

New Approaches in Laser Photolysis Measurements

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Introduction

The last four decades have seen remarkable progress in the development of techniques for time-resolved spectroscopy. It is common to think of this progress solely in terms of the great improvement in time resolution of spectroscopic measurements that have been achieved over the years. The introduction of flashlamps made it possible to use flash photolysis techniques to measure absorption spectra of transient species with millisecond¹ and microsecond lifetimes² in the early 1950s. With the advent of laser technologies, the time resolution of photolysis studies increased from nanoseconds in the 1960s^{3,4} to picoseconds in the 1970s.⁵ Finally, in the 1980s, photolysis studies with femtosecond time resolution have become possible.⁶ Further advances in time resolution of these experiments is unlikely as they could be achieved only at the expense of reasonable spectral resolution due to limitations imposed by the uncertainty principle.

Improvement in time resolution of 12 orders of magnitude is certainly impressive and has made it possible to study many processes that would be inaccessible by other means. However, the basic concept behind these photolysis experiments, and the kind of information available from them, has been similar regardless of the time scales in which they are applied. An intense light source (the pump source) excites a sample to induce a change in state of the absorbing molecule and, in some cases, to initiate a photochemical process. The changes induced in the sample are then monitored by passing the beam of a second, weaker light source (the probe source) through the sample. The change in intensity of the probe beam transmitted through the sample is then measured as a function of time and/or wavelength. This information can then be used to determine kinetic and spectral characteristics of new species produced subsequent to excitation.

This Account will not concentrate on new developments in improving time resolution of the type of laser photolysis techniques described above. Instead, it will discuss alternative techniques that make it possible to obtain information about short-lived species or rapid photoprocesses that is not available from traditional time-resolved absorption studies. For example, photoacoustic techniques make measurements of time-resolved spectra more sensitive, and diffuse reflectance techniques make it possible to measure time-resolved absorptions in opaque samples. Other techniques

combine laser photolysis with various forms of spectroscopy, such as natural and magnetic circular dichroism and X-ray studies, which are more sensitive to structural properties of molecules than standard absorption measurements. Techniques have also been developed in recent years to combine photolysis techniques with magnetic resonance spectroscopies. Space restrictions prohibit discussion of these latter techniques in this Account. To learn about them, the reader is thus referred to recent articles describing flash photolysis ESR techniques^{7,8} as well as to articles describing the use of similar methods applied to photolysis NMR studies.⁹⁻¹¹

Alternative Methods for Probe Detection

Improvements in time resolution of laser photolysis measurements have primarily been driven by the availability of new pump sources with higher intensities and shorter pulse widths. Aside from these improvements in time resolution, new developments in time-resolved spectroscopy that will be discussed here have generally involved different ways of producing or detecting the probe beam. In some cases, standard methods of monitoring changes in transmission of a probe beam in laser photolysis measurements may not be appropriate. For example, more sensitive methods may be needed for detecting weak signals, or samples to be studied may not be sufficiently transparent to make transmission measurements. Several methods for more indirectly monitoring spectral changes can be useful in such cases.

Photoacoustic Detection. Photoacoustic spectroscopy (PAS) has been used for some time as a sensitive tool for measuring small absorption changes.¹² After a ground-state molecule absorbs light and becomes excited, it may return to its ground state through a radiationless transition. In the process, the sample will be heated, and this will result in localized density changes in the sample. As a result, a pressure wave, or acoustic wave, will be produced. By detection of this wave with a microphone, extremely sensitive measurements of light absorption can be made.

PAS techniques can also be used to measure time-resolved spectra. This was first suggested in 1977 when

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David Kliger was born in Newark, NJ, on Nov 3, 1943. He received a B.S. degree in chemistry from Rutgers University in 1965 and a Ph.D. in physical chemistry from Cornell University in 1970. After spending a postdoctoral period at Harvard University, he joined the faculty at the University of California, Santa Cruz, where he is currently Professor of Chemistry. He pioneered the use of the thermal lensing technique in multiphoton absorption studies and the use of photoacoustics in time-resolved absorption studies and developed the time-resolved CD and MCD techniques described in this review. He has applied these techniques and other spectroscopic tools to a wide variety of studies in photochemistry and biophysics.

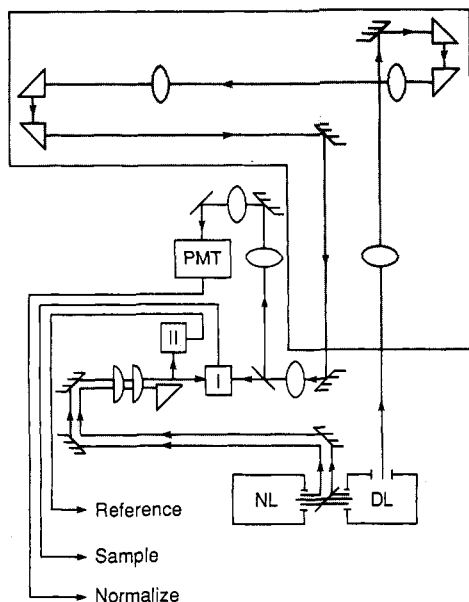


Figure 1. Schematic of an apparatus to measure transient absorption spectra by using a differential photoacoustic method. Light from the pump nitrogen laser, NL, excites the photoacoustic sample and reference cells, I and II, as well as a dye laser, DL, probe source. The probe light passes through an optical delay line for timing probe pulses relative to pump pulses before entering cell I. Signals from cells I and II are subtracted in a differential amplifier and are normalized to probe intensity as detected by a photomultiplier, PMT. See ref 15 for details.

Rockley and Devlin saw nonlinear photoacoustic signals in rose bengal due to an excited-state absorption.¹³ Actual time-resolved measurements of transient absorptions were observed soon after this observation in picosecond measurements of excited-state absorptions.¹⁴ In each of these studies, absorption kinetics were observed at a single wavelength. A variation of the photoacoustic method developed by Yee and Kliger¹⁵ made nanosecond time-resolved spectral measurements more tractable.

A difficulty in using PAS to measure transient absorptions is that in the initial absorption process, which generates the species under study, a large photoacoustic signal is often produced which may conceal smaller signals resulting from absorptions by the transients of interest. To avoid this problem, Yee and Kliger used a differential PAS technique as shown in Figure 1. Here two identical sample cells are excited by equal-intensity pump beams with a second probe beam passing through only one of the cells. PAS signals from these two cells are subtracted in a differential amplifier so the resulting PAS signal is due solely to absorption of the probe.

This approach for measuring time-resolved photoacoustic spectra was used to measure excited-state absorption spectra as well as decay times. Figure 2 shows, for example, excited singlet and triplet absorptions seen in 1,2:3,4-dibenzanthracene by the PAS technique¹⁵ and by standard methods.¹⁶ This PAS method has the

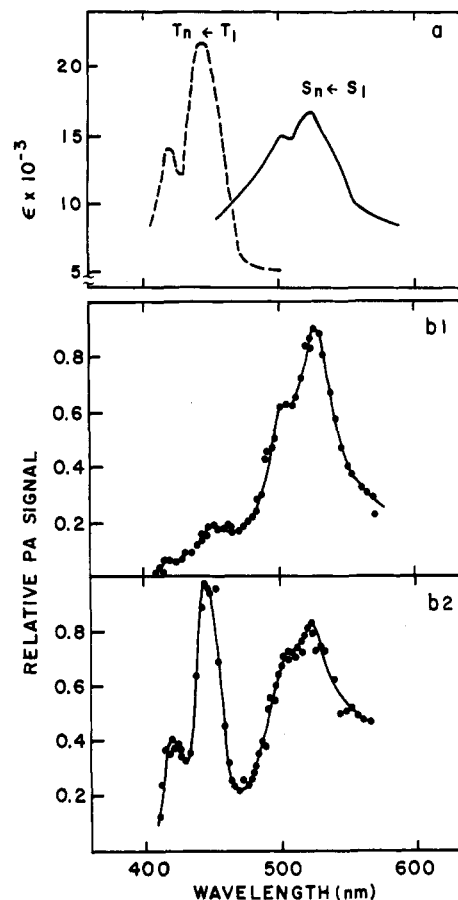


Figure 2. Time-resolved absorption spectra of 1,2:3,4-dibenzanthracene in cyclohexane solution as measured with the apparatus shown in Figure 1¹⁵ and by standard photolysis techniques.¹⁶ Top: Excited singlet state and triplet state absorptions measured by conventional techniques. Middle: Transient PAS signal obtained when pump and probe pulses enter the sample cell simultaneously. This signal is due primarily to excited singlet state absorption, with some contribution by triplet absorption. Bottom: Transient PAS signal obtained when probe beam is delayed 25 ns relative to the pump pulse. Here some of the singlets have decayed to produce more triplet states so the spectrum is a combination of absorptions from both states. Reprinted with permission from *J. Phys. Chem.* 1983, 87, 1887. Copyright 1983 American Chemical Society.

disadvantage of requiring point-by-point time as well as wavelength measurements. However, the measurements were shown to be 40 times more sensitive than standard laser photolysis absorption measurements. The PAS technique should also be particularly useful for studying transient absorptions of highly fluorescent species. While fluorescence interferes with direct optical absorption measurements, efficient fluorescence means there will be smaller photoacoustic signals from the initial absorption process to interfere with PAS signals from transient absorptions.

An interesting variation of the photoacoustic method for determining excited-state absorptions combines photoacoustic techniques with transient grating techniques.¹⁷ In transient grating experiments,¹⁸ two crossed, time-coincident laser pulses absorbed by a sample induce an interference pattern in the sample with fringe spacing determined by the wavelength of the beams and their crossing angle. This generates a

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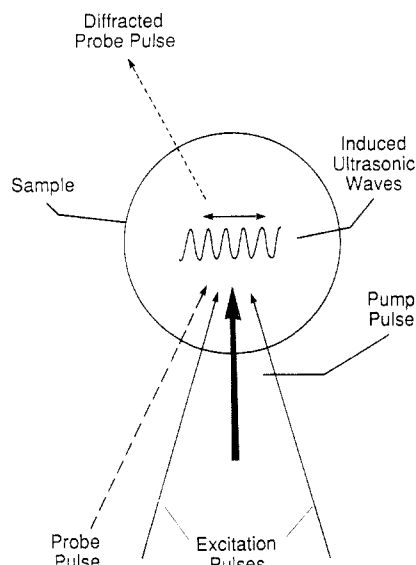


Figure 3. Schematic representation of how the transient grating technique can be used to measure excited-state spectra. A pump pulse initially populates the excited states. Two excitation pulses, with wavelength λ at which the excited state absorbs, are crossed in the sample at some later time. This produces an acoustic grating whose amplitude is proportional to the absorption probability of the excited state at λ . The amplitude of this grating is then measured by Bragg diffraction of the probe pulse.

spatial periodicity in the refractive index of the sample that mimics the periodicity of the optical interference pattern that produces it. The sample then acts as a Bragg diffraction grating. Thus when a probe pulse is passed through the sample, part of the probe pulse is diffracted off in a unique direction as a collimated beam.

Figure 3 shows how the transient grating technique can be modified to yield transient spectra by using a sequence of four laser pulses.¹⁷ First a pump pulse excites the molecules from their S_0 to S_1 states. Following this creation of the excited states, two excitation beams at a wavelength corresponding to an excited-state absorption ($S_n \leftarrow S_1$) are crossed in the sample. Absorption of the light from the optical interference pattern of these pulses results in a grating pattern of high-lying S_n excited states. Relaxation of the S_n states produces local heating of the sample with a pattern that mimics the grating pattern and launches an ultrasonic wave. This wave is then monitored by the Bragg diffraction of the probe beam whose amplitude is proportional to the magnitude of the $S_n \leftarrow S_1$ absorption. Since only the excited-state absorption, and not the ground-state absorption, produces the grating pattern in this technique, the acoustic wave produced by S_0 absorption does not contribute to the measured signal.

Another interesting aspect of time-resolved PAS is that one can use it for time-resolved calorimetry measurements. By using transducers having different frequency responses to measure photoacoustic waveforms produced from samples excited by pulsed lasers, one can determine enthalpies of various intermediates with lifetimes from 10 ns to 50 μ s.¹⁹ Such measurements can also be used to determine volume changes that take

place as a result of photochemical reactions.²⁰ Volume changes that contribute to PAS signals can result from thermal effects from the reaction which induce solvent volume changes and from volume changes in the reactants themselves. Since the former are strongly temperature dependent while the latter are nearly temperature independent, one can determine both enthalpy changes and volume changes of photochemical reactions by analyzing PAS signals obtained at different temperatures.^{20,21}

Diffuse Reflectance Measurements. As discussed above, most laser photolysis measurements monitor changes in sample transmission and thus must be carried out with transparent samples. For highly scattering samples, the standard methods must be modified. The photoacoustic technique works better than transmission methods on opaque samples, but it is not ideal, since scattered laser light striking the acoustic detector can cause spurious signals. Furthermore, inhomogeneities which cause light scattering can often also result in poor acoustical transmission in samples.²²

Fortunately, a method has been developed that works optimally with highly scattering samples. This diffuse reflectance laser photolysis technique was first reported by Kessler and Wilkinson in 1982.²³ As the name suggests, this method takes advantage of the fact that opaque samples cause diffuse scattering as well as specular reflection of incident light. Specular reflection is a surface phenomenon and results in reflection of incident light at an angle equal to the angle of incidence. In diffuse reflection, however, incident light significantly penetrates the sample, undergoes multiple reflections, and is scattered from the sample over a broad range of angles. While specular reflection will not be significantly affected by sample absorptions, the intensity of diffusely reflected light will depend on sample absorption. Thus, time-resolved measurements can be made by monitoring the intensity of probe light diffusely reflected from opaque samples.

Most diffuse reflectance studies have measured absorption kinetics one wavelength at a time. However, a recent study introduced a detection system using a spectrograph and optical multichannel analyzer²⁴ to the diffuse reflectance technique to make time-resolved studies of opaque samples with high spectral resolution possible.²⁵ An example of spectra measured with this technique is shown in Figure 4. There, absorption spectra of the lowest excited triplet state of anthracene at 12 K in 3-methylpentane glass and in an *n*-heptane Shpol'skii matrix are shown.

The Shpol'skii effect has often been used to obtain high-resolution emission and excitation spectra of molecules that normally have relatively broad, unstructured ground-state spectra.²⁶ Yee and Kliger looked at the triplet spectrum of anthracene to see if similar sharpening would be found in excited-state

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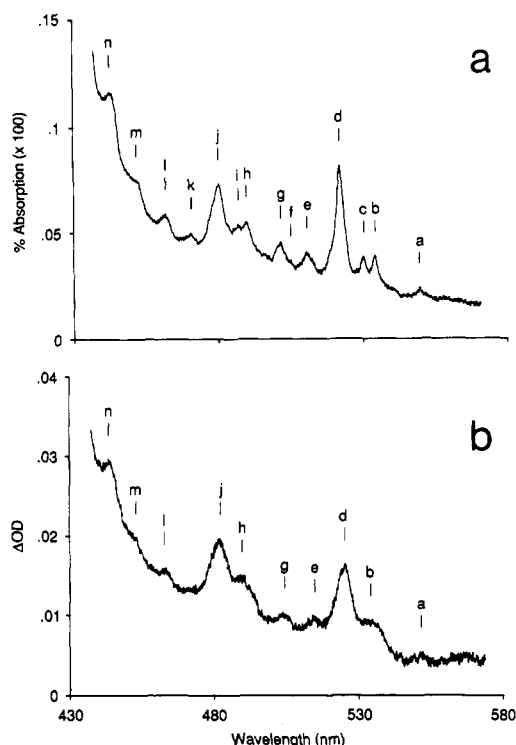


Figure 4. Triplet-triplet absorption spectra of the weak ${}^3B_{3u} \leftarrow {}^3B_{2u}$ ($T_5 \leftarrow T_1$) and ${}^3A_{1g} \leftarrow {}^3B_{2u}$ ($T_4 \leftarrow T_1$) transitions of anthracene at 12 K measured by (a) a diffuse reflectance technique in an *n*-heptane Shpol'skii matrix and (b) a standard transmission laser photolysis technique in a 3-methylpentane glass.

transitions. Standard laser photolysis techniques could not be used since Shpol'skii matrices are highly light scattering. The PAS technique did not work well on such samples because of problems discussed above. As Figure 4 shows, however, the diffuse reflectance technique makes it possible to obtain very nice time-resolved spectra of these opaque samples. Bands shown in Figure 4 are due to the very weak $T_4 \leftarrow T_1$ and $T_3 \leftarrow T_1$ transitions and show a weak Shpol'skii effect in which bands sharpen by about a factor of 2. The strong $T_5 \leftarrow T_1$ band at higher energy (1 order of magnitude more intense) showed no sharpening in the Shpol'skii matrix.

Methods Sensitive to Molecular Structures

The techniques discussed above allow the measurement of time-resolved spectra with increased sensitivity or in unusual types of samples. However, most of them basically yield absorption spectra of transient species just as more traditional flash photolysis experiments do. Recent advances also make it possible to combine laser photolysis techniques with various forms of spectroscopy that are more sensitive to molecular structures. While time-resolved Raman spectroscopy has been used for some time,²⁷ only recently has it become possible to make circular dichroism (CD), magnetic circular dichroism (MCD), electron spin resonance (ESR), nuclear magnetic resonance (NMR), and X-ray measurements with high time resolution.

Time-Resolved Circular Dichroism. Circular dichroism is valuable as a spectroscopic tool that is sensitive to certain molecular structures. Natural CD is only exhibited by asymmetric molecules, and CD

spectra often yield valuable information about the nature of the asymmetry in the spatial structures of these molecules or about asymmetric molecular environments. CD spectra have played important roles, for example, in determining the helical content of biologically important molecules. Magnetic circular dichroism (MCD), the circular dichroism induced in spectral transitions by a magnetic field, can also be used as a tool that is sensitive to molecular structure. The physical basis of MCD is different from that of natural CD, and it is not necessary for molecules to be asymmetric to exhibit MCD. All molecules will yield MCD spectra in the presence of a sufficiently large magnetic field. The form and intensities of MCD spectra are strongly affected by near degeneracies in electronic energy levels. As a result, MCD spectra are exquisitely sensitive to subtle changes in the structures or environments of chromophores.²⁸ In samples that exhibit both natural and magnetic CD, the two techniques can yield complementary information. In hemoglobin, for example, the visible-band MCD reflects the coordination symmetry and spin state of the heme group while the natural CD spectrum in the Soret region reflects the asymmetry of the heme protein environment.

CD and MCD effects tend to be rather small and thus difficult to measure. For a spectral transition with extinction coefficient ϵ on the order of 10^4 , a typical natural CD, the difference in extinction coefficients for left and right circularly polarized light, $\Delta\epsilon = \epsilon_l - \epsilon_r$, would be on the order of 1–10. Determining the CD by directly measuring absorption of left and right circularly polarized light and taking the difference would thus require a very high signal-to-noise ratio. The standard method for measuring CD uses a photoelastic modulator to produce alternately right and left circularly polarized light with kilohertz modulations, and the alternating difference in absorption is measured directly as the differential ac signal. Since these modulators are resonant devices, their frequency cannot be significantly increased (to do so would mean reducing their size significantly, thus reducing optical throughput), and this has restricted time-resolved CD measurements to the millisecond or longer time regime. Recently, a new time-resolved CD technique (TRCD) has been developed which makes it possible to increase greatly the time resolution of CD measurements.²⁹

The TRCD technique, employing an apparatus shown schematically in Figure 5, measures CD by determining the change in ellipticity of elliptically polarized light which passes through a circularly dichroic sample. The principles behind these measurements have recently been described in detail.³⁰ The technique uses a typical laser photolysis approach except that the polarization properties of the probe beam are modified, and it is the changes in these polarization properties caused by the sample that are monitored rather than merely the changes in probe intensity. After passing through a linear polarizer, the probe light goes through a strained fused silica plate which acts as a retarder whose fast axis is oriented at $+45^\circ$ or -45° to the light-polarization axis. This produces elliptically polarized light whose major axis is aligned along the ori-

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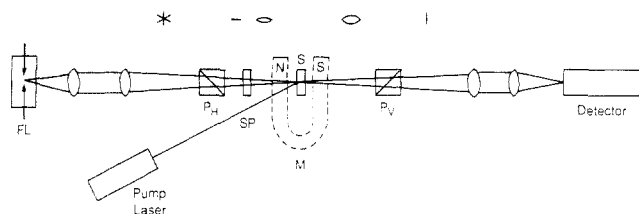


Figure 5. Apparatus used for time-resolved CD measurements. For photoinitiated reactions, the pump laser is used as a photolysis source. Probe light from a xenon flashlamp (FL) is collimated and focused into the sample (S). Before entering the sample cell, the probe beam passes through a polarizer (P_H) and a strained fused silica plate (SP), which acts as a retarder to produce elliptically polarized light. For MCD measurements, the sample is surrounded by a magnet (M). After the beam passes through the sample, the change in the polarization ellipticity is determined by passing the beam through another polarizer (P_V) oriented perpendicularly to P_H . The probe light is then recollimated and focused into a detection system consisting of either a monochromator and photomultiplier to measure the kinetics of CD changes or a spectrograph and optical multichannel analyzer to measure CD spectra at fixed times following photolysis. Polarization properties at various points along the optical train are indicated above the optical train.

ginal linear-polarization axis and whose major-axis intensity is about 10^4 times the minor-axis intensity. Elliptically polarized light can be thought of as comprising components of left and right circularly polarized light of different intensities with a greater difference in the intensities of the two components yielding smaller ellipticity.

When the elliptically polarized probe beam passes through a circularly dichroic sample, the differential absorption results in changes in the relative intensities of the two circular components, and this results in a change in the polarization ellipticity. For example, if left elliptically polarized (LEP) light passes through a sample with positive CD (i.e., $\epsilon_l > \epsilon_r$), the left circular component will be absorbed more than the right circular component. The emerging light will then have greater polarization ellipticity (larger major component relative to minor component). For a sample with negative CD, the polarization ellipse would become fatter.

From the above it should be clear that monitoring the change in polarization ellipticity of the probe will enable one to determine the CD of a sample. To do this, the probe beam is sent through a second polarizer oriented perpendicularly to the first. This passes light oriented only along the minor axis of the ellipse. One can measure this intensity for a right elliptically polarized (REP) probe, I_r , and an LEP probe, I_l . The quantity $S = (I_r - I_l)/(I_r + I_l)$ is then directly proportional to $\Delta\epsilon$ of the sample. Furthermore, for a sample with a $\Delta\epsilon/\epsilon$ of $1/10\,000$, this vertical-component intensity will change by $1/10$. This greatly reduces the signal-to-noise ratio in intensity measurements that one needs for CD measurements and makes it possible to make CD measurements much more rapidly.

This method for measuring CD is, by its nature, quite sensitive to linear birefringence effects. Thus, excessive strain in elements along the optical path can affect the polarization ellipticity, just as the strained fused silica plate does, and can cause artifacts. Interestingly, excitation from the laser pulse itself can cause such an artifact in some cases. A linearly polarized laser will excite only a subset of molecules with specific orientations due to the photoselection effect. For molecules

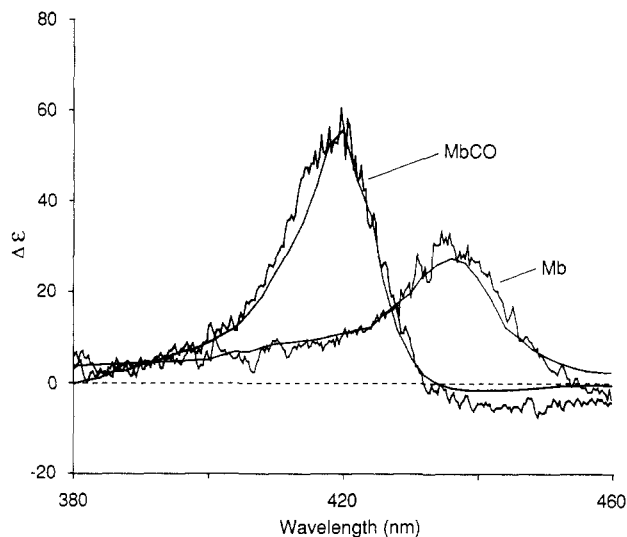


Figure 6. CD spectra of MbCO and Mb. Smooth curves are spectra of the stable species taken on a conventional CD spectrophotometer. Noisier curves are spectra measured by using the TRCD technique with OMA detection. The OMA gate width was 500 ns. The MbCO spectrum was obtained from an MbCO sample without laser excitation. The Mb spectrum was obtained from the same sample with the OMA gate monitoring the probe light beginning 200 ns after the 7-ns, 532-nm photolysis pulse.

with rotation times comparable to or longer than the time scale of the CD measurement, this can result in a time-dependent birefringent sample that can interfere with CD measurements. Such effects have been analyzed in detail and can be avoided with proper control of the pump-laser polarization.³¹

As implied above, time-resolved MCD (TRMCD) measurements can be made with the same technique as TRCD measurements by applying a magnetic field to the sample. Static magnets with fields of 5–20 kG can be used, as in commercial MCD instruments, or pulsed magnets can be obtained that produce fields on the order of 100 kG for periods of several milliseconds. In addition to other birefringence effects, one must account for another possible artifact in TRMCO measurements, due to Faraday rotation caused by the magnetic field. This rotation affects the solvent and cell windows as well as the solute molecules and can be large enough to overwhelm MCD signals of interest. To avoid this problem, two methods have been shown to be successful. One is to use a reference cell (cell plus solvent) as well as a sample cell (including the molecule of interest) along the optical path. These are placed inside two matched magnets with opposite field orientations. The reference cell then produces a Faraday rotation which cancels that produced from the sample cell. Alternatively, a sugar solution with the proper concentration can be used to cancel the Faraday rotation effect to yield a pure MCD spectrum.

This TRCD technique has successfully been used to measure CD spectra of molecular excited states with lifetimes as short as 800 ps³² and CD kinetic changes with time resolution as short as 1 ns.^{31,33} An example of a time-resolved CD spectrum measured by this

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technique is shown in Figure 6. Here the CD spectra of (carbonmonoxy)myoglobin (MbCO) and deoxy-myoglobin (Mb) are shown. The smooth curves show CD spectra taken of the stable species with a conventional CD spectrophotometer. These are compared to CD spectra obtained by using a TRCD apparatus using OMA detection with a 500-ns observation period. The MbCO spectrum is from an unphotolyzed MbCO sample, and the Mb spectrum is from the same sample 200 ns after photolysis resulted in ligand dissociation.³³ The TRMCD technique has also been demonstrated to be capable of measuring MCD signals with nanosecond resolution. For example, both ground and excited triplet state MCD spectra of the free base tetraphenylporphyrin and zinc tetraphenylporphyrin have been measured with nanosecond resolution.³⁴

Time-Resolved X-ray Measurements. X-ray diffraction and spectrographic measurements have been considered for many years to be one of the best means for molecular structural determinations. To be able to make time-resolved X-ray measurements would thus be an exciting prospect. Two advances in X-ray light sources promise to make this prospect a reality. First, synchrotron light sources are available that provide intense pulses of X-rays with durations on the order of 10^{-10} s and repetition rates of megahertz or greater. Use of synchrotrons as X-ray probe sources together with laser pump sources makes time-resolved structural studies possible. The first demonstration of this technique involved a study of the changes in X-ray absorption of (carbonmonoxy)myoglobin upon photolysis.³⁵ This study showed the promise of time-resolved X-ray studies as well as limitations of the currently available systems. High-quality X-ray absorption spectra of (carbonmonoxy)myoglobin and deoxy-myoglobin were obtained, but even though nanosecond laser pulses and subnanosecond synchrotron X-ray pulses were used, the time resolution of the system was on the order of 300 μ s. This is because the repetition rate of the synchrotron pulses (391 kHz), was much greater than that of the laser pulses (20 Hz) so the two sources could not effectively be synchronized. Instead, a system was developed to collect a number of picosecond X-ray probe pulses after each laser photolysis pulse, and this limited time resolution of the experiment.^{35,36}

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In principle, one could achieve much greater time resolution and sensitivity in the above experiments if the repetition rate of the laser and synchrotron pulses were matched. Unfortunately, lasers with sufficient intensity for pump pulses and with sufficient repetition rates to match those of synchrotrons are not available. An alternative approach to time-resolved X-ray studies that holds a great deal of promise involves the use of laser pulses to produce X-rays.³⁷ X-rays can be produced by laser excitation of carbon fibers, by producing plasmas, or by generating electrons, which are then accelerated and directed to an X-ray target. Pulses of X-rays in the 0.5–2-Å region and with durations in the picosecond time regime have been generated that are suitable for X-ray diffraction measurements. Combining such an X-ray source with a second pump laser should soon make it possible to perform X-ray diffraction measurements with picosecond time resolution.

Conclusions

Recent advances in our ability to make spectral measurements with high time resolution have been truly impressive. Absorption and emission studies are now possible with time resolution on the order of femtoseconds. A number of novel innovations have also made it possible to combine laser photolysis techniques with a variety of other spectral techniques. With these innovations it is now possible to perform time-resolved spectral studies with a high degree of sensitivity and on samples that are opaque. It is also possible to measure thermodynamic properties such as enthalpies and volumes with high time resolution as well as to measure the kinetics of molecular structural changes by carrying out time-resolved CD, MCD, ESR, NMR, or X-ray measurements. These capabilities, and other innovations likely to be developed in the coming years, make it possible to obtain an incredibly rich pool of information from laser photolysis measurements that will enhance our understanding of the dynamics of chemical reactions.

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