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-
monoxyhemoglobin, 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 appear-
ing 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 μs 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

TABLE 1:   Growth of the Number of Publications* in Areas Related to, and in, Raman Spectroscopy

		(minimum) Average Annual Number of Published Papers* in			
Period	Lasers	Spectroscopy	Raman Spectroscopy	Time-Resolved Raman	Time-Resolved
1967-1971	1057	21,735	465	0	22
1972-1976	3393	31,808	883	0.6	50
1977-1979	5583	38,761	1213	8	111
1980 and 1981	7259	44,220	1487	16	164
1982-June 1984	9006	44,442	1498	23	242

*
From computer search for papers with titles containing the words heading the different columns and abstracted by Chemical Abstracts during the different periods given.

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. Newer 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 (TR^3) EXPERIMENT:

Suppose we have the following change:

$$P(t_A) \qquad t_B \qquad t_C$$
$$A \text{------}\rightarrow \quad B \text{~~~~~}\rightarrow \quad C \text{~~~~~}\rightarrow \qquad \cdots \cdots \cdots \cdots \qquad (1)$$

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 γ rays (as in pulse

radiolysis), a heat pulse, a sudden pH change, mixing of another reagent, or a pulse of light to induce the A~~→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, λ_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, λ_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
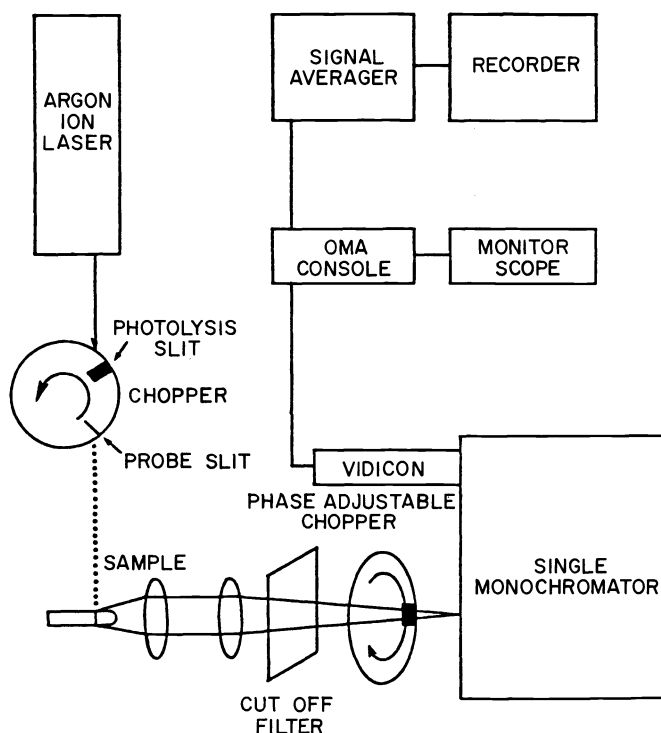

Fig. 1. An experimental setup for determining the resonance Raman spectra of transients on the millisecond time scale using mechanical modulation of a c.w. laser (Ref. 4).

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 photo-
synthetic 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.
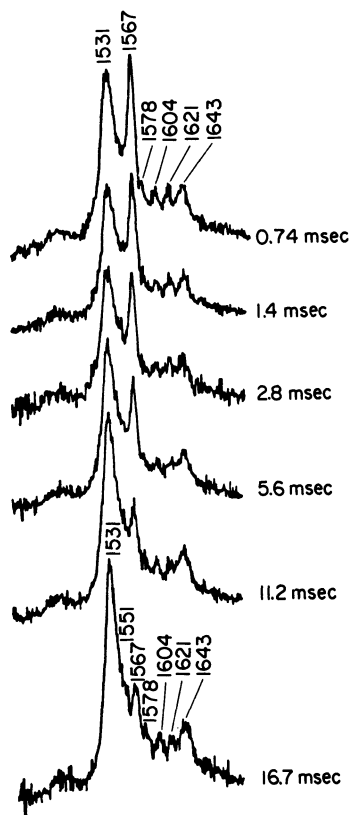

Fig. 2. The results of using the
apparatus in Fig. 1 on the M_{412}
intermediate in the bacteriorhodopsin
photocycle in the C=C stretching
vibration frequency region. As the
separation between the pump and probe
slits in the rotating disks increases,
the band intensity of the vibration
at 1567 cm^{-1} decreases giving a decay
time for this species of ~ 7 ms, in
agreement with previous optical studies
in the 400 nm region. The times given
on the side are calculated from the
angular separation of the two slits
and the motor rotation frequency.

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 suf-
   ficiently 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 TR[3] 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 synchro-
   nously 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
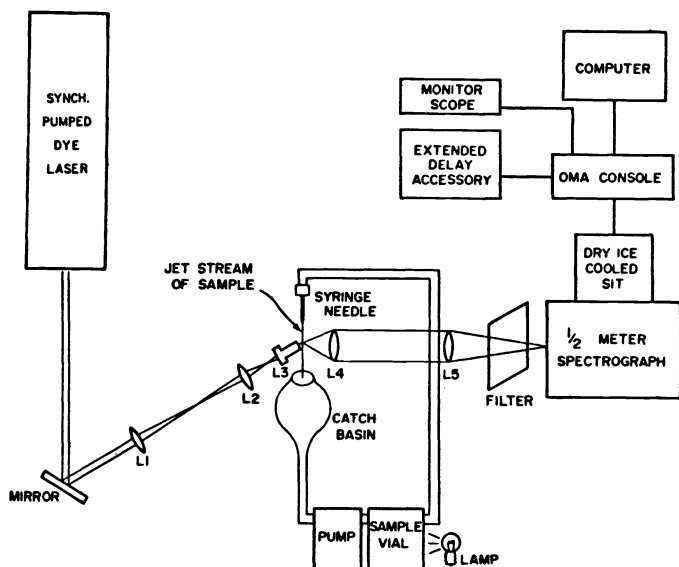   sample flow rates of 10-40 m/s, the latter condition can be satisfied.

Fig. 3. The microbeam flow tech-
nique 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 synch.
pump 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
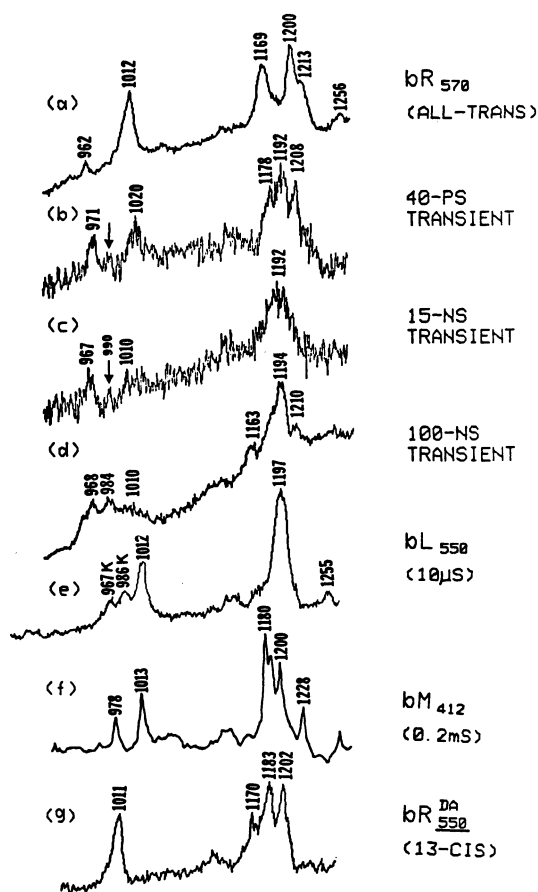sample exposed to one pulse
before the arrival of the next
one.

Fig. 4. A comparison of the time-resolved Raman spectra of the different transients (spectra b→f) appearing in different time scales in the configuration sensitive region (1000–1200 cm^{-1}) during the photocycle of bacteriorhodopsin. The spectra for the parent molecule (containing all trans-retinal; spectrum a) and that for bR$^{DA}_{550}$ (containing 13-cis-retinal; spectrum g) are also shown. These spectra show the time history of the retinal changes during the photocycle (Ref. 15).

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 carbon-monoxyhemoglobin 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 deoxy-hemoglobin (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 wave-length of the pump and the probe can be changed independently and can be delayed from one another by variable amounts of delay times.

REFERENCES

1. R. G. W. Norrish and G. Porter, Nature 164, 658 (1949).
2. For reviews see:
   a. M. A. El-Sayed, "Time Resolved Resonance Raman Spectroscopy in Photochemistry and Photobiology," in Multichannel Image Detectors in Chemistry, ACS SYMPOSIUM SERIES Bk. 102, Chpt. 10, pp. 215-227 (1979).
   b. G. H. Atkinson, Time-Resolved Raman Spectroscopy, Vol. 9, Eds. R. J. H. Clark and R. E. Hester, Heyden, London (1982) pp. 1-62.
   c. J. Terner and M. A. El-Sayed, "Time-Resolved Resonance Raman Spectroscopy of Transients in Photochemistry and Photobiology," Accounts of Chemical Research, to be published.
3. P. Pagsberg, R. Wilbrandt, K. B. Hansen and K. V. Weisberg, Chem. Phys. Letters 39, 538 (1976).
4. J. Terner, A. Campion and M. A. El-Sayed, Proc. Natl. Acad. Sci. USA 74, 5212 (1977).
5. A. Campion, M. A. El-Sayed and J. Terner, Biophys. J. 20, 369 (1977).
6. L. R. Dosser, J. B. Pallix, G. H. Atkinson, H. C. Wang, G. Levine and M. Szwarc, J. Chem. Phys. 62, 555 (1979).
7. M. A. Marcus and A. Lewis, Science 195, 1382 (1977).
8. J. Terner, C.-L. Hsieh, A. R. Burns and M. A. El-Sayed, Proc. Natl. Acad. Sci. USA 76, 3046 (1979).
9. a. A. Campion, J. Terner and M. A. El-Sayed, Nature 265, 659 (1977).
   b. K. B. Lyons, J. M. Friedman and P. A. Fleury, Nature 275, 565 (1978).
   c. R. F. Dallinger, J. R. Nestor and T. G. Spiro, J. Amer. Chem. Soc. 100, 6251 (1978).
   d. W. H. Woodruff and S. Farquharson, Science 201, 831 (1978).
10. a. J. M. Friedman and K. B. Lyons, Nature 284, 570 (1980).
    b. S. Beck and L. Brus, J. Chem. Phys. 75, 4934 (1981).
11. J. Terner, T. G. Spiro, M. Nagumo, M. F. Nicol and M. A. El-Sayed, J. Amer. Chem. Soc. 102, 3238 (1980).
12. M. Coopey, H. Tourbez, P. Valat and B. Alpert, Nature 284, 568 (1980).
13. J. Terner, J. D. Stong, T. G. Spiro, M. Nagumo, M. Nicol and M. A. El-Sayed, Proc. Natl. Acad. Sci. USA 78, 1313 (1981).
14. C.-L. Hsieh, M. Nagumo, M. Nicol and M. A. El-Sayed, J. Phys. Chem. 85, 2714 (1981).
15. C.-L. Hsieh, M. A. El-Sayed, M. Nicol, M. Nagumo and J.-H. Lee, Photochem. Photobiol. 38, 83 (1983).
16. T. L. Gustafson, D. M. Roberts and D. A. Chernoff, J. Chem. Phys. 79, 1559 (1983).