TRIPLET STATE ODMR SPECTROSCOPY

TECHNIQUES AND APPLICATIONS TO BIOPHYSICAL SYSTEMS

Edited by
Richard H. Clarke

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Preface

Since the first EPR experiments on photoexcited triplet states of organic molecules done in the late 1950s by Clyde Hutchison and his group at the University of Chicago, magnetic resonance research on the organic triplet state has been a steadily increasing field of interest among a diverse group of physicists, chemists, and biologists. The emergence of optically detected magnetic resonance (ODMR) spectroscopy over the past 10 years has served to both increase interest in the paramagnetic properties of the organic triplet state and to expand considerably the scope of triplet-state systems that may be investigated. Nowhere is this expansion more readily apparent than in the recent emergence of ODMR in the field of biology, where the properties of the photoexcited triplet state as revealed by ODMR are now utilized as a probe into the environment and interactions experienced by the photoinduced paramagnetic center in its natural surroundings.

In organizing the present volume—the first book focused exclusively on the field of ODMR spectroscopy—we have attempted both to provide a centralized reference to the various experimental approaches of zero-field and high-field ODMR and to present an introduction to the newly emerging areas of application of ODMR spectroscopy to problems of biological interest. The first six chapters provide a detailed development of experimental techniques and theoretical background, written at a level that will permit researchers not presently working in ODMR to integrate ODMR into their present laboratory operation and to acquaint those presently involved in ODMR with the range of approaches available. The techniques described include several not yet utilized in the investigation of biological systems, but whose potential for such applications can be appreciated from their presentation. Chapters 7–11 are in areas of application chosen to display the most actively pursued fields of ODMR in biophysical systems. The applications chapters by no means attempt to review those areas exhaustively nor to encompass all problems in biology that may be successfully pursued by ODMR. Rather, the applications are presented to illustrate the power of ODMR spectroscopy and to stimulate interest in its adoption in solving new problems.

ODMR spectroscopy is essentially one technique with several different approaches. We have attempted to preset in one volume all these approaches, some of which have been the subject of extensive review articles in separate locations, often written exclusively for the researcher already familiar with the field. Here we expect that those newly interested in ODMR can find sufficient background information and technical detail to initiate experiments, as well as examples drawn from ongoing research programs to illustrate both the capabilities and limitations of ODMR spectroscopy when applied to biological problems.

The enthusiasm displayed by all the contributors to this volume suggests that we may be at the beginning point of significant development of ODMR in the understanding of biophysical problems. Our ultimate aim in effecting a worldwide collaboration of active researchers interested in ODMR spectroscopy is to transmit this enthusiasm to other research groups, including those not familiar with ODMR. To that end I consider the collaborative efforts of the contributing authors to have been highly successful. I am grateful to them all for making this project both worthwhile and enjoyable.

Over the course of the editorial preparation of this volume I have benefited greatly from the past and present efforts of my research group, whose hard work and helpful discussion did much to shape the efforts of my laboratory in ODMR. Further, the support of the U.S. Department of Energy, the U.S. Army Research Office, and the National Institutes of Health is gratefully acknowledged for their role in the development of our research program. Finally, the sincere efforts of the collaborating authors, who worked graciously on this project while under extreme demands on their time from countless other sources, are acknowledged with my personal gratitude.

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Boston, Massachusetts
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Contents

1

Zero-Field ODMR Techniques—Phosphorescence Detection

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1 INTRODUCTION

Since the introduction of optically detected zero-field magnetic resonance in 1968 (1) the ODMR technique has evolved into a many-faceted, versatile set of experimental methods. As most organic molecules phosphoresce from their lowest triplet state, it is most natural to extract the triplet magnetic resonance information through their phosphorescence. Indeed, phosphorescence detection (PDMR) is the basic technique, if not the mainstay, of ODMR. In this chapter we will give a systematic description of the standard PDMR methodology. Several variations and modifications of the basic technique of Maki and his co-workers, specifically developed to handle biophysical problems, will be discussed in subsequent chapters.

PDMR is based on the generally unequal population, as well as the phosphorescence radiative rate, of the triplet spin sublevels. In the presence of resonant microwaves some population may be transferred from one spin sublevel to another. The affected molecules are therefore rendered more (or less) radiative. Accordingly, the phosphorescence output level will change, indicating the occurrence of a magnetic resonance. The condition for a PDMR experiment is favorable if the difference in radiative rate and/or population between two spin sublevels is large. The former arises from a preferential mixing of a spin sublevel with the singlet manifold. The latter depends on more complicated triplet spin kinetics, and is more easily realized at low temperatures. Partly owing to the contribution of the ODMR technique, the physical basis for these interactions is now generally understood (2).

We will give a general description of the experimental technique in Section 2. In Section 3 some practical aspects of microwave helix and cavity designs are given. These components, lying at the heart of the apparatus, are usually not commercially available. Section 4 is a general formalism of the triplet state problem relevant to zero-field ODMR. Section 5 presents the methodology in PDMR study of triplet state kinetics. Finally, in Section 6 we describe a new technique that enables PDMR experiments at higher temperatures.

2 THE BASIC SPECTROMETER

In this section we describe in some detail the basic configuration of a PDMR spectrometer. It is written in the belief (not necessarily shared by the author) that ODMR is now a mature technology, in the sense that investigation of it as a phenomenon has been completed and that it is ready to be exploited by workers in other fields who have no previous exposure to this technique. The experimental scheme presented below is not unique, nor most elegant. Instead it contains sufficient practical information for a first-timer to make a modest beginning.

A basic PDMR spectrometer is schematically shown in Fig. 1. A sample mounted inside a microwave helix or cavity is situated at the lower section of a cryostat. An optical system delivers the desired exciting light to the sample through a bottom window of the cryostat. The ensuing phosphorescence of the sample is collected by a lightpipe and conducted to the top of the apparatus, where it is detected by a photomultiplier (PM) after proper filtering. Meanwhile, a microwave system generates, conditions, and delivers microwaves into the helix or cavity. The occurrence of a magnetic resonance is monitored by a change of the phosphorescence output and retrieved by appropriate detection electronics. It should be emphasized that this is a very simple experiment. Among the major advances in the methodology of physical chemistry in the last decade, ODMR is relatively capital-nonintensive.

We will describe some of the subsystems in more detail below. Before we go into specifics, however, a general comment is in order. The apparatus shown in Fig. 1 features vertical in-line optics, in which the exciting and detecting light paths are in line and vertical. Another popular scheme utilizes horizontal light paths with perpendicular exciting and detecting beams. This configuration is inherently superior in exciting-light rejection, but, with the constraint of the helix or cavity, it usually has a decreased phosphorescence collection efficiency. This may be an important consideration in weakly emitting systems such as biological molecules. Since there is usually a rather large wavelength gap between the exciting light and the phosphorescence, effective rejection of

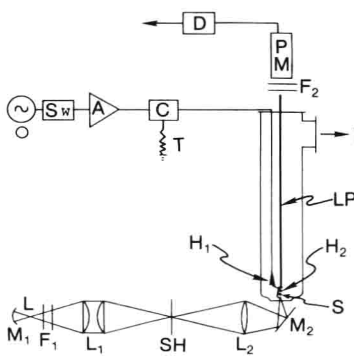

Figure 1 Simplified schematic of a basic PDMR spectrometer. A, microwave amplifier; C, circulator; D, detection electronics; F, filter; H_1, microwave horn; H_2, the helix; L, lamp; M, mirror; C, microwave oscillator; PM, photomultiplier; S, sample; SH, shutter; SW, microwave switch; T, termination.

scattered exciting light is seldom a problem. An important advantage of the vertical in-line configuration (besides cheaper cryogenics) should be mentioned: when the apparatus is properly designed (see Section 2.1), it is possible to change samples while it is immersed in liquid helium. Depending on the research objectives, this could be an important operational freedom.

2.1 Sample Assembly

Because of its wide-band characteristics, a helix design is frequently used in zero-field PDMR to generate microwave magnetic fields. We will defer discussion of the helix design to later. It suffices to say here that the sample should be placed approximately one-quarter wavelength up from the open end of the helix and it should fill the inner diameter of the helix as much as possible. Furthermore, in order to achieve effective collection of phosphorescence the lightpipe should be close to the sample. These two requirements lead to a practical design of mounting the sample at the end of a lightpipe, which, during an experiment, is inserted into the helix. The sample holder is a very-thin-shell Teflon cylinder, at the bottom of which is mounted a thin (0.5–1 mm) quartz window. The Teflon cylinder is machined to be a snug fit for the lightpipe at room temperature. At lower temperatures Teflon contracts against the (quartz) lightpipe, thereby holding the sample by friction. A good machinist can find

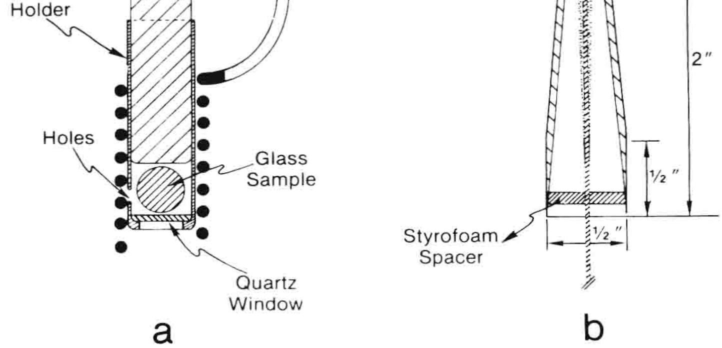

Figure 2 Detail view of the apparatus. (*a*) A glass sample assembled in the helix; (*b*) a broad-band microwave horn useful in coupling microwaves into the helix.

the optimal combination of inner diameter and wall thickness in a few trials. It is important to cut a few holes at the lower section of the Teflon cylinder so that liquid helium makes direct contact with the sample. Note that this design allows sample changing during a liquid helium experiment.

Quite often in chemical or biophysical applications one uses low-temperature glass samples. It may be appropriate to mention a most helpful trick in handling these samples that was devised by Dr. Richard Neumann in our laboratory. Outgassed sample solution is placed with an eyedropper onto the quiescent surface of liquid nitrogen in a small dewar, usually inside a nitrogen-filled glove box to exclude oxygen. Owing to the ball-boiling effect, the sample solution remains as a sphere, rolling on the nitrogen surface. If more than one drop is added, the drops tend to coalesce into one, providing a convenient way to control sample size. The sample sphere gets cooled down evenly before it sinks to the bottom. It is then transferred into the Teflon holder and mounted (under liquid nitrogen). In this way we obtained perfectly transparent samples, even for alcoholic glasses. A mounted glass sample inside a helix is shown in Fig. 2*a*.

2.2 Microwave System

It is no coincidence that the zero-field ODMR technique was born at a time when frequency-agile microwave sweep oscillators became commercially available. Deprived of the freedom of Zeeman tuning, the zero-field ODMR technique requires convenient frequency tuning over an extended frequency range. These microwave sources are basically classified in three types: (*a*) YIG-tuned oscillators, (*b*) voltage-tuned thermionic tubes or solid-state diode oscillators, and (*c*) microwave synthesizers. The YIG oscillators are currently the most popular. Since they change frequency by tuning the magnetic field on a YIG crystal, they are inherently slower and more nonlinear in sweep. The voltage-tuned vacuum tubes, backward-wave oscillators and voltage-tuned magnetrons, do not have these shortcomings, but require high-voltage supplies. Varactor-tuned Gunn or Impatt diode oscillators have limited tuning ranges. Microwave synthesizers have the obvious advantage of precision and stability, but they also have slow switching speeds and a prohibitive cost. This analysis leaves YIG oscillators as the choice for general-purpose ODMR microwave sources.

The inherent advantage of ODMR versus conventional magnetic resonance, as it is so frequently cited, lies in its detection of a more energetic photon. For workers baptized in the painstaking art of low-level microwave detection, the real delight of ODMR is its more tolerant "brute-force" microwave requirement. For basic detection of an ODMR transition a full oscillator output of $\geqslant 20$ mW is usually applied to the sample. For triplet kinetics or more elegant coherent experiments microwave powers up to 30 W are common practice. These power levels are obtained by using microwave amplifiers. For frequencies below 2 GHz, solid-state FET amplifiers are practical. For higher frequencies traveling-wave tube (TWT) amplifiers still find no substitutes. These units are commercially available at considerable cost. For a worker with some experience with high voltages, purchasing the TWT alone and building the system could mean substantial savings.

Transmitting microwaves into the helix is not a trivial problem. By and large, 50-Ω impedance-matched components and transmission lines should always be used. Common mistakes by beginners include using unmatched between-series adaptors and using unmatched tees for splitting microwave power. The oscillator and amplifier output should be protected by isulators, which absorb the reflected waves. Inside the cryostat we prefer using RG141 cables with stainless steel outer conductor and silver-plated SS inner conductor. Last but not least, we mention the impedance mismatch between the RG141 cable and the helix. Without going into sophisticated microwave engineering, some reflection at this point probably cannot be avoided. We have found that a linear horn such as shown in Fig. 2*b* increases the microwave power delivered to the helix by at least a factor of 2, thereby largely alleviating the problem of microwave transmission. It was designed to match impedances between 50 and 175 Ω. This linear horn can be made in most machine shops, and it is soft soldered to the outer conductor of the RG141 cable. The center conductor should be supported *coaxially* by a foam plastic disk cut from package materials of the supermarket variety. Perfectionists will also taper the dielectric material of the cable, but the difficulty in shaping the Teflon dielectric properly makes this endeavor of limited value.

2.3 Optical System

For most ODMR experiments an arc lamp is a suitable exciting source. Except under special circumstances, using a laser for excitation is troublesome and costly. Xe or Xe–Hg lamps are often used, the latter being more convenient if the presence of Hg lines does not interfere with the experiment. Lamp wattage ranges from 150 to 1000 W. The larger lamps provide more photons over a larger image area, with no advantage in brightness. Arc wandering is the greatest source of lamp instability. This problem is minimized by driving the lamp with a highly regulated constant-current power supply (for Xe or Xe–Hg lamps only). The high loop gain of these power supplies makes them unable to cope with the enormous impedance fluctuation of the lamp during starting; it is therefore necessary to start the lamp with a crude power supply, then to gradually switch the load over to a more sophisticated one. Most electronic shops can devise a practical scheme for this operation. The arc lamps in our laboratory have less than 1/1000 amplitude noise. This is an important asset in some kinetic experiments.

It is obvious that filters should be used to isolate the desired exciting band, and detection filters to reject the exciting light to the desired ratio. Monochromators may, of course, be employed in more refined experiments at the expense of reduced throughput. A 5-cm aqueous solution filter should be mounted next to the lamp to remove infrared waves. Kasha's classic paper serves as a starting point (3). It should be mentioned that most long-wavelength-passing filters fluoresce at longer wavelengths upon absorbing at the stop band, thereby defeating the function of filtering to some extent. Indeed, the practicality of the vertical in-line scheme rests upon the availability of a series of very-low-fluorescence filters (Schott FG10, GG4, GG10).

Many PDMR experiments need a relatively fast shutter. Most electrical shutters are not fast and their solenoid transients are constant menaces to integrated circuits. It is better to get the fastest photographic shutter and to use only the minimum aperture until past the image of the lamp (see Fig. 1). Shutter actions of about 0.5 ms may be obtained.

An important element of the vertical in-line apparatus is the lightpipe. It may be a fire-polished suprasil rod. Schott offers a class of coated lightpipes with superior apertures. The flint glass variety however, fluoresces strongly upon blue or UV irradiation. Flexible optical fiber bundles usually have a low throughput, owing to the limited two-dimensional packing efficiency of circles.

2.4 Detection Electronics

Detection electronics is a subject that defies general description. Besides the common need of a photomultiplier, ODMR detection systems depend on the specific experimental goal, the accessibility of electronic equipment, and personal taste. Some specific comments on experimental techniques may be offered as we present the methods for kinetic studies. Here we will only describe the elementary scheme for the most basic PDMR mission: observing a resonance line.

The most straightforward PDMR scheme utilize lock-in detection. The microwaves are, say, amplitude modulated while slowly scanning the frequency region of interest, and the PM output is then phase analyzed in a lock-in amplifier. For PDMR purposes the maximum effect is achieved by total (or on–off) amplitude modulation. This is usually done within the microwave oscillator or externally with a microwave diode switch. The period of the modulation frequency should be long compared with the spin-lattice relaxation time (T_1) or the lifetime of the shortest-living spin substate, whichever is shorter. For organic molecules in the triplet state at liquid helium temperatures this amounts to an upper limit of about 500 Hz.

2.5 Cryogenic Requirement

Most ODMR experiments are conducted in the absence of spin-lattice relaxation. This condition is usually achieved by working below the λ point of liquid helium. While the cryogenic technique is by now well developed for this operation, a few rules of thumb may help in setting up a new apparatus. The helium reservoir should hold ≈ 2 l. A pumping line of at least 2 in. in diameter, leading through a minimum convenient length to a pump of commensurate volume capacity, is recommended. Since this is an optical experiment, plumbing and operating procedures should be designed to exclude air or moisture lest the optical window be fouled up.

3 HELIX AND CAVITY DESIGN

If there is any psychological deterrent to a prospective newcomer in the ODMR field, it is perhaps the helix or cavity design. Performing the most crucial role in ODMR, they are the few components not commercially available. Since microwave engineers get the job done by trial and error themselves, it is little wonder that an uninitiated scientist views it with some apprehension. Writing as a scientist who has more important scientific issues in mind, the author will present some practical schemes for designing and fabricating this central piece of ODMR apparatus. What follows does not claim to be elegant or even legitimate in microwave engineering; its adequacy, however, is guaranteed by the relatively tolerant nature of the ODMR technique.

3.1 The Helix

The helix is the basic device for coupling microwaves to the sample in ODMR. It provides a moderate enhancement of the microwave magnetic field for a given power. More importantly, it is basically a broad-band device, compatible with the ODMR requirement of a wide frequency coverage. Indeed, if field inhomogeneity can be tolerated, the helix may be considered a universal device. This is actually the general practice in ODMR: the same helix is used from 0.5 to 8 GHz and beyond for general applications, while more refined experiments over a small range are performed with cavities.

In engineering jargon a helix is a slow-wave structure. Consider an infinite helical arrangement of conductor. Imagine that the phase vector of maximum current travels along the conductor with the speed of light. The accompanying electromagnetic wave propagating along the helical axis will have to travel at a reduced speed, being therefore a "slow wave." Meanwhile the distance between adjacent repeating field patterns, or the guided wavelength, is shorter than that in free space. Since the energy stored in one wavelength is the same, this compression of wavelength enhances the maximum magnetic field attainable with a given power. A mathematical solution has been made on a model "sheath helix," which is a thin cylinder conducting only along a helical path. For such an idealized helix the compression factor is approximately $2\pi a/p$, where a and p are the radius and pitch of the helical conducting path, respectively. Half of the microwave power is inside the helix. The magnetic field contains both longitudinal and transverse components, even in the lowest mode. Field patterns for a real life helix constructed of finite-size wires are necessarily more complicated. For more technical information the reader may consult ref. 4.

Except for the rare opportunity to use a helix from an expired microwave tube such as a traveling wave tube (TWT) or backward wave oscillator (BWO), most ODMR workers wind their own helix. The usual material is No. 18 to No. 20 copper wire, No. 19 being the inner-conductor size of the RG141 cable. Ribbon-type wires (common for magnet winding) may also be used, thereby approaching more closely the sheath-helix model. For the experimental configuration described in Section 2 the helix diameter is dictated by the size of the lightpipe. We usually use a metal rod of the same diameter as the lightpipe for helix winding. The spring-back of the wires will provide enough clearance. A slot is cut on top of the rod to retain the wires during the winding operation. Two straightened wires of the same size are wound on the rod side by side to make a tight bifilar winding, in which each incoming pair touches the previous pair. With this guideline the pitch of the helix is predetermined and approaches a constant value after a few turns. After the winding is completed one of the wires is unwound while keeping the other from being distorted. The early (or excess) turns are then cut away, resulting in an open-ended helix. A crooked helix is indicative of uneven pitch. For a typical inner diameter of 5 mm, a No. 19 wire leads to a helix with a compression ratio of ~ 20. This helix will give a fairly uniform microwave field below 2 GHz but may have cylindrical nodes at higher frequencies. For experimental configurations with perpendicular viewing of phosphorescence a more open helix of trifilar construction may be preferred. The smaller compression ratio is partially compensated for by the freedom to reduce helix diameter. This approach tends to provide better field homogeneity at high frequencies. The length of the helix is not a crucial parameter. For an open-ended helix, microwaves reflect at the end, creating a standing wave with maximum field one-quarter of a (compressed) wavelength back. For the above cited helix any length beyond 2 cm serves no useful purpose.

This simplistic scheme of helix fabrication is adequate in the sense that any imperfections (pitch nonuniformity, ohmic loss on a work-hardened conductor, etc.) cause smaller problems than coupling the helix to the transmission line. By and large, the helix should be attached to the coaxial cable through a short, smooth section of wire. Gradually increasing the pitch at the first turn of the helix will help, although the improvement has not been quantified. The solder connection between the helix and the transmission line should be carefully made. This problem of attachment is avoided if the inner conductor of the coaxial cable itself is used to wind the helix. The assembly should be tested with a small antenna sampling the microwave field inside the helix. A good coupling will produce a microwave power spectrum mimicking that of the generator. An improperly made connection may show a few sharp dips in the power-versus-frequency spectrum. At these dips the helix acts as a low-grade cavity.

3.2 Cavity

Beyond the basic operation with a helix, an ODMR worker may have specific missions requiring higher-quality microwave magnetic fields. For high field homogeneity with well-defined polarization it is most straightforward to utilize