibria are perturbed by rapid T or P changes and their relaxation to new equilibrium states tracked by TRRS)
- Pulse radiolysis. (High energy electron beam or γ-ray irradiation of solutions initiates reactions)
- Flash photolysis. (High intensity flash lamp discharges or pulsed lasers initiate reactions)
- Electrochemical. (Rapid changes of electrode potentials in an electrochemical cell induce redox reactions)

Experimental Arrangements and Instrumentation for TRRS

Fig 1 illustrates one of the ways in which state-of-the-art TRRS data are obtained from photochemical reaction intermediates in the picosecond time regime

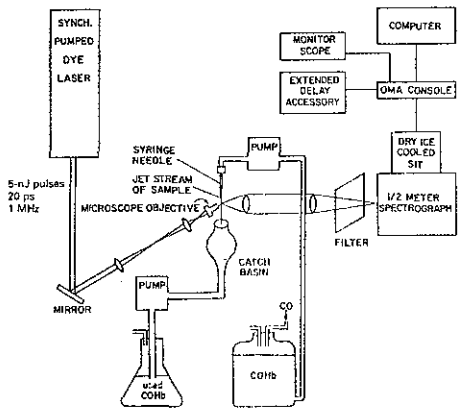

Fig 1 Block diagram of experimental apparatus for detecting chemical reactions taking place in a few picoseconds. In this work each 30 ps pulse of a tightly focused laser beam simultaneously photolyzes and excites the Raman spectrum of carbonmonoxyhemoglobin (COHb). The rapidly flowing liquid stream positions a new sample under each pulse, thus assuring that, as the spectra from multiple pulses are averaged, the same compounds are monitored. The Raman scattered light is dispersed by a spectrograph onto a silicon intensified target (SIT) detector and the resulting spectrum is registered in an optical multichannel analyzer (OMA). A limitation of this technique, associated with its dependence on only one laser, is its inability to discriminate among different reactions occurring within the time frame of a single pulse. (Diagram from Terner *et al.*, Ref. 1).

[1]. This shows a diagram of an experimental set-up for studying the mechanism of photodissociation of CO from carbonmonoxyhemoglobin, COHb. Here the light source for both photolysis and excitation of Raman spectra is a cavity-dumped dye laser (Spectra Physics model 375) synchronously pumped by a mode-locked argon ion laser (Spectra Physics model 171). The combination produces a quasi-continuous train of light pulses, each pulse releasing only 5 nJ of energy with ~ 30 ps duration but with a pulse repetition rate as high as 0.8 MHz. Although the pulse energy is low, peak power is high so it is desirable to change the sample between successive pulses. To this end the laser beam is focused very tightly to a diameter of ~ 1 μm and directed into a vertically flowing free jet of the sample solution. With a flow rate of only 0.2 cm^3/s through a 30-gauge syringe needle, having a bore diameter of 150 μm, a linear flow velocity of 10 m/s through the laser focus is achieved. The 1.2 μs interval between pulses thus corresponds to a flow distance of 12 μm. Residence time of the sample in the 1 μm focal zone is 0.1 μs, a long time compared with the duration of a single laser pulse.

Scanning a spectrum is, of course, inconceivable in only 30 ps. For this type of experiment to be successful, a polychromator or spectrograph and a multichannel detector are required in place of the spectrometer and PMT. With the image intensifiers and high sensitivity television-type or diode-array detectors which have been developed for low-light-level (LLL) military applications, excellent multichannel detectivity is now attainable. Nonetheless, due to the intrinsic weakness of the Raman effect, it is necessary to integrate the received signal over many individual pulsed events. Thus a high laser repetition rate is of great advantage. For the system shown in Fig 1 a silicon-intensified target (SIT) vidicon tube served as the integrating detector and this was controlled and read by an optical multichannel analyser (OMA) manufactured by Princeton Applied Research Corporation (PARC). The spectrograph was made by SPEX. We shall return to this apparatus and look at some experimental results later in this article.

Commonly, lasers with high individual pulse energies but low repetition rates are preferable for TRRS work. Commercially available lasers in this category are most often solid-state Nd-YAG or Nd-glass. However, these are infrared lasers and have therefore to be frequency multiplied by non-linear crystals to generate visible or uv light. Nd-YAG, for example, lases at a wavelength of 1.06 μm. Its frequency doubled output is green light at 532 nm; tripling gives 355 nm, and quadrupling gives 266 nm. Of course, considerable losses occur in these multiplication processes but, in spite of this, several tens of millijoules per pulse may be had at 266 nm with a 10-30 Hz repetition rate from an appropriate commercial laser.

An alternative pulsed uv laser source which is rapidly growing in popularity is the gas excimer laser. Filled with a mixture of a noble gas (Ar, Kr or Xe) and a halogen (F or Cl) as the active medium, an excimer laser delivers relatively high energy pulses (greater than 250 mJ from one standard commercial system) directly in the uv region, typically at a rep rate of 10-50 Hz.

Some of the essential characteristics of these two important laser types are summarized in Table 1. Either type can

Table 1 Some characteristics[a] of Nd/YAG and excimer lasers.

Nd/YAG: Rep rate 2-40 Hz, Linewidth < 1 cm^{-1} (<0.2 cm^{-1} with intra-cavity etalon), Beam divergence < 0.5 mrad.

Wavelength, nm	1064	532	355	266
Energy/pulse, mJ	280	110	50	20
Pulse width, ns	9	7	6	5
Peak power, MW	30	15	8	4
Average power, W (at 10 Hz)	2.8	1.1	0.5	0.2

Excimer: Rep rate 0.1-100 Hz, Beam divergence 5-10 mrad, Beam size 10x25mm, Linewidth 10-30 cm^{-1}.

Laser medium	XeF	N_2	XeCl	KrF	KrCl	ArF	F_2
Wavelength, nm	351	337	308	249	222	193	157
Energy/pulse, mJ	400	16	500	1000	100	500	15
Pulse width, ns	14	6	10	16	9	14	6
Peak power, MW	28	3	50	60	11	35	2.5
Average power, W (at 10 Hz)	4	0.1	5	10	1	5	0.1

a — Representative figures for good commercially available lasers.

pump a dye laser and thus produce narrow linewidth, tunable pulsed laser radiation. Dyes are available to generate laser light throughout the visible range and, again with frequency doubling, even throughout most of the non-vacuum uv. Given that there are still other dye pumps, such as the nitrogen laser or even direct flashlamp discharges, it is clear that the photo-physicist, -chemist, -biologist or -spectroscopist has pulsed laser light sources of tremendous versatility close at hand. Nowadays the difficulty mainly is one of selection and then, of course, affording one's choice.

In spite of the relatively high cost implied, several research groups are combining two separate lasers in TRRS experiments. A good example of such an arrangement is shown in Fig 2 [2]. Here the third harmonic of a Nd-YAG laser (355 nm) photolyzes the sample and, after a controlled time delay, a second Nd-YAG laser is fired to generate a resonance Raman probe beam. This probe is processed by a frequency doubler and a dye laser before arrival at the sample. In one particular application of this pump-then-probe arrangement the uv laser pumped anthracene (10^{-2} molar in benzene) to its electronically excited triplet state. In the presence of a 10^{-4} molar concentration of a carotenoid molecule, such as β-carotene, a bimolecular energy transfer reaction occurred in $\sim 1\ \mu s$ to produce the carotenoid triplet state. This was then examined by the Raman probe laser. The pulse sequence of pump (7 ns laser pulse length), delay (1 μs), probe (7 ns) was repeated at $\sim$ 10 Hz with the sample solution flowing through the observation cell such that each laser pulse pair impinged on a separate volume of sample.

As shown in Fig 2 an alternative method of creating a time delay between the photoexcitation pump pulse and the Raman probe pulse is with an optical delay line. This requires only one laser and a beam splitter to divide the laser output into two beams, one of which (the pump) is directed to the sample while the other (the probe) travels a longer route — perhaps around the lab a couple of times. Since light travels at $\sim 3 \times 10^8$ m/s, a path difference between pump and probe beams of 3 meters corresponds to a time delay of 10^{-8}s, i.e. 10 ns. Clearly, generating microsecond time delays by this scheme is inconvenient.

With low rep rate ($\sim$ 10 Hz) lasers of short (ns or ps) pulse duration the laser is effectively off for much longer times than it is on. Accordingly, when long sequences of pulse pairs are employed in order to accumulate readable signal levels at the detector it makes sense to limit the dark noise of the detector. This is readily achieved by turning off the detector when the Raman probe pulse is off. Specifically a high voltage pulser is connected to the multichannel detector

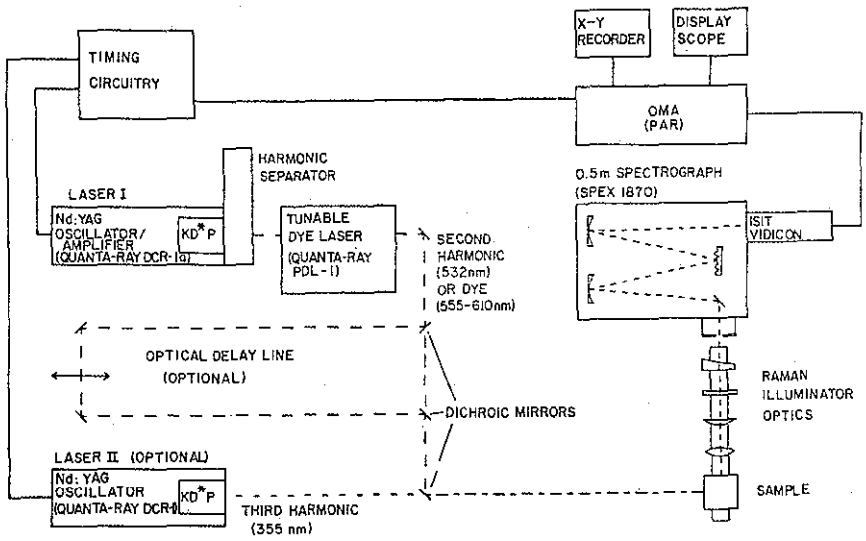

Fig 2 Equipment for pump-then-probe TRRS studies of excited states. An advantage of this arrangement is its movable "time windows" through which the Raman spectra of reaction intermediates may be seen some time after the initial photolysis event. When restricted to one laser, an optical delay provides the time delay for the Raman probe (3 m $\cong$ 10 ns). For longer delays between pump and probe laser pulses a second laser may be used; this probe laser also may be tuned to a wavelength different from that of the photolysis pump laser so as to coincide with an absorption band of a reaction intermediate, thus providing enhanced sensitivity through the resonance Raman effect. In this illustration an intensified SIT (i.e. an ISIT) detects the Raman spectrum. (Dallinger *et al.*, Ref. 2).

to provide synchronized gating of the image intensifier stage. Since the dynamic range of multichannel detectors is severely limited, as compared with photomultiplier tubes, this can be an important factor. For, depending on the sample and experimental arrangement, it may be necessary to accumulate hundreds or even thousands of signal pulses in order to achieve dependable S/N ratios.

Very few laboratories have claimed success in generating measurable Raman spectra from single laser pulses. Since spontaneous Raman band intensities scale with the intensity of the excitation source it might appear that single pulse experiments could be done simply by raising the intensity of the laser. However, there is a problem here, not only in getting higher laser beam intensities but, more seriously limiting, also in the amount of power that can be concentrated into a sample before the onset of non-linear processes or even dielectric break down. A 10 mJ pulse delivered in 10 ns and focused down to a 10 μm diameter spot size generates rather more than a terawatt of power per square centimeter (10^{12} W/cm^2), enough to blast a hole through any sample cell wall!

Perhaps the most successful single-shot TRRS experiments have been those done in Risφ, Denmark, where a flashlamp-pumped dye laser has served admirably as a resonance Raman probe of reaction intermediates generated by electron-beam pulse radiolysis [3]. A diagram of the Risφ pulse radiolysis TRRS set-up is shown in Fig 3. The similarity to the pulsed laser photolysis TRRS arrangement in Fig 2 is immediately apparent, with the Febetron electron accelerator replacing the photolysis laser as the reaction initiator. The fact that the Febetron is essentially a single-shot device dictates the need for single-pulse TRRS in Risφ and the considerable success of these pioneering experiments is a tribute to the experimental skills of

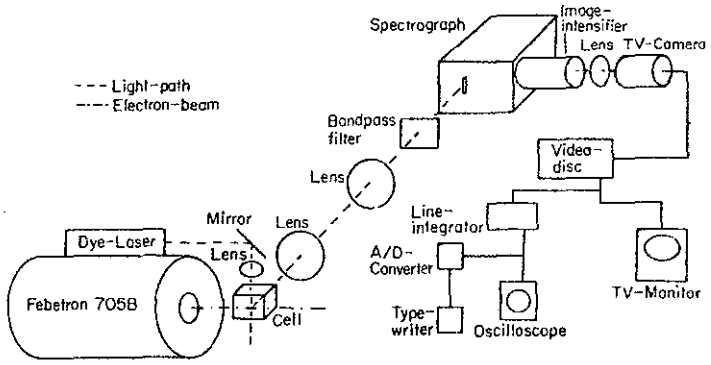

Fig 3 Equipment for TRRS studies of transient species formed in pulse radiolysis. Similar in principle to the apparatus shown in Fig 2, here the pump (radiolysis) pulse is generated by a Febetron electron accelerator rather than a laser. Firing of the Raman probe laser pulse is delayed electronically to provide the variable "time window." (Diagram from Wilbrandt *et al.*, Ref. 3)

the engineers and physicists there.

A final point to be made about instrumentation for pulsed-laser multichannel TRRS experiments concerns the spectrograph. As is always the case for Raman spectroscopy, the instrument ideally should have good luminosity and resolution (vibrational bands are commonly just a few wavenumbers wide and ~ 1 cm^{-1} resolution is desirable for solution studies; better than this for solids or for gaseous samples), and superb stray light rejection. However, for compatibility with a multichannel detector a spectrograph or polychromator must be substituted for the usual monochromator that is the heart of any single-channel scanning instrument. The rejection of stray light through the coupling together of two monochromators with additive dispersion and narrow intermediate slits is impractical for a polychromator which must pass a wide band of frequencies throughout. If a conventional double monochromator *is* selected, its intermediate slits must be opened wide and the final exit slit removed altogether. These conditions are far from optimal for stray light rejection, particularly so since in order to attain a respectable band pass (say 1000 cm^{-1}) it usually is necessary to fit low groove density (low dispersion) diffraction gratings in the instrument and these are unlikely to be of the holographic type. Another shortcoming of adapting monochromators to multichannel detectors is that, unless they are designed to output to a camera as well as to an exit slit and photomultiplier, they usually will produce a distorted image with curved and/or out-of-focus and/or vignetted spectral lines in off-center parts of the detector.

To counter these limitations the Spex TRIPLEMATE (Fig 4) and other 3-stage instruments were designed. In these a double monochromator with subtractive dispersion and a wide intermediate slit becomes at once an efficient stray light filter and band-pass selector. The narrow exit slit serves also as the entrance to the third stage, a spectrograph having a good quality image plane, flat over at least 2 cm. Designers of the three commercial triple systems currently have opted for different ways of varying the effective dispersion of the spectrograph stage so as to allow easy access to either a wide range of frequencies at low resolution or a narrower range at higher resolution. However, only the Spex instrument appears to be suitable for work at short uv wavelengths (to 200 nm) as well as throughout the visible region and into the near infrared (1000 nm). This is because the other commercial instruments incorporate complex glass lens systems which do not transmit at wavelengths shorter than ~ 350 nm. Although most of the multichannel TRRS

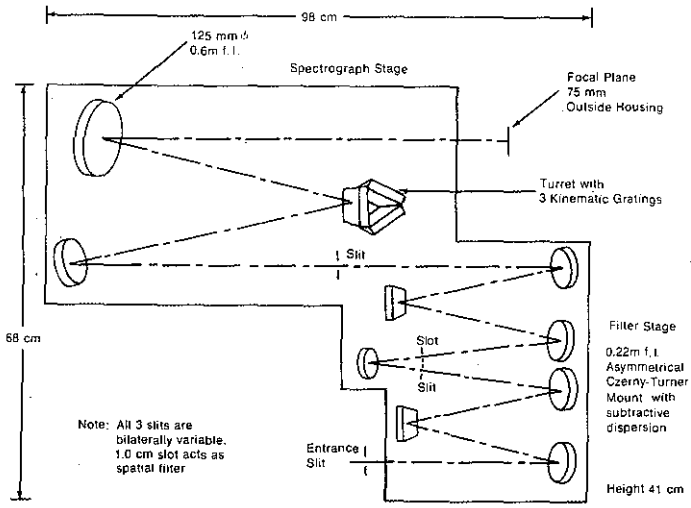

Fig 4 Optical layout of the SPEX TRIPLEMATE triple spectrograph. Its front end comprises a double spectrometer arranged for subtractive dispersion. This yields a variable, homogeneous (non-dispersed) spectrum devoid of disrupting Rayleigh (elastic) scatter from the sample. The third stage of the instrument disperses this light onto a flat focal plane which can be readily coupled to a sensitive detector such as an ISIT (Intensified Silicon Intensified Target) Vidicon or a diode array. A trio of gratings permits operation throughout the uv and visible regions as well as providing selectable bandpasses and resolutions.

work reported to date has, in fact, been done with simple spectrographs preceded by cut-off filters, the new generation of 3-stage instruments is obviously superior in important respects and is therefore making an impact [4].

Lest the technical sophistication of the foregoing experiments seem to render them too exotic for most aficionados of the Raman method perhaps it is time to point out that much worthwhile TRRS work, can, in fact, be done with wholly conventional cw lasers, double monochromators and single-channel photomultiplier instrumentation. Indeed the flow system illustrated in Fig 1 can serve well with such conventional instrumentation to yield TRRS data in the submicrosecond time regime. As shown earlier, a continuous flow of only 0.2 cm^3/s of a sample through a 150 μm jet is equivalent to a residence time in a 1 μm laser focus of only 0.1 μs. The laser may be operating cw and the Raman spectrum may be scanned quite slowly. Such an experimental arrangement has been found suitable for Raman studies of reaction intermediates in the photocycle of bacteriorhodopsin [5]. With reversible photoreactions of this type the sample solution may be recycled to avoid heavy consumption of reagents.

When the flow system is combined with an efficient rapid mixing device, the TRR spectra of any reaction intermediates that are formed may be studied. The diagram of such an arrangement (Fig 5) illustrates the versatility of this method for characterizing reaction mechanisms. The observation delay after mixing is readily varied by adjusting the flow rate or the position of the laser beam downstream from the mixing chamber; the relative flow rates of the reactants may be varied, as may their concentrations. A sizable number of organic redox reactions have been studied by this continuous-flow TRRS technique and a variety of transient free radicals characterized [6].

The familiar stopped-flow technique, which usually is coupled with uv-visible absorption measurements for the determination of reaction kinetics, has also been successfully adapted to TRRS

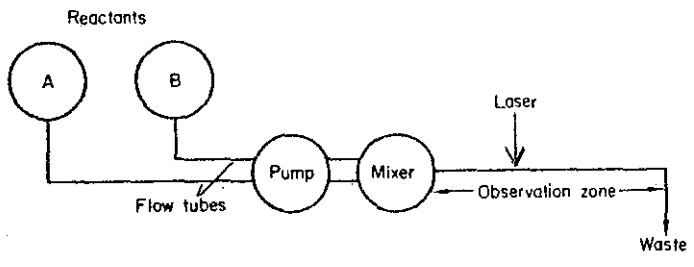

Fig 5 Schematic diagram of a continuous fast-flow apparatus for obtaining TRRS data from reacting systems under steady-state conditions. In this scheme, two or more substances may be reacted. The status of the reaction can be monitored at different times merely by repositioning the Raman laser beam or by changing the reactant flow rates (Hester Ref. 6).

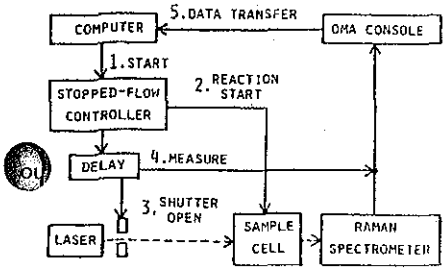

Fig 6 The small amount of reagents required constitutes one advantage of this stopped-flow approach over the continuous fast-flow apparatus depicted in Fig 5. For both methods, however, the mixing process is rather sluggish and this restricts the shortest delay time before the "time window" of the Raman probe laser pulse can be "opened" to a few milliseconds after mixing. (Diagram from Shindo and Hiraishi, Ref. 8).

studies [7, 8]. The main advantage of this method as compared with continuous flow lies in the lower consumption of reagents. Because the reaction progresses entirely within the spectrometer sample cell, a multichannel detector is needed to follow the time course of a reaction. A typical experimental arrangement is diagrammed in Fig 6. Since it takes about a millisecond to mix together two solutions and flow them a few millimeters to an observation zone or cell, chemical mixing flow systems are suitable only for reaction studies in the time scale of several milliseconds or longer.

Other ways in which conventional cw laser, single-channel spectrometer equipment may be adapted for TRRS experiments include electrochemical and matrix isolation methods. Unstable electro-reduction or -oxidation products may be generated at electrode surfaces by rapid modulation of potential and then, under the appropriate conditions, electrode-surface-enhancement of the Raman spectra may increase the sensitivity of the technique. Surface-enhanced Raman scattering (SERS) is most applicable to the coinage metals, particularly silver, after subjecting them to an electrochemical anodization treatment. Raman spectra from molecular species (pyridine is the most extensively studied) adsorbed at such metal surfaces become several orders of magnitude more intense than normal. Although the mechanism of the effect remains subject to controversy there is no doubting its efficacy in enhancing the sensitivity of the Raman probe. Its potential for studies of electrogenerated reaction intermediates was recognized several years ago [9] and a recent study of the electrogenerated free radical cation of methylviologen [10] (Fig 9) serves to illustrate one of the more recent applications.

Continuous flow of a sample solution through a polarized electrochemical cell offers another pathway to redox intermediates which has gained widespread acceptance in electron spin resonance (esr) spectroscopy for the study of free radicals in solution. The method is easily modified for Raman studies. In order to achieve sufficient sensitivity in the Raman measurements to match that of esr (typically, 10^{-4}-10^{-6}M radical concentrations are studied) it is necessary to achieve resonance and/or surface-enhancement of the spectra. TRRS data may also be obtained however from non-radical reaction intermediates and the Raman method claims a further advantage over esr: it is not influenced by the line-broadening mechanisms that often interfere with esr measurements.

Matrix isolation entails the trapping of chemical species at low temperature in rigid media. The matrix medium may be a frozen noble gas or a glassy frozen solvent. Photolysis of molecules trapped in matrices neatly generates stable reaction intermediates which would be very short lived under normal room temperature conditions. At cryogenic temperatures they are preserved in a frozen, unreactive state in the matrix. Spectroscopic studies of such photochemical reaction intermediates are thus possible with ordinary, non-exotic instrumentation. Indeed, this is an area in which infrared spectroscopy has proved outstandingly successful and many articles have been written attesting to this fact [11].

Although less widely publicized, Raman spectroscopy of matrix isolated species has also proved worthwhile in diverse areas and recently has been well reviewed [12]. In Fig 7 some Raman spectra from the carcinogenic substance benzidine illustrate the type of result obtained from uv photolysis of a glassy substance frozen (at 77K, the temperature of liquid nitrogen) in ethanol solution [13]. At $\sim 10^{-3}$ M concentration the colorless benzidine solution gives rise to only a weak spontaneous Raman spectrum with 457.9 nm excitation (spectrum A). However, photolysis results in photo-ionization of the corresponding free radical cation which absorbs strongly at the laser wavelength and hence yields a resonance Raman spectrum. This enhanced spectrum of the free radical photoproduct is shown as C, the result of computer subtraction of the before-photolysis (A) from the after-photolysis (B) spectrum. Astonishingly, this resonance-enhanced radical spectrum is far more intense than even that of the ethanol solvent which is four orders of magnitude more concentrated. Several other unstable organic free radicals have been studied by similar low temperature photolysis methods combined with a Raman probe [14].

Although TRRS is a relatively new field of endeavour, there already are far too many papers in the scientific literature to attempt a comprehensive review of them here. Such a review of TRRS published work has, however, very recently been published [15] and I thoroughly commend this to anyone interested in exploring the field in greater depth. The main thrust of this present article is towards acquainting you, the reader, with the methods of

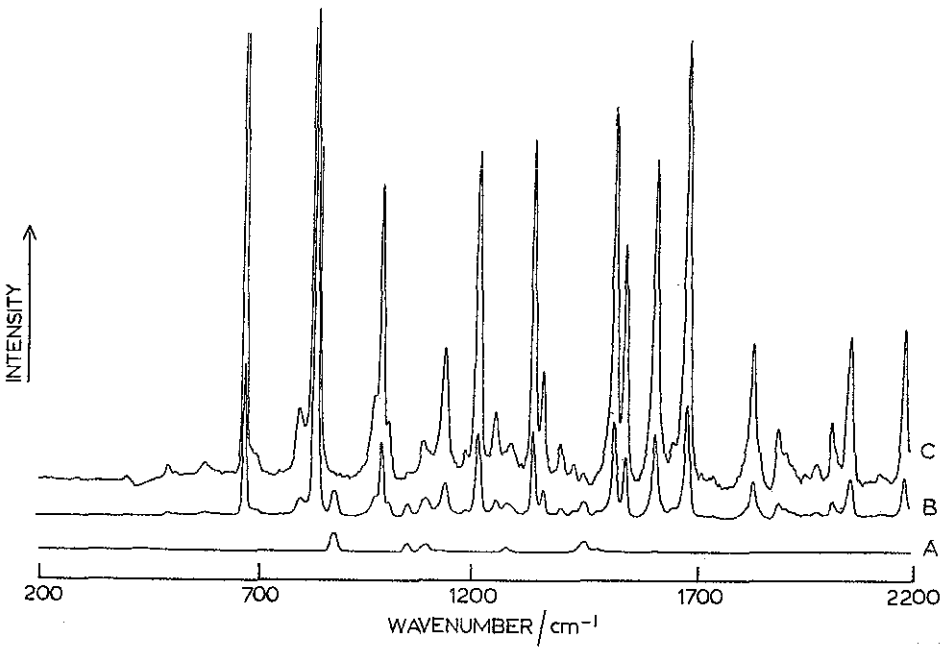

Fig 7 A well-established way of identifying reaction intermediates is to freeze them at liquid nitrogen temperatures and below. At 77K the Raman spectra of a 10^{-3}M benzidene solution before and after photolysis have been taken under 457.9 nm excitation. Only solvent bands appear in A, the spectrum taken before photolysis; B is the spectrum resulting from photolysis. Scale expanded by a factor of four, C equals B minus A; it shows the bands of the benzidine free radical cation. Absorbing strongly at the selected laser wavelength, the free radical cation yields an intense resonance Raman spectrum. (Spectra from Hester and Williams, Ref. 13)

time-resolved Raman spectroscopy and providing some indication of their strengths and weaknesses. Nevertheless, it does seem to be appropriate now to illustrate the methods further with a few specific examples in the hope of persuading you that it all is, somehow, worthwhile!

Some Applications of TRRS

Of late the energy crisis has spawned much interest in photochemical reactions which might some day convert solar energy into other more readily utilized forms. In this context the ruthenium trisbipyridyl complex dication, $[Ru^{II}(bpy)_3]^{2+}$, has become a popular photosensitizer. Absorption of blue light by this molecule results in its excitation to a chemically reducing triplet state. Under the usual (oxygen-free) conditions in which it is deployed, its lifetime in aqueous solution, is ~ 600 ns. The mechanism of this photoexcitation is depicted by the Jablonski diagram of Fig 8 [16]. The electronically excited singlet state (A_2) is much shorter lived (~ 0.1 ns) than the triplet (A_3) state. By taking advantage of the $A_3 \rightarrow A_4$ triplet-triplet absorption, the resonance-enhanced TRRS spectrum of the active (A_3) state has been measured by both a pulsed laser-multichannel method [17] and a cw laser single-channel method [16]. Detailed analysis of the vibrational spectrum has established quite conclusively the structure of this photo-excited species. Unexpectedly it is asymmetric and may be depicted as the free radical $[Ru^{III}(bpy)_2\ bpy^{\cdot-})]^{2+}$. Evidently, the $[Ru^{II}(bpy)_3]^{2+}$ metal ligand charge-transfer (MLCT) absorption transition is localized and results in the creation of a bipyridyl free radical anion coordinated to an oxidized metal atom. In the solar energy context, the excited $[Ru(bpy)_3]^{2+*}$ species may reduce electron donors such as methylviologen, MV^{2+} (1,1'-dimethyl-'4,4'-bipyridine dication), to their free radical counterparts. $MV^{+\cdot}$ may then act as an electron relay and, in the presence of a suitable catalyst, reduce water to hydrogen.

Fig 9 illustrates some Raman spectra from the various species involved in this scheme of reactions [18]. They demonstrate how photoexcited states can be generated in measurable concentrations by tightly focusing a laser beam and also show quite clearly the presence of the free radical intermediate form of the viologen electron relay. As with excited $[Ru(bpy)_3]^{2+*}$, detailed vibrational analysis of the free radical $MV^{+\cdot}$ spectrum provides fundamental information on its structure and bonding [18].

As stated earlier, pump-then-probe TRRS methods have been applied by various investigators as a means of getting a handle on photochemical

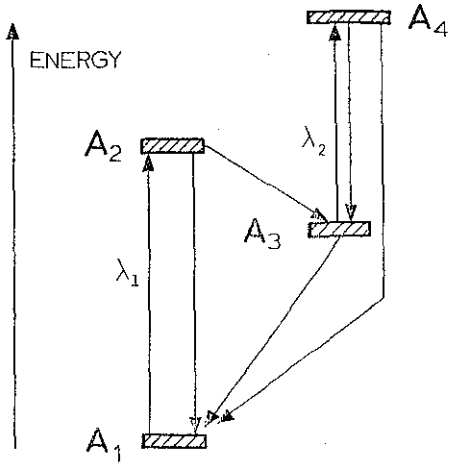

Fig 8 Idealized four-level Jablonski diagram showing electronic singlet (A_1 and A_2) and triplet (A_3 and A_4) states. The $A_1 \rightarrow A_2$ transition is induced by the photolysis pump laser, λ_1. The excited singlet state, A_2, may return to the ground state, A_1, by fluorescence emission or undergo inter-system crossing to the lowest triplet state A_3. The probe laser, λ_2, may excite resonance Raman spectra from A_3 via the excited triplet-triplet transition $A_3 \rightarrow A_4$. Phosphorescence emission may accompany the return to A_1 from A_3 or A_4. (Forster and Hester, Ref. 16.)

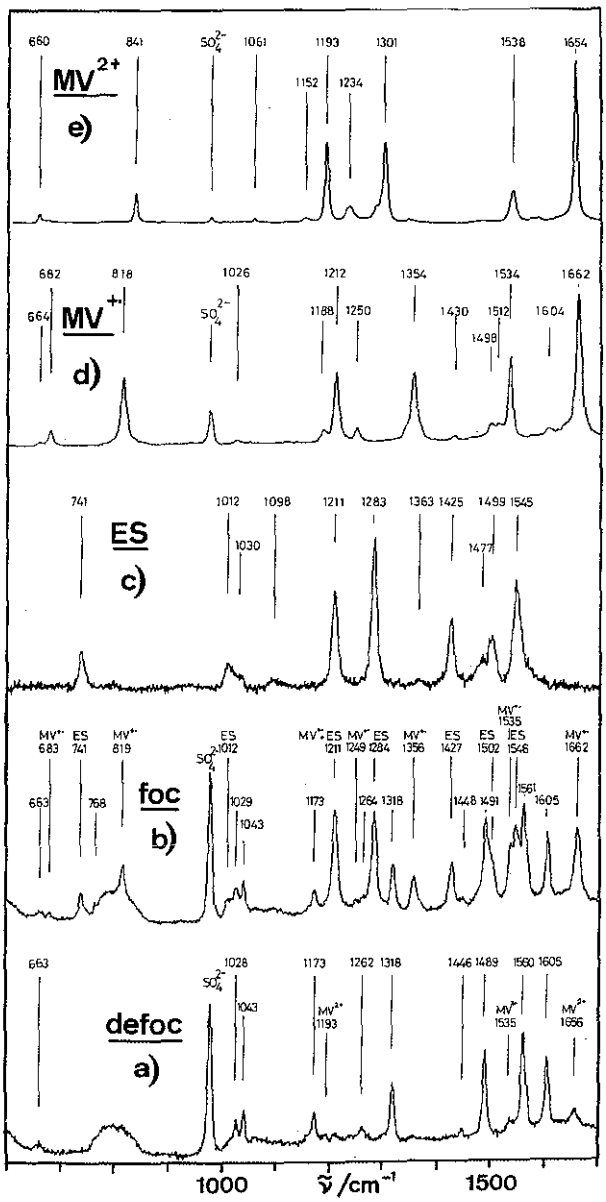

Fig 9 By controlling the power density of a laser impinging on a sample, it is possible either to generate only its Raman spectrum or to both photolyze the sample and generate the Raman spectrum of the reaction intermediate(s). In (a), a defocused laser beam at 350.6 nm was directed into an aqueous solution containing 10^{-3}M of the photoseneitizer $[Ru(bpy)_3]^{2+}$ and MV^{2+} together with 0.5M SO_4^{2-}. In (b), the same solution was photolyzed and Raman excited by a focused laser beam. By scaling and then subtracting the two spectra, the spectrum in (c) of the excited compound $[Ru(bpy)_3]^{2+*}$ was generated.

The top two spectra are of an electron relay, methyl viologen. Spectrum (d) is of the material when reduced to a free radical, $MV^{+\cdot}$; spectrum (e) is of the material in its normal state, MV^{2+}.

The energy crisis has stimulated interest in reactions involving this ruthenium compound (and analogs) and methyl viologen. In the presence of suitable catalysts, sunlight can induce the conversion of the solvent (water) to hydrogen fuel and oxygen via redox reactions of the water with $MV^{+\cdot}$ and the oxidized photosensitizer. (Spectra from Forster and Hester, Ref. 18.)

reaction intermediates. A couple of examples from the recent literature are the studies of photostimulated electron transfers from organic amines to *trans*-stilbene [19] and the characterization of transient intermediates in the photo-Fries isomerization of phenyl acetate [20]. Each of these studies may be considered as illustrating the state of the art. However, perhaps a more familiar molecule is hemoglobin (Hb). It recently has been the subject of intensive study by TRRS methods. As mentioned previously, the photodissociation (deligation) of CO from COHb has been examined by picosecond TRRS methods relying on the same laser pulse as both pump and probe. The Raman spectrum of the photoproduct is similar to that of deoxyhemoglobin but there are significant band frequency shifts in both high and low frequency regions. These indicate complex time-dependences which have been interpreted in terms of various relaxations of the electronic structure, the iron-porphyrin conformation and the tertiary and quaternary structures of the globin protein. O_2Hb and other ligated hemoglobins have also been studied resulting in a rich rapidly growing literature around this subject [21,22]. Fig 10 shows some results from a TRRS study of O_2Hb [22].

Finally, since I suspect that much of the future growth of the TRRS literature will be centered in the area of molecular biology, let me mention one further piece of work from my own laboratory. This involves the characterization of an intermediate redox state of cytochrome c which is formed in alkaline solution by the reaction between ferricytochrome c and the dithionite ion as reducing agent. Fig 11 portrays a few of our spectra; the middle ones (b) we believe are associated with a short-lived intermediate species containing iron (II) in a coordination condition which normally characterizes iron (III). The spectra were obtained with the multichannel detector in combination with cw laser excitation of a flowing reaction mixture [23].

In conclusion, perhaps it should be emphasized that, although great strides have been taken already in making time-resolved vibrational spectroscopy a reality through the exploitation and development of laser Raman methods, more — much more — remains to be done. Awaiting development are a great many ways in which TRRS data can be obtained without recourse to confusing non-linear techniques. Soon we may expect that these will begin to make an impact. From this article it may be seen that Raman spectroscopists have not been slow to capitalize on technological developments in pulsed lasers and sensitive electronic multichannel detectors. Such high technology continues to grow at an ever-increasing rate and recent experience suggests that today's state-of-the-art, seemingly exotic, experiment probably will become commonplace tomorrow. Raman spectroscopic research evidently remains quite a distance from a dead end and the future for such time-resolved studies as have been described in this article looks very inspiring indeed.

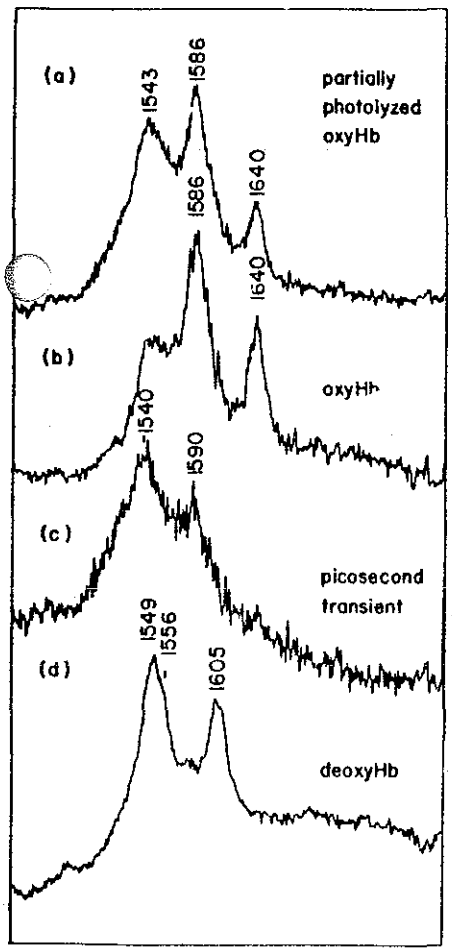

Fig 10 Raman spectra of oxyhemoglobin (O_2 Hb) excited by and photolyzed by 30 ps laser pulses at 532 nm. (a) partially photolyzed O_2Hb, obtained with tight focusing of the laser (~ 100 μm spot); (b) unphotolyzed O_2Hb, obtained with loose focusing; (c) O_2Hb photoproduct, obtained by subtracting (b) from (a) with scaling adjusted to eliminate the 1640 cm^{-1} band; (d) deoxy Hb. Comparison of (c) and (d) shows the effects of molecular structure relaxation following this photo-deligation reaction. (Terner *et al.*, Ref. 22.)

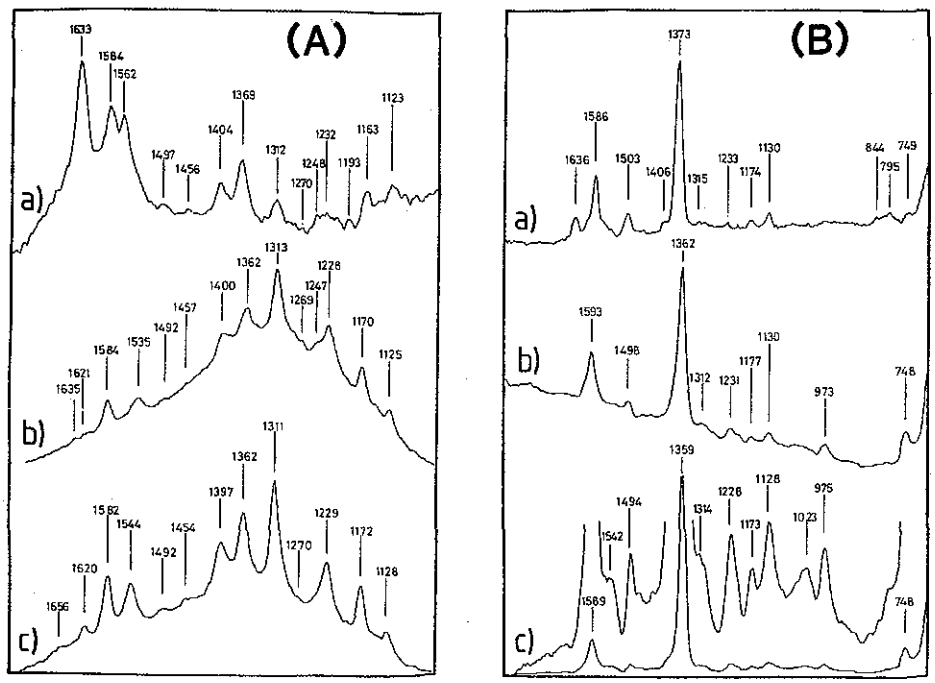

Fig 11 Raman spectra of cytochrome c at pH 10.5 excited at 514.5 nm (A) and 413.1 nm (B). Capillary flow cell and SIT Vidicon detector. 0.1 mM cyt. c in oxidized (a), reduced (c) and intermediate (b) forms; (b) obtained ~ 55 ms after mixing ferricyt. c with a large excess of dithionite. These spectra establish the existence of a short-lived intermediate species containing iron (II) in the coordination condition which normally characterizes iron (III). (Forster *et al.*, Ref. 23.)

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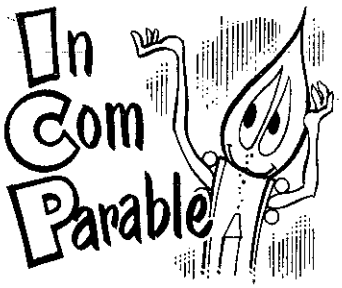

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