Techniques of time-resolved resonance Raman spectra of photochemical transients in the milli- to picosecond time scale

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Abstract — The number of annual publications since 1967 in the field of Raman Spectroscopy as well as related fields such as spectroscopy, lasers and time-resolved Raman is tabulated and compared. Time-resolved Raman is indeed growing at a very rapid rate. The different techniques in this field that are developed in our laboratories over the past decade are discussed and some sample results of the application of these techniques on two important photobiological systems, bacteriorhodopsin and carbon-monoxymyehemoglobin, are briefly given.

INTRODUCTION

In order to accurately understand natural changes, it is imperative that we determine the corresponding structural changes occurring during the course of time over the period during which these changes are taking place. Due to technical limitations, time-resolved study was limited to studying either slow changes or to the slow part of the change. In the absence of time-resolved structural studies with x-ray techniques on the short time scale, optical spectroscopy (flash photolysis (Ref. 1)) was the main tool to study rapid changes in the fifties and sixties. It developed from the millisecond to the fraction of microseconds as flash lamps of short duration became available. Of course, the laser revolution had assisted this field to expand into the nano, picosecond (and now the femto) time domain in the seventies and eighties.

While the field of flash or laser time-resolved optical spectroscopy has greatly assisted in determining the kinetics of many rapidly occurring photochemical changes, they are frequently limited to studying systems with broad optical absorption spectra. The rates of formation and decay of many transients have been carefully determined with these techniques. Due to the lack of vibration structure, structural information concerning these transients are rarely obtainable. For this reason, time-resolved vibrational spectroscopy becomes one of the most useful tools presently available for investigating the mechanisms of rapid physical and chemical changes. Due to large resonance Raman enhancements, high laser photon flux and short laser pulses, the Raman (vibration) spectra, and thus the inferred molecular structures, of short-lived transients are now determined in the subpicosecond to the second time scale. This has led to a more accurate description of the mechanisms involved in a number of photochemical, photophysical and photobiological processes (Ref. 2).

During the past couple of decades, lasers have had a strong positive impact not only on time-resolved spectroscopy but also on science in general, engineering and medicine. New research fields have opened up in which light is used, e.g., laser fusion, laser-mass spectroscopy, holography, CARS, light induced gratings, short time domain research (in the pico and femtosecond regions), resonance Raman and other scattering spectroscopy, high resolution spectroscopy, state-to-state dynamic studies, laser surgery, laser communication, ..., etc.

This review has two different sections, A and B. In section A, the number of papers appearing annually in the different fields related to Raman spectroscopy (e.g., spectroscopy, lasers, time-resolved studies and time-resolved resonance Raman) and abstracted by Chemical Abstracts during 1967 to mid-1984 is tabulated and compared. The results show that while studies in time-resolved Raman spectroscopy were nonexistent before the seventies, they have been growing rapidly over the past eight years. It is because of the properties of lasers (high light intensity, coherence and short pulses) that the Raman spectrum of, and thus structural information on, short-lived transients can be studied.

In Section B of this article we describe some of the different time-resolved (incoherent) resonance Raman techniques that our research group has developed over the past eight years to study transients in the milli, micro, nano and picosecond time scale. Both c.w. and pulsed lasers are used. With c.w. lasers, the use of amplitude modulation of the laser or the microbeam-sample flow techniques enabled us to study transients down to the 0.1 ns time scale for liquid samples. Lasers of short pulse duration are used with the flow techniques...
to obtain the Raman spectra of pico-to-nanosecond transients. The use of optical multi-channel detection is found to be important in all these studies.

A. PROGRESS IN THE RAMAN AND RELATED FIELDS:

A computer search in Chemical Abstracts is carried out to find out the number of papers having in their title the following words: Lasers, Spectroscopy, Raman Spectroscopy, Time Resolved and Time-Resolved Raman. The search was carried out in the files which are divided into the following periods: 1967-1971, 1972-1976, 1977-1979, 1980-1981 and 1982-June '84. Table 1 gives the "minimum" average number of papers per year in each category during these different periods. Assuming that Chemical Abstracts gives an accurate representation, not of the absolute number of papers but rather of the relative growth of the different areas represented, one may reach the following conclusions:

1. All fields have grown during the past period. Never fields have grown at a faster rate than established fields. Thus the fastest growing area is time-resolved Raman (has grown by 3800% since 1972-1976 until now). This is followed by the time-resolved area (grown by 1100% since the 1967-1971 period) and the use of lasers in chemically oriented problems (grown by 850% since the 1967-1971 period). The field of Raman spectroscopy has grown by 322% and the field of spectroscopy has grown by 200% since the 1967-1971 period.

2. In spite of the large growth of the time-resolved Raman and time-resolved fields, the number of papers per year is relatively small (in the latest period this is 23 and 242 papers, respectively).

3. The percentage of the number of papers published annually in Raman spectroscopy to the corresponding number of papers in the mother field of spectroscopy is ~ 2% in the 1967-1971 period, 3% in the 1972-1976 period, and ~ 3.3% since 1976. This might suggest that Raman spectroscopy has benefited relatively more from laser use than the rest of the fields of spectroscopy.

4. The percentage of the annual number of papers in time-resolved Raman to the corresponding total time-resolved papers has increased from 0% in 1967-1971, to 1% in the 1972-1976 period, 7% in the 1977-1979 period and 9% since 1980.

5. The percentage of the annual number of papers in time-resolved Raman to the corresponding number of Raman papers has increased from 0% in 1967-1971; < 0.1% in 1972-1976; 0.7% in 1977-1979; 1.1% in 1980-1981; to 1.5% in 1982-1984.

B-I. THE IDEAL TIME-RESOLVED RESONANCE RAMAN (TR³) EXPERIMENT:

Suppose we have the following change:

\[ P(t_A) \rightarrow t_B \rightarrow t_C \]

where \( t_i \) are characteristic times of the different transformations. The first transformation is induced by a sudden perturbational pulse of duration short compared to the characteristic times \( t_i \). The perturbing pulse could be a pulse of electrons or \( \gamma \) rays (as in pulse
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radiolysis), a heat pulse, a sudden pH change, mixing of another reagent, or a pulse of light to induce the \( A \rightarrow B \) change photochemically. In the latter case, which we shall focus on in this review, transients can be an excited state of \( A \) or a new chemical species. Studies on transients, e.g., free radicals, anion and cation radicals, unstable isomers, intermediates in photobiological transformations, excited states of organic and inorganic systems, constitute the largest fraction of research in the TR\(^3\) field so far.

The most ideal change is that for which \( t_A < t_B < t_C \) and for which the absorption wavelength, \( \lambda_i \), is different for the different species \( i \). The ideal experiment would be performed by using two laser pulses, photolysis and probe, of short pulse durations, whose separation in time and frequency can be varied continuously and independently to record the time profile of each species by following its resonance Raman spectrum at its characteristic resonance absorption wavelength, \( \lambda_i \). Unfortunately, not every laboratory is equipped with two tunable picosecond pulsed lasers. We used only one (c.w. or pulsed laser). We selected systems where the parent and intermediates have overlapping strong absorption bands. Thus the same laser is used as the photolysis and the probe source. The difference between the Raman spectra taken at low and high powers yields information about the Raman spectrum of the transient(s), produced during the characteristic time scale of the experiment.

B-II. TIME-RESOLVED RAMAN WITH A C.W. LASER

1. Transients in the Millisecond-Microsecond Time Scale

One of the first TR\(^3\) experiments on transients was carried out in 1976 by Pagsberg et al. (Ref. 3) in this time scale by using pulse radiolysis and a laser. In our work, the intensity of a c.w. laser is mechanically modulated (Ref. 4) with one of a pair of rotating disk choppers around the sample and rotating synchronously in frequency. This disk has two slits, one large and one small. As they rotate the continuous wave laser light is chopped into a repeating sequence, of an intense photolysis pulse (large slit) followed by a weak probe pulse (from the narrow slit), separated by a delay that depends on the angular separation of the two slits as well as the rotation frequency of the chopper. The second chopper, facing a monochromator (see Fig. 1), has only one slit which is in phase with the

\[ \text{ARGON ION LASER} \]

\[ \text{PHOTOLYSIS SLIT} \]

\[ \text{CHOPPER} \]

\[ \text{PROBE SLIT} \]

\[ \text{SAMPLE} \]

\[ \text{CUT OFF FILTER} \]

\[ \text{SINGLE MONOCHROMATOR} \]

\[ \text{SIGNAL AVERAGER} \]

\[ \text{RECORER} \]

\[ \text{OMA CONSOLE} \]

\[ \text{MONITOR SCOPE} \]

\[ \text{VIDICON PHASE ADJUSTABLE CHOPPER} \]

\[ \text{Fig. 1. An experimental setup for determining the resonance Raman spectra of transients on the millisecond time scale using mechanical modulation of a c.w. laser (Ref. 4).} \]
probe slit in the first chopper and thus acts as a mechanical gate, blocks the scattered radiation from the photolytic flash but allows the Raman scattering from the probe pulse to reach the detector. By varying the delay between the photolytic and probe flashes, kinetic rates can be obtained.

Figure 2 shows the decay of the ethylenic stretch (1567 cm\(^{-1}\)) of the M\(_{412}\) intermediate in the photocycle of bacteriorhodopsin, the other photosynthetic system in nature besides chlorophyll. It has a half life of 7 msec, in agreement with the transient optical absorption measurements. While a multichannel detection system is desirable, these experiments can be performed with a conventional scanning monochromator. Mechanical factors limit the above technique to the millisecond time domain.

The above technique can be modified to obtain the spectra for microsecond transients (Ref. 5) by using one disk with one slit. Comparing spectra at low and high powers gives the spectrum of the transient. Like all the one pulse experiments, scattering from transients formed from zero time up to the resolution time of the experiment contributes to the Raman spectrum. The time resolution of this method is limited by the size of the slit as well as by the motor rotation frequency. In principle, the size of the slit can be as small as the laser beam waist at the diffraction limited focus of the laser beam. With 30 Hz motors, a practical time resolution of this method is in the microsecond time scale (Ref. 5). Species with longer rise times can be detected by increasing the slit width and by changing the laser wavelength.

The use of two pulsed lasers, one of nanosecond and the other of microsecond pulses for studies of microsecond transients was first carried out by Dosser et al. (Ref. 6).
2. **Raman of Micro- and Nanosecond Transients by Using Microbeam Flow Techniques (Ref. 7,8)**

When flow techniques (Ref. 7) are combined with microbeam methods, c.w. lasers can be used (Ref. 8) to determine the spectra of transients in the micro- and nanosecond time domains. With a microscope lens, the laser is focused to a size of a few microns on a flowing liquid sample having a velocity of up to 40 m/s (or much faster for gaseous samples, e.g., from a nozzle beam). The scattered radiation is focused on the slit of a spectrometer equipped with an optical multichannel detector. As the laser power increases, the contribution to the observed spectra from transients formed during the residence time of the flowing sample within the laser focus increases. The residence time of the sample within the laser focus, and thus the resolution time of the experiment, can be as short as 80 ns for liquid samples and can be changed by changing the flow rate or the focus size of the laser on the flowing sample.

**B-III. TIME-RESOLVED RAMAN WITH PULSED LASERS**

1. **Transients in the Micro-Nanosecond Time Scale**

Nanosecond pulsed lasers are found to be useful for resonance Raman of transients in this time domain (Ref. 9). Usually the rep rate is sufficiently slow that good stirring of a large volume insures a fresh sample for each pulse. The change in the spectrum with the laser power is studied and the Raman spectrum of the transient(s) formed within the pulse width (several nanoseconds) is extracted. The use of two tunable nanosecond lasers, one for photolysis and one to probe transients formed at different delay times has been very fruitful (Ref. 10).

2. **Transients on the Picosecond Time Scale**

The number of resonance Raman of photochemical transients formed on the picosecond time scale remains small. As more reliable picosecond lasers become available, more studies in this time scale will undoubtedly be made. To the best of the author's knowledge, the first two independent reports on TR3 of picosecond transients were in 1980 (Ref. 11, 12).

For obtaining the resonance Raman of picosecond transients, we have used the same microbeam-flow setup described in B-II:2, except that a synchronously pumped mode locked Argon ion cavity dumped dye laser was used instead of the c.w. laser (see Fig. 3). This laser produced a train of 30-50 ps pulses of 10 nJ/pulse which are separated by a microsecond. In order to obtain the spectrum of transients formed within the pulse width (from the power dependence studies), the sample has to flow out of the laser focus in between pulses. Focusing with a microscope lens and using sample flow rates of 10-40 m/s, the latter condition can be satisfied.

**Fig. 3.** The microbeam flow technique which is used to determine transients in different time scales. If a c.w. laser is used, the Raman of the intermediates appearing within the residence time of the flowing sample within the laser focus can be determined (down to 80 ns). If a synchronously pumped laser is used, the Raman spectrum of picosecond transients can be recorded if the flow rate of the sample across the laser focus is sufficient to remove the sample exposed to one pulse before the arrival of the next one.
This technique was used to answer two important questions in photobiology. In the first (Ref. 11,13), we have used it to find out the spin state of the Fe$^{2+}$ in the transient formed on the picosecond time scale after carbonmonoxymoglobin photodissociates and loses its CO. This was done by observing the Raman spectrum of the porphyrin system whose stretching vibrations are sensitive to the spin state of Fe$^{2+}$. It was found that the picosecond transient has a spectrum which closely resembles that of deoxyhemoglobin (high spin) rather than the carbonmonoxy complex (low spin). This suggests that spin conversion took place amongst the electronic excited states of the carbonmonoxy complex prior to dissociation (Ref. 13).

In the second photobiological system we studied on the picosecond time scale, bacteriorhodopsin, the question was raised as to whether or not the first step in the photocycle involves the storage of the absorbed photon energy into chemical energy by a configuration change of the retinal (antenna) on the picosecond time scale (Ref. 14). Fig. 4 compares the fingerprint region (1000-1200 cm$^{-1}$) of the resonance Raman spectra of the parent molecule with the different transients (spectra b+f) formed at different time scales after the absorption of light by the parent molecule. The largest changes are observed in the first step (a+b) on the picosecond time scale due to retinal configuration changes and in the last step (e+f) when the retinal Schiff base loses a proton (Ref. 15).

More recently, a pulse and probe technique on the picosecond time scale was developed by Gustafson et al. (Ref. 16). In this system, the wavelength of the pump and the probe can be changed independently and can be delayed from one another by variable amounts of delay times.

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2. For reviews see:


