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'THEORETICAL AND EXPERIMENTAL STUDIES IN ELECTRON
PARAMAGNETIC RESONANCE'

An Investigation into the Design and Construction of 34GHz and 55GHz
EPR Spectrometers and their use in the EPR Study of the Ferric Ion in
Myoglobin, Myoglobin Fluoride and Hemoglobin.

by

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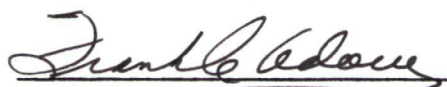
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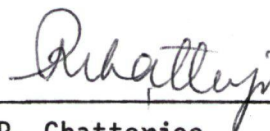
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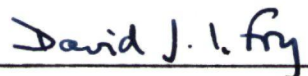
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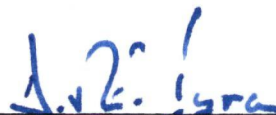
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ABSTRACT

This thesis describes the development of a 34GHz homodyne electron paramagnetic resonance (EPR) spectrometer, a 34GHz superheterodyne EPR spectrometer and a 54-60GHz EPR spectrometer. These instruments were used in the study of the EPR spectra of the ferric ion in metmyoglobin, metmyoglobin fluoride and methemoglobin.

A brief introduction to EPR and the EPR study of metmyoglobin is given, followed by three major sections:

1. A description of the basic theory of EPR and the theory of the detection process of an EPR transition. The instrumentation of conventional and high frequency spectrometers is also discussed.
2. A description of the 34GHz EPR spectrometers and the 54-60GHz EPR spectrometer constructed during the research project. The serrodyne technique used to convert the 34GHz homodyne spectrometer into a superheterodyne spectrometer is described together with comparative sensitivity results.
3. The results of 34GHz and 55GHz EPR experiments are given for single crystals of metmyoglobin, metmyoglobin fluoride and methemoglobin and polycrystalline samples of metmyoglobin and methemoglobin. The results are discussed in terms of a strong crystalline electric field model.

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To my parents,
Mr. and Mrs. A. Gray
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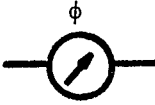
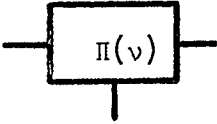
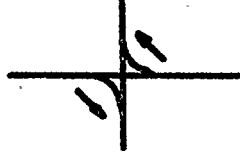
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EXPLANATION OF SYMBOLS

	Oscillator of frequency ν
	Variable attenuator
	Isolator
	Variable phase shifter
	Directional coupler
	Resonant Cavity
	Amplifier
	Synchronous demodulation of frequency ν
	Hybrid or magic tee
	Microwave diode
	Microwave short circuit
	Tunable short circuit
	Cross-guide coupler

CHAPTER I

INTRODUCTION

This chapter discusses the physical origin of electron paramagnetism and provides an introduction to Electron Paramagnetic Resonance or EPR. The application of the technique of EPR is surveyed with emphasis on biological systems. An introduction is also given to the EPR study of metmyoglobin, methemoglobin and their derivatives.

1.1 Background

The magnetic properties of a substance can be classified in terms of the magnitude and sign of its susceptibility χ which relates the induced magnetic moment $\vec{M}$ to the applied magnetic field $\vec{H}$.

$$\chi = (\vec{M}) (\vec{H})^{-1} \quad (1.1.1)$$

where χ and M can be defined referred to unit volume, unit mass or to a gram molecule of the substance.

Substances which have a temperature independent negative magnetic susceptibility are diamagnetic. The induced magnetic moment establishes a magnetic field which is antiparallel to the applied magnetic field. Diamagnetism is the atomic or microscopic manifestation of Lenz's Law. Paramagnetic substances have a positive value for χ . All substances exhibit diamagnetism, however $|\chi_{\text{dia}}| \ll |\chi_{\text{para}}|$ for paramagnetic substances.

Paramagnetism arises when an atom, molecule or ion has a permanent magnetic moment which may be electronic or nuclear in origin. Paramagnetic substances do not have a net magnetic moment in the absence of a magnetic field because the magnetic dipoles of the atoms or ions are randomly

oriented due to thermal motion. Ferromagnetic substances have much larger values for the susceptibility. Ferromagnetism can be explained by assuming that strong magnetic exchange interactions exist between the atoms or ions because the paramagnetic atoms or ions are in close physical proximity.

This thesis is concerned with the electron paramagnetism due to the unfilled d-electron shell of the iron atom in metmyoglobin and methemoglobin. The filled shells of the iron atom, or of the other atoms in the molecule, do not contribute to the paramagnetism.

EPR is a branch of radio frequency and microwave spectroscopy which uses the resonant absorption of microwave energy between quantum levels of the ground state of the paramagnetic species to study its physical environment. EPR is observed normally when the ground state of the paramagnetic species is degenerate in the absence of a magnetic field.

1.2 Survey of EPR Applications

EPR was first observed experimentally by Zavoisky (1945). The experimental observation of EPR requires that a paramagnetic substance be immersed in both an applied magnetic field, to produce the Zeeman splitting of the ground state, and a rotating magnetic field. Transitions are induced if the energy separation of the Zeeman levels is equal to the energy of the electromagnetic radiation supplying the rotating magnetic field. The EPR resonance is detected by the absorption of power from the electromagnetic radiation. The detection of EPR transitions is described in detail in Chapter III.

TABLE I

TRANSITION GROUPS OF THE PERIODIC TABLE

<u>Group</u>	<u>Atomic Numbers</u>	<u>Incomplete Shell</u>	<u>n</u>
Iron	21(Sc) to 30(Zn)	$3d^n$	$n = 0 \text{ to } 10$
Palladium	39(Y) to 48(Cd)	$4d^n$	$n = 0 \text{ to } 10$
Lanthanide	57(La) to 71(Lu)	$4f^n$	$n = 0 \text{ to } 14$
Platinum	71(Lu) to 80(Hg)	$5d^n$	$n = 0 \text{ to } 10$
Actinide	89(Ac) to 103(Lw)	$5f^n$	$n = 0 \text{ to } 14$
		$6d^{n'}$	$n' = 1 \text{ or } 2$

The technique of EPR is an important analytical tool in physics, chemistry and the life sciences. EPR has been used to study the transition metal ions in the crystalline field of a large number of host lattices. This research plays an important role in checking bonding theories of the transition metal ions and in elaborating on the nature of the magnetic and electronic interactions of atoms and ions. The transition metal ions comprise five groups of elements in the periodic table. The incomplete shell in the five groups of elements is given in Table I.

EPR can also be used to study molecules which have a ground state with a spin angular momentum quantum number $s = 1$. The paramagnetism of the oxygen molecule O_2 results from its $s = 1$, or triplet ground state.

Any molecule which has an odd number of electrons will be paramagnetic due to the spin angular momentum of the unpaired electron. Nitric oxide (NO) and nitrogen dioxide (NO_2) are two examples of molecules which are stable with an odd number of electrons and are consequently paramagnetic.

EPR can be used in the study of chemical reactions and molecular structure. A stable molecule, such as $DPPH^*$, which possesses an unpaired electron is called a free radical. Unstable, short lifetime free radicals are often produced as intermediate steps in a chemical reaction. The EPR spectra of stable free radicals can consist of a number of closely spaced resonances as a function of the magnetic field. The closely spaced resonances are known as hyperfine structure and are due to the internal magnetic field of the magnetic nuclei on the unpaired electron. The hyperfine structure in the EPR spectra of free radicals can be

* Diphenyl-picryl hydrazyl.

relatively complicated indicating that the unpaired electron is free to move in a "delocalized" molecular orbital and interact with several magnetic nuclei. The analysis of such spectra can be used to study molecular structure.

Semiconductor samples can be studied using EPR. The spectra arise from the unpaired electrons or holes associated with donor or acceptor atoms. The conduction electrons in metals have also been studied by EPR. The application of EPR in the study of colour centres in crystals has provided detailed information on the structure of these centres not obtainable from optical means alone.

The remainder of this section reviews briefly the application of EPR to biological substances. Commoner, Townsend and Pake (1954) obtained the first EPR spectra from samples of freeze-dried tissues. The spectra were attributed to the presence of unpaired electrons associated with free radicals. Subsequent studies with improved EPR instrumentation by Commoner, et al., (1961) and Hollocher, et al., (1961) showed that similar spectra could be obtained from fresh tissue samples. The origin of the free radical spectra was shown to be the redox enzymes associated with mitochondria. EPR has also revealed that significant differences exist in the concentration of free radicals in normal and malignant tissue. This is a potentially important field of application because it is possible that EPR can be used as a diagnostic tool to detect the early onset of cancer. Ultra-violet light and ionizing radiation can damage or kill biological systems. EPR has demonstrated that free radicals are created by the action of the radiation and that the concentration is related to the amount of damage.

A technique has been developed in which a stable free radical or "spin label" can be bonded to a macromolecule and used as a paramagnetic

probe of its environment. It has been possible to detect and study conformational changes in the macromolecule under a variety of conditions by studying changes in the EPR spectra of the "spin label."

Transition metal ions occur in nature and these can be studied by EPR. The information obtained relates to valence, bonding character and the local symmetry of the crystalline electric field of the paramagnetic ion. Iron is the most important transition metal occurring in nature. It plays a key role in the cytochromes of the respiratory mechanism and is required for heme synthesis, oxygen transport and in the enzymes peroxidase and catalase. Cobalt (vitamin B₁₂) and copper (ceruloplasmin) are also important in living systems as are trace amounts of manganese, zinc, chromium, nickel and molybdenum.

1.3 The EPR of Myoglobin and Hemoglobin

Bennett and Ingram (1955) initiated the EPR study of polycrystalline samples of the met^{*} forms of myoglobin (MbH₂O) and hemoglobin (HbH₂O). This, and subsequent work, established that the EPR signal originated from a single transition with a highly anisotropic g-value ranging from $g_{||} = 2$ to $g_{\perp} = 6$. The theoretical interpretation was given by Griffith (1956). The EPR signal was assigned to the Zeeman splitting of the $|\pm \frac{1}{2}\rangle$ levels of a high spin ferric ion with a large zero field splitting between the Kramers doublets. Bennett, et al. (1957) used the large g-value anisotropy to determine the orientation of the heme normals with respect to the myoglobin and hemoglobin crystalline axes.

* The iron atom is in the ferric state in metmyoglobin and methemoglobin.

More detailed EPR studies of type A crystals of metmyoglobin were then performed by Eisenberger and Pershan (1966, 1967) and Helcké, et al., (1968). This research was complemented by the detailed structural analysis of myoglobin by Kendrew, et al., (1960) using x-ray crystallography.

One of the important parameters describing the EPR spectra and related to the crystalline electric field of the ligands surrounding the iron atom is the zero field splitting parameter D . This parameter describes the zero field separation of the doublets in the ground state sextet of the ferric ion. The experimental determination of the magnitude of D is important for comparison with theoretical calculations of the zero field splitting based on various models. The EPR experiments of Eisenberger and Pershan (1966) and Slade and Ingram (1969) on MbH_2O yield $2D = (8.76 \pm 1.20)cm^{-1}$ and $2D = (8.8 \pm 0.9)cm^{-1}$, respectively. These values are in good mutual agreement but do not agree with the results of magnetic susceptibility experiments. Uenoyama, et al. (1968) and Morimoto, et al. (1965) have given $2D = (21.0 \pm 1.0)cm^{-1}$ for MbH_2O and $2D = (13.0 \pm 0.5)cm^{-1}$ for metmyoglobin fluoride (MbF) on the basis of magnetic susceptibility experiments. Mössbauer spectroscopy can also be used to determine the parameter D under certain conditions. Lang and Marshall (1966) have obtained $2D \approx 14cm^{-1}$ for hemoglobin fluoride (HbF) which is close to the value of $2D$ obtained by Uenoyama et al. (1968) for MbF . Richards, et al. (1967) measured the zero field splitting of some iron porphyrin compounds directly using far infra-red spectroscopy. These compounds are similar to the heme group in MbH_2O , although there is an additional bond between the heme iron and a histidine amino acid in the proteins. The experimentally

determined zero field splitting in the iron porphyrin compounds varied from $2D = 11\text{cm}^{-1}$ to $2D = 24\text{cm}^{-1}$ depending largely on the fifth, out of plane ligand bonded to the ferric iron. These values can only be taken as an approximate indication of the magnitude of the D-value to be expected from MbH_2O because the iron atom in Mb and Hb is six-coordinated while the iron atom in the porphyrin compounds is five-coordinated.

This thesis includes a re-examination of the EPR determination of the zero field splitting of MbH_2O and HbH_2O because the direct measurement of the zero field splitting of MbH_2O and HbH_2O by far infra-red spectra or millimetre EPR spectroscopy remains beyond the state of the art.

Bennett et al. (1957) have studied the EPR spectra of the ferric iron in crystalline HbH_2O . They reported approximate g-values and gave no information on line widths since the aim of their research was to determine the orientation of the heme-plane normals with respect to the crystalline axes. Part of the experimental work described in this thesis examines and compares the EPR spectra of single crystal and polycrystalline samples of MbH_2O , MbF and HbH_2O .

CHAPTER II

BASIC THEORY OF EPR

The condition for the observation of EPR is derived in Sec. (2.1) of this chapter. The spin-lattice and spin-spin interactions, and their importance in the detection of EPR, are discussed in Sec. (2.2). The behaviour of the magnetization of a macroscopic sample containing a number of paramagnetic ions is described in the derivation of the Bloch equations given in Sec. (2.3). Section (2.4) summarizes EPR line broadening mechanisms.

2.1 The Resonance Condition

The electronic magnetic moment of a paramagnetic ion, atom or molecule arises whenever there is a resultant angular momentum which can be either pure orbital or spin angular momentum or a combination of both. The magnetic moment $\vec{\mu}$ of a paramagnetic ion is proportional to the resultant angular momentum $\vec{J}$.

$$\vec{\mu} = \gamma \hbar \vec{J} \quad (2.1.1)$$

where γ is the magnetogyric ratio

and $\hbar$ is Planck's constant divided by 2π

The torque $\vec{T}$ experienced by a magnetic dipole moment $\vec{\mu}$ when it is subject to a magnetic flux density $\vec{B}$ is

$$\vec{T} = \vec{\mu} \times \vec{B} \quad (2.1.2)$$

The torque is equal to the time rate of change of angular momentum

$$\frac{d\vec{J}}{dt} = \vec{\mu} \times \vec{B} \quad (2.1.3)$$

$$= \gamma \hbar (\vec{J} \times \vec{B}) \quad (2.1.4)$$

or
$$\frac{d\vec{\mu}}{dt} = \gamma \hbar (\vec{\mu} \times \vec{B}) \quad (2.1.5)$$

The solution to Eq. (2.1.5) when the z-axis is parallel to the magnetic flux density $\vec{B}$ is given by Abragam and Bleaney (1970).

$$\begin{aligned} \mu_x &= \mu \sin \alpha \cos (\omega_L t + \epsilon) \\ \mu_y &= \mu \sin \alpha \sin (\omega_L t + \epsilon) \\ \mu_z &= \mu \cos \alpha \end{aligned} \quad (2.1.6)$$

where μ_x , μ_y and μ_z are the components of the magnetic moment vector $\vec{\mu}$ along the x, y and z axes,

α is the angle between the direction of the magnetic moment vector $\vec{\mu}$ and the z axis,

ϵ is a phase factor

and ω_L is the frequency of precession of the vector $\vec{\mu}$ about the magnetic field direction; it is given by

$$\omega_L = -\gamma \hbar B \quad (2.1.7)$$

The negative sign in Eq. (2.1.7) indicates that the precession is in the direction of a left-handed screw advancing along the magnetic field direction. In general, the sense of the precession of the angular momentum vector is unimportant in EPR experiments and is not determined. Koepp (1969) has described a special EPR spectrometer which does give the polarization of the absorbed microwave radiation.

Equations (2.1.6) show that the z-component of $\vec{\mu}$ is constant. The precessional motion of $\vec{\mu}$ about the magnetic field direction produces an oscillating magnetic field in any direction perpendicular to the magnetic field. If an oscillating magnetic field $H_1 \cos \omega t$ is

applied in the plane perpendicular to the magnetic field direction, where $H_1 \ll |B|$, then the motion of the precessing magnetic moment is relatively undisturbed unless $\omega = \omega_L$. Pake (1962) has shown that if $\omega = \omega_L$, then the component $\mu \cos \alpha$ of the dipole can "resonate" between the positions of being parallel and antiparallel to the applied magnetic field. The resonant change in the z-component of the magnetic moment is referred to as magnetic resonance.

This description has been given using readily visualized classical arguments. Slichter (1963) has shown that identical results are obtained from a more detailed quantum mechanical approach.

The energy of a magnetic dipole moment in a magnetic field is given by

$$W = -\vec{\mu} \cdot \vec{B} \quad (2.1.8)$$

$$= -\gamma \hbar |J| |B| \cos \alpha \quad (2.1.9)$$

The quantum mechanically allowed values of $J \cos \alpha$ along the magnetic field direction are given by $M, (M-1), \dots, -M$, where M is equal to $|J|$ and is integral or half-integral. The energy of the state with magnetic quantum number M is given by

$$W_M = \gamma \hbar B M \quad (2.1.10)$$

The allowed transitions are governed by the selection rule $\Delta M = \pm 1$ so that the resonance condition is given by

$$\begin{aligned} \hbar \omega &= W_M - W_{M-1} \\ &= \gamma \hbar B \end{aligned} \quad (2.1.11)$$

where ω is the angular frequency of the oscillating magnetic field.

EPR experiments are normally described using the alternative resonance condition

$$\hbar\omega = g\beta B \quad (2.1.12)$$

where g is the spectroscopic splitting factor
and β is the Bohr magneton.

The spectroscopic splitting factor, or g -factor is an experimentally determined dimensionless quantity of order unity and is related to the Landé splitting factor. The g -factor of a "free spin" is 2.0023.

The sign of g is not normally determined in an EPR experiment because a linearly polarized oscillating magnetic field is used. This magnetic field can be resolved into two circularly polarized components one of which is in the sense of the precession of the magnetic moment and which leads to the resonance phenomenon.

2.2 Spin-Lattice and Spin-Spin Interactions

The interaction of the precessing magnetic moment system with the oscillating magnetic field produces both absorption and induced emission between the magnetic energy levels with equal probability. Since the probability of spontaneous emission is negligible at radio and microwave frequencies the resonance phenomenon tends to equalize the populations of the two energy levels. There are, however, non-radiative processes which attempt to restore the paramagnetic spin system to thermal equilibrium. One such process couples the spin system with the thermal fluctuations of the surrounding lattice. The lattice vibrations or phonons in a solid can induce transitions between the Zeeman levels of the ground state of a paramagnetic ion. The interaction between the

paramagnetic system and the phonon spectra of a solid can be characterized by a spin lattice relaxation time T_1 .

Following Abragam and Bleaney (1970) the rate equation for approach to thermal equilibrium for a simple two level system is given by

$$\frac{d(n_a - n_b)}{dt} = \frac{1}{T_1} \left\{ (N_a - N_b) - (n_a - n_b)_0 \right\} \quad (2.2.1)$$

where N_a and N_b are the level populations at thermal equilibrium,

n_a and n_b are the populations without equilibrium,

and $(n_a - n_b)_0$ is the initial population difference.

The solution of Eq. (2.2.1) is given by

$$(n_a - n_b) = \left\{ (n_a - n_b)_0 - (N_a - N_b) \right\} \exp. \left(\frac{-t}{T_1} \right) + (N_a - N_b) \quad (2.2.2)$$

which demonstrates that there is an exponential return from the initial population difference $(n_a - n_b)_0$ to $(N_a - N_b)$ with time constant T_1 .

If the level populations are also altered by the resonance phenomena, then

$$\frac{dn_b}{dt} = - \frac{dn_a}{dt} = w_e (n_a - n_b) \quad (2.2.3)$$

where w_e is the rate at which electronic transitions are induced per paramagnetic centre by the oscillating field.

Therefore

$$\frac{d(n_a - n_b)}{dt} = -2w_e (n_a - n_b) \quad (2.2.4)$$

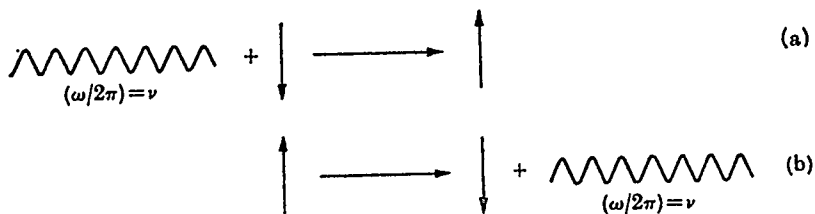


Figure (2.1).

Illustration of the direct spin-lattice relaxation process. A phonon of frequency ν is absorbed in (a) by a spin which makes a transition to the upper state. In (b) the spin makes a transition to the lower state emitting a phonon at the resonant frequency.

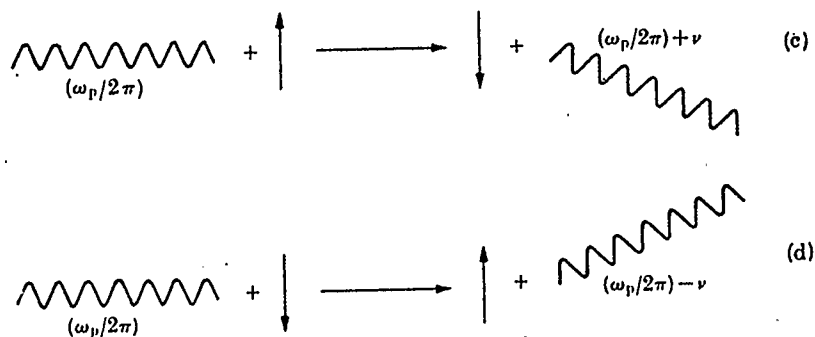


Figure (2.2).

Illustration of the Raman spin-lattice relaxation process. In (c) a phonon of frequency $(\omega/2\pi)$ is scattered as a phonon of frequency $(\omega/2\pi)+\nu$ accompanied by a "down" transition. This process can be involved in an "up" or "down" transition.

Combining Eq. (2.2.4) with Eq. (2.2.1) yields

$$\frac{d(n_a - n_b)}{dt} = \frac{1}{T_1} (N_a - N_b) - \left(\frac{1}{T_1} + 2w_e \right) (n_a - n_b) \quad (2.2.5)$$

The steady state solution of Eq. (2.2.5) is given by

$$\frac{(n_a - n_b)}{(N_a - N_b)} = \frac{1}{(1 + 2w_e T_1)} \quad (2.2.6)$$

where now $(n_a - n_b)$ is the equilibrium population difference in the presence of an EPR transition. The difference in the energy level populations during a magnetic resonance transition is therefore a function of the magnitude of $w_e T_1$. If $2w_e T_1 \ll 1$ then power saturation will limit the amplitude of the EPR signal.

The nature of the spin-lattice interaction was first discussed by Waller (1932). He distinguished two spin-lattice interaction processes, the "direct process" and the "Raman process." The direct process, which involves a phonon of energy equal to that required to induce the resonant transition, is illustrated in Fig. (2.1). A phonon may be absorbed by the paramagnetic system in a spin transition upwards in energy, "up" transition, or may be emitted in a "down" transition. The Raman process, which involves the interaction of a phonon of arbitrary energy with the paramagnetic spin system together with the emission of another phonon of the appropriate energy to satisfy energy conservation, is illustrated in Fig. (2.2). The Raman process can also be involved in an "up" transition or "down" transition.

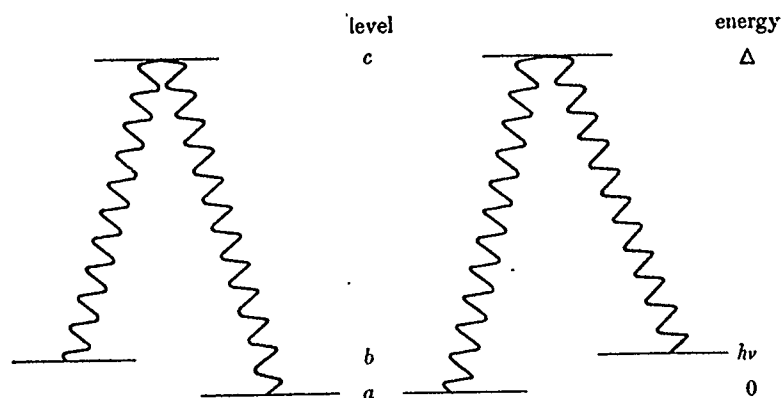


Figure (2.3).

Illustration of the Orbach spin-lattice relaxation process. The EPR transition occurs between the levels (a) and (b). Phonons at frequencies $\Delta, \Delta - h\nu$, are absorbed or emitted by direct processes causing transitions (a)-(c) and (b)-(c) respectively.

At temperatures of $0(4^{\circ}\text{K})^*$ the number of phonons that have the appropriate energy to interact with the spin system, via the direct process, is small compared to the total number of phonons. Consequently, the Raman process is the dominant non-radiative spin-lattice relaxation process under these conditions. At lower temperatures, the total number of phonons decreases and it is likely that the direct process will become more important. The mechanism whereby the phonons interact with the paramagnetic ion is associated with the modulation of the crystalline electric field due to lattice vibrations. The varying electric field interacts indirectly with the electronic spin system through the electronic orbital motion via the spin-orbit interaction. This explains the fact that s-state ions ($L = 0$) often have relatively long spin-lattice relaxation times. Orbach (1961) extended the quantitative description of spin-lattice relaxation of Kronig (1939) and Van Vleck (1940) and has proposed a further mechanism for spin-lattice relaxation. In this process a phonon is absorbed by a direct process and the paramagnetic system is excited to a much higher energy level from which it decays by emission of a further phonon and the "up" or "down" transition between the EPR levels. This process is illustrated in Fig. (2.3).

The spin-spin interaction between the paramagnetic ions in a diamagnetic solid is an important relaxation process. The simplest spin-spin interaction is the magnetic dipole interaction of one paramagnetic ion with the magnetic field produced by an adjacent ion.

* For $0(4^{\circ}\text{K})$ read order of magnitude 4°K .

This effect can be calculated if the ionic separation is known. The local magnetic field, at the site of a paramagnetic ion, must be added vectorially to the external field in any calculation of the resonance condition. Consequently, the spin-spin interaction can lead to a broadening of the EPR line through variations in the local field of all the paramagnetic ions. The result of this interaction is referred to as "inhomogeneous" broadening and it is equivalent to the line broadening obtained by using a magnetic field which is inhomogeneous over the sample volume.

The lifetime of the paramagnetic ion, in the various Zeeman levels produced by the external magnetic field, can be shortened by the effect of the additional oscillating magnetic field arising from the precession of neighbouring paramagnetic ions. If the paramagnetic ions are subject to the same applied magnetic field then they have identical precessional frequencies. The oscillating magnetic field produced by one ion is at the correct frequency to induce a transition or 'spin flip' in another ion, and vice versa. This spin-spin interaction increases the transitional probability of a spin flip and shortens the lifetime of the individual ion in a given quantum state. Because the lifetime is shortened the uncertainty principle leads to a broadening of the energy level and, consequently, to a broadening of the EPR transition. This spin-spin interaction leads to the concept of the spin-spin relaxation time T_2 . The spin-spin relaxation time does not describe the linewidth of an inhomogeneously broadened line but it does describe the linewidth of a 'spin packet' which does have a unique value for the precessional frequency of the spins.

There is a further purely quantum mechanical spin-spin interaction that becomes important whenever the separation of the paramagnetic ions is less than $\sim 5\text{\AA}$. This is the exchange interaction arising from the overlap of the electronic charge clouds of the paramagnetic ions. Isotropic exchange interaction can lead to 'exchange narrowing' of the EPR lineshape, however, the normal doping of the paramagnetic ions in the diamagnetic lattice separates them sufficiently to produce negligible exchange interactions.

2.3 The Bloch Formalism of Magnetic Resonance

The preceding discussion has described the phenomenon of magnetic resonance in terms of the behaviour of a simple paramagnet in a magnetic field and the non-radiative relaxation processes that allow the observation of the magnetic resonance. In practice, one must consider a large aggregation of paramagnets. A minimum of 10^{10} spins is normally required for observation by EPR. Bloch (1946) introduced a set of phenomenological equations that describe the behaviour of the magnetization of the sample in terms of the spin-lattice and spin-spin relaxation times for the macroscopic system. These equations were used originally to describe nuclear paramagnetic resonance but they can also be used to describe EPR.

The Zeeman energy of the magnetization $\vec{M}$ in a magnetic flux density $\vec{B}$ is given by

$$\begin{aligned} W &= - \vec{M} \cdot \vec{B} \\ &= - M_z |B| \end{aligned} \tag{2.3.1}$$

where the z direction is parallel to that of $\vec{B}$.

If the z -component of the magnetization is not equal to the equilibrium value then it will approach this value exponentially

$$\left(\frac{dM_z}{dt} \right) = \left[\frac{-(M_z - M_0)}{T_1} \right] \quad (2.3.2)$$

where M_0 is the equilibrium value of the magnetization

and T_1 is the longitudinal or spin-lattice relaxation time.

The components of the magnetization in the plane perpendicular to the applied magnetic field are influenced by the spin-spin interaction between the paramagnetic centres. Bloch assumed that the spin-spin relaxation could also be described by a relaxation time T_2 so that

$M_{x,y}$ obey the equation

$$\left(\frac{dM_{x,y}}{dt} \right) = \left[- \frac{M_{x,y}}{T_2} \right] \quad (2.3.3)$$

T_2 is also known as the transverse relaxation time. The equation of motion of the magnetization $\vec{M}$ is given by

$$\left(\frac{d\vec{M}}{dt} \right) = \vec{M} \times \vec{B} \quad (2.3.4)$$

This equation describes the motion of the magnetization vector in the laboratory frame of reference at rest with respect to the applied magnetic field. Equations (2.3.2) and (2.3.3) describe the relaxation of M_z and $M_{x,y}$ with respect to a frame of reference rotating about the z -direction at the Larmor frequency ω_L . Pake (1962) has shown that the Bloch equations can be obtained by transforming Eqs. (2.3.2) and (2.3.3) into the laboratory frame of reference

$$\left[\frac{dM_{x,y}}{dt} \right] = \gamma (\vec{M} \times \vec{B})_{x,y} - \left[\frac{M_{x,y}}{T_2} \right] \quad (2.3.5)$$

$$\left[\frac{dM_z}{dt} \right] = \gamma (M \times B)_z + \left[\frac{M_0 - M_z}{T_1} \right] \quad (2.3.6)$$

The steady state solution of these equations for electrons was derived by Pake (1962). In the presence of an oscillating* magnetic field, $B_x = 2B_1 \cos \omega t$, the magnetization M_x can be resolved into two components in-phase and out-of-phase with B_x . It is convenient to introduce a complex susceptibility $\chi = \chi' - i\chi''$.

Then

$$M_x = \chi' \left(\frac{1}{\mu\mu_0} \right) \cdot (2B_1 \cos \omega t) + \chi'' \left(\frac{1}{\mu\mu_0} \right) \cdot (2B_1 \sin \omega t) \quad (2.3.7)$$

where μ_0 is the permeability of free space

and μ is the relative permeability of the medium.

The solutions of Eqs. (2.3.5) and (2.3.6) with the oscillating magnetic field B_x added to the steady magnetic field B are given by

$$M_z = \chi_0 \left(\frac{1}{\mu\mu_0} \right) B \left\{ \frac{1 + T_2^2 \Delta\omega^2}{1 + T_2^2 \Delta\omega^2 + \gamma^2 B_1^2 T_1 T_2} \right\} \quad (2.3.8)$$

and

$$M_x = \frac{1}{2} \chi_0 \left(\frac{1}{\mu\mu_0} \right) B T_2 \left\{ \frac{T_2 \Delta\omega 2B_1 \cos \omega t + 2B_1 \sin \omega t}{1 + T_2^2 \Delta\omega^2 + \gamma^2 B_1^2 T_1 T_2} \right\} \quad (2.3.9)$$

where χ_0 is the static susceptibility which is related to M_0

$$\text{by } M_0 = \left(\frac{1}{\mu\mu_0} \right) \chi_0 B$$

* An oscillating field can be resolved into two counter-rotating magnetic fields of equal amplitude one of which has the same sense as the Larmor precession.

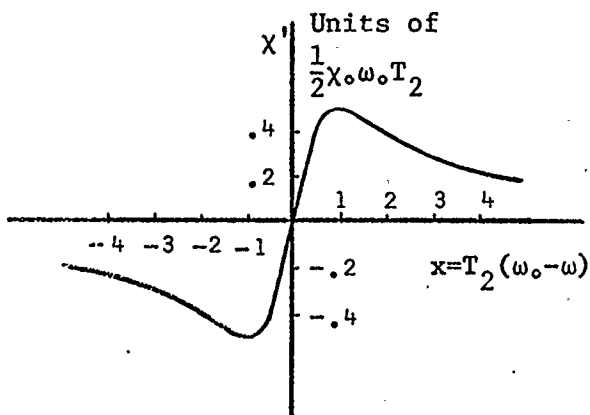
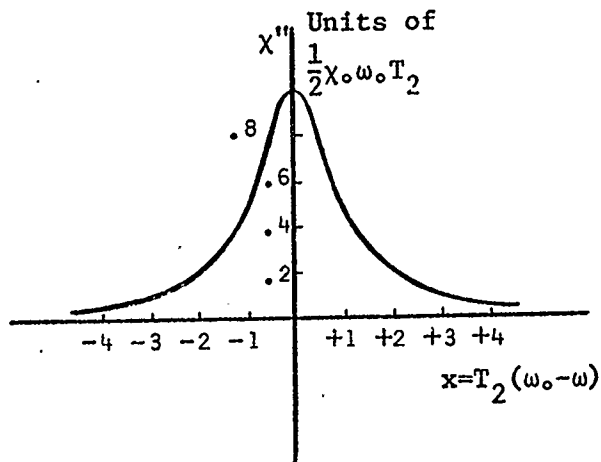


Figure (2.4).

Graphs showing the imaginary (χ'') and real (χ') components of the complex magnetic susceptibility, as a function of the dimensionless variable $x = T_2(\omega_0 - \omega)$, with negligible saturation.

Comparing Eq. (2.3.7) with Eq. (2.3.9) yields

$$\chi' = \frac{1}{2}\chi_0\omega_L T_2 \left\{ \frac{T_2\Delta\omega}{1 + T_2^2\Delta\omega^2 + \gamma^2 B_1^2 T_1 T_2} \right\} \quad (2.3.10)$$

$$\text{and } \chi'' = \frac{1}{2}\chi_0\omega_L T_2 \left\{ \frac{1}{1 + T_2^2\Delta\omega^2 + \gamma^2 B_1^2 T_1 T_2} \right\} \quad (2.3.11)$$

Pake (1962) has shown that the average energy absorbed per unit volume by the sample from the oscillating magnetic field is dependent on χ'' . Consequently, the out-of-phase component of the susceptibility χ'' , which is a maximum at resonance, is referred to as the "absorption" component. The in-phase component of the susceptibility χ' is zero at resonance and is referred to as "dispersion." Figure (2.4) gives the graphs of χ' and χ'' as a function of $\Delta\omega$ when saturation effects are neglected ($B_1^2 T_1 T_2 \ll 1$).

2.4 Line Broadening Mechanisms

Some of the mechanisms producing a finite linewidth in the observation of an EPR transition have been described in Sec. (2.2). This section summarizes these and other possible line broadening mechanisms. The discussion of the observed linewidth effects in crystals of MbH_2O and HbH_2O is reserved until Chapter VIII.

The Bloch equations describe the resonance of an assembly of paramagnets and predict that the lineshape will be Lorentzian. This model is correct for many liquid or exchange-narrowed systems but, in general, it is not correct for paramagnetic ions in a crystalline lattice. In solids, the various interactions of the paramagnetic ion with its surroundings tend to broaden the line. This broadening can be classified as either homogeneous or inhomogeneous.

(i) The resonance is said to be homogeneously broadened if the linewidth arises from the finite lifetime of the states involved in the transition.

(ii) The resonance is said to be inhomogeneously broadened when the individual paramagnets are precessing at different Larmor frequencies.

The relaxation time T_1 , described in Sec. (2.2), can lead to a broadening of the resonance. If this is the dominant line broadening process then the linewidth will be temperature dependent. In some cases the spin-spin relaxation time T_2 can produce a broadening of the resonance. If this process is dominant then the linewidth is temperature independent.

Inhomogeneous broadening of an EPR line in a solid can be due to dipolar interaction with similar or dissimilar magnetic moments. The interaction of a paramagnetic ion with adjacent nuclear spins can cause either further splitting of the Zeeman lines and hyperfine structure, or it may broaden the EPR line.

Experimental sources can also lead to EPR line broadening. This is associated with either large frequency instabilities in the microwave radiation or as a result of inhomogeneities in the magnetic field across the sample volume. In some circumstances it is also possible to obtain a line broadening by the use of high frequency magnetic field modulation. Saturation of an EPR line decreases the peak of the curve of χ'' as a function of B to a greater extent than the wings of this curve and this effect can also lead to a line broadening.

Two further sources of line broadening are associated with statistical variations in the crystal structure containing the paramagnetic ion. Assuming that the spectrum of the paramagnetic ion is

anisotropic, then the variations in the orientation of each unit cell can lead to a statistical variation in the g -values from paramagnetic ions in different unit cells. The magnitude of this effect is related to the rate of change of the g -value with orientation. The second line broadening mechanism associated with the crystal structure is the possible variation in the crystalline electric field surrounding each paramagnetic ion. The crystalline electric field is described by parameters which may have a statistical variation.

CHAPTER III

The Detection of Absorption and Dispersion Components of an EPR Signal

This chapter describes and relates the observed EPR signals to the components of the complex susceptibility, χ' and χ'' arising from the Bloch formalism of magnetic resonance.

The sample resonant cavity system is analysed in Sec. (3.1) using a lumped parameter LCR equivalent circuit. A derivation of the resonant cavity reflected wave is given in Sec. (3.2). The Smith chart is used in Sec. (3.3) to develop the relationship between the components of the cavity reflected wave, derived in Sec. (3.2), and the microwave voltage due to an EPR resonance. The Smith chart is used because a minimum number of approximations are required and it is also useful in the discussion of the effects of frequency modulation noise in the microwave power. The latter is one of the most important limitations to spectrometer sensitivity. Section (3.4) includes a discussion of the demodulation of a modulated microwave carrier frequency and a description of the spectrometer demodulation system adopted in this laboratory. Section (3.5) discusses the use of magnetic field modulation.

3.1 An Equivalent Circuit Analysis of the Sample Resonant Cavity System

In EPR spectrometers the sample is normally located inside a resonant microwave cavity in the region where the microwave magnetic field is a maximum. In the absence of saturation, the microwave power absorbed by the sample at resonance is proportional to the square of the amplitude of the microwave magnetic field. The microwave magnetic field in a resonant structure exceeds that in a non-resonant structure by the Q factor of the cavity.

In the equivalent circuit description the sample is located in the oscillating magnetic field of the inductor. The effective inductance, L , is increased due to the presence of the sample.

$$L = L_0 (1 + \eta \chi) \quad (3.1.1)$$

where η is the filling factor,

L_0 is the inductance in the absence of a sample

and $\chi = \chi' - i\chi''$ is the complex susceptibility.

The impedance of an LCR circuit at frequency ω is

$$Z = R_0 + i \left(\omega L - \frac{1}{\omega C} \right) \quad (3.1.2)$$

where R_0 is the equivalent d.c. resistance

and C is the capacitance.

Substituting Eq. (3.1.1) into Eq. (3.1.2) and using the complex form for χ yields

$$Z = \left(R_0 + \omega L_0 \eta \chi'' \right) + i \left[\omega L_0 (1 + \eta \chi') - \frac{1}{\omega C} \right] \quad (3.1.3)$$

At resonance, $\omega = \omega_r$ and $\omega_r L_0 = \frac{1}{\omega_r C}$

$$Z = \left(R_0 + \omega_r L_0 \eta \chi'' \right) + i \left(\omega_r L_0 \eta \chi' \right) \quad (3.1.4)$$

Consequently, a change in the imaginary component of the susceptibility leads to a change in the effective resistance of the LCR circuit. This is referred to as "absorption" because the change in the resistance produces a change in the power absorbed by the cavity at resonance. The change in the resistance δR with a change in the susceptibility χ'' is given by

$$\frac{\delta R}{R_0} = Q_n \chi'' \quad (3.1.5)$$

where $Q = \frac{\omega L_0}{R_0}$ is the unloaded Q of the resonant circuit.

Similarly, a change in the real component of the susceptibility will change the reactance of the equivalent circuit altering the resonant frequency of the sample cavity system. This is experimentally observable as the phenomenon of "dispersion." The change in reactance δX is given by

$$\frac{\delta X}{R_0} = Q_n \chi' \quad (3.1.6)$$

Most EPR spectrometers are adjusted to detect changes in the imaginary, or "absorption" component of the susceptibility.

3.2 A Derivation of the Resonant Cavity Reflection Coefficient

A more complete theory of the detection of EPR transitions can be developed using the concept of the voltage reflection coefficient. Slater (1950) has shown that the normalised impedance at the input plane of a one-port cavity is given by

$$\frac{Z}{Z_0} = \frac{\left(\frac{1}{Q_e} \right)}{\left(\frac{1}{Q_u} \right) + i \left(\frac{\omega}{\omega_r} - \frac{\omega_r}{\omega} \right)} \quad (3.2.1)$$

where Z is the complex impedance of the cavity,

Z_0 is the characteristic impedance of the waveguide mode, and the loaded Q of the resonant cavity Q_L , excited in a specific mode is given by

$$\frac{1}{Q_L} = \frac{1}{Q_u} + \frac{1}{Q_e} \quad (3.2.2)$$

where Q_u is the unloaded (intrinsic) Q , which is determined by losses due to the finite conductance of the walls

and Q_e is the external Q which is determined by losses arising from power leakage out of the cavity.

The voltage reflection coefficient Γ is defined by

$$V_i = \Gamma V_r \quad (3.2.3)$$

where V_i is the incident voltage,

V_r is the reflected voltage

and $\Gamma = \Gamma' + i\Gamma''$

$$= |\Gamma| \exp(i\phi)$$

where ϕ is the relative phase of the input and output voltages.

The voltage reflection coefficient and the impedance are related by

$$\Gamma = \frac{Z - Z_0}{Z + Z_0} \quad (3.2.4)$$

Combining Eq. (3.2.1), Eq. (3.2.2) and Eq. (3.2.4) yields

$$\Gamma = \left(\frac{\Gamma_0 + i\omega' Q_L}{1 + i\omega' Q_L} \right) \quad (3.2.5)$$

where $\Gamma_0 = \left(\frac{Q_u - Q_e}{Q_u + Q_e} \right)$ is the reflection coefficient at the resonant cavity frequency ω_r

$$\text{and } \omega' = \left(\frac{\omega}{\omega_r} - \frac{\omega_r}{\omega} \right)$$

The amplitude and phase of the reflected wave from the one-port cavity can be calculated as a function of ω , Q_L , Q_u and V_i using Eq. (3.2.5) and Eq. (3.2.3). The phase of the reflected wave is given relative to that obtained when the resonant cavity is detuned and acts like a real short. Equation (3.2.5) and the definition of Γ_0 show that the reflected wave is either in phase with the incident wave if $Q_u < Q_e$, or π radians out of phase with it if $Q_u > Q_e$. The cavity is said to be overcoupled if $Q_u > Q_e$ and undercoupled if $Q_u < Q_e$.

Equation (3.2.5) becomes

$$\Gamma = \left\{ \frac{\Gamma_0 + (\omega' Q_L)^2}{1 + (\omega' Q_L)^2} \right\} + i \left\{ \frac{(1 - \Gamma_0) \omega' Q_L}{1 + (\omega' Q_L)^2} \right\} \quad (3.2.6)$$

If $\Delta\omega = (\omega - \omega_r) \ll \omega_r$, then $\omega' \approx \frac{2\Delta\omega}{\omega_r}$ and $(\omega' Q_L)^2 \ll 1$

so that Eq. (3.2.6) becomes

$$\Gamma = \left\{ \Gamma_0 + \left(\frac{2\Delta\omega Q_L}{\omega_r} \right)^2 \right\} + i \left\{ (1 - \Gamma_0) \frac{2\Delta\omega}{\omega_r} Q_L \right\} \quad (3.2.7)$$

Equation (3.2.7) demonstrates that when $\Delta\omega \ll \omega_r$, Γ' is a function of $\left(\frac{\Delta\omega}{\omega_r} \right)^2$ and Γ'' is a function of $\left(\frac{\Delta\omega}{\omega_r} \right)$.

The decomposition of the voltage reflection coefficient into the two components, given by Eq. (3.2.6) or Eq. (3.2.7), is important because of their phase relationship to the microwave voltages arising from the absorption or dispersion components of the complex susceptibility of an EPR sample at resonance. Equation (3.2.6) or

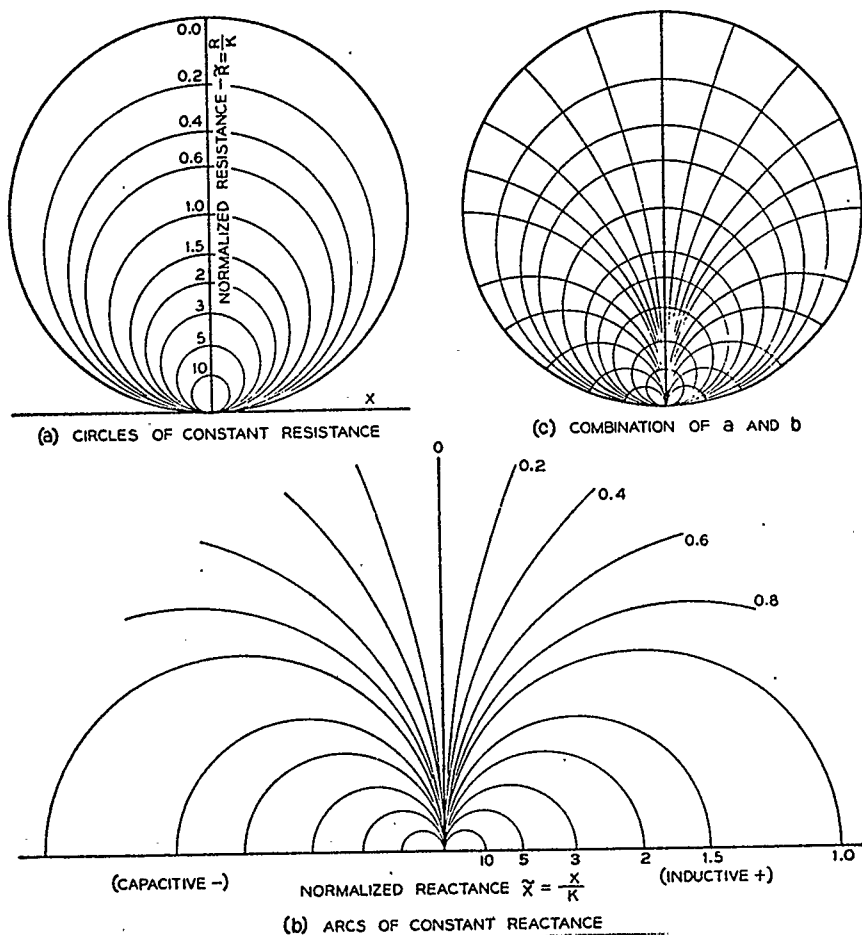


Figure (3.1).

An illustration of the impedance grid of the Smith chart. The circles of constant resistance are given in (a) and the arcs of constant reactance are given in (b). These grids are combined in (c) to give the impedance grid of the Smith chart.

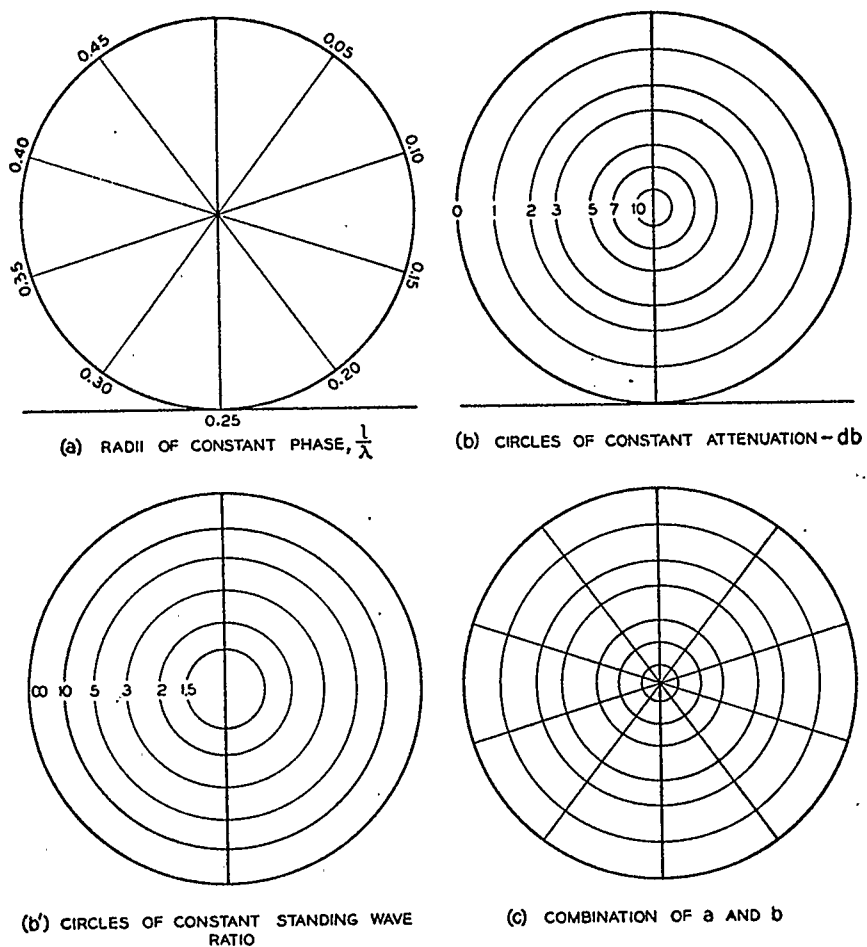


Figure (3.2)

An illustration of the propagation grid of the Smith chart. Contours of constant phase, attenuation and standing wave ratio are given in (a), (b) and (b'). These are combined in (c) to give the propagation grid.

Eq. (3.2.7) can then be used in the analysis of the effect of frequency modulation noise in the detection of either absorption or dispersion in an EPR spectrometer using a one-port reflection cavity. The Smith chart representation is a convenient way of showing the phase relationship between the components of the cavity reflection coefficient and the components of an EPR resonance.

3.3 The Smith Chart Representation of a Sample Cavity System

The mathematical relationships between the reflected wave and the complex impedance for a resonant microwave cavity containing an EPR sample are involved and difficult to visualise. The Smith chart is one of a number of graphical methods which help in the determination of normalised impedance, standing wave ratio, phase and reflection coefficient. The Smith chart representation of a one-port cavity gives the phase and amplitude of the reflection coefficient if the complex impedance or admittance of the sample cavity is known. The Smith chart representation was first applied to the analysis of EPR spectrometers by Wilmshurst, et al. (1962).

The Smith chart includes a mapping in an impedance plane defined by the overlapping resistive and reactive coordinate systems given in Fig. (3.1c). The impedance grid, given in Fig (3.1) and discussed by Southworth (1950), can be used to represent the resistive and reactive components in line terminations. The relative phase and attenuation along the transmission line to, or reflected from, the line termination can be represented on the propagation grid given in Fig. (3.2). The propagation grid is constructed from contours of constant phase, attenuation and standing wave ratio. The Smith chart is the impedance grid given in

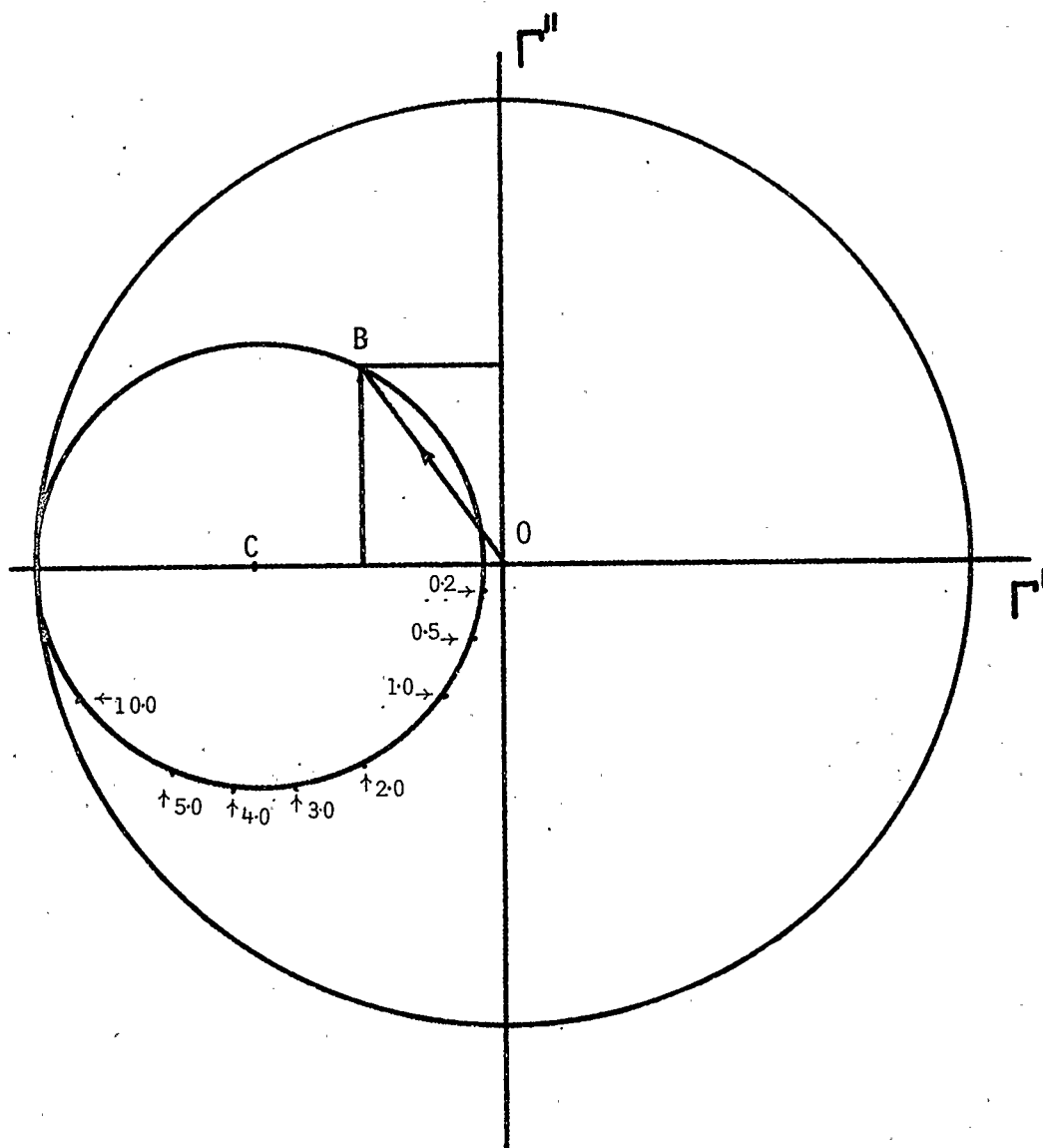


Figure (3.3).

The Smith chart diagram illustrating the reflection coefficient of an undercoupled resonant cavity as a function of frequency. The circle with centre C represents the loci of the reflection coefficient, as a function of frequency, for an undercoupled resonant cavity for which $Q_L \approx 5000$, $Q_e/Q_u \approx 1.1$ and $\omega_r \approx 35\text{GHz}$. The coordinates on the lower half of the circle of constant conductance are expressed in MHz from the cavity resonant frequency. These coordinates are repeated on the top half of the circle and the phasor OB represents the reflected wave when the frequency is 2MHz removed from the cavity resonant frequency.

Fig. (3.1c) together with coordinates around the unit circle, and along the real (r') axis, giving relative phase, attenuation and standing wave ratio as shown in Fig. (3.2). The importance of the Smith chart stems from the ease with which transformations can be made between normalized impedance, standing wave ratio, phase and attenuation.

The Smith chart can be used to give the voltage reflection coefficient of a one-port resonant cavity as a function of frequency. The normalized admittance $\left(\frac{Y}{Y_0}\right)$ is obtained from Eq. (3.2.1)

$$\left(\frac{Y}{Y_0}\right) = \left(\frac{Z_0}{Z}\right) = \left(\frac{Q_e}{Q_u}\right) + i \left(\frac{\omega}{\omega_r} - \frac{\omega_r}{\omega}\right) Q_e \quad (3.3.1)$$

Consequently, the real component of the normalized admittance, the normalized conductance, is independent of the microwave frequency and is a constant for any particular cavity. Southworth (1950) has shown that the lines of constant conductance are circles in the Smith chart. The imaginary component of the admittance, the susceptance, approaches $-\infty$ for $\omega \rightarrow 0$ and $+\infty$ for $\omega \rightarrow \infty$. Therefore, as the microwave frequency is varied from zero to ∞ , the loci of the reflected wave from a one-port cavity traces out a circle in the Smith chart. Equation (3.3.1) neglects the fact that the cavity will be resonant at more than one frequency. EPR spectrometers use low mode number, high Q cavities and the effect of other cavity resonances can be neglected.

Figure (3.3) illustrates the loci of the reflection coefficient as a function of frequency for an undercoupled resonant cavity for which $Q_L \approx 5,000$, $\left(\frac{Q_e}{Q_u}\right) \approx 1.1$ and $\omega_r \approx 35$ GHz. The coordinates on the circle of constant conductance are expressed in terms of the frequency

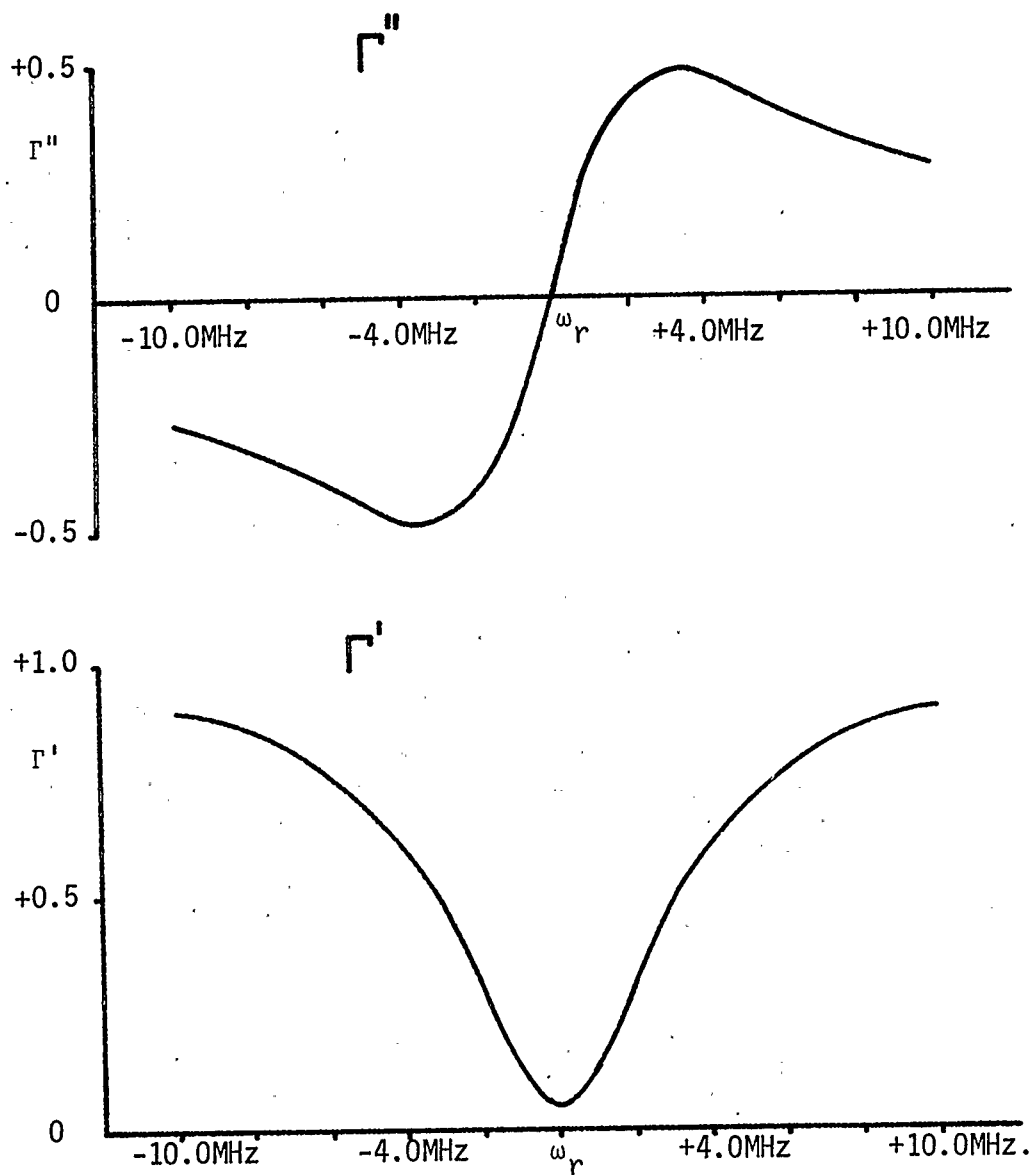


Figure (3.4).

Graphs showing the frequency dependence of the Γ' and Γ'' components of the cavity reflected wave. The frequency coordinates are centred on and relative to the cavity resonant frequency ω_r .

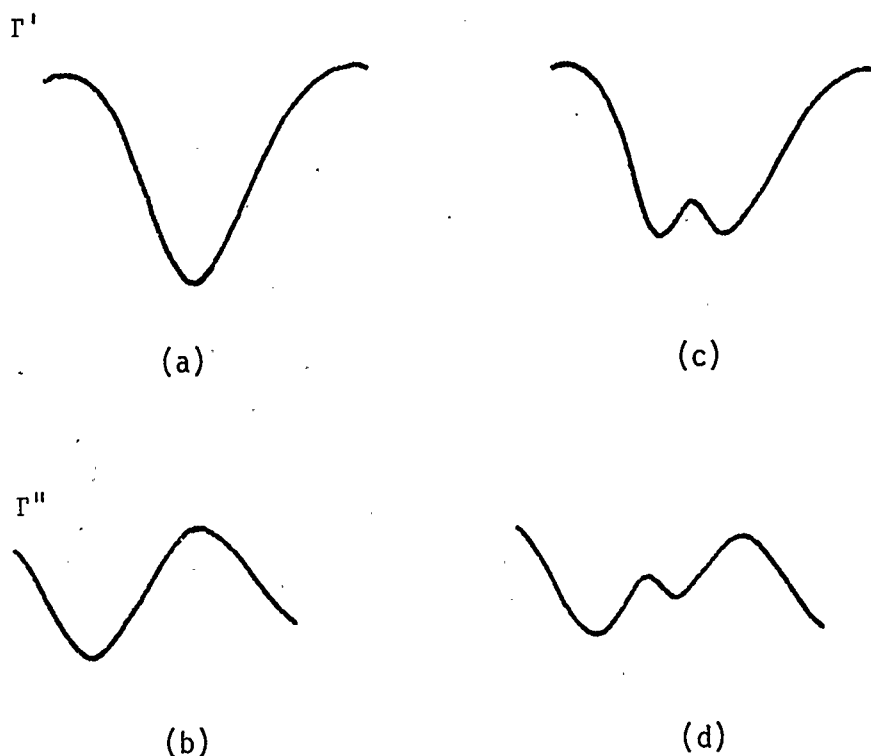


Figure (3.5a,b,c and d).

Curves illustrating the Γ' and Γ'' components of the cavity reflected wave with and without an EPR signal. These curves are tracings of oscilloscope photographs of the components of the cavity reflected voltage in phase with the Γ' and Γ'' axes. The klystron frequency and oscilloscope time base were swept by the same sawtooth voltage. The real and imaginary components of the cavity reflected voltage are given in (a) and (b) respectively. Figures (c) and (d) demonstrate that the absorption component (χ'') of an EPR transition is in-phase with Γ' and dispersion (χ') is in-phase with Γ'' .

deviation from the cavity resonant frequency. The relative magnitude and phase of the reflected wave when $\omega - \omega_r = +2$ MHz is represented by the phasor OB which can be resolved into orthogonal components parallel to the r' and r'' axes. The frequency dependence of these components is plotted in Fig. (3.4). Experimentally it is difficult to determine the frequency variation of the r' and r'' components because, in general, the output power of a microwave oscillator is also frequency dependent. Figures (3.5 a and b) show the superposition of these two effects.

The loci of the reflected wave arising from an EPR resonance, as the magnetic field is varied from zero to infinity, is a complicated function of the sample cavity parameters, the fundamental lineshape of the resonance and the degree of saturation of the sample. The simplest case is an unsaturated Lorentzian EPR resonance. It is shown in Appendix A that the locus of the reflected wave in this case will trace out a circle in the Smith chart as the magnetic field is varied from zero to infinity. It is experimentally demonstrated in Fig. (3.5 c and d) that the absorption component of an EPR transition (χ'') is in-phase* with r' , and dispersion (χ') is in-phase with r'' . A single crystal of DPPH was used as the EPR sample at a cavity power of $\sim 2\mu\text{W}$ to obtain Fig. (3.5 c and d).

3.4 Signal Demodulation

It has been shown in Sec. (3.3) that the EPR signal obtained using a resonant cavity can be resolved into two orthogonal signal voltages.**

* This is used in the sense that the phase difference is 0 or π radians.

** A wide band cavity-lock will effectively remove the dispersion component.

This section describes the demodulation of a modulated microwave carrier and shows that the detection of either pure absorption or pure dispersion can be accomplished by "synchronous demodulation."

An amplitude modulated microwave voltage can be demodulated by a microwave semiconductor diode in basically four modes of operation. The output voltage V_{out} from the semiconductor diodes depends on the peak value of the microwave electric field which is proportional to the microwave input voltage V_{in} at the diode. The characteristic curve of V_{out} as a function of V_{in} for semiconductor diodes includes two regions in which the demodulation is known as quadratic and linear demodulation.

(i) Quadratic Demodulation

Quadratic or square law demodulation arises when the demodulator output voltage is related to the microwave input voltage by

$$V_{out}(t) \propto V_{in}^2(t) \quad (3.4.1)$$

Torrey and Whitmer (1948) have shown that a typical silicon point-contact microwave diode operated at an incident power $< 5\mu W$ is a quadratic demodulator.

(ii) Linear Demodulation

The relation describing linear demodulation is

$$V_{out}(t) \propto V_{in}(t) \quad (3.4.2)$$

A silicon point-contact microwave diode operates as a linear demodulator at an incident microwave power $> \sim 50\mu W$.

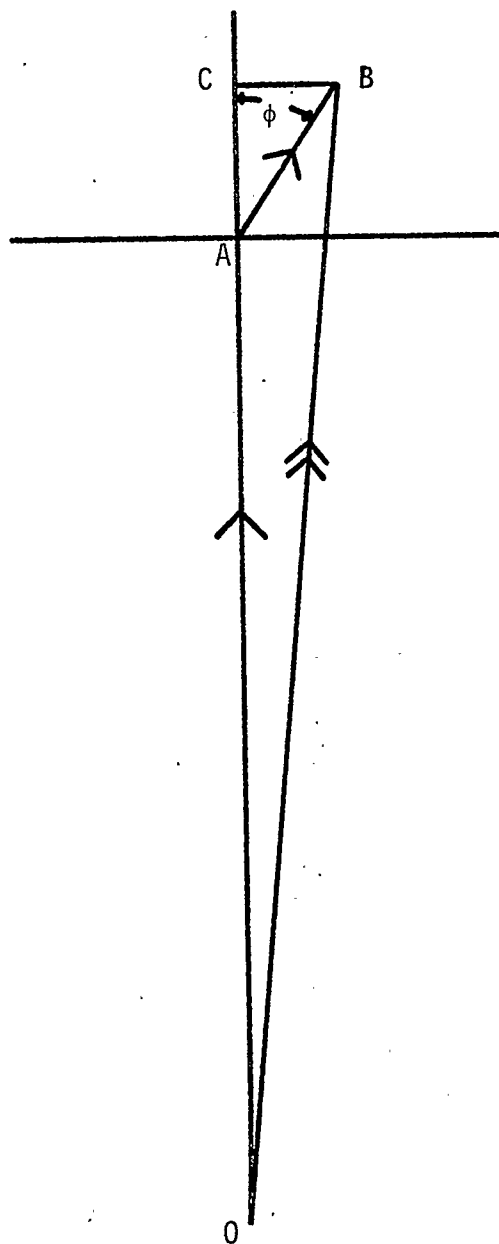


Figure (3.6).

The phasor diagram for the combination of a reference voltage OA and a signal voltage AB which are coherent, i.e. ϕ is a constant independent of time. For $OA \gg AB$ the demodulator output depends on OB which is approximately equal to $CO = (OA + AB \cos \phi)$.

The output voltage in Eq. (3.4.1) and Eq. (3.4.2) is a d.c. voltage with an a.c. component if $v_{in}(t)$ is amplitude modulated. The output voltage in Eq. (3.4.1) or Eq. (3.4.2) does not contain information about the instantaneous phase of the microwave radiation. Also, the quadratic and linear demodulation techniques cannot be used to demodulate a particular component of a microwave voltage.

(iii) Synchronous Demodulation

Synchronous demodulation is used to demodulate an amplitude modulated component of a microwave voltage in phase with a reference microwave voltage. Synchronous demodulation can be used in the detection of absorption and dispersion. The basic principle of the synchronous demodulation of a weak signal is illustrated in Fig. (3.6). The phasor OA represents the reference or homodyne voltage v_{ref} and the phasor AB represents the signal voltage v_{sig} at a relative phase angle of ϕ . When v_{ref} is coherent with v_{sig} so that ϕ is a constant independent of time, and v_{ref} is $\gg v_{sig}$, then the microwave demodulator output will depend on the value of the phasor OB which is approximately equal to $(v_{ref} + v_{sig} \cos\phi)$. Consequently, the output of the demodulator is dependent on the particular component of the signal voltage in phase with v_{ref} .

The EPR homodyne spectrometers operated in this laboratory use a balanced mixer to obtain balanced synchronous demodulation. The matched microwave diodes terminate the symmetric arms of a hybrid or magic tee, the reference voltage is incident on the E arm of the tee and the signal voltage is incident on the H arm of the tee. It is shown in Appendix B that, under these circumstances, the output voltage is given

by
$$V_{out}(t) \propto V_{in}(t) \cos \phi$$

where ϕ is the phase angle between $V_{in}(t)$ and the reference or homodyne voltage V_{ref} . V_{out} is independent of time variations in V_{ref} (a.m. noise) and is independent of the magnitude of V_{ref} if the microwave demodulators are operated as linear demodulators. In an EPR homodyne spectrometer V_{ref} and V_{sig} are coherent because they are derived from the same microwave source. This mode of demodulation can lead to the detection of either absorption or dispersion by altering the phase of microwave homodyne voltage.

(iv) Superheterodyne Demodulation

Superheterodyne demodulation is used to extract audio frequency modulation of a microwave carrier while rejecting the demodulator noise at the audio modulation frequency. The microwave signal voltage is mixed with a reference voltage of a different frequency and the initial demodulation is performed at the difference or intermediate frequency. The intermediate frequency should exceed the frequency of the "knee" of the demodulator's flicker noise spectrum. Amplitude modulation of the signal voltage produces amplitude modulation of the intermediate frequency voltage which can then be demodulated by (i), (ii) or (iii). Synchronous demodulation at the intermediate frequency in a superheterodyne EPR spectrometer is desirable because of superior noise rejecting properties, and because either absorption or dispersion can be detected easily. Synchronous demodulation at the intermediate frequency was first introduced in an EPR superheterodyne spectrometer by Teaney, Klein and Portis (1961).

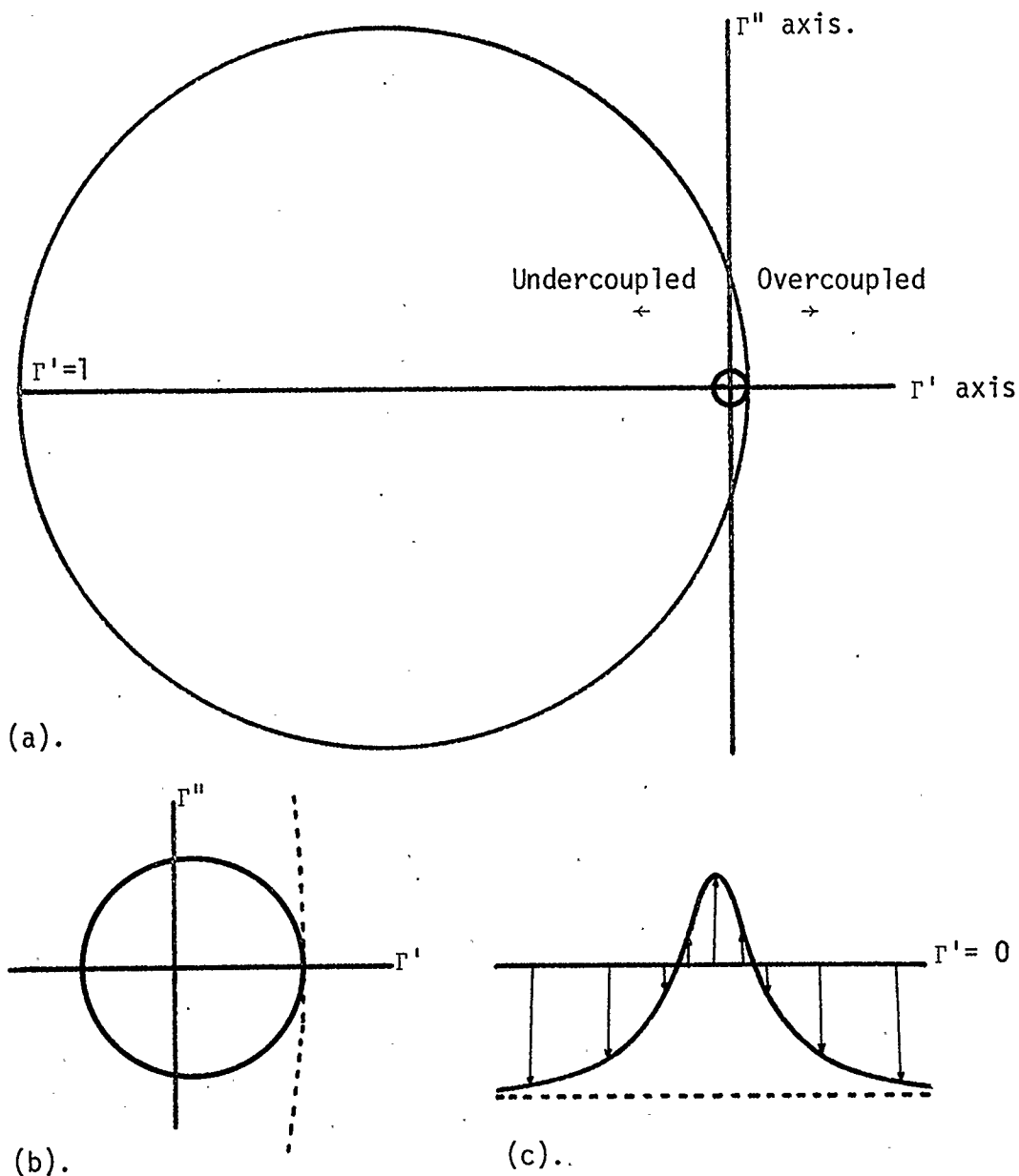


Figure (3.7.a.b and c).

An illustration of the Smith chart diagram, (a), of a sample cavity whose coupling is changed, as shown, on going through an EPR resonance. The small circle including the point $\Gamma' = \Gamma'' = 0$ represents the variation in reflected wave as a function of magnetic field due to a Lorentzian EPR signal. The small circle is enlarged in (b). The absorption component (χ'') of the reflected wave, due to the EPR signal, goes through zero as shown in (c). The χ'' phasors at various values of B are shown in (c).

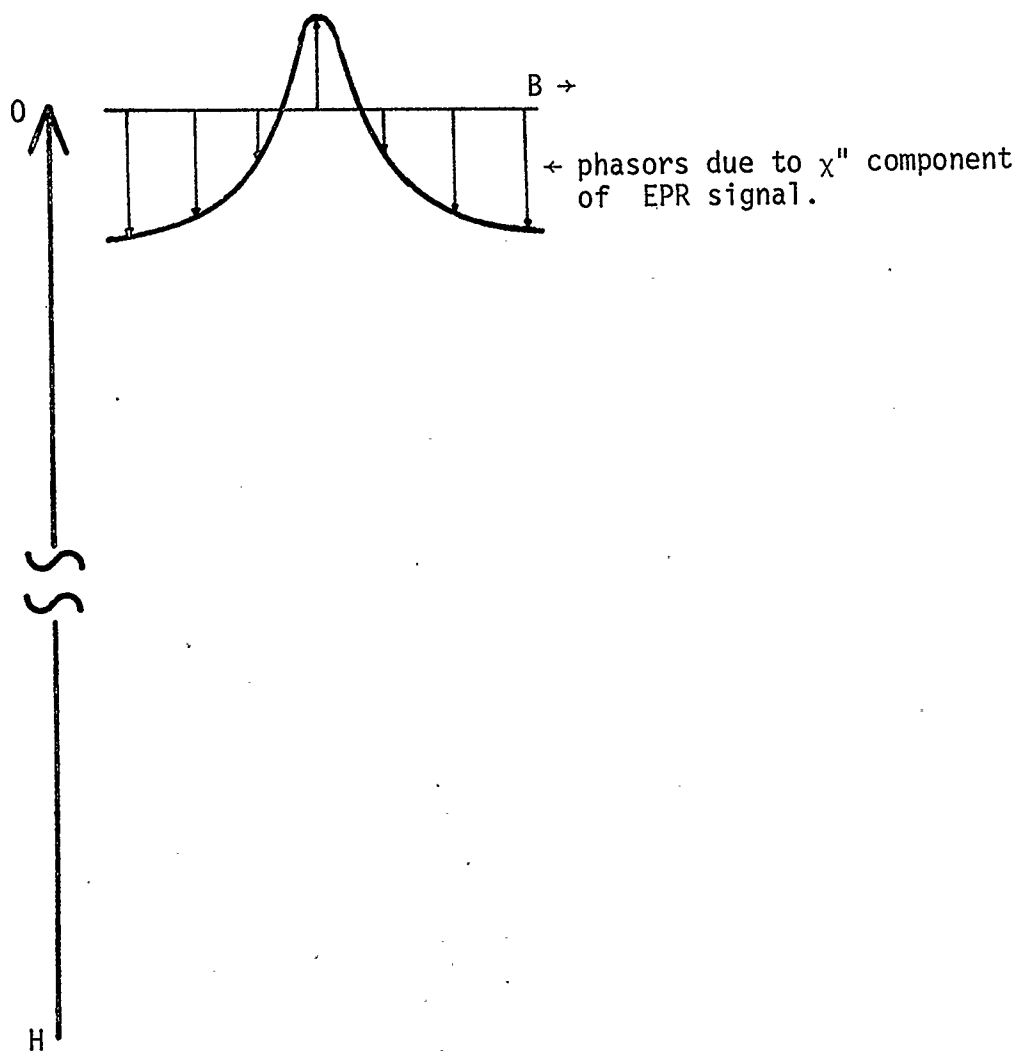


Figure (3.8)

The phasor diagram for the combination of a homodyne or reference voltage H_0 with the phasors representing the χ'' component of the EPR signal at various values of magnetic flux density B . Despite the phase discontinuity in the χ'' component due to the EPR signal no distortion is produced in the detected lineshape.

Feher (1957) observed that if the sample cavity is very slightly overcoupled then an EPR signal could change the cavity coupling and produce a distorted absorption line. This observation has been repeated by Poole (1967), amongst others. It applies only to a simple bridge EPR spectrometer without a homodyne voltage applied to the microwave demodulator, and it does not apply to a homodyne spectrometer. This can be explained in terms of a Smith chart representation of the EPR resonance.

The Smith chart representation of the reflected wave from a sample cavity containing an EPR sample is given in Fig. (3.7 a and b). The large circle gives the cavity reflected wave as a function of frequency. The microwave frequency is kept constant in spectrometer operation and only the small circle, enlarged in Fig. (3.7b), representing the variation of the reflected wave as a function of magnetic field need be considered. When this circle includes the point $\Gamma' = \Gamma'' = 0$ on the Smith chart the cavity coupling will be changed by the magnetic resonance. Figure (3.7 c) shows the variation in the absorption component of the magnetic resonance as a function of magnetic field. The absorption component includes a phase discontinuity but not a voltage discontinuity as implied in Feher (1957). The absorption component Fig. (3.7 c) can be detected without distortion if the conditions for synchronous demodulation are satisfied. The combination of the homodyne voltage and signal voltage is represented on the phasor diagram Fig. (3.8). It is apparent that there is no voltage discontinuity and no distortion of the absorption lineshape, at the spectrometer output, due to the phase discontinuity in the absorption reflected wave.

3.5 The Use of Magnetic Field Modulation

It is advantageous to obtain amplitude modulation of the EPR signal voltage by modulation of the magnetic field. High sensitivity is obtained using narrow band amplification and synchronous demodulation at magnetic field modulation frequency. The non-linear variation in χ' or χ'' with magnetic field, illustrated in Fig. (2.4), produces signal voltages at harmonics of the modulation frequency. The relationship between the shape and amplitude of the Fourier coefficients of the n^{th} harmonic of the absorption a_n and dispersion d_n components of an EPR line and the amplitude of the field modulation has been studied extensively by Buckmaster et al. (1966, 1968, 1969, 1971). Most studies employ the detection of a_I , however, improved resolution of complex resonances can be obtained if a_{III} rather than a_I is detected.

Buckmaster and Dering (1968) have shown that

$$\lim_{(H_\omega/H_\Delta \rightarrow 0)} \{a_n, d_n\} \propto H_\omega^n \cdot \frac{d^n(\chi'', \chi')}{dH_\omega^n} \quad (3.5.1)$$

if the magnetic field modulation amplitude (H_ω) is small compared to the half resonance width at half maximum amplitude H_Δ . It is typical in the literature to refer to these Fourier coefficients as being the derivatives of χ' and χ'' independent of the amplitude of H_ω and H_Δ .

CHAPTER IV

THE INSTRUMENTATION OF EPR SPECTROSCOPY

The basic theory of the detection of an EPR signal, given in Chapter III, applies specifically to the case of a sample suitably located in a resonant microwave cavity. The use of a single mode resonant cavity has been almost universally adopted in commercial and laboratory built spectrometers because of its simplicity, high inherent sensitivity and versatility. Comparable results can be achieved with other specialized techniques, e.g. the use of a microwave helix (Wilhmurst, Gambling and Ingram, 1962) or a bimodal cavity (Teaney, Klein and Portis 1961; Koepp, 1969). These latter techniques will not be described in detail here.

Section (4.1) discusses the advantages and disadvantages of particular operating frequencies and the need, in some cases, to obtain EPR spectra from a particular sample at different microwave frequencies. The theoretical and experimentally achievable sensitivity of an EPR spectrometer, as a function of the operating microwave frequency, is discussed. The designs of various homodyne and superheterodyne spectrometers are described and compared in Sec.(4.2) and Sec.(4.3). Section 4.4 discusses the factors which limit the sensitivity of the EPR spectrometers described in Secs. (4.2) and (4.3).

4.1 The Choice of Spectrometer Operating Frequency

EPR spectrometers are normally constructed to operate in a frequency range in one of the three microwave frequency bands; 8-12 GHz,

18-26GHz and 26-40GHz. The reasons for the development of EPR spectrometers in these bands are: (i) the microwave components in these bands are readily available and of high quality and reasonable cost; (ii) the resonant cavity size is convenient for most samples; (iii) the magnetic fields of the correct magnitude and homogeneity can be obtained in a low temperature dewar; and (iv) microwave oscillators and diodes are inexpensive, readily available and of high quality in comparison to their millimetre wave counterparts.

Measurements of EPR spectra at frequencies above 40GHz are useful in the following situations: (i) when the zero field splitting of the ground state of a paramagnetic ion exceeds 40GHz; (ii) in the study of very small samples such as tiny single crystals of biological material; (iii) in the study of magnetic field dependent g-values; and (iv) the study of the magnetic field dependence of spin-Hamiltonian crystalline electric field parameters.

The discussion of the first three situations can be illustrated with respect to the study of the EPR spectra of ferric iron in crystals of MbH_2O and HbH_2O . Many other examples exist.

The direct measurement of the zero field splitting in the ground state of a paramagnetic ion requires that the energy of the microwave quanta is comparable to the energy separation in the zero field splitting under investigation. Ideally, the investigation of the ground state of the ferric ion in MbH_2O , HbH_2O and similar high spin heme compounds

would include the study of all five allowed transitions of the sextet ground state. The zero field splitting between the three doublets

($s_z = \pm \frac{1}{2}, \pm \frac{3}{2}, \pm \frac{5}{2}$) is so large that a spectrometer operating at approximately 300 GHz with magnetic fields of up to approximately 15T would be required to observe the five allowed transitions. The application of EPR in the region of the electromagnetic spectrum between approximately 100 GHz and 1000 GHz is very difficult. Mock (1966) has shown that it is possible to use waveguide techniques in EPR measurements up to 140 GHz. Boettcher, Dransfeld and Renk (1968) have shown that EPR is possible using essentially optical techniques at 1000 GHz.

No direct experimental measurement of the zero field splitting in MnH_2O has been made, however Slade and Ingram (1969) have shown that the experimental $g_{\perp}$ -value obtained from the lowest doublet is frequency dependent to an extent which is a function of the zero field splitting between the lower two doublets. The study of the frequency dependence of the $g_{\perp}$ -value of MnH_2O is an example of the situation (ii) above in which it is important to use EPR measurements made at different microwave frequencies.

The third situation in which it may be advantageous to use a high frequency EPR spectrometer occurs when the EPR sample size is limited. The volume of the resonant cavity decreases as the frequency increases so that the same sample will occupy a proportionally larger fraction of the cavity volume at higher frequencies. The ratio of the sample to cavity volume occurs in the expression for the filling factor η . This factor was introduced in Sec.(3.1) where it was shown that the EPR signal amplitude is proportional to η .

The fourth situation in which it is important to obtain EPR results at different frequencies can be illustrated by the work of Buckmaster, Chatterjee and Shing (1971). They showed that the largest crystalline electric field parameter for Gd^{3+} in lanthanum ethyl sulphate single crystals changed by approximately 0.2% between 5 GHz and 35 GHz. The experimental data from both the 5 GHz and the 35 GHz EPR spectrometers was obtained at room temperature.

Theoretical calculations of the variation in EPR spectrometer sensitivity with frequency have been given by Feher (1957), Hyde (1961) and Alger (1968). The theoretical sensitivity improvement factor as a function of the frequency is proportional to the seven-halves power of the frequency ratio when the sample is of limited size but not saturable, and to the one-half power of this ratio when the sample size is not limited. In practice, these sensitivity improvement factors are difficult to obtain at frequencies in excess of 40 GHz because the noise factor and conversion gain of presently available microwave detectors degrades significantly with increasing frequency.

It is appropriate to consider the experimental difficulties that, to the present, have prevented the extensive application of EPR above 100 GHz. The magnetic field required to observe a " $g = 2$ " EPR resonance at frequencies in the range 70-300 GHz varies from 2.5T to 11T. The best iron core laboratory electromagnets have a maximum magnetic field of approximately 3.5T in a 2.5 cm air gap. This magnetic field limits the maximum frequency at which one could observe a " $g = 2$ " EPR signal to approximately 100 GHz. It is possible to generate larger magnetic fields by using either superconducting magnets or high current solenoids, however both are expensive in comparison to the iron core electromagnet when capital and operating costs are considered and when comparable magnetic

field homogeneities are required. Superconducting magnets are capable of producing approximately 10-15T over a volume suitable for a high frequency EPR cavity and high power solenoids can produce approximately 18T in a 2.5cm axial bore. In laboratories with liquid helium facilities the use of a superconducting magnet is the best method to obtain magnetic fields in excess of 3.5T. An EPR spectrometer operating in the frequency range 100-300 GHz but without the magnetic field capability of observing a " $g = 2$ " EPR resonance would still be a valuable instrument to measure directly large zero field splittings.

The development of microwave technology was limited initially to the lower frequency bands. Components in these bands, in particular the 8-12 GHz band, are a convenient size for construction to the required tolerances. Components in the cm wavelength region (3-30 GHz) are now readily available and reasonable in price. Higher frequency, millimeter wavelength components (30-300 GHz) are available from a limited number of manufacturers. The specifications of these components are frequently inferior to those possible in the 8-12 GHz band. The limitations for millimeter components arise because of the achievable dimensional tolerances in the much smaller waveguide and component dimensions. The current technological high frequency limit for TE mode waveguide is approximately 220 GHz which is close to the theoretical limit for some components as shown by Kroon and Van Nieuiland, (1967). Quasi-optical or oversize waveguide are necessary for efficient microwave propagation above ~ 100 GHz. In general, it can be stated that the cost of high frequency components increases with frequency and their availability and performance decrease.

Three specific problems exist in the design of high frequency EPR spectrometers. These are: the selection of the most suitable source of

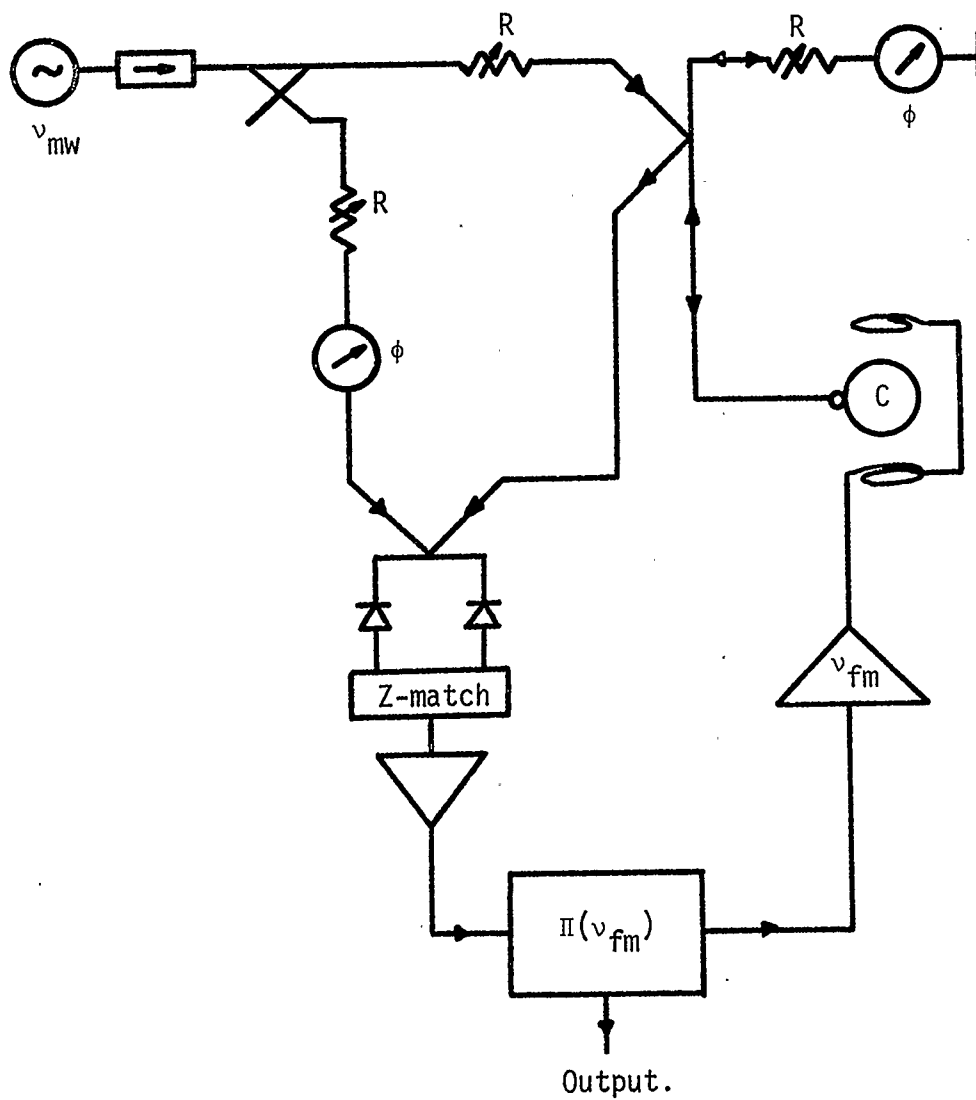


Figure (4.1).
Block diagram of the basic homodyne EPR spectrometer configuration.

microwave radiation; the selection of the detector with the best noise factor and conversion gain under operating conditions; and the selection of the most efficient microwave radiation propagation mode. These problems have been discussed by Slade and Ingram (1969) with particular emphasis on the 4 mm (75 GHz) wavelength region. They constructed a 4 mm EPR spectrometer using conventional techniques except that oversize waveguide was used between the bridge and the sample cavity and a confocal resonator was used for the a.f.c. circuit. This 4 mm EPR spectrometer used a superconducting magnet with a maximum field of 5T. Van der Boom (1969) also constructed a homodyne EPR spectrometer at 70 GHz using essentially conventional techniques. The millimeter EPR spectrometer constructed and operated to obtain the results described in this thesis operated between 54-60 GHz. This EPR spectrometer was used in conjunction with an iron core electromagnet with 10 cm diameter tapered pole faces rated to 2.08T in a 3 cm air gap.

4.2 Homodyne EPR Spectrometers

The most popular spectrometer configuration is based on the homodyne principal. The basic homodyne EPR spectrometer configuration is illustrated in Fig. (4.1). The microwave power is normally derived from a reflex klystron and the majority of the output power is incident on the microwave sample cavity bridge. Some power is extracted via a 10 or 20 dB directional coupler to provide the coherent homodyne power necessary for microwave synchronous demodulation. The power incident on each microwave diode is adjusted so that they are operated under conditions for optimum signal to noise ratio. A precision attenuator is used to vary the power incident on the sample cavity. Buckmaster and Dering

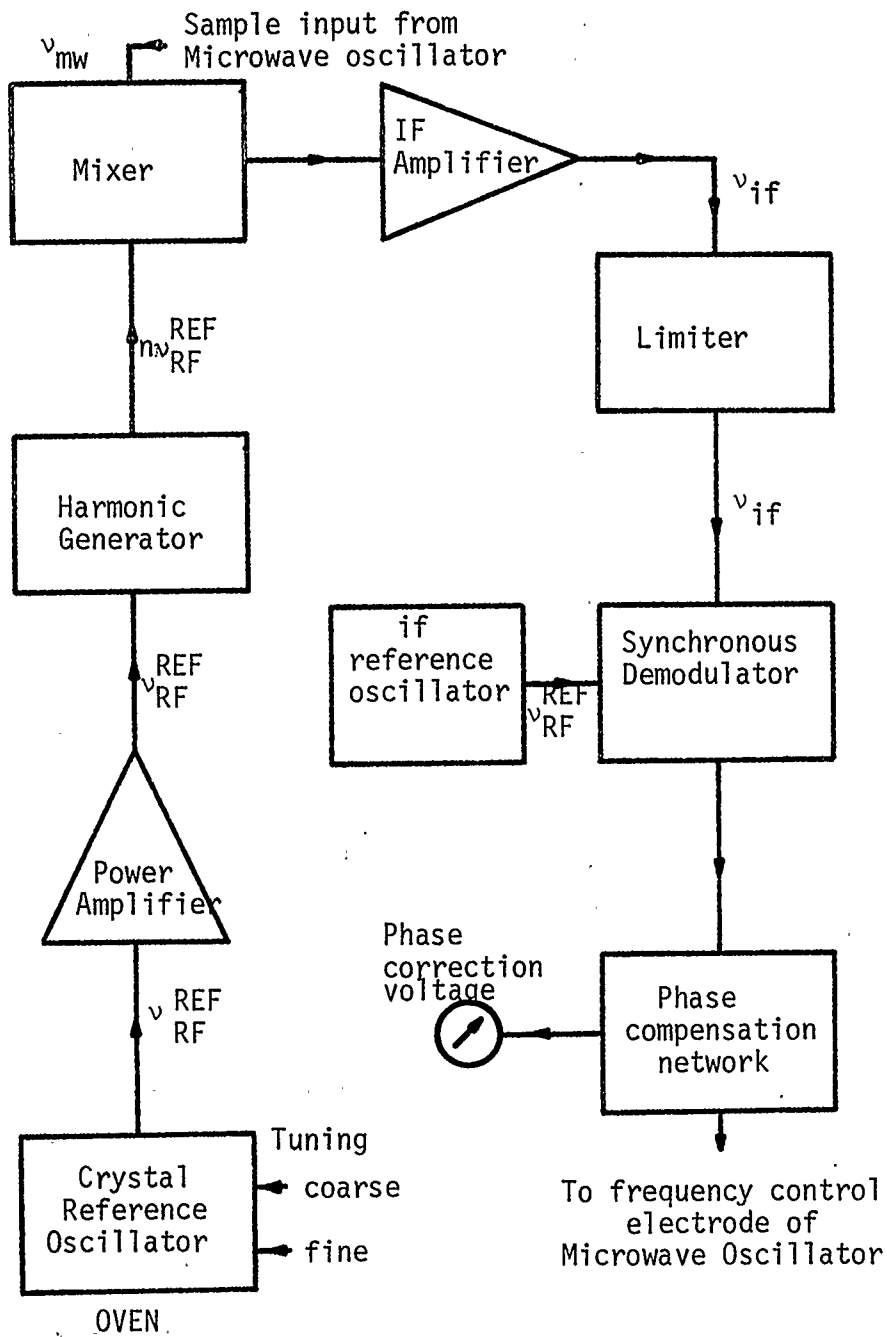


Figure (4.2).
Block diagram of a microwave oscillator synchronizer.

(1967) have shown that the most stable bridge balance can be achieved with a balance arm including a rotary vane precision attenuator, a rotary vane precision phase shifter and a short as shown in Fig. (4.1). A hybrid tee, constructed from two rhodium plated aluminum blocks was used in this study because of its mechanical stability. Any reflection coefficient can be obtained with a minimum of adjustments with this bridge balancing system. When the resonance condition for a sample is satisfied the microwave bridge is slightly unbalanced and an EPR signal voltage reaches the microwave diodes. The desired absorption or dispersion component of the signal voltage is selected using the microwave homodyne phase shifter.

Demodulation of the EPR signal is accomplished using a matched pair of microwave silicon point-contact diodes in a balanced mixer. This configuration has the advantage that amplitude-modulation (a.m.) noise, originating in the klystron and incident on the diodes via the homodyne arm, is cancelled and does not contribute to the overall noise factor of the EPR spectrometer. Balanced synchronous demodulation also reduces the effect of the klystron f.m. noise at cavity powers in excess of ~ 10 mW. The transformation of f.m. noise into a.m. noise by the sample cavity is discussed in detail in Sec (4.4).

Buckmaster and Dering (1967) have shown that the spectral purity of the microwave power and the degree of coincidence of the microwave and sample cavity frequencies play important roles in determining the long-term sensitivity and stability of an EPR spectrometer. The various methods for improving the spectral purity of the microwave radiation have been reviewed and discussed by Dering (1967). The method adopted in this laboratory and illustrated in Fig (4.2) has been to phase-lock the frequency of the klystron to a harmonic of a tunable, high spectral

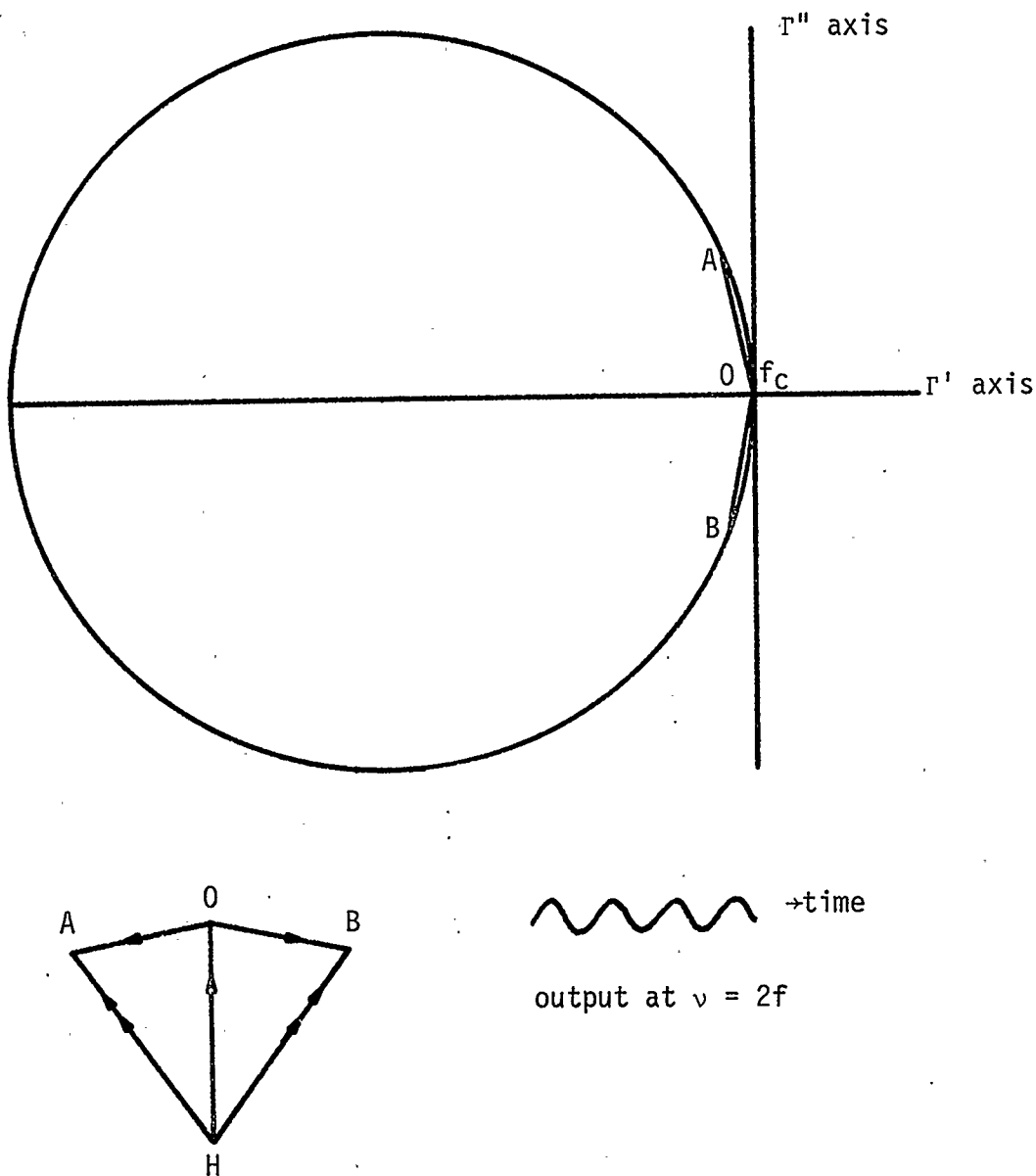
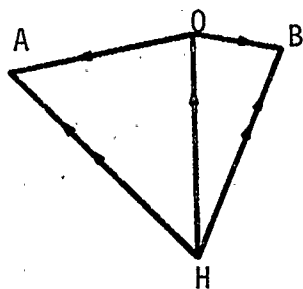
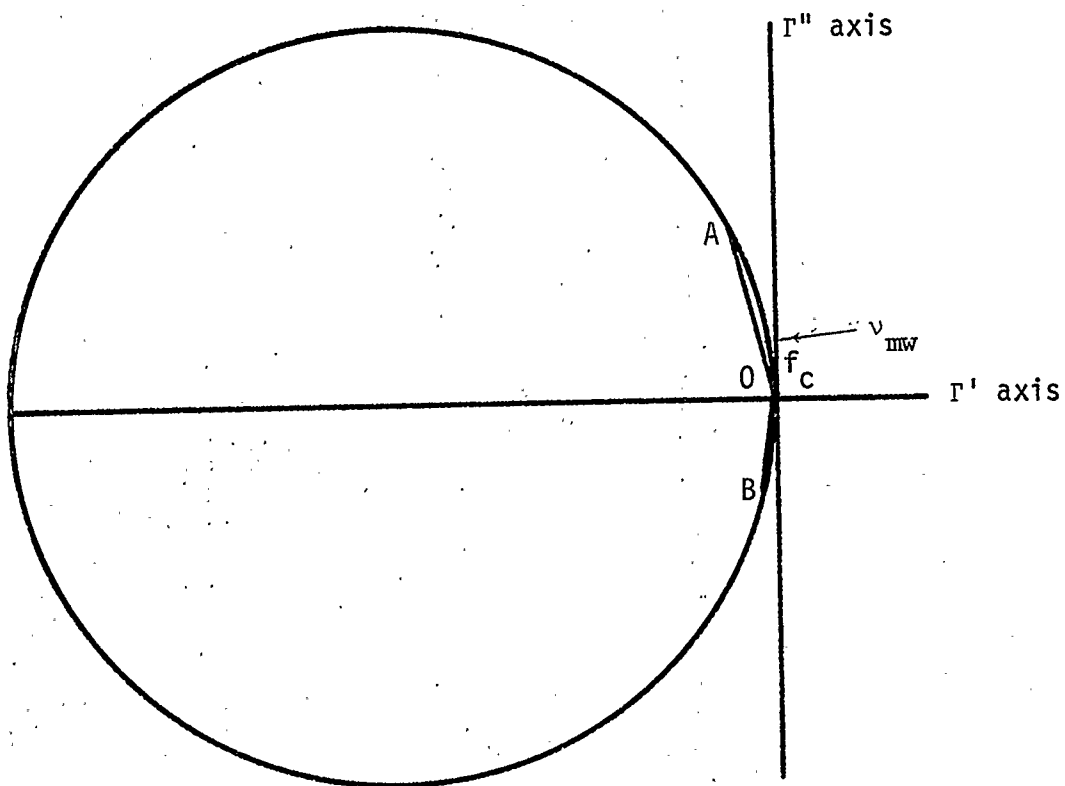


Figure (4.3a).

Smith chart and phasor diagrams illustrating the operation of a cavity frequency lock circuit which uses klystron frequency modulation. The klystron is frequency modulated at $\nu = f$ with a peak frequency deviation Δf . The phasors OA, zero and OB correspond to the output voltage of a balanced EPR bridge when the frequency is $\nu_{mw} + \Delta f$, ν_{mw} , and $\nu_{mw} - \Delta f$ and the klystron frequency ν_{mw} is equal to the cavity resonant frequency f_c . The combination of the signal phasor and the homodyne phasor HO leads to an a.m. signal at $2f$ as shown.



output contains a component at $\nu=f$.

Figure (4.3b)

Smith chart and phasor diagram illustrating the effect of a frequency error between the klystron frequency ν_{mw} and the cavity resonant frequency f_c . The spectrometer output now contains a component at the klystron modulation frequency which can be used in a feedback circuit to reduce the frequency error.

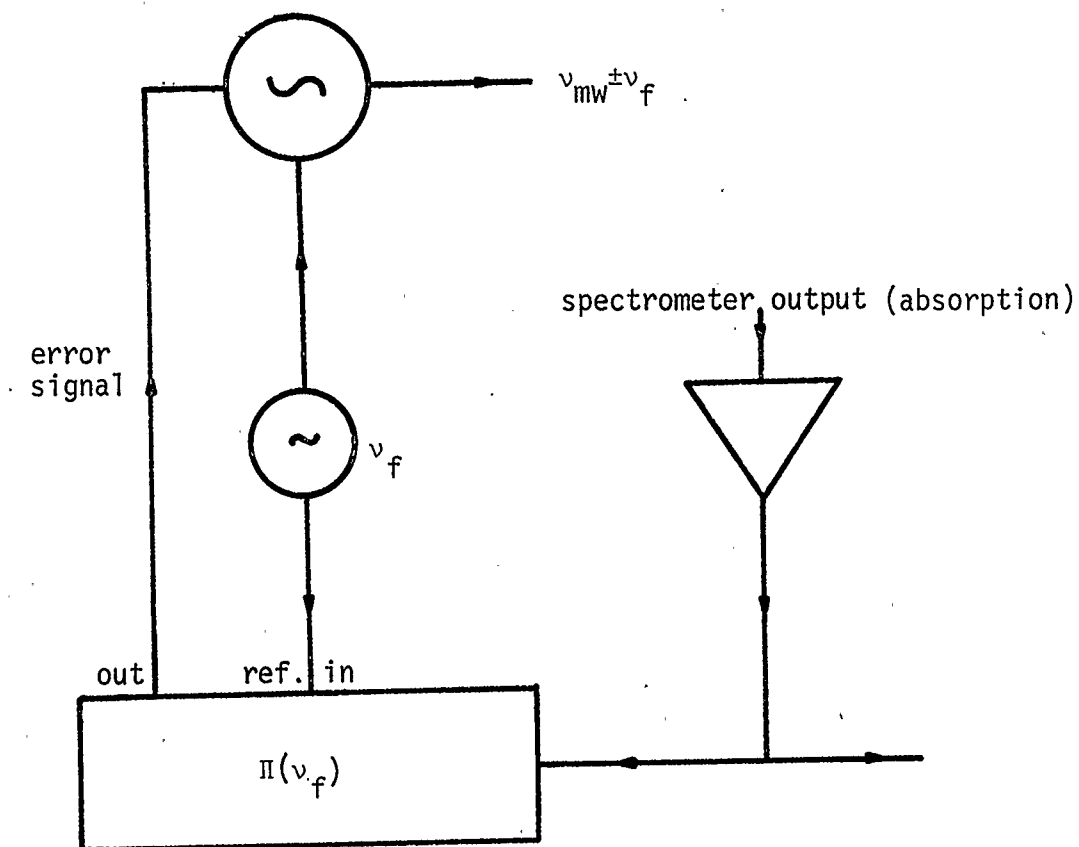


Figure (4.4)
Block diagram of the klystron frequency modulation type
cavity frequency lock circuit.

purity crystal oscillator. Near continuous microwave frequency coverage over GHz bandwidths is achieved in the klystron output frequency by using ten tunable crystal oscillators with overlapping frequency ranges in the phase lock synchronizers. The crystal oscillators have centre frequencies spaced 12.5 kHz apart in the range 15.000 to 15.113 MHz. Commercial units are available in all frequency bands from 1 GHz to 40 GHz (Microwave Systems Inc.).

The frequency of the klystron can be locked to the sample cavity resonant frequency in a number of ways. Commercial spectrometers often use frequency modulation of the klystron. When the klystron is tuned to the cavity resonant frequency and the spectrometer is adjusted to detect absorption, then the second harmonic but not the fundamental of the modulation frequency appears at the output of the spectrometer. The operation of this system is described in the Smith Chart diagram in Fig (4.3 a and b). The microwave frequency is modulated between the points A and B on the Smith chart at a rate f . The lines OA and OB represent the phasors at the extremes of the frequency modulation. The phasor representing the cavity reflected wave is added to that representing the homodyne voltage adjusted to detect absorption. The resultant is amplitude modulated at $2f$ unless there is a frequency error between the microwave power and the cavity resonant frequency. A frequency error will lead to amplitude modulation at the frequency f in the spectrometer output as shown in Fig (4.3b). Synchronous demodulation at the frequency f is used to convert the a.c. signal to a quasi d.c. error voltage which will reduce the frequency error as shown in Fig (4.4).

The disadvantage of this system is that it is possible to observe

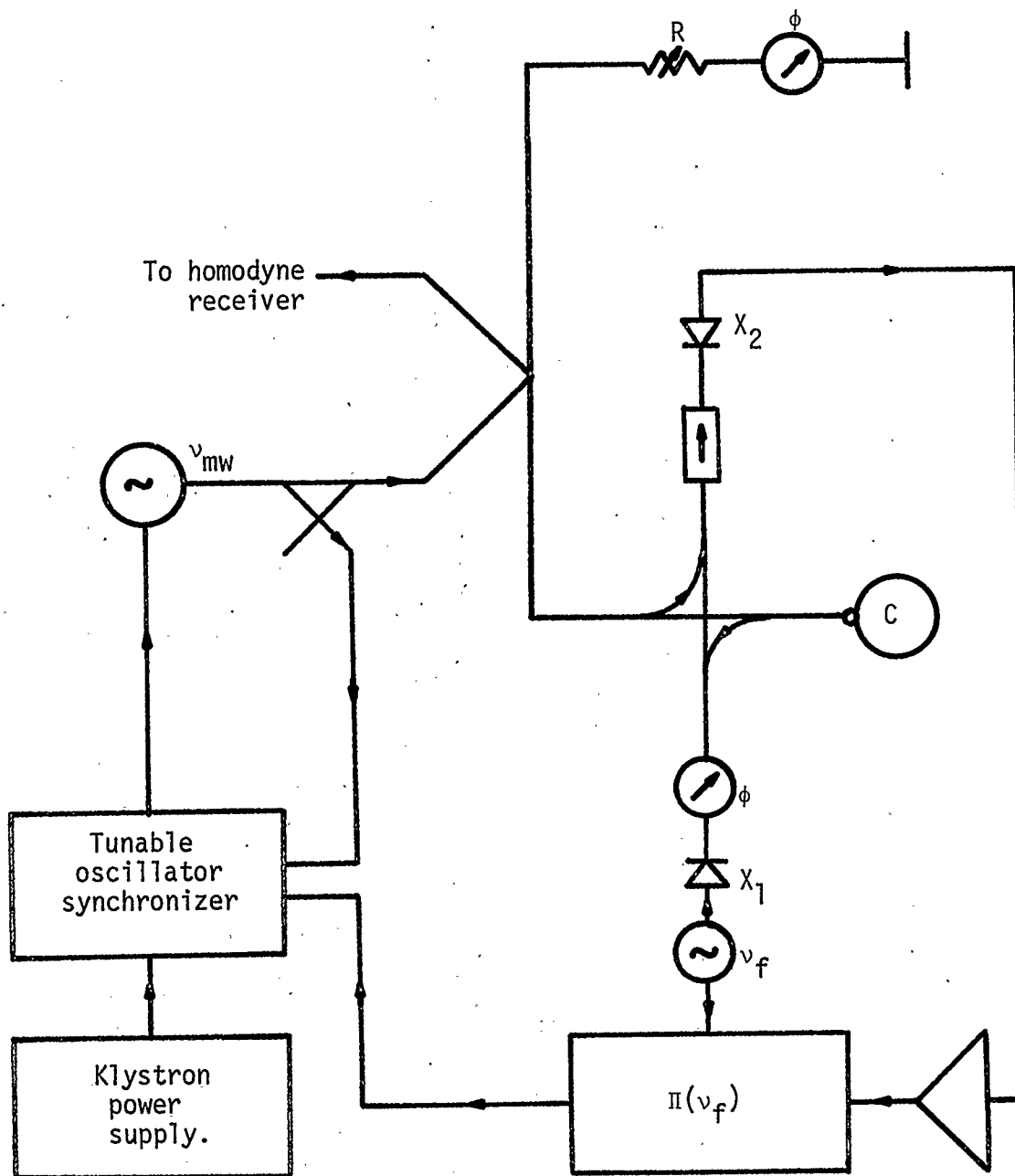


Figure (4.5)

Block diagram of the Owston (1964) cavity frequency lock circuit.

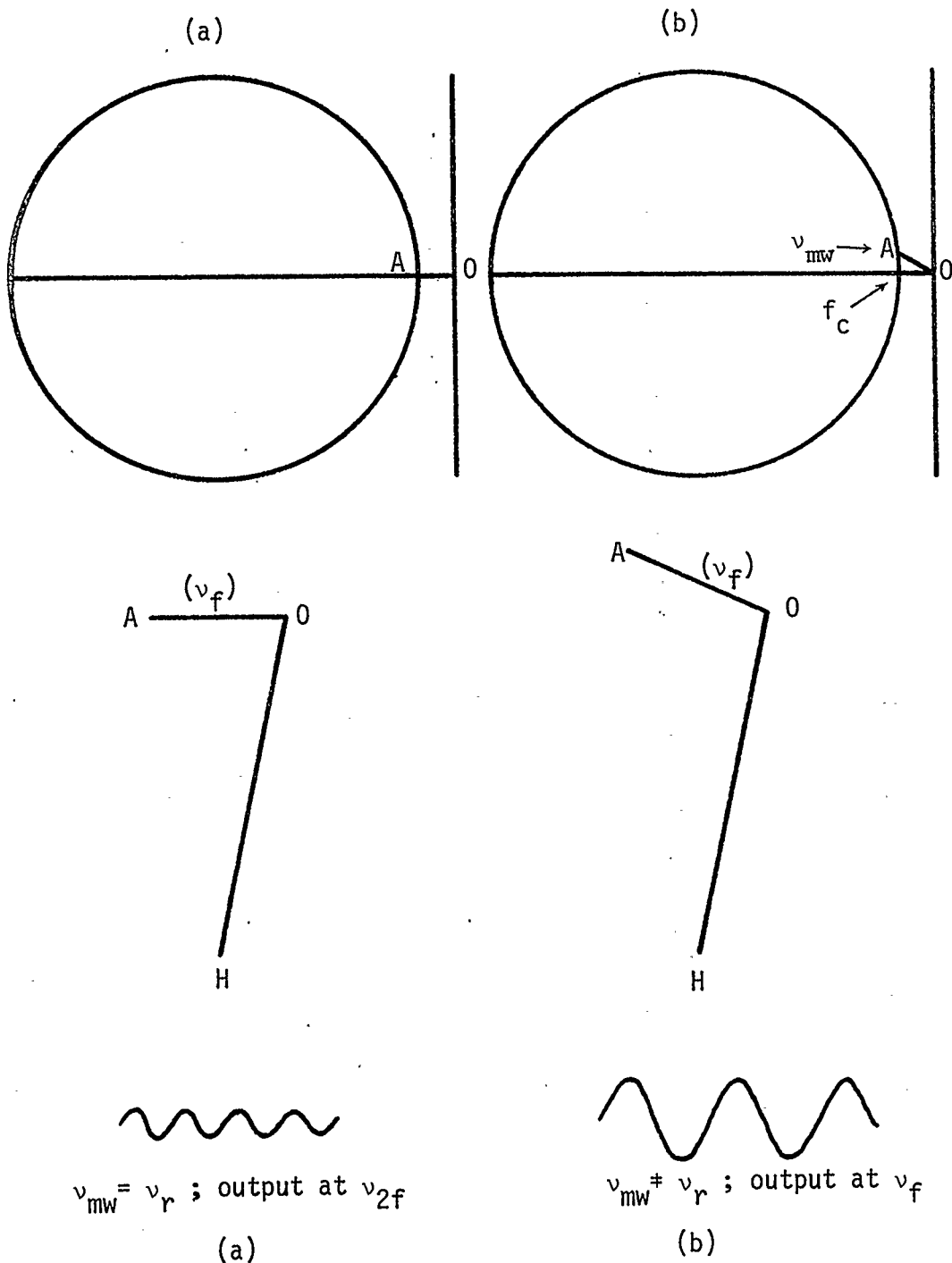


Figure (4.6a and b)

Smith chart illustrating the operation of the Oswton circuit, (a), with no frequency error and, (b), with a frequency error $v_{mw} \neq f_c$. The amplitude modulation of A_0 by X_1 at a frequency v_f in Fig.(4.5) produces an output at f only if a frequency error exists. The a.m. voltage at f can be used as shown in Fig.(4.5) to reduce the frequency error.

spurious satellites or broadening of a very narrow EPR resonance when the magnetic field corresponds to the resonance condition for the frequency sidebands. This is unimportant for X-band spectrometers, but because of the increased cavity bandwidth at higher frequencies, Van den Boom (1969) has shown that it may be necessary to increase the frequency of the klystron modulation to a point where the spurious signals are detectable. This type of cavity frequency lock frequently fails at cavity power levels of less than $\sim 10 \mu\text{W}$. Adjustment of the microwave homodyne phase-shifter will detune the cavity and the demodulated signal will consist of some of the dispersion component mixed into the absorption component. The cavity lock circuit will be inoperative if the homodyne phase setting differs from that required for pure absorption by more than $\sim \frac{1}{4}\pi$ radians.

The cavity lock system used in this laboratory is a version of the Pound (1946) system originally described by Owston (1964). The additional microwave and electronic components required in this cavity locking system are illustrated in Fig (4.5). The Smith chart representation of its operation is given in Fig (4.6). A fraction of the voltage reflected from the cavity at resonance is incident on the microwave diode x_1 in Fig (4.5). An a.c. voltage at a frequency f is applied to x_1 , this amplitude modulates the reflected voltage from x_1 represented by OA in Fig (4.6). The phase of the reflected voltage is adjusted so that when the microwave frequency is equal to the cavity resonant frequency then the output of x_2 is zero at f although a small a.c. signal at $2f$ is present. This is illustrated in the phasor diagram in Fig (4.6). The phasor HO represents the voltage incident on x_2 derived from the power

incident on the cavity by the cross guide coupler. Whenever the cavity frequency changes, the phasor OA rotates relative to HO and an a.c. signal at f is obtained. This a.c. voltage at f can be used, as before, to lock the microwave frequency to the cavity.

The disadvantages of the Owston (1964) system are that it will not work if the cavity is very close to critical coupling and the power incident on the cavity is less than $\sim 1\text{mW}$. Buckmaster and Dering (1966) have described a version of this system which will work when the cavity power level is $\sim 1\mu\text{W}$. In this version, and the Owston (1964) circuit described above, the microwave homodyne phase shifter determines the amplitude of the absorption signal. The cavity lock is operative for all settings of the homodyne phase shifter. Dispersion can be detected with the appropriate homodyne phase if the bandwidth of the cavity resonant frequency-lock loop is narrowed to exclude the magnetic field modulation frequency. The klystron will then be locked to the cavity resonant frequency averaged over a number of periods of the magnetic field modulation frequency. Simultaneous phase and cavity locking can be achieved using the Owston (1964) circuit because the reference crystal oscillator in the synchronizer can be tuned electronically at rates up to 1 kHz.

A homodyne EPR spectrometer requires relatively straightforward electronic circuitry. The static magnetic field is amplitude modulated by passing an a.c. current of frequency f_m through a pair of Helmholtz modulation coils. When the resonance condition is satisfied the magnetic field modulation produces amplitude modulation of the voltage reflected from the cavity and narrow band synchronous demodulation at the magnetic field modulation frequency f_m is possible. The normal magnetic field modulation frequencies used in homodyne EPR spectrometers are either around

400 Hz or around 100 kHz. In the former case the cavity can be constructed from metal with relatively thick walls. In the later case however, the cavity conducting surface must be greater than the skin depth at the microwave frequency and less than the skin depth at the modulation frequency. The use of high frequency magnetic field modulation reduces the contribution of inverse ($1/f$) frequency detector noise to the overall noise figure of a homodyne EPR spectrometer.

4.3 Superheterodyne and Homodyne EPR Spectrometers Using High Frequency Amplitude Modulation of the Signal Power

Superheterodyne demodulation and high frequency (>100 kHz) field modulations have been used to minimize the effects of the $1/f$ noise arising from the microwave diode demodulators. Recently an additional technique has been used which is essentially a homodyne EPR spectrometer with high frequency amplitude modulation of the signal power. With the improvement in noise properties of microwave diodes, particularly at ~ 10 GHz, the superheterodyne design is no longer used extensively to obtain high sensitivity. There are circumstances, however, in which it is still advantageous to use superheterodyne detection. These are:

- (i) when studying an EPR resonance with very narrow linewidths ($<\sim 10\mu\text{T}$) or very broad linewidths ($>\sim 10\text{mT}$),
- (ii) when it is difficult or impossible to introduce high frequency modulation magnetic fields inside the sample cavity,
- (iii) in ENDOR studies where low magnetic field modulation frequencies are preferable but high sensitivity is important,
- (iv) when a good cavity frequency lock system is required for low power operation ($<1\mu\text{W}$) and

(v) when the recording of the absorption curve a_0 is required.

The fifth requirement may be necessary because the Fourier coefficient a_I of a composite EPR signal gives poor relative intensities when lines of different widths are present and broad lines in the presence of narrow lines may even be overlooked.

The early superheterodyne EPR spectrometers used two klystrons and were patterned after World War II radar. One klystron supplied the sample cavity power and the other the local oscillator power. The frequency of the local oscillator klystron was usually offset from that of the signal klystron by 30 MHz. Signal detection was accomplished first at the 30 MHz intermediate frequency (i.f.) to minimize the $1/f$ contribution of the microwave detector noise in the overall noise figure. The detection of EPR signals was accomplished by synchronous demodulation of the output of the i.f. amplifier at the magnetic field modulation frequency. Frequency modulation (f.m.) noise in the microwave power to the sample cavity degraded the sensitivity of the early two-klystron superheterodyne spectrometer, particularly at high power levels. A complicated frequency locking system was also required to ensure that the difference frequency remained within the pass band of the intermediate frequency amplifier. Teaney, Klein and Portis (1961) introduced synchronous demodulation at the i.f. into the two klystron superheterodyne EPR spectrometer and showed that this reduced the effects of klystron f.m. noise. They also demonstrated that this source of noise could be effectively eliminated by the use of a bimodal cavity. Despite this advantage, the bimodal cavity has not been widely adopted in EPR spectroscopy due to additional complexity in tuning, construction and sample positioning.

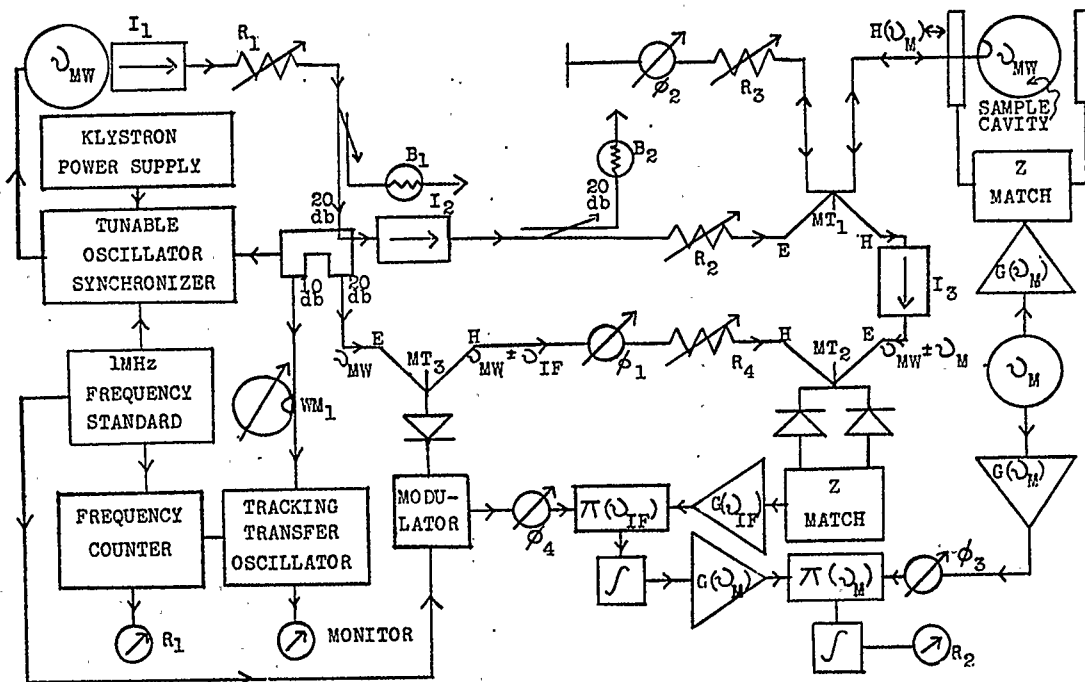


Figure (4.7)

Block diagram of the Buckmaster and Dering (1967) single klystron superheterodyne EPR spectrometer.

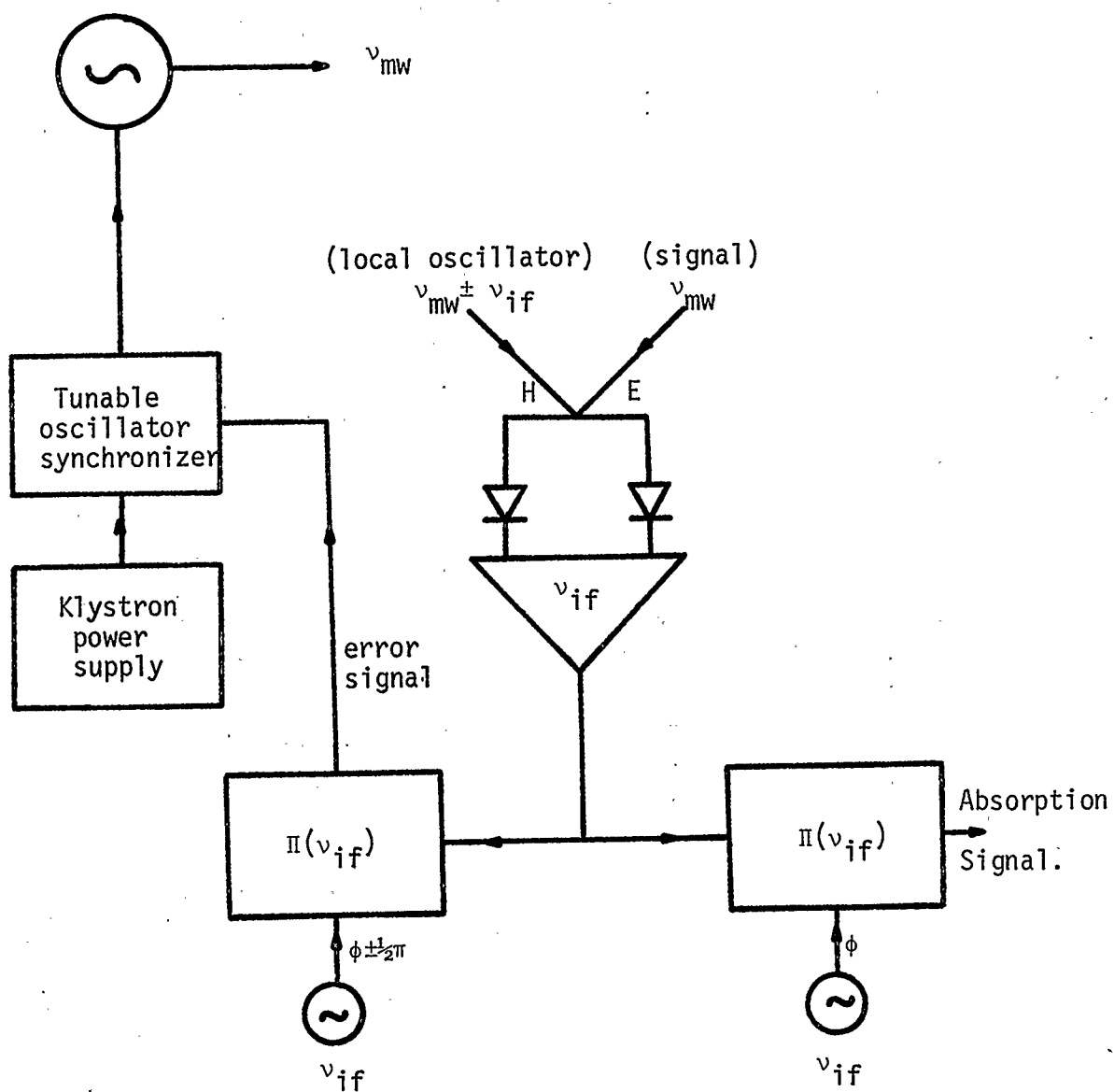
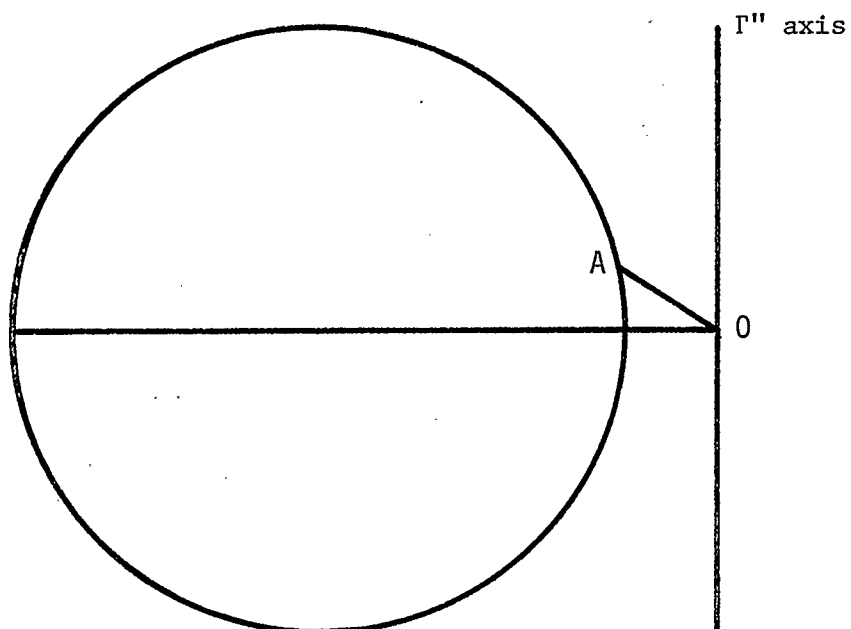
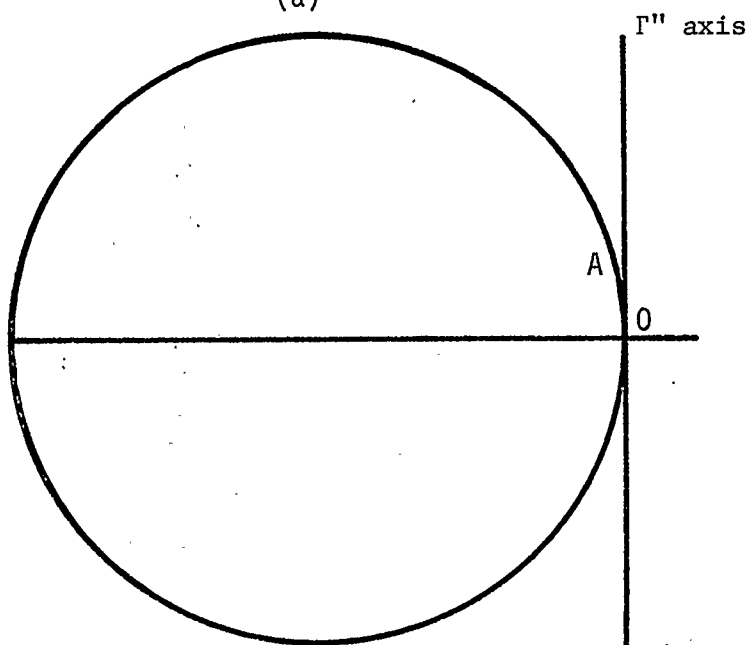


Figure (4.8)

Block diagram of a superheterodyne cavity frequency lock circuit.



(a)



(b)

Figure (4.9a and b)

Smith chart diagrams (a), an undercoupled resonant cavity and (b), the reflection coefficient in the signal arm of the balanced spectrometer bridge.

Schmid and Gunthard (1966) also designed a K-band superheterodyne EPR spectrometer using two klystrons and synchronous demodulation at the intermediate frequency. This is a versatile, high sensitivity instrument, however it is also relatively expensive to build and complicated to operate. Buckmaster and Dering (1967) developed a single klystron superheterodyne EPR spectrometer which is cheaper to build and easier to operate than a comparable two klystron superheterodyne. A block diagram of this spectrometer is given in Fig (4.7). The local oscillator power is obtained by using a single sideband generator (Rantec MX200) to displace the frequency of the microwave radiation by up to 150 kHz from that of the klystron. Synchronous demodulation of the spectrometer i.f. signal can be performed at the driving frequency to the single sideband generator because it is coherent with the i.f. signal. One of the advantages of the single klystron superheterodyne spectrometer is the ease with which a high sensitivity cavity frequency-lock can be obtained without any additional microwave components. The block diagram for this type of cavity frequency-lock is given in Fig.(4.8). Figure (4.9a) illustrates the variation in the cavity reflected wave as a function of frequency for an undercoupled cavity. The bridge balancing elements are adjusted to introduce a voltage in the signal arm which cancels that due to the imperfect cavity coupling. The Smith chart of the microwave voltage in the signal arm of the EPR bridge as a function of frequency is given in Fig.(4.9b). The point O represents the spectrometer operating point when the klystron frequency coincides with the cavity resonant frequency and the EPR bridge is perfectly balanced. If the microwave frequency changes to the point A on the Smith chart then the phasor representing the

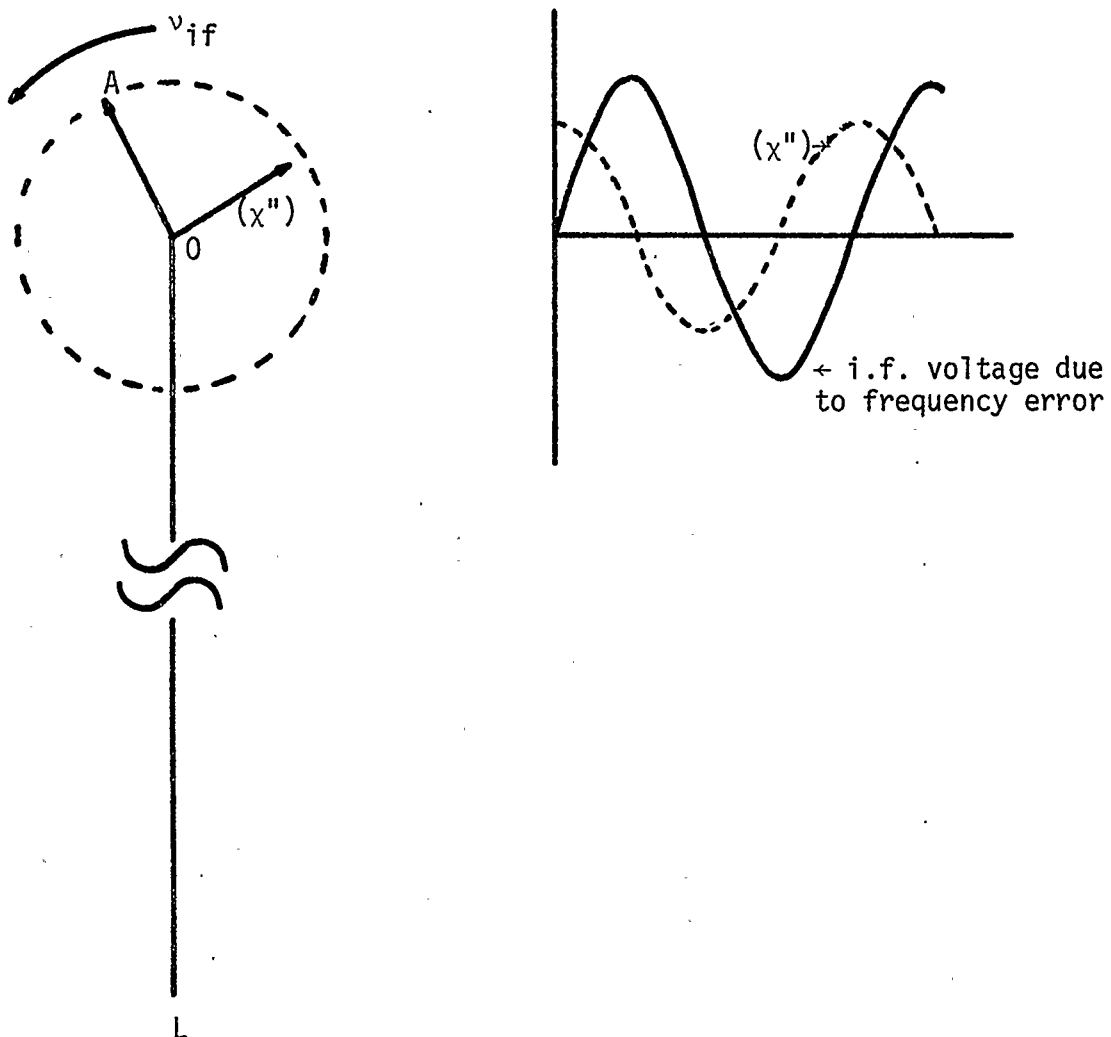


Figure (4.10).

Phasor diagram illustrating the creation of the i.f. cavity frequency lock signal in a superheterodyne EPR spectrometer.

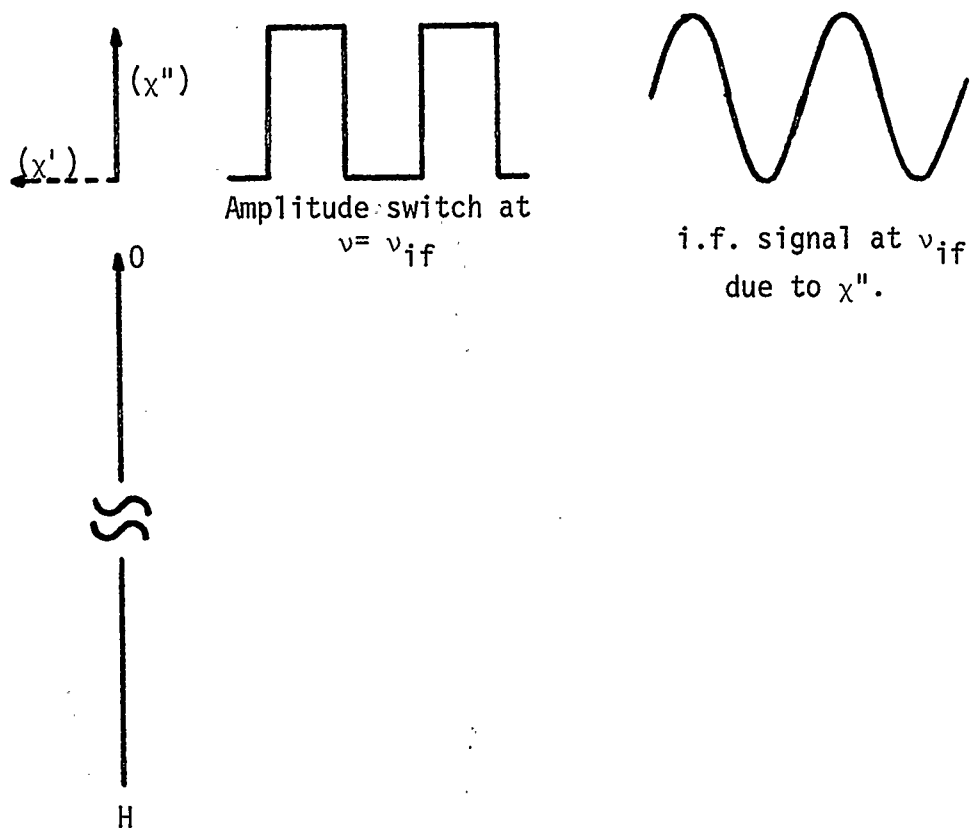


Figure (4.11).

Phasor diagram illustrating the operation of an amplitude switching homodyne EPR spectrometer.

microwave voltage in the signal arm due to the frequency error is given by OA. Figure (4.10) shows that the combination of the phasor OA and the local oscillator phasor LO leads to an i.f. signal which is $\sim \frac{1}{2}\pi$ radians out of phase with that due to an absorption signal. Consequently, the reference phase to the synchronous demodulator used to create the error signal is $\frac{1}{2}\pi$ radians out of phase with that required in the signal channel tuned to detect absorption.

Chapter V includes a description of a new type of single klystron superheterodyne EPR spectrometer based on the serrodyne principle of frequency translation. The serrodyne superheterodyne EPR spectrometer is particularly suitable for Q-band and higher frequencies.

A homodyne EPR spectrometer can be adapted to have some of the advantages of the superheterodyne EPR spectrometer. This is achieved by high frequency amplitude modulation of the signal voltage. Praddaude (1967), Faulkner and Whippey (1966) and Takao and Hayashi (1967) have described homodyne EPR spectrometers using high frequency amplitude modulation of the signal voltage. The detection process can be represented by the phasor diagram given in Fig. (4.11) if it is assumed that no phase modulation accompanies the amplitude modulation. A transmission ferrite modulator was used by Praddaude (1967) to produce 100 kHz amplitude modulation of the signal voltage. Synchronous demodulation was performed first at 100 kHz and then at the magnetic field modulation frequency. Faulkner and Whippey (1966) used a circulator and a reflecting microwave diode switch to produce 425 kHz amplitude modulation of the signal voltage. The analysis of the spectrometer built by Takao et al. (1967) is more complicated because the amplitude modulator also introduced some phase modulation. The phasor diagram for the operation of the

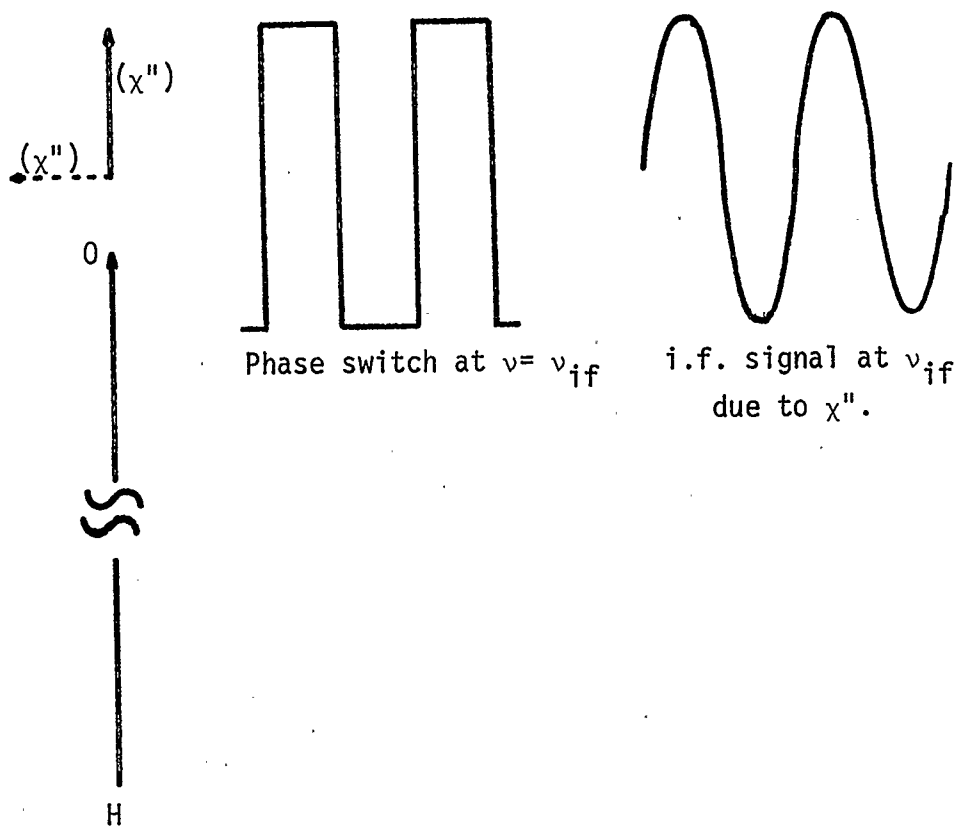


Figure (4.12).

Phasor diagram illustrating the operation of a phase switching EPR spectrometer.

switching homodyne is given in Fig. (4.11). The information for the cavity frequency lock loop cannot be obtained from the EPR signal demodulators in a spectrometer adjusted to detect absorption because of the fixed phase relationship between the error signal voltage and the homodyne voltage H_0 in Fig. (4.11). For this reason additional microwave components are required. The operation of the cavity lock feedback loop is not as efficient as the superheterodyne system because of the additional directional coupler required in sampling the cavity reflected wave. A -20 dB coupler was used in the Faulkner and Whippey (1966) EPR spectrometer design.

Wilmshurst (1967) proposed that a p-i-n diode could be used instead of the microwave point-contact diode in the Faulkner and Whippey (1966) spectrometer to produce π radians phase-switching of the signal voltage. The phasor diagram for this 'phase-switch' is given in Fig. (4.12). The efficiency of the phase switch and amplitude switch can be compared using Fig. (4.11) and Fig. (4.12). There is a theoretical loss of 6 dB in the signal voltage using the reflecting amplitude switch driven by a square wave which does not occur in the superheterodyne design or in the phase switching design suggested by Wilmshurst (1967). There appears to be no experimental evaluation of the use of a p-i-n diode as a π radians phase switch and the application of the technique is restricted to the lower frequency bands for which p-i-n diodes are available.

The main advantages of the superheterodyne EPR spectrometer over the high frequency amplitude modulation EPR spectrometer are the greater efficiency with the available signal power and the ease with which a high sensitivity cavity-lock loop can be obtained. An amplitude

modulation technique will produce a 3 dB loss in signal power even if it is 100% efficient. Praddaude (1967) evaluated the performance of his 400 Hz magnetic field modulation homodyne EPR spectrometer, with and without the 100 kHz ferrite amplitude modulator, and found that the improvement factor was 36--73% of that expected theoretically even at very low power levels. The predominant noise source was assumed to be $1/f$ noise in the theoretical estimation of the improvement factor. No explanation of this effect was given beyond mention of the fact that the poorer improvement factor was obtained with better quality microwave diodes. It is possible that this variation in improvement factor with the quality of the microwave diodes was due to an additional noise source in the receiver subsequent to the microwave diodes. The signal-to-noise ratio would then be dependent on the quality of the diodes at low power levels, as described by Praddaude. In a similar evaluation of the serrodyne superheterodyne EPR spectrometer, the experimental sensitivity improvement obtained with the serrodyne, in comparison to the homodyne configuration, was independent of the quality of the microwave diodes. The experimental sensitivity results reported in this thesis were obtained with a matched pair of 1N53B silicon point-contact microwave diodes.

4.4 Noise Limitations on the Sensitivity of an EPR Spectrometer

Ideally, the performance of an EPR spectrometer should be evaluated in terms of its noise figure and the efficiency of conversion of an EPR resonance into a detectable signal voltage. The performance of an EPR spectrometer can also be given in terms of the minimum detectable signal as a function of cavity Q , cavity power, etc. This problem has been analysed by Buckmaster and Dering (1967) and Buckmaster and Skirrow (1971).

This section discusses the major sources of noise in EPR spectrometers, the situations in which they are particularly important and the ways in which they may be minimized.

4.4.1 Microwave Demodulator and Preamplifier Noise

Semiconductor diodes of various designs are used extensively as microwave demodulators in EPR spectrometers. All diode demodulators exhibit a $1/f$ noise spectrum component, however the "knee" of this component is very sensitive to their design. The first demodulation frequency of an EPR spectrometer is usually chosen to minimize the effect of this noise source.

Buckmaster and Dering (1965) have shown that point-contact diodes of contemporary design exhibit a $1/f$ noise spectrum component which becomes of comparable magnitude to that of their shot noise spectrum component at approximately 100 kHz. Schottky barrier and backward diodes have a $1/f$ noise spectral component which is comparable to the shot noise spectral component at ~ 10 kHz. Buckmaster and Rathie (1971) have experimentally compared the noise spectra of these three types of microwave diodes and their application in EPR spectrometers.

The optimum conditions for microwave bias power, matching and i.f. impedance of the demodulator diodes in an EPR spectrometer have been studied by Dering (1965) for point-contact diodes, and by Rathie (1970) for Schottky barrier and backward diodes. A transformer is used to match the output of the microwave demodulator to the high impedance input of a low noise preamplifier. The noise contribution from the preamplifier depends on its source impedance, centre frequency and bandwidth about the demodulation frequency. Normally, the preamplifier is followed

by a narrow band synchronous demodulator so that the bandwidth of the preamplifier is relatively unimportant, provided that odd harmonics of the modulation frequency are not passed by the preamplifier. The preamplifier should have a good dynamic range to avoid saturation effects.

4.4.2 The Effects of Frequency Modulation Noise in the Microwave Power

The microwave oscillator frequency modulation (f.m.) noise is converted by the sample resonant cavity into amplitude modulation (a.m.) noise. The degree to which this can degrade the performance of a spectrometer has been studied by Teaney, Klein and Portis (1961) and Buckmaster and Dering (1967), amongst others. Buckmaster and Dering (1967) have studied experimentally the effects of variation of cavity Q, the r.m.s. deviation of the klystron oscillator frequency and the power incident on the microwave sample cavity. They showed that this noise source was usually the ultimate limitation to the sensitivity of the spectrometer unless high spectral purity microwave power was used. The treatment of the effects of f.m. noise given here is an attempt to give a more detailed analysis using the Smith chart representation of the sample cavity and the model of oscillator f.m. noise given by Ondria (1968) and Shields (1969). The rapid advances in microwave detector technology and the use of microwave parametric and maser preamplifiers have increased the importance of this noise source in relation to demodulator noise. A more detailed analysis of this process is appropriate.

Wilmshurst (1968) has stated that the spectrometer sensitivity S_{fb} to f.m. noise for a spectrometer using a balanced synchronous demodulator is given by

$$S_{fb} = 2kV_c \frac{\delta f}{(df)^2} \quad (4.4.1a)$$

and the spectrometer sensitivity S_{fu} to f.m. noise for a spectrometer using an unbalanced synchronous demodulator is given by

$$S_{fu} = kV_c \left(\frac{V_c}{V_d} \right) \pm 2 \cdot \left(\frac{\delta f}{(df)^2} \right) \quad (4.4.1b)$$

where k is a constant for the demodulators

V_c is the voltage transfer from the klystron to the sample cavity

V_d is the voltage transfer to the microwave demodulators in the homodyne arm

δf is the steady degree of mistuning of the klystron from the resonant cavity frequency

and df is the unloaded bandwidth of the cavity

Equations (4.4.1a and b) were stated without a detailed proof, however, good experimental verification was provided. A complete derivation of these equations is given in Appendix C. The method of derivation, the approximations used and the limitations of their applicability are now discussed.

Wilmshurst (1968) assumed that the noise output of the EPR spectrometer klystron can be separated into a.m. and f.m. components and treated separately. Ondria (1968) has shown that this assumption is justified. Following Shields (1969) the output voltage v of an oscillator with zero amplitude modulation noise can be described by

$$v = A \sin [2\pi f_0 t + \theta(t)] \quad (4.4.2)$$

where $\beta = [2\pi f_0 t + \theta(t)]$ is the instantaneous total phase of the oscillator in radians.

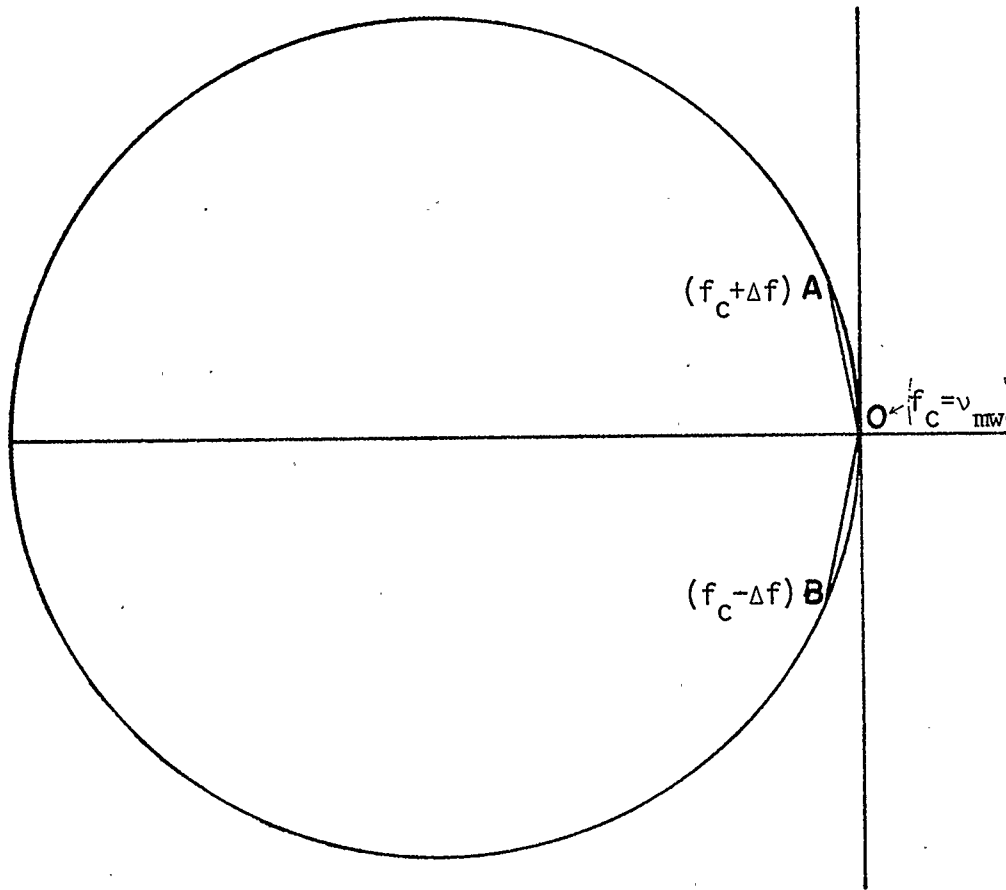


Figure (4.13).

Smith chart of the cavity reflected wave showing the phasors OA and OB at the extremes of the frequency deviation of the f.m. noise at the final demodulation frequency f_m .

The frequency modulation in Eq. (4.4.2) is described by the time dependent phase variations $\theta(t)$ about the linearly progressing phase $2\pi f_o t$. The instantaneous frequency f is defined as

$$f = \frac{1}{2\pi} \cdot \left(\frac{d\theta}{dt} \right) = f_o + \frac{1}{2\pi} \left(\frac{d\theta(t)}{dt} \right) \quad (4.4.3)$$

Wilmshurst (1968) assumed that only the component of the klystron output frequency spectrum displaced from the peak power frequency f_o by the final demodulation frequency is important in the derivation of the effects of f.m. noise. The final demodulation frequency is normally the field modulation frequency f_m . Consequently the important component of the f.m. noise spectrum can be represented by

$$\theta(t) = \Delta\phi \sin (2\pi f_m t) \quad (4.4.4)$$

where $\Delta\phi$ is the peak phase deviation.

Substituting Eq. (4.4.4) into Eq. (4.4.2) yields

$$V = A \sin [2\pi f_o t + \Delta\phi \sin (2\pi f_m t)] \quad (4.4.5)$$

The instantaneous frequency f is given by

$$f = f_o + \Delta f \cos 2\pi f_m t \quad (4.4.6)$$

where $\Delta f = f_m \Delta\phi$ and is the peak frequency deviation.

The Smith chart representation of the reflection coefficient as a function of frequency is given in Fig (4.13). The phasors representing the amplitude and phase of the reflected wave from a matched cavity with resonant frequency f_c are given by the lines OA, zero reflected wave, and OB, when the frequency is $f_c + \Delta f$, f_c and $f_c - \Delta f$ respectively.

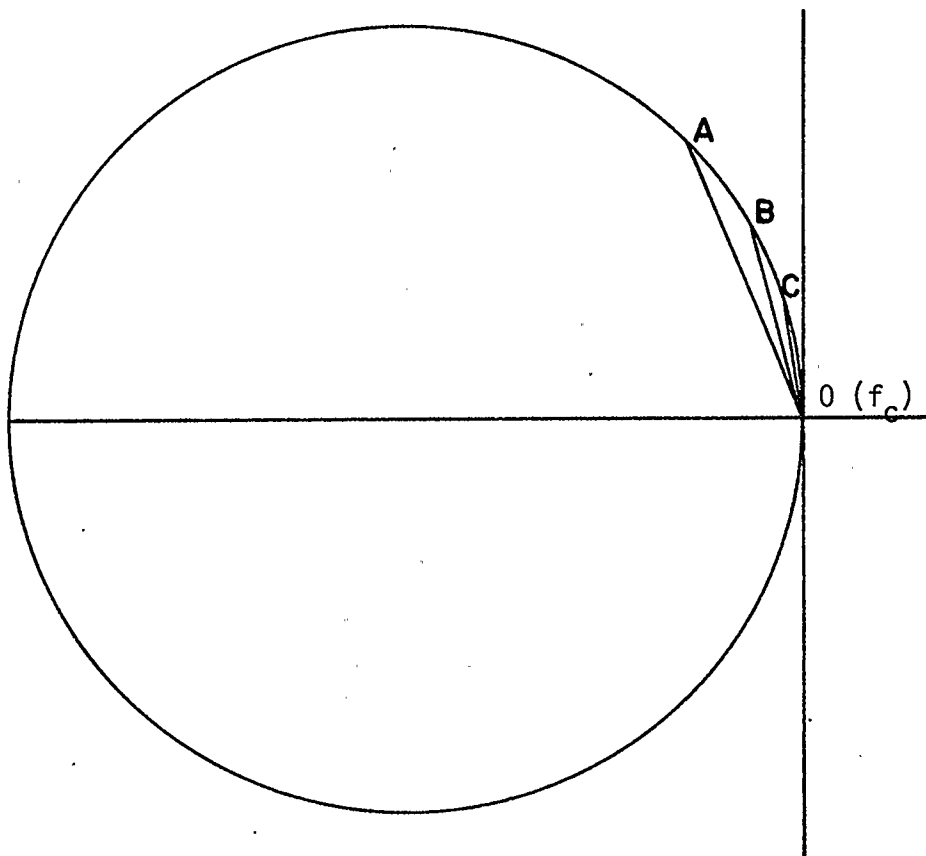


Figure (4.14).

Smith chart of the cavity reflected wave showing the phasors at the extremes of the frequency deviation in the f.m. noise when there is a frequency error between the klystron centre frequency and the cavity resonant frequency. The phasors, OA, OB and OC represent the amplitude and phase of the reflected wave when the frequency is $(f_o + \Delta f)$, f_o and $(f_o - \Delta f)$. The frequency error δf between the klystron peak power frequency f_o and the cavity resonant frequency f_c is greater than the peak frequency deviation Δf at the frequency f_m .

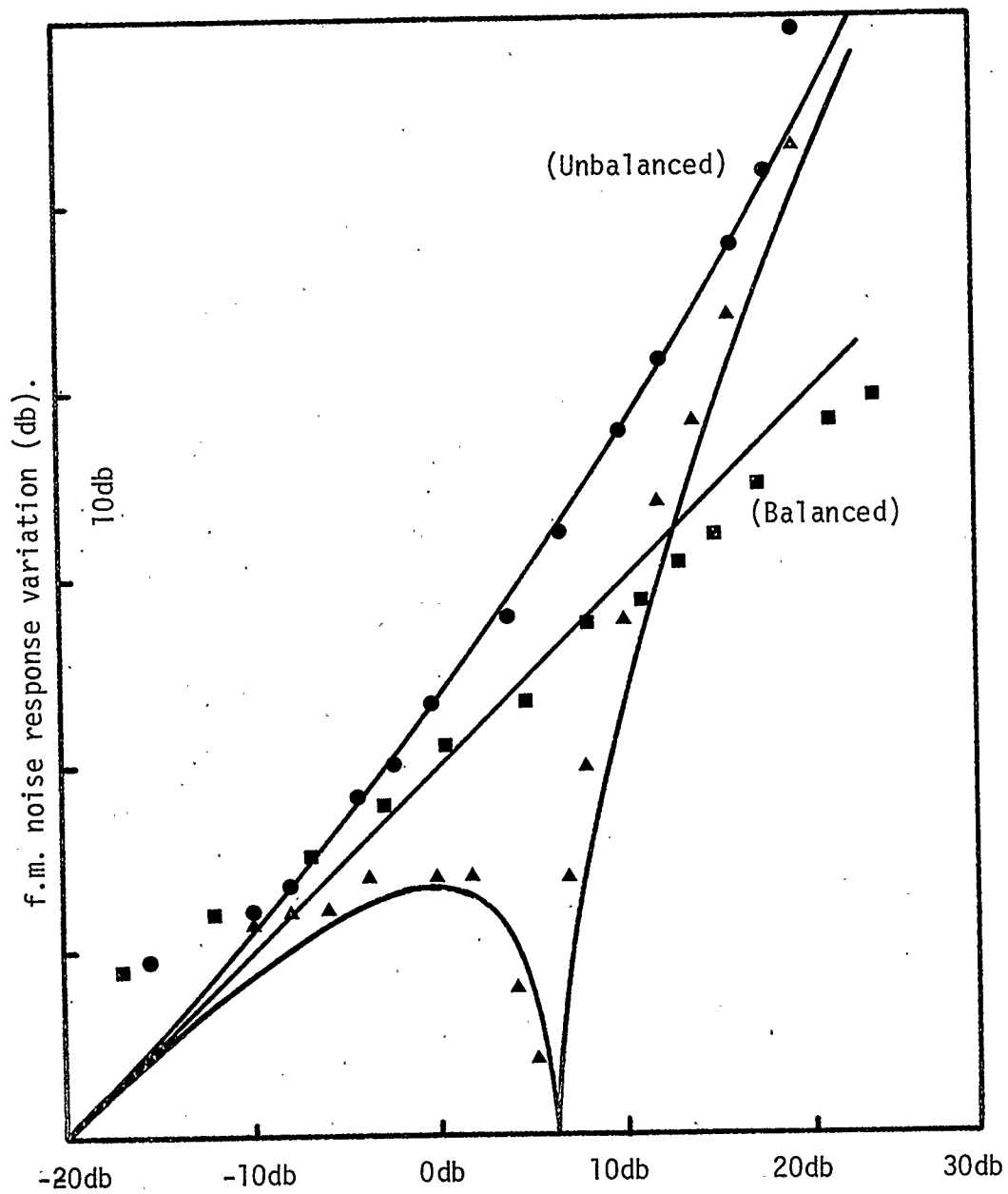


Figure (4.15).

Spectrometer response to klystron f.m. noise. Reproduced from Wilmshurst (1968).

This diagram can also be used to describe the amplitude and phase of the a.m. signal in the signal arm of the balanced EPR bridge.

The peak power frequency f_o , may not correspond exactly to the cavity resonant frequency f_c . In his derivation of the sensitivity of an EPR spectrometer to f.m. noise, Wilmshurst (1968) considered only the situation in which the error frequency δf is greater than the peak frequency deviation Δf at f_m . The phasors, OA, OB and OC in Fig. (4.14) represent the amplitude and phase of the reflected wave when the frequency is $[f_o + \Delta f]$, f_o and $[f_o - \Delta f]$. Figures (4.13) and (4.14) indicate that the original frequency modulation will be transformed into an amplitude modulated wave by the discriminating action of the resonant cavity. If the signal arm of the bridge was terminated by a matched microwave detector then the amplitude of the a.m. signal at a frequency f_m , arising from the frequency modulation, would be proportional to $|AO| - |OC|$. Account must be taken, however, of the relative phase of the phasors AO, CO and the phase of the homodyne voltage when a balanced synchronous demodulator is used.

Wilmshurst (1968) has considered the amplitude and phase modulation of the cavity reflected wave in his treatment of the effects of f.m. noise. Figure (4.15) reproduces Wilmshurst's Fig. 2 and shows the experimental points and theoretical curves for balanced and unbalanced synchronous demodulation. The two possible phasings for the unbalanced condition are shown. The advantage of a balanced synchronous demodulator over an unbalanced demodulator at high cavity power levels is apparent.

Equation (4.4.1a) shows that the voltage sensitivity to f.m. using a balanced synchronous demodulator for a homodyne spectrometer tuned to

absorption is proportional to the microwave voltage incident on the sample cavity. In the absence of saturation the magnitude of the EPR absorption signal will also increase at a rate proportional to the microwave voltage incident on the cavity. Consequently, if discriminated f.m. noise is limiting the sensitivity of an EPR spectrometer then the signal to noise ratio becomes independent of the cavity power level. This has been experimentally demonstrated by Buckmaster and Dering (1967).

No account has been taken in the derivation of S_{fb} and S_{fu} , given in Appendix C, of the effect of the cavity frequency lock loop. These expressions demonstrate, however, the importance of minimizing the error frequency, δf , between the klystron peak power frequency and the cavity resonant frequency. Wilmshurst's analysis is also applicable when a narrow band cavity lock loop is used but it becomes more complicated when a broad band cavity lock loop is used.

4.4.3 Amplitude Modulation Noise

Ondria (1968) showed that a.m. noise in the klystron output can be converted to f.m. noise by the action of a microwave bridge containing a resonant cavity. The EPR bridge is balanced only at the cavity resonant frequency and is not perfectly balanced at the a.m. noise sideband frequencies. The a.m. sidebands can then appear in the signal arm of the bridge. Because the carrier is removed in the bridge balance the voltage incident on the microwave demodulators in the signal arm has two components, one at frequency $f_o + f_m$ and the other at $f_o - f_m$. These components recombine with the homodyne voltage to give an amplitude modulation output at the frequency f_m to the preamplifier.

Balanced demodulators cancel the a.m. noise in the local oscillator power both in the homodyne and superheterodyne spectrometer. Wilmshurst (1968) has derived an expression for the voltage sensitivity to a.m. noise (S_{ab}), discriminated by the sample cavity for a balanced synchronous demodulation homodyne spectrometer. This is given by

$$S_{ab} = \frac{kV_c \left(\frac{f_m}{\Delta f} \right)}{\left[1 + \left(\frac{f_m}{\Delta f} \right)^2 \right]^{1/2}} \quad (4.4.3)$$

The analysis given in Sec. (4.4) and in Appendix C, is applicable to a homodyne EPR spectrometer. This analysis is also directly applicable to the superheterodyne spectrometer with synchronous demodulation at the intermediate frequency.

Microphonics can also lead to a degradation of the spectrometer sensitivity. In this laboratory specially designed shock mounts have been used to support the spectrometer. This has been successful in minimizing this source of noise.

4.4.4 Summary

Buckmaster and Dering (1965, 1966) have shown that the limiting sensitivity of an EPR spectrometer is given by

$$\chi_{\min}'' = \frac{4}{nQ_u} \left[\frac{F k T B}{P_{in}} \right]^{1/2} \quad (4.4.4)$$

where $\chi_{\min}''$ is the minimum detectable value for the absorption component of an EPR resonance,

F is the noise figure of the spectrometer system,

k is Boltzmann's constant,

B is the bandwidth of the measurement system,

and P_{in} is the incident power on the microwave bridge.

Buckmaster and Skirrow (1971) have given a detailed expression for the noise figure F of an EPR spectrometer, this includes terms arising from the noise sources considered previously in Sec.(4.4).

$$F = N_d + G(N_f + N_a) + N_p - 1 \quad (4.4.5)$$

where N_d is the noise temperature ratio of the microwave demodulators,

G is the conversion gain of the microwave demodulators,

N_f is the noise figure due to f.m. noise,

N_a is the noise figure due to a.m. noise,

N_p is the noise figure due to the preamplifier.

N_p is less than N_d and normally can be neglected. The effect of microphonics in the cavity arm of the EPR bridge can be included in N_f and N_a because the microphonics lead to phase and amplitude fluctuations in the EPR bridge which are equivalent to f.m. and a.m. noise in the microwave power. N_d is a constant for particular microwave demodulator diodes operating at a fixed demodulation frequency under optimum conditions for the product of the conversion loss and noise temperature ratio. When N_d is the largest term in the spectrometer noise figure then

$$\chi_{\min}'' \propto \frac{1}{Q_u P_{\text{in}}^{1/2}} \quad (4.4.6)$$

At high sample-cavity power levels with the best available microwave demodulators GN_f , or possibly GN_a , is the dominant term in the noise figure expression, [Eq.(4.4.5)]. Buckmaster and Dering (1966) have demonstrated and experimentally verified that under these conditions the signal-to-noise ratio becomes independent of the cavity power level. When the spectrometer sensitivity is limited by either f.m. or a.m. noise, the only way to improve the signal-to-noise ratio is to improve

the spectral purity of the microwave source so that N_d remains the dominant term in the spectrometer noise figure. The use of a parametric or maser preamplifier is justified only if N_d is the dominant noise source of the EPR spectrometer at the maximum power and with the maximum cavity Q that are compatible with the experiment. Buckmaster and Skirrow (1971) have given a detailed expression for the noise factor of an EPR spectrometer.

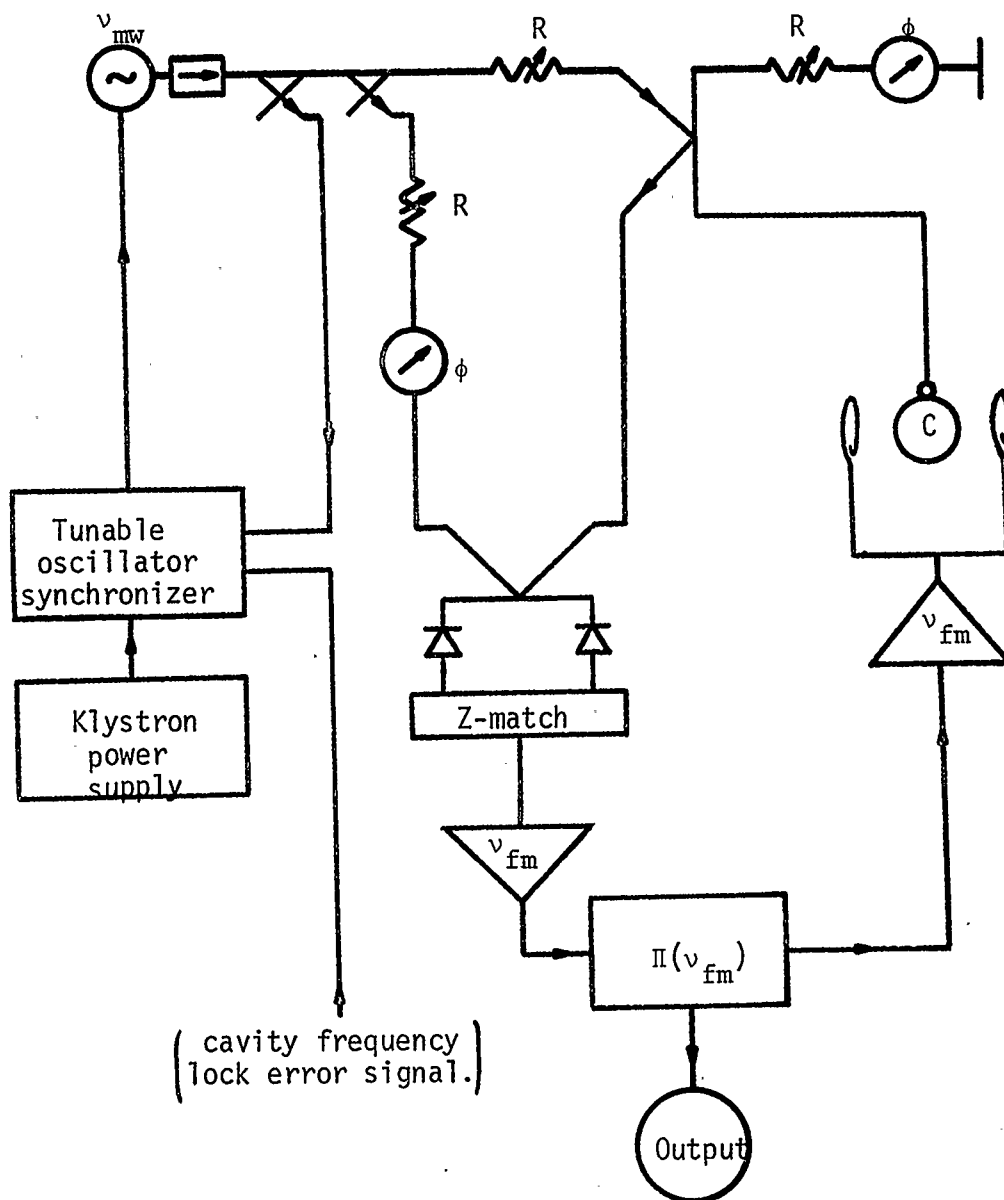


Figure (5.1).
Block diagram of the 34 GHz EPR spectrometer.

CHAPTER V

A DESCRIPTION OF THE EPR SPECTROMETERS

This chapter describes the three EPR spectrometers built as part of the research described in this thesis. The 34 GHz homodyne EPR spectrometer described in Sec.(5.1) is essentially a high frequency version of the 9-10 GHz EPR spectrometer described by Buckmaster and Dering (1965, 1967a). The 34 GHz homodyne EPR spectrometer was modified by the addition of a ferrite phase modulator which served as a serrodyne frequency translator. The principle of serrodyne frequency translation and the 34 GHz serrodyne superheterodyne EPR spectrometer are described in Sec.(5.2). The 54-60 GHz EPR spectrometer was built to provide additional data on the frequency dependence of the g-value results obtained from the hemoprotein crystals. This spectrometer is described in Sec.(5.3) and represents the beginning of the development of a high sensitivity EPR spectrometer to operate in the frequency range 54-60 GHz.

5.1 The 34 GHz Homodyne EPR Spectrometer

A block diagram of the 34 GHz homodyne EPR spectrometer is given in Fig. (5.1). The microwave power was supplied by a 300 mW reflex klystron (Varian 313K) driven by a low voltage klystron power supply (HP - 716B). The output frequency of the klystron is mechanically and electronically tunable within the range $34 \text{ GHz} \pm 350 \text{ MHz}$. This tuning range is inadequate for use with a fixed tuned sample cavity if samples with varying losses are studied at different temperatures. Consequently, tunable cylindrical cavities excited in the TE_{011} and TE_{111} modes were used.

The microwave frequency was phase locked to a harmonic of a high stability, MHz crystal oscillator using a 26-40 GHz microwave oscillator synchronizer (Microwave System MOS-V). As described in Chapter IV, the phase-locked oscillator has an improved spectral purity and can be manually and electronically tuned. A further important advantage of the use of the synchronizer at 34 GHz is the ease with which the microwave frequency can be measured to high accuracy. The microwave frequency was calculated using the relation

$$\nu_{MW} = 12n\nu_s \pm 0.040884 \quad (5.1.1)$$

where ν_{MW} is the microwave frequency in GHz,

ν_s is the tunable synchronizer reference oscillator frequency in GHz,

n is the harmonic number

and 0.040884 is the i.f. of the synchronizer in GHz.

The synchronizer reference oscillator frequency, ν_s , was measured with a frequency counter to an accuracy of $1:10^8$. The harmonic number n was determined by comparison of the reading of a wavemeter with the possible values of $12n\nu_s$. The sign in Eq. (5.1.1) was determined using the i.f. frequency meter on the front panel of the synchronizer. The klystron was locked to the desired frequency, it was then unlocked and the klystron frequency altered to see whether ν_{MW} was greater or less than $12n\nu_s$. In practice, no ambiguity in n exists. Normally, the microwave frequency is measured by using an additional reference EPR sample, usually polycrystalline DPPH, for which the g -value is known to an accuracy of 0.01%. The microwave frequency measurement procedure

outlined above is more accurate by a factor of approximately ten thousand. Consequently, the g -values determined in an experiment using this technique will normally be more accurate because the error involved in the measurement of the magnetic field occurs only once and not twice as occurs when a calibrating sample is used.

The isolator adjacent to the klystron minimized the possible effects of load variation and other spurious reflections on the performance of the klystron. The microwave power incident on the spectrometer bridge was adjusted by a rotary vane precision attenuator. The cavity arm of the bridge was made vacuum tight by the introduction of a vacuum seal. It was necessary to evacuate the waveguide and cavity for operation at 77°K to prevent the freezing of oxygen inside the system. The vacuum seal was made from a UG599/U square flange with two pieces of Q band waveguide on either side of a thin rectangular piece of mica. Epoxy resin was used to seal the mica in the flange. This unit provided a good vacuum seal and its insertion in the waveguide system introduced a VSWR of less than 1.15 at 34 GHz.

The hybrid-tee used in the bridge was constructed from two blocks of metal (Phillips 7260 QA) so that its mechanical rigidity minimized the effects of microphonics which degrade the bridge balance. The bridge balance was obtained with a rotary vane precision attenuator, a rotary vane precision phase shifter and a short.

The microwave demodulators employed in the balanced synchronous demodulator were a carefully matched pair of 1N53B silicon point-contact diodes. A transformer was used to impedance-match the demodulators to the preamplifier. The microwave diodes were connected to the primary of the transformer through a d.c. resistance of 100 Ω . A 100 k Ω

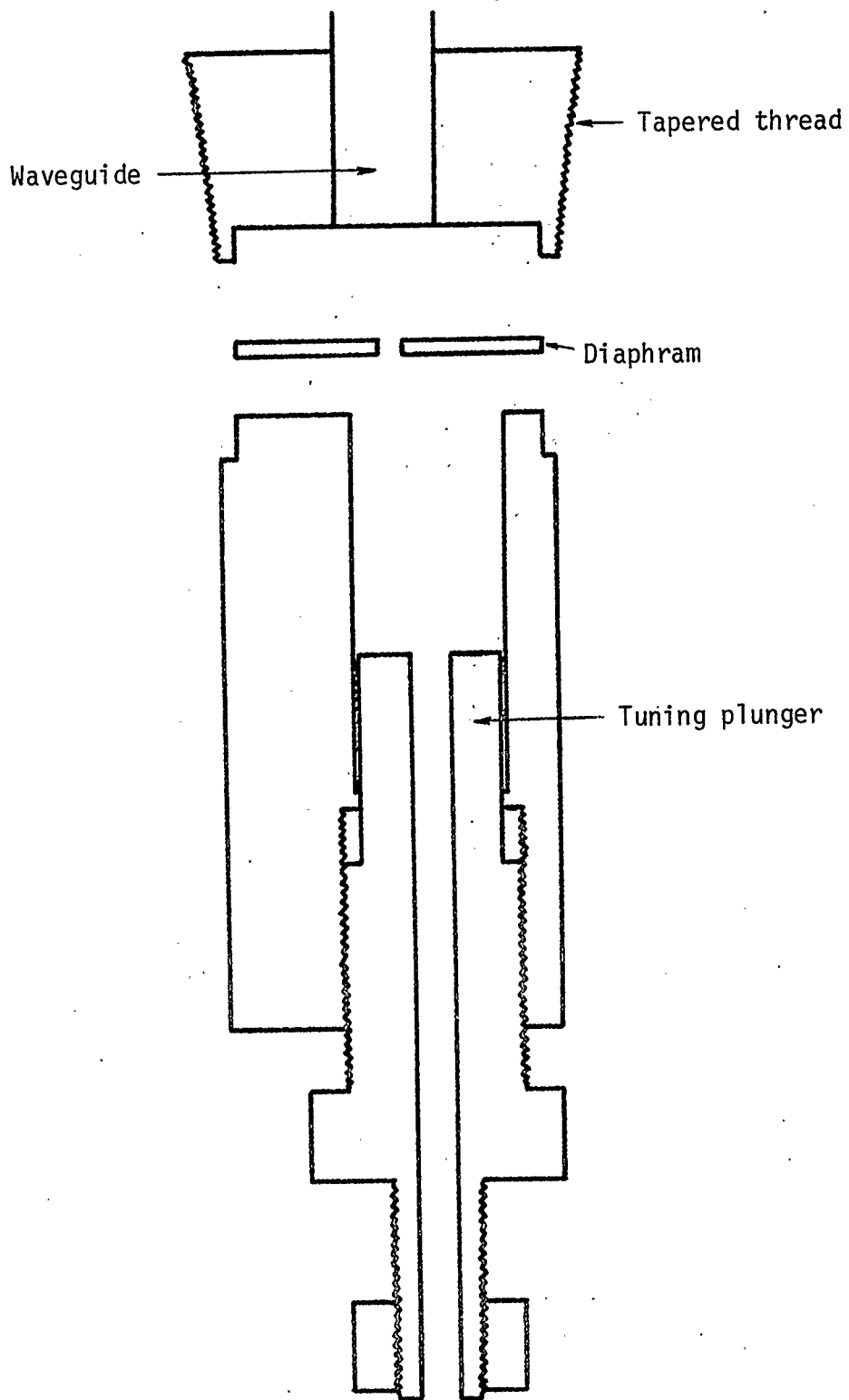


Figure (5.2).

Diagram of the 34GHz tunable TE_{011} cylindrical resonant cavity.

resistance was connected across the secondary of the transformer so that it presented an a.c. impedance of $200\ \Omega$ to each microwave diode. The diode operating conditions were established by Dering (1966) for the 1N23WE diodes used in the 9-10 GHz spectrometer and were found to be optimum also for the 1N53B diodes. A low noise preamplifier (PAR CR4) with a variable bandwidth was used to amplify the signal voltage.

The homodyne voltage incident on the microwave demodulators was derived from the klystron voltage incident on the EPR bridge using a directional coupler. The homodyne voltage was adjustable in both amplitude and phase.

Cylindrical, tunable resonant sample cavities excited in the TE_{011} and TE_{111} modes were used with the 34 GHz spectrometer. Both designs were tunable over a frequency range that was centred on and greater than the tuning range of the klystron. The cylindrical TE_{011} cavity was used in the majority of the work. It was found that the higher Q of the TE_{011} cavity more than compensated the better filling factor of the TE_{111} cavity and an improved signal to noise ratio could be obtained with the TE_{011} cavity. The microwave magnetic field is always orthogonal to the static magnetic field with the TE_{011} cavity and, consequently, angular rotation studies are easier to perform. A diagram of the design of this cavity is given in Fig. (5.2). It is relatively easy to construct as there are no wall currents at the corners so that a carefully designed choke on a non-contact tuning plunger is not necessary. The loaded Q of the brass TE_{011} cavity was 5,000. Quartz sample tubes were used to support the sample in the centre of the cavity. A narrow band version of the Owston (1964)

cavity frequency lock was used with this spectrometer.

5.2 The 34 GHz Serrodyne Superheterodyne EPR Spectrometer

This section describes both the use of a ferrite phase modulator as a serrodyne and its application in an EPR spectrometer. Serrodyne frequency translation is achieved by the generator of a linear sawtooth of phase or time delay.

The time variation of the voltage or electric field E at any point in a waveguide can be represented by

$$E = A \sin \left[2\pi f t + \theta_o \right] \quad (5.2.1)$$

Where A is the maximum value of E ,

f is the frequency of the microwave radiation

and θ is the phase.

The introduction of a phase modulator into the waveguide allows phase, and consequently frequency modulation of the output radiation with respect to the input radiation. The output frequency can be "translated" with respect to the input frequency by sawtooth phase modulation. The amplitude of the sawtooth must be 2π radians. Representing the linear change in phase in the sawtooth phase modulation by

$$\theta(t) = \pm \left(\frac{2\pi}{T} \right) t + \theta_o \quad \text{for } 0 \leq \theta(t) \leq 2\pi$$

where T is the period of the sawtooth phase modulation.

Equation (5.2.1) becomes

$$\begin{aligned} E &= A \sin \left[2\pi f_{in} t \pm \left(\frac{2\pi}{T} \right) t + \theta_o \right] \\ &= A \sin \left[2\pi (f_{in} \pm f_{mod}) t + \theta_o \right] \end{aligned} \quad (5.2.8)$$

Eq. (5.2.2) shows that the frequency translation is equal to the frequency of the sawtooth phase modulation and the sense of the translation depends on the slope of the linear change in phase. This elementary theory predicts that ideal sawtooth phase modulation produces 100% efficient frequency translation. The i.f. obtained by mixing the input and output frequencies will be coherent with a sine wave of the same frequency as the serrodyne driving voltage.

Cumming (1957) first studied the serrodyne technique. He developed a theory describing the factors which limit the performance of a serrodyne and carried out an experimental evaluation of serrodynes using klystrons and travelling wave tubes. The design of a single klystron superheterodyne EPR spectrometer demands that the phase modulation be independent of the microwave radiation source. O'Hara and Schaffman (1959) demonstrated that a ferrite phase modulator could be operated as a serrodyne to produce a frequency translation of ~ 100 kHz with 20 dB suppression of the carrier and unwanted sidebands. This type of phase modulator is suitable for inclusion in an EPR homodyne spectrometer to convert it to the superheterodyne mode of demodulation.

The factors which limit the performance of a serrodyne can be studied either by using a spectrum analyser or by mixing the input and output frequencies and studying the beat frequencies generated. The serrodyne output can be described by the suppression of the carrier and the unwanted sidebands, (S) and by the conversion loss, (L). These have been defined by Cumming (1965).

$$S = \frac{\text{output power (modulated and measured at the desired frequency)}}{\text{output power (modulated and measured at an undesired frequency)}}$$

$$L = \frac{\text{output power (when the device is operating unmodulated)}}{\text{output power (modulated and measured at the desired output freq.)}}$$

Good serrodyne performance is achieved when the suppression is high and the conversion loss is small. The performance of the serrodyne using a ferrite phase modulator is limited by

- (i) non-zero flyback time
- (ii) non-linear phase modulation
- (iii) incidental amplitude modulation
- (iv) non-optimum sawtooth amplitude

The first two limitations are particularly important and normally limit the performance of the serrodyne. The third limitation can arise in a ferrite phase modulator by the variation in insertion loss with current in the field coil. The fourth limitation can arise if operation is performed over a band of frequencies using a constant sawtooth amplitude. Cumming's (1965) theory predicts that the conversion loss will be less than 1 dB and the suppression of the carrier and all the unwanted sidebands will be 20 dB if the flyback time is approximately equal to one tenth of the period of modulation. A ferrite phase modulator serrodyne can be inserted in the signal arm of an EPR spectrometer since its conversion loss is less than 1 dB. The operating frequency of the ferrite phase modulator (TRG A 150) was chosen to be 50 kHz since the 2π radians flyback time was 2μ secs. It was found that the measured conversion loss and suppression were in good agreement with those predicted theoretically.

The ferrite phase modulator consists of a ferrite rod supported in the centre of a rectangular waveguide and magnetized by the magnetic

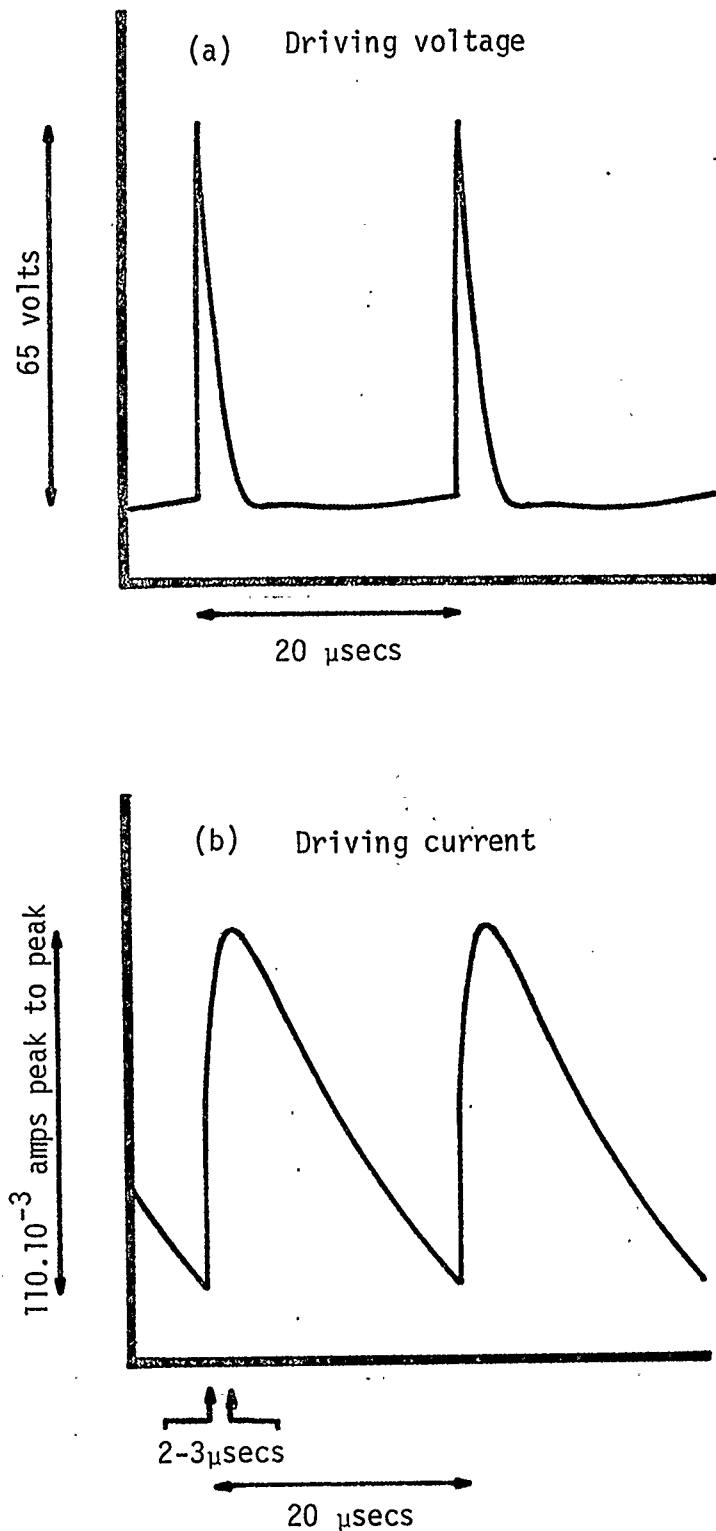


Figure (5.3a and b)

Illustration of the a.c. current and voltage waveforms used to drive the serrodyne phase modulator.

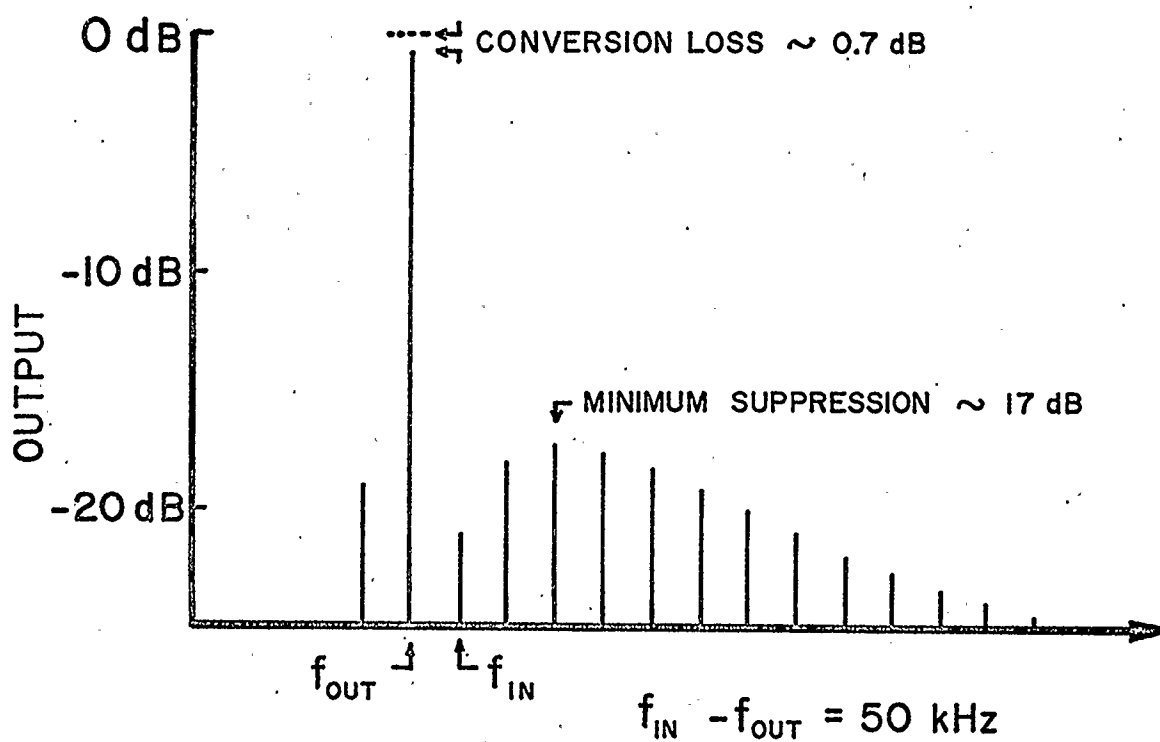


Figure (5.4)

Diagram showing the measured output frequency spectrum of an optimally adjusted 34GHz serrodyne when the frequency shift is 50kHz.

field produced by a current through a coil wrapped around the waveguide. The d.c. resistance of the coil was $6.25\ \Omega$ and the inductance was $0.55\ \text{mH}$. A phase change of 2π radians could be obtained by changing the current through the coil by $\sim 110\ \text{mA}$. A d.c. biasing current of $\sim 70\ \text{mA}$ was used to obtain serrodyne operation about a convenient portion of the phase shift against current characteristic. The a.c. current and voltage waveforms that have been used are given in Fig. (5.3 a and b). The driving voltage was essentially a spike voltage followed by a variable decay. The magnitude and decay rate of the voltage can be varied to optimize the output spectrum observed using a spectrum analyser (Tektronix IL 40). The resulting current is shown in Fig. (5.3b) and the spectrum obtained with this current waveform is shown in Fig. (5.4). The linear portion of the phase shift as a function of time is negative so that the desired sideband frequency is lower than the input frequency by $50\ \text{kHz}$. The upper sidebands are produced during the flyback period. The suppression of the carrier and wanted sidebands is greater than $16\text{--}17\ \text{dB}$ and the conversion loss is $\sim 0.7\ \text{dB}$. The suppression of the unwanted sidebands could be improved slightly by using higher power circuitry. The improvement in the conversion loss, however, would be minimal and this is the more important consideration in the EPR spectrometer configuration. The driving circuitry used was similar to that employed by Ernst (1967) in a phase modulated microwave interferometer.

A block diagram of the Q band serrodyne superheterodyne EPR spectrometer is given in Fig. (5.5). The design is a simple modification of the homodyne EPR spectrometer described in Sec (5.1). The serrodyne frequency translator can be included in the signal arm of the spectrometer

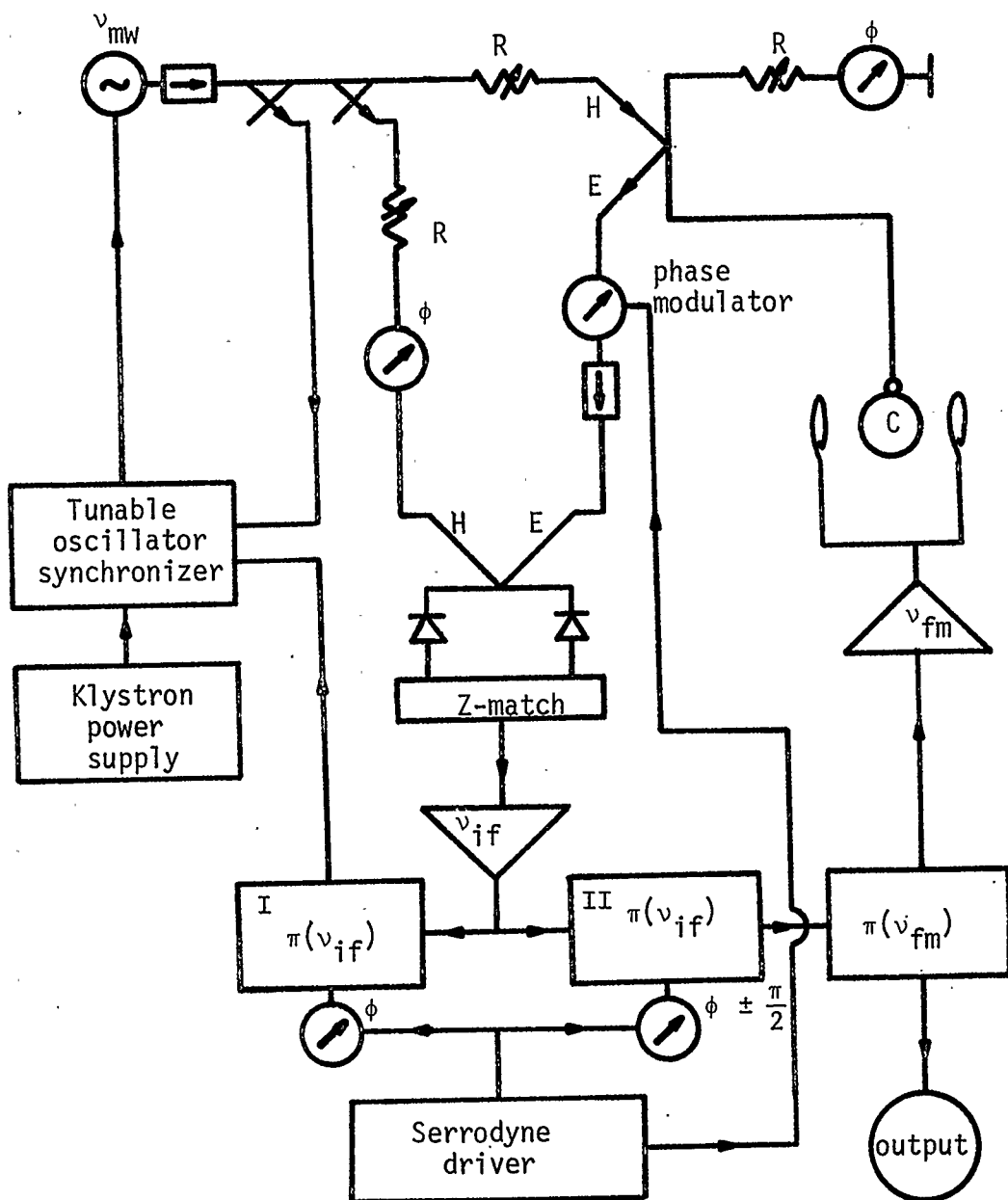


Figure (5.5).
Block diagram of the 34 GHz Serrodyne Superheterodyne spectrometer.

because of the high efficiency of the serrodyne technique. The presence of the unwanted harmonics in the output spectrum do not affect the performance of the spectrometer because the power level in the signal arm (~ 1 pW) is very small in comparison to that incident on the demodulators in the local oscillator arm (~ 1 mW). If the serrodyne had been included in the local oscillator arm of the spectrometer then the unwanted sidebands (~ 10 μ W) would mix to create a 50 kHz i.f. voltage in the absence of any signal power. An isolator has been included in the signal arm following the serrodyne to minimize any leakage from the local oscillator arm to the cavity bridge.

The cavity-lock information can be obtained from the i.f. output of the preamplifier. The signal arising from an error of frequency between the cavity resonance frequency and the klystron frequency is orthogonal to the voltage arising from an absorption signal. Consequently the reference phase of the i.f. synchronous demodulator II in Fig. (5.5) is adjusted to be $\frac{1}{2}\pi$ radians out of phase with that of synchronous demodulator I.

This spectrometer can be used to detect either the absorption or the dispersion components of an EPR signal. The dispersion component can be detected if the bandwidth of the cavity lock synchronous demodulator is sufficiently narrow to exclude the magnetic field modulation frequency sidebands. The cavity lock feedback loop then eliminates the effect of low frequency cavity drift but it will not remove either the sidebands arising from the dispersion signal or the effects of the klystron f.m. noise sidebands at the magnetic field modulation frequency. The dispersion signal will be recorded if the reference phase of the i.f. synchronous demodulator I is shifted by $\frac{1}{2}\pi$ radians

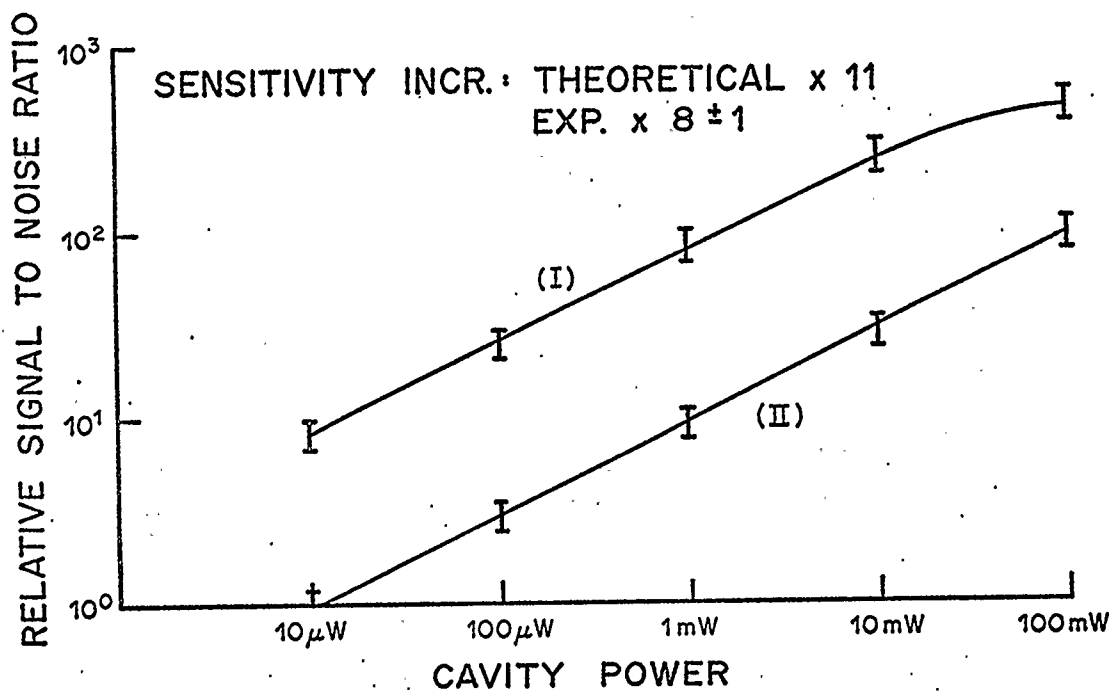


Figure (5.6)

Graphs of the experimentally determined sensitivity of a 34GHz EPR ferrodyne spectrometer as a function of the microwave cavity power with (I), and without (II), application of the driving voltage to the phase modulator.

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from the normal position for the detection of the absorption signal.

The results of an experimental comparison of the spectrometer performance are given in Fig. (5.6). To obtain homodyne detection, the i.f. synchronous demodulator was bypassed and the spectrometer was operated without a cavity frequency lock loop. The performance of the spectrometer was not affected by this procedure during the measurement time interval. The theoretical sensitivity improvement is the square root of the ratio of the initial demodulation frequencies, assuming that the dominant spectrometer noise source in both cases is $1/f$ demodulator noise. The theoretical improvement factor is eleven when the i.f. is 50 kHz and the magnetic field modulation frequency is 400 Hz. Experimentally a factor of eight was obtained for both absorption and dispersion components at power levels below $\sim 10\text{mW}$. The improvement factor decreased slightly at sample cavity power levels above $\sim 10\text{mW}$. These results were obtained with a cylindrical TE_{011} cavity with a loaded Q of 5,000. The additional noise source at cavity power levels of $\sim 100\text{mW}$ appeared to be independent of the reference phase to the i.f. synchronous demodulator. Consequently, the origin of the additional noise is probably a combination of both a.m. and f.m. noise in the klystron power. The output amplitude of the noise source would be dependent on the reference phase to the i.f. synchronous demodulator if the dominant additional noise was due to either a.m. or f.m. noise in the klystron power.

One disadvantage of the serrodyne superheterodyne spectrometer is that the phase modulator is sensitive to changes in the stray magnetic field. Rotation of the electromagnet in angular rotation experiments or a magnetic field sweep over a wide range may produce a phase change in

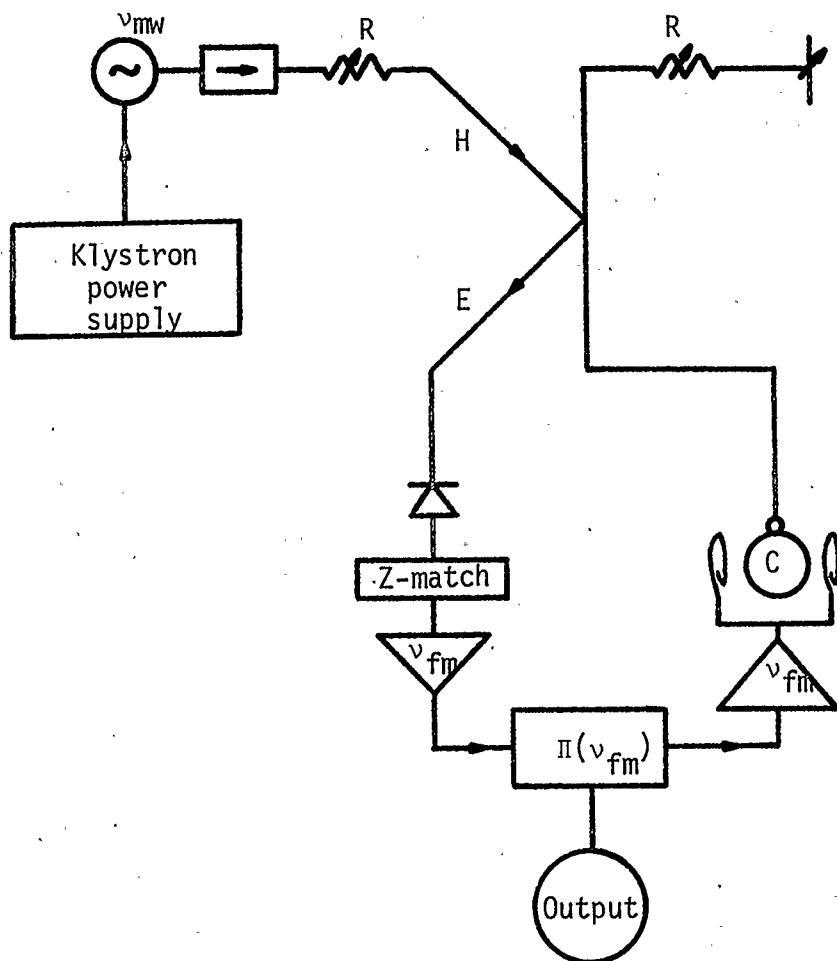


Figure (5.7).

Block diagram of the 54-60 GHz EPR spectrometer.

the i.f. signal and consequently the line shape may be distorted. This disadvantage can be eliminated using magnetic shielding.

5.3 The 54-60 GHz EPR Spectrometer

This spectrometer was built to perform EPR measurements on metmyoglobin crystals at a frequency higher than 34 GHz. The spectrometer with which the majority of this data has been obtained is of simple design, however it possessed adequate sensitivity for even small crystals of volume (10^{-4} -- 10^{-5} cc). It also proved to be very valuable in assessing the difficulties in using 50-75 GHz components in an EPR spectrometer.

A block diagram of the simple version of the spectrometer is given in Fig. (5.7) and is similar to the design used by Slade and Ingram (1969) and by Baer and Wessel (1968). The microwave power was derived from a reflex klystron (OK1 55V10) with a centre frequency of 55 GHz, a tuning range of approximately ± 5 GHz and an output power of ~ 120 mW. The tuning range eliminated the necessity for using tunable cavities, however the facility of having a removable tuning plunger allowed relatively accurate and easy sample positioning. The majority of the experiments have been performed with a tunable TE_{111} mode cylindrical cavity with a loaded Q of approximately 1500.

The waveguide run to the cavity was kept as short as possible to minimize the microwave attenuation and long line effects. The length of the waveguide run from the cavity to the matched hybrid tee of the bridge was ~ 50 cm. For lengths greater than ~ 100 cm line resonances were observed which tended to obscure the cavity resonance and which made spectrometer adjustment difficult.

The theoretical attenuation per unit length of rectangular 50-75 GHz TE_{10} mode waveguide, calculated from a formula given by Moreno (1948), is approximately forty times that for the same mode at 8.2-12.4 GHz. Slade and Ingram (1968) minimized this effect by using oversize waveguide with tapered transitions to the cavity and to the bridge. Oversize waveguide was not used in the 55 GHz spectrometer because the attenuation of the ~50 cm of silver 50-75 GHz waveguide was ~1 dB. The added complexity of oversize waveguide and two waveguide tapers were not considered to be justified for the small improvement possible.

The microwave bridge used a magic tee (Hitachi M3701) constructed from gold plated brass similar in construction to the hybrid tee (Phillips 7260 QA) used in the 26-40 GHz spectrometer. The bridge balancing and diode biasing were performed using an adjustable flap attenuator and a movable short. This arrangement was found to be superior to either; a variable flap attenuator, a variable flap phase shifter and a short; or an E-H tuner and a termination, because the phase and amplitude adjustments could be made more easily and independently with the attenuator and movable short.

The tunable crystal mount (DBB-313) used a 1N53B silicon point-contact diode. The tunable crystal mount matching was found to be very frequency dependent since readjustment was necessary if the klystron frequency was changed by as little as 50 MHz. The microwave diode demodulator was biased to produce a current of approximately 80 μ A through the 100 Ω resistance in series with the primary of the transformer.

The magnetic field modulation was supplied by an a.c. current in a pair of specially designed coils mounted on each magnet pole piece.

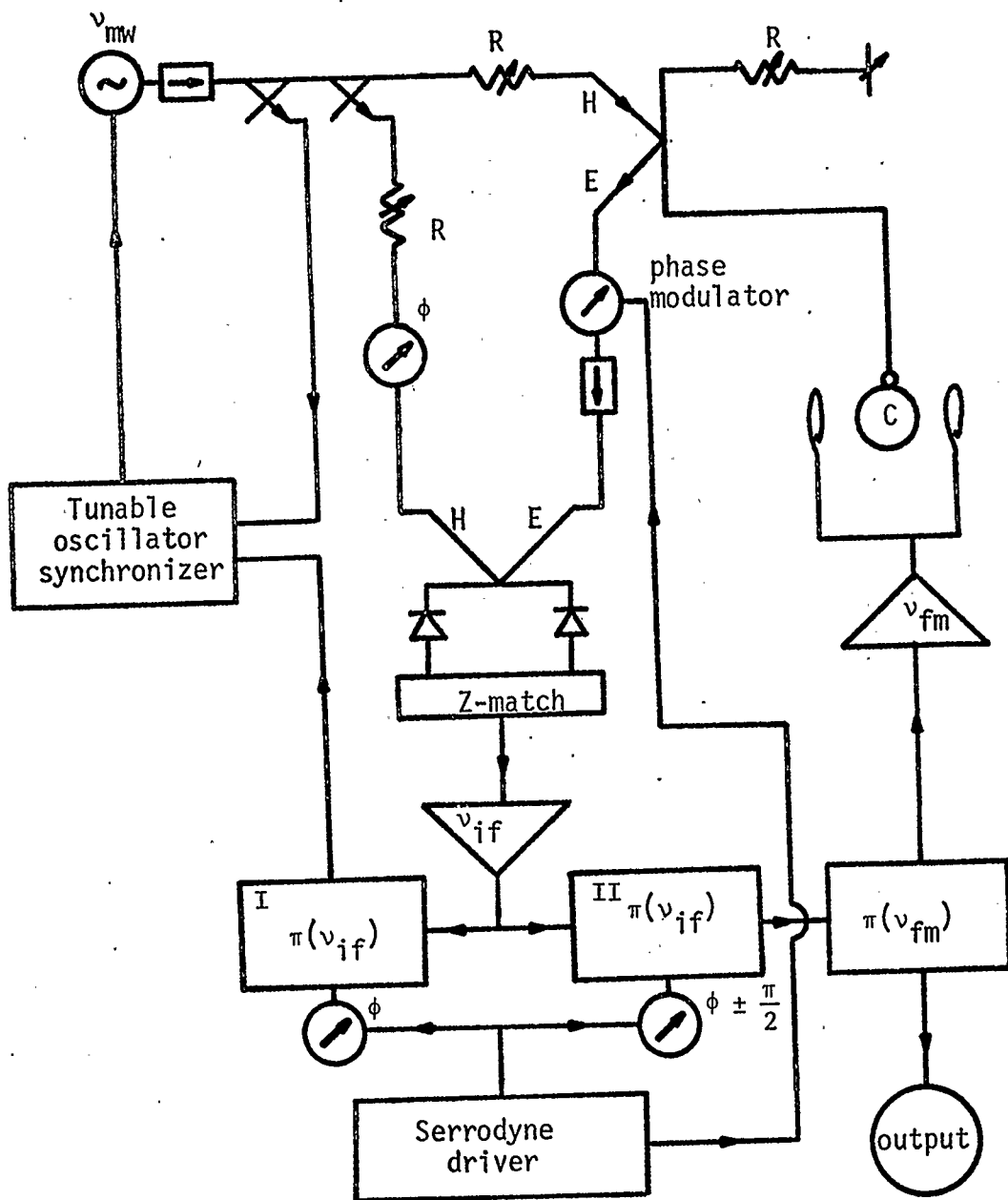


Figure (5.8)
Serrodyne version of the 54-60 GHz EPR spectrometer.

An aluminum former was machined to have the same shape as the tapered pole pieces. The former was then teflon sprayed and approximately 600 turns of 22 gauge coated copper wire were wound on the former and embedded in epoxy resin. The coils were easily removed from the aluminum former because of the teflon coating. They had a d.c. resistance of 8Ω and an inductance of ~ 5 H depending on the magnitude of the steady magnetic field produced by the electromagnet. The coils were driven by a power amplifier with $2 \mu\text{F}$ capacitance in series with the coils to reduce the reactive impedance. Peak-to-peak magnetic fields of 10^{-2} T could be produced easily with these coils at frequencies between 200 and 400 Hz.

The microwave frequency was determined using a calibrating polycrystalline DPPH sample and a high frequency (60-91 MHz) proton magnetic resonance field meter. Earlier experiments used lower frequency (8-15 MHz) deuterium magnetic resonance to calibrate the settings of a Varian Mark II Fieldial between 1.2 Tesla and 2.0 Tesla. The measurement of the magnetic field is described more fully in Sec.(5.4)

Commercial phase modulators are available in a wide range of frequency bands up to 140 GHz so that the serrodyne technique could be used in millimeter EPR spectroscopy. Figure (5.8) gives the block diagram of one version of a 54-60 GHz serrodyne spectrometer which is under development in this laboratory. A further sophistication of this spectrometer would involve phase locking the klystron. This however would require considerable extra expense for an additional oscillator, phase lock synchronizer and mixer which initially would not be justified until an experimental evaluation of a simple system illustrated in Fig.(5.8) is complete.

5.4 Measurement of the Magnetic Field

The magnetic field was measured using the nuclear magnetic resonance of protons or deuterium nuclei. All the measurements on the 34 GHz EPR spectrometer were performed with a version of the transistor marginal oscillator designed by Donnally and Saunders (1960). This NMR gaussmeter was used with proton samples but the lithium nuclei signal in saturated lithium chloride was observable. A heavy water sample was also used with this circuit for calibration of the Fieldial used with the high field magnet for the 54-60 GHz spectrometer. The Fieldial calibration however changed with time and reliable, accurate results could be obtained only by recalibrating the Fieldial for each EPR experiment. The electromagnet pole gap of 3 cm was too narrow to permit simultaneous EPR and NMR experiments because the inhomogeneity of the magnetic field around the cavity dewar broadened the NMR signal excessively. It was necessary to remove the dewar with the waveguide and sample cavity and replace it with the NMR probe.

A high frequency proton magnetic resonance gaussmeter, similar to that described by Robinson (1965), was constructed. This circuit used varactor tuning, a very short (7.5 cm) brass tube to the probe and the circuitry was contained in a piece of cylindrical brass tubing 2.5 cm in diameter. The proton meter could be used to 91 MHz with a 5-turn coil. Consequently the necessity of using the deuterium samples was eliminated as the proton signal is both narrower and more intense. The minimum signal to noise ratio with the high frequency proton resonance probe was approximately ten. The 2.5 cm brass tube enclosing the electronics was mounted in a plexiglass holder and could be moved

to a central position in the pole gap with accurate reproducibility.

The gyromagnetic ratio of the proton was taken to be equal to

$(42.5776 \pm .0006 \text{ MHz/T})$.

CHAPTER VI

CRYSTALLINE FIELD THEORY AND THE SPIN-HAMILTONIAN

This chapter includes a description, in Sec. (6.1), of the magnetic and electric interactions of a free paramagnetic ion in an external magnetic field. The point charge crystalline electric field theory used to determine the low lying energy levels of a paramagnetic ion in a crystalline lattice is described in Sec. (6.2). The effect of a "strong" crystalline field with tetragonal symmetry on the d^5 electron configuration is discussed in detail in Sec. (6.3) because this calculation can be used as a model for the description of the properties of the ground state of high spin ferric heme compounds.

The spin-Hamiltonian formalism is developed in Sec. (6.4) and discussed in Sec. (6.5) in relation to the experimental EPR and far-infrared data for the ferric ion in high spin heme compounds. The magnetic field dependence of the EPR $g_{\perp}$ -value of the lowest doublet of the ground state of the ferric ion is discussed in Sec. (6.6) as a means of estimating the zero field splitting of the ground state.

6.1 The Hamiltonian for a Free Ion in a Magnetic Field

Abragam and Pryce (1951) have shown that the Hamiltonian for a free ion $\mathcal{H}_F$ is

$$\mathcal{H}_F = V_C + V_{LS} + V_{SS} + V_N + V_Q + V_B + V_b \quad (6.1.1)$$

where V_C is the Coulomb interaction of the electrons with the nuclear charge and between the electrons themselves.

V_{LS} is the interaction between the magnetic moment due to the spin angular momentum and that due to the orbital angular momentum.

V_{SS} is the magnetic spin-spin interaction between the electron magnetic dipole moments.

V_N is the interaction between the electron and the nuclear magnetic moments.

V_Q is the electrostatic interaction between the nuclear electric quadrupole moment and the electric field gradient.

V_B is the magnetic interaction between an applied magnetic field and the electron magnetic dipole moment.

V_b is the interaction of the nuclear magnetic moment with the external magnetic field.

Detailed expressions for the potential terms in the free ion Hamiltonian have been given by Low (1960). For an n -electron atom with a nuclear charge Ze , the term V_C is given by

$$V_C = \sum_{k=1}^n \left(\frac{\vec{p}_k^2}{2m} - \frac{Ze^2}{|\vec{r}_k|} \right) + \sum_{j < k}^n \left(\frac{e^2}{|\vec{r}_{jk}|} \right) \quad (6.1.2)$$

where $\vec{p}_k$ is the momentum of the k th electron,

$\vec{r}_k$ is the radius vector of the k th electron,

m is the electronic mass,

e is the electronic charge magnitude,

and $\vec{r}_{jk}$ is the radius vector between the j th and k th electrons.

The expression for V_C assumes that the nucleus is at rest and the electrons and nucleus are point charges. The first term in Eq. (6.1.2) is the sum of the kinetic energy of all the electrons minus the

Coulomb electrostatic attraction between the electrons and the nucleus. The second term in Eq. (6.1.2) represents the mutual repulsion of the electrons. An exact solution of the multi-electron problem is not possible and the central field approximation has been used by Condon and Shortley (1935), amongst others, to obtain the eigenvalues of the operator equivalent form of Eq. (6.1.2).

The term V_{LS} describes the interaction between the magnetic moment due to the spin angular momentum and that due to the orbital angular momentum. This interaction is referred to as the spin-orbit coupling and is given by

$$V_{LS} = \sum_{j,k} \left[a_{jk} (\vec{l}_j \cdot \vec{s}_k) + b_{jk} (\vec{l}_j \cdot \vec{l}_k) + c_{jk} (\vec{s}_j \cdot \vec{s}_k) \right] \quad (6.1.3)$$

where $\vec{l}_j$ is the orbital angular momentum of the j th electron

$\vec{s}_k$ is the spin angular momentum of the k th electron

and a_{jk} , b_{jk} and c_{jk} are coupling constants

Russell-Saunders spin-orbit coupling arises when the total orbital angular momentum $\vec{L}$ interacts with the total spin angular momentum $\vec{S}$. Equation (6.1.3) then takes the operator equivalent form

$$V_{LS} = \lambda \vec{L} \cdot \vec{S}$$

where λ is the spin-orbit coupling constant for the ion.

The term V_{SS} describes the spin-spin interaction between the magnetic dipoles arising from the electronic spin.

$$v_{SS} = \sum_{j,k} 4\beta^2 \left[\frac{\vec{s}_j \cdot \vec{s}_k}{|\vec{r}_{jk}|^3} - \frac{3(\vec{r}_{jk} \cdot \vec{s}_j)(\vec{r}_{jk} \cdot \vec{s}_k)}{|\vec{r}_{jk}|^5} \right] \quad (6.1.4)$$

where β is the Bohr magneton

Equation (6.1.4) is equivalent to the classical expression for the interaction energy of two magnetic dipole moments. The operator equivalent form of v_{SS} has been given by Pryce (1950).

The term v_N describes the magnetic dipole interaction between the electron spins and the nuclear spin I , the magnetic dipole-dipole interaction between the nuclear spin and the electronic orbital moments and the contact interaction between s-electrons and the nuclear spin. The expression for v_N is

$$v_N = 2\gamma_N\beta_N\beta \sum_k \left\{ \frac{(\vec{I}_k - \vec{s}_k) \cdot \vec{I}}{|\vec{r}_k|^3} + \frac{3(\vec{r}_k \cdot \vec{s}_k)(\vec{r}_k \cdot \vec{I})}{|\vec{r}_k|^5} + \frac{8\pi\delta(r_k)\vec{s}_k \cdot \vec{I}}{|\vec{r}_k|^3} \right\} \quad (6.1.5)$$

where γ_N is the nuclear magnetogyric ratio

β_N is the nuclear magneton

$\delta(r_k)$ is the Dirac delta function

The magnetic dipole-dipole interaction between the electron spins and the nuclear spin I is equivalent to the classical dipole interaction. The interaction between the nuclear spin and the orbital moment of the electrons is similar to spin-orbit interaction. The last term in Eq. (6.1.5) arises because s-electrons have a finite probability of 'contact' with the nucleus. The operator equivalent form of v_N has been given by Abragam and Pryce (1951).

The term v_Q describes the electrostatic interaction between the nuclear quadrupole moment Q and the electric field. Bleaney and Stevens (1953) have shown that the quadrupole interaction is given by

$$V_Q = \frac{e^2 Q}{2I(2I-1)} \sum_k \left\{ \frac{I(I+1)}{|\vec{r}_k|^3} - \frac{3(\vec{r}_k \cdot \vec{I})^2}{|\vec{r}_k|^5} \right\} \quad (6.1.6)$$

The quadrupole interaction arises when $I \geq 1$. The electrostatic interaction between the nucleus and the electrons varies with nuclear orientation whenever there is an electric field gradient at the nuclear site because the nuclear charge distribution is not spherically symmetrical.

The term V_B describes the Zeeman interaction of the electronic magnetic moment with the external magnetic field B . The electronic Zeeman effect is given by

$$V_B = \sum_k \beta (\vec{l}_k + 2\vec{s}_k) \cdot \vec{B} \quad (6.1.7)$$

The term V_b describes the interaction of the nuclear magnetic moments with the external magnetic field B .

The nuclear Zeeman effect is given by

$$V_b = -\gamma_N \beta_N \vec{I} \cdot \vec{B}$$

The magnitudes of these interactions are $V_c \approx 10^5 \text{ cm}^{-1}$, $V_{LS} \approx 10^2 \text{ cm}^{-1}$ (iron group ions), $V_{SS} \approx 1 \text{ cm}^{-1}$, $V_N \approx (10^{-1} - 10^{-3}) \text{ cm}^{-1}$ and $V_Q \approx 10^{-3} \text{ cm}^{-1}$.

The magnitude of the electronic and nuclear Zeeman interactions for conventional EPR experiments are $\approx 1 \text{ cm}^{-1}$ and $\approx 10^{-3} \text{ cm}^{-1}$ respectively.

The iron group transition metal series has the electronic configuration $(1s)^2 (2s)^2 (2p)^6 (3s)^2 (3p)^6 (3d)^n$ where n is five for the ferric (Fe^{3+}) ion and six for the ferrous (Fe^{2+}) ion. The most abundant isotope of iron, Fe^{56} , has zero nuclear spin and, consequently,

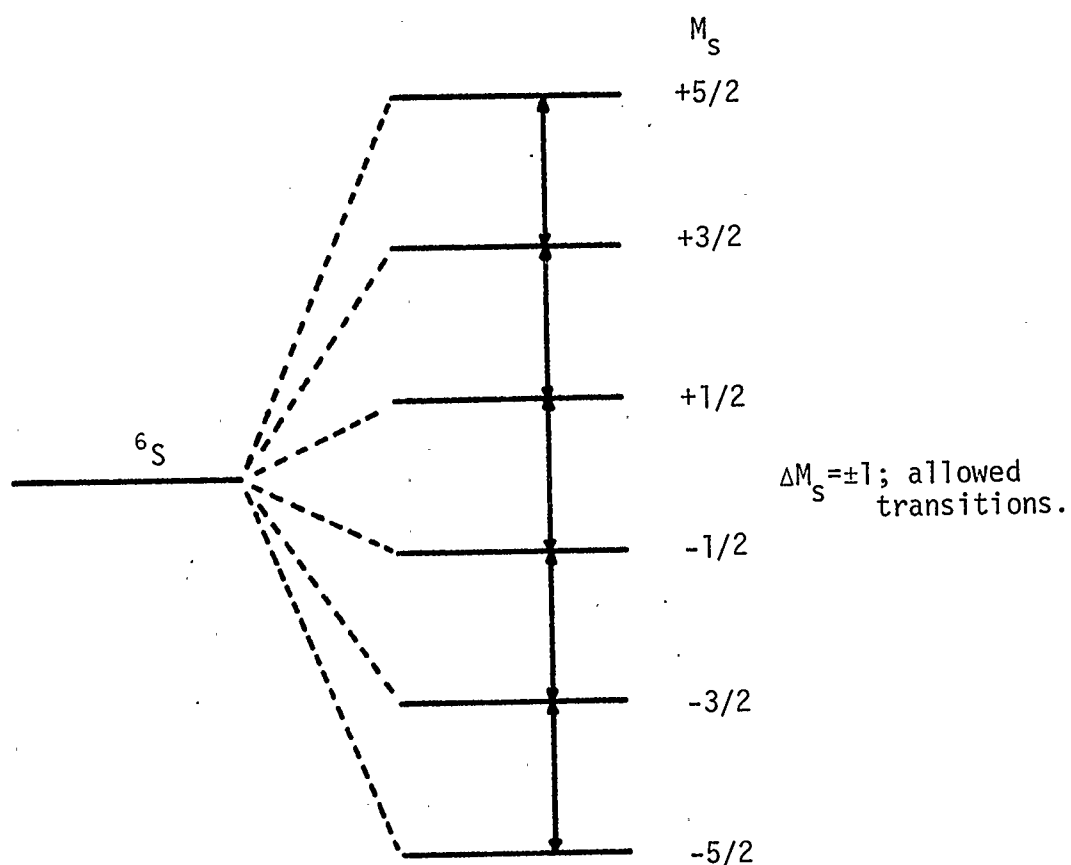


Figure (6.1).

Illustration of the splitting of a $6S$ state in a magnetic field.

the terms v_n and v_Q in $\mathcal{H}$ are zero. The electronic configuration d^5 gives rise to sixteen 'terms' which are the eigenvalues of v_c . The term values arise from the different possible assignments of the five d orbitals consistent with the Pauli Exclusion principle. The ground state can be assigned, for five d electrons, on the basis of Hund's rules. The five d -electrons occupy the five different d orbitals to give a spherically symmetrical state with zero orbital angular momentum. The electronic spin are aligned parallel to one another to give $s = \frac{5}{2}$. The label 6s is used for the ground state. Excited states correspond to electron configurations in which either two electrons with parallel spins share a d orbital and the remaining three electrons have parallel spins, ($s = \frac{3}{2}$ or quartet states), or two pairs of electrons having anti-parallel spins share two d -orbitals ($s = \frac{1}{2}$ or doublet states).

In EPR experiments at or below room temperature the Boltzmann factor ensures that the majority of the paramagnetic ions are in the ground state. The free ion ground state for Fe^{3+} is degenerate in the absence of a magnetic field and the paramagnetism arises from the spin angular momentum. Figure (6.1) shows the splitting of the 6s ground state in a magnetic field. A single isotropic magnetic resonance transition will be observed corresponding to the five allowed transitions. The EPR of the ferric ion in metmyoglobin and in iron porphyrin compounds differs considerably from the EPR of the free ion described above. The explanation for the difference must lie in the influence of the molecular or crystalline field despite the fact that, theoretically, the energy levels of a pure s -state ion are not split by a crystalline electric field.

6.2 The Point Charge Model of the Crystalline Electric Field

The point charge model first discussed by Van Vleck (1932) describes the ions or atoms surrounding the paramagnetic ion as point electric monopoles or dipoles located at the appropriate lattice sites. The electrostatic potential energy at (r, θ, ϕ) due to the surrounding point charges is given by

$$V(r, \theta, \phi) = \sum_i \frac{q q_i}{|\vec{R}_i - \vec{r}|} \quad (6.2.1)$$

where q_i is the charge of the i th nearest neighbour

q is the net electronic charge of the paramagnetic ion

and $\vec{R}_i$ is the radius vector of the i th nearest neighbour

Equation (6.2.1) can be simplified using the expansion

$$\frac{1}{|\vec{R}_i - \vec{r}|} = \sum_{\ell=0}^{\infty} \frac{r^{\ell}}{R_i^{\ell+1}} P_{\ell}(\cos \omega) \quad (6.2.2)$$

where ω is the angle between $\vec{R}_i$ and $\vec{r}$

and $P_{\ell}(\cos \omega)$ is the Legendre polynomial of order ℓ .

Expanding $P_{\ell}(\cos \omega)$ in terms of spherical harmonics yields

$$P_{\ell}(\cos \omega) = \frac{4\pi}{(2\ell+1)} \sum_{m=-\ell}^{+\ell} (-1)^m Y_{\ell}^{-m}(\theta_i, \phi_i) Y_{\ell}^m(\theta, \phi) \quad (6.2.3)$$

where $Y_{\ell}^m(\theta_i, \phi_i)$ is the spherical harmonic

(θ_i, ϕ_i) are the polar and azimuthal orientation of $\vec{R}_i$

and (θ, ϕ) are the polar and azimuthal orientation of $\vec{r}$

Substituting Eqs. (6.2.3) and (6.2.2) into Eq. (6.2.1) yields

$$V = \sum_{\ell=0}^{\infty} \sum_{m=-\ell}^{\ell} \sum_i \left(\frac{4\pi}{2\ell+1} \right) \left(\frac{q q_i r^\ell}{R_i^{(\ell+1)}} \right) \cdot (-1)^m Y_{\ell}^{-m}(\theta_i, \phi_i) \cdot Y_{\ell}^m(\theta, \phi) \quad (6.2.4)$$

which can be simplified to give

$$V(r, \theta, \phi) = \sum_{\ell=0}^{\infty} \sum_{m=-\ell}^{+\ell} A_{\ell}^m r^{\ell} Y_{\ell}^m(\theta, \phi) \quad (6.2.5)$$

$$\text{where } A_{\ell}^m = \sum_i \left(\frac{4\pi}{2\ell+1} \right) \cdot \frac{q q_i}{R_i^{(\ell+1)}} \cdot (-1)^m Y_{\ell}^{-m}(\theta_i, \phi_i) \quad (6.2.6)$$

The number of terms in the expansion of $V(r, \theta, \phi)$ is determined by the symmetry of the crystalline electric field. The number of matrix elements of the crystalline electric field between the electronic wave functions is limited due to the angular variation and parity of the wave functions. The matrix elements of the crystalline potential are of the form $\left\langle \chi_{\ell_1}^{m_1} \left| Y_{\ell}^m \right| \chi_{\ell_2}^{m_2} \right\rangle$. It can be shown that the crystalline potential matrix elements between wavefunctions arising from the same configuration are zero when $(\ell_1 + \ell_2) < \ell$. For iron group ions ℓ_1 and ℓ_2 have a maximum value of 2 so that ℓ has a maximum value of 4. Only those crystalline electric field terms with even ℓ need to be considered because the parity product of the wavefunctions must be even as the matrix elements for odd parity crystalline electric field terms are zero. The point group symmetry of the crystalline electric field surrounding the paramagnetic ion must be known to apply this theory. The specific form of the crystalline potential for various cubic point group symmetries has been tabulated by Low (1960) and Hutchings (1964).

The Hamiltonian for a paramagnetic ion in a crystalline electric field $\mathcal{H} = \mathcal{H}_F + v$ can be solved using perturbation theory. The crystalline potential v is introduced into the calculation at the appropriate stage depending on the relative magnitude of v , the Coulomb interaction v_C and the spin-orbit interaction v_{LS} . Three possibilities exist.

1. Weak Field ($v < v_{LS}$)

The weak field case applies particularly to the lanthanide and actinide transition metal groups because the f electrons are partially screened from the crystalline electric field by the outer s and p electrons of the paramagnetic ion. The crystal field interaction is less than the spin-orbit interaction so that it can be introduced as a perturbation after the spin-orbit coupling has been considered.

2. Intermediate Case ($v_{LS} < v < v_C$)

In crystals containing iron group transition metal ions as impurities the crystalline electric field interaction is stronger than the spin-orbit interaction. The crystal field interacts with the orbital angular momentum and the $(2L + 1)$ possible orientations are split in energy by approximately 10^4 cm^{-1} . Consequently, only the lowest level is appreciably populated and the orbital motion is said to be "quenched."

3. Strong Field ($v > v_C$)

If the crystalline potential v is comparable to, or greater than the Coulomb interaction v_C then the coupling between the individual electrons is broken down and the effect of the crystalline electric field on each orbital must be considered separately.

The strong field case is discussed in greater detail because the

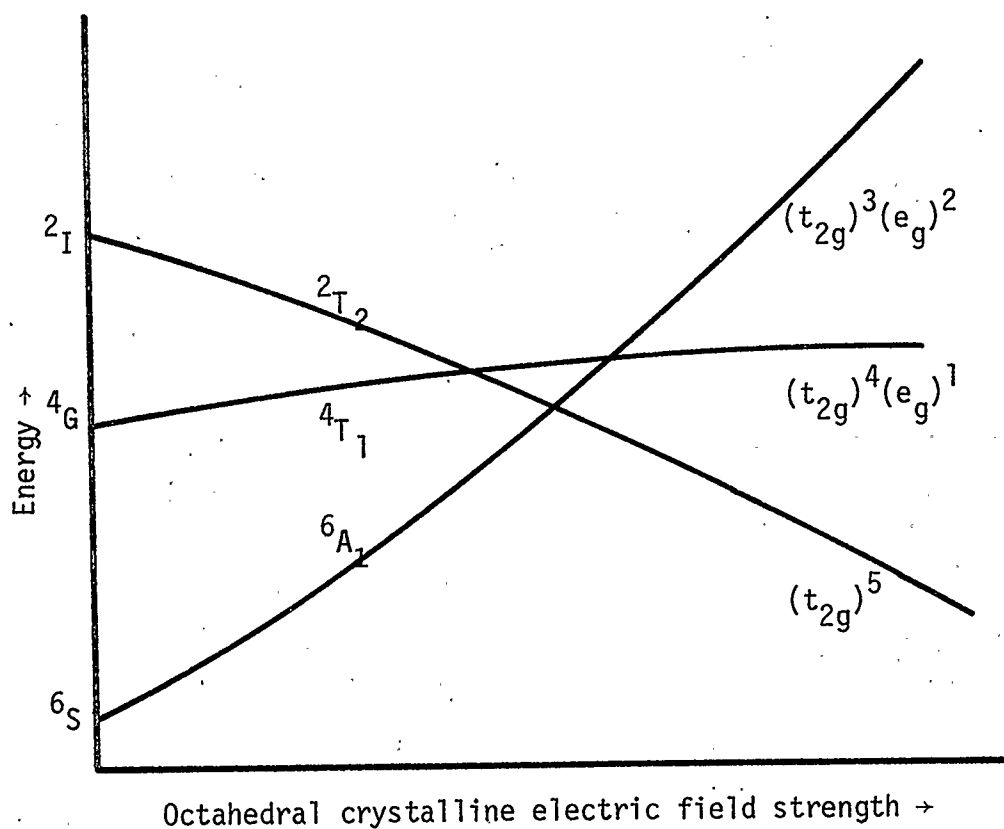


Figure (6.2).

Illustration of the variation in energy of the three lowest levels of the ferric ion as a function of the strength of an octahedral crystalline electric field. Reproduced from Kotani (1968).

bonding of the iron atom in hemoproteins and heme compounds appears to be consistent with a strong crystalline electric field. The degeneracy of the d-electrons is lifted to give two sublevels in a cubic crystalline electric field with six-fold co-ordination (octahedral symmetry). The sublevels are normally labelled using the group theoretic notation t_{2g} and e_g . The solution of the Hamiltonian $\mathcal{H} = V_c + V$ has been given for the iron group ions in octahedral symmetry by Tanabe and Sugano (1954). Following Kotani (1968) the variation in energy of the three lowest levels of the ferric ion as a function of the strength of the octahedral crystalline field is shown qualitatively in Fig. (6.2). The energy of the crystalline electric field at which the 6A_1 (6s) state crosses the 2T_2 state is known as the critical field strength. Group theoretic labels are used to distinguish the energy levels in the crystalline electric field from the free ion energy levels. Hartree (1946) has shown that the magnetic susceptibility from hemoprotein samples changes significantly when the ligand bonding to the sixth co-ordination position of the iron is altered. In order to explain the magnetic susceptibility and EPR results the magnitude of the crystalline electric field in the hemoproteins must be sufficiently large to be in the ground state cross-over region. The fluoride and met derivatives of hemoglobin and myoglobin are high spin ($s = \frac{5}{2}$, 6A_1 ground state) and the azide and cyanide derivatives are low spin derivatives ($s = \frac{1}{2}$, 2T_2 ground state). An experimental value for the magnetic moment intermediate between that obtained for the high spin and low spin compounds is observed for a number of derivatives. These are referred to as "spin-mixed" derivatives. The magnetic susceptibility of hemoproteins is reviewed in Chapter VII.

6.3 The Effect of a Strong Crystalline Electric Field with Tetragonal Symmetry on the d^5 Configuration

Harris (1968a, 1970) has shown that a strong crystalline electric field calculation can be used to account for the magnetic properties of the ferric ion in high and low spin hemoprotein, and in a number of iron porphyrin compounds. An outline of this calculation is included in Sec. (6.3). The notation and derivation of the crystalline field parameters follows that of Harris (1968b).

6.3.1 The d-Electron Energies

The direct effect of the crystal-line electric field on the degenerate free ion d-orbital is calculated. The d-orbital wavefunctions can be expressed as the product of a radial function (R_{3d}) and a spherical harmonic $Y_2^m(\theta, \phi)$.

$$\psi_m = R_{3d}(r) Y_2^m(\theta, \phi) \quad (6.3.1)$$

It is more convenient to use the alternate orthonormal set of wave-functions given with respect to a cartesian system of axes. The labels; d_{z^2} , $d_{x^2-y^2}$, d_{xy} , d_{yz} , and d_{zx} are normally used for these wave-functions.

The crystalline potential v for tetragonal D_{4h} symmetry can be derived using the methods outlined in Sec. (6.2).

$$v(D_{4h}) = \text{constant term} + A_2^0 r^2 Y_2^0 + A_4^0 r^4 Y_4^0 + A_4^4 r^4 (Y_4^4 + Y_4^{-4}) \quad (6.3.2)$$

$$\text{where } A_2^0 = -4\sqrt{\frac{\pi}{5}} \left(\frac{q}{a^3} - \frac{p}{b^3} \right)$$

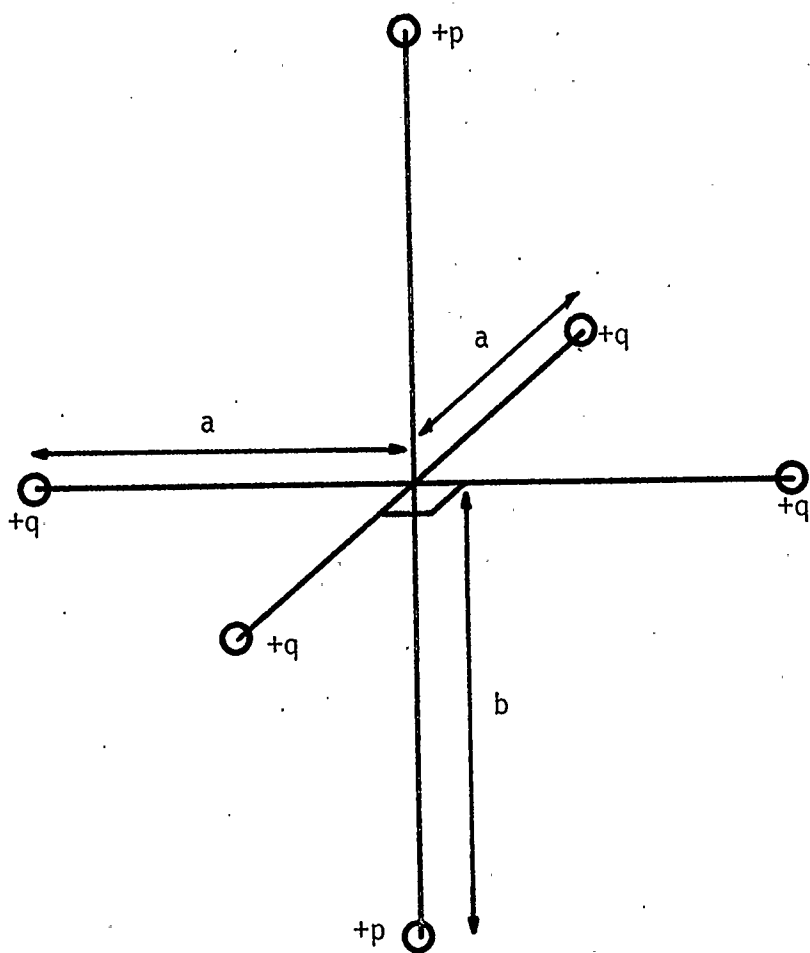


Figure (6.3).

Illustration of the point charge model for a crystalline electric field of tetragonal symmetry. $2b$ is the distance along the tetragonal axis.

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$$\begin{aligned}
 A_4^0 &= + \sqrt{\frac{\pi}{3}} \left(\frac{3q}{a^5} - \frac{4p}{b^5} \right) \\
 A_4^4 &= + \sqrt{\frac{35}{2}} \left(\frac{q}{a^5} \right)
 \end{aligned} \tag{6.3.3}$$

The symbols for the lattice distances a and b and the electric charges p and q for this distribution are illustrated in Fig. (6.3). The terms A_2^0 , A_4^0 and A_4^4 are not normally calculated from this elementary model because even in the weak field case the theoretical values for the A coefficients are not in good agreement with the experimentally determined A 's. In some cases, e.g. Chatterjee and van Ormondt (1970), theoretical values for the A coefficients can be calculated by lattice sum calculations but normally the A coefficients are treated phenomenologically and fitted to the experimental data. Symmetry considerations are used to determine which terms are necessary in the expansion of the crystalline potential.

Harris (1968b) has shown that the d orbital energies are

$$E_d = \langle \psi | V(D_{4h}) | \psi \rangle \tag{6.3.4}$$

$$E_d = (\text{constant term}) + aA_2^0 \langle r^2 \rangle + bA_4^0 \langle r^4 \rangle + cA_4^4 \langle r^4 \rangle \tag{6.3.5}$$

$$\text{where } \langle r^n \rangle = \int R_{3d}^* r^n R_{3d} r^2 dr \tag{6.3.6}$$

and a , b and c are constants given in Table 1 of Harris (1968b).

The resulting energy level diagram is given in Fig. (6.4).

Because of their spatial distribution the e_g orbitals in octahedral symmetry are of higher energy than the t_{2g} orbitals. In D_{4h} symmetry the t_{2g} orbitals are split into e and b_2 orbitals and the e_g orbitals are split into a_1 and b_1 orbitals as shown in Fig. (6.4).

The energy of the four orbitals is given in Fig. (6.4) in terms of the

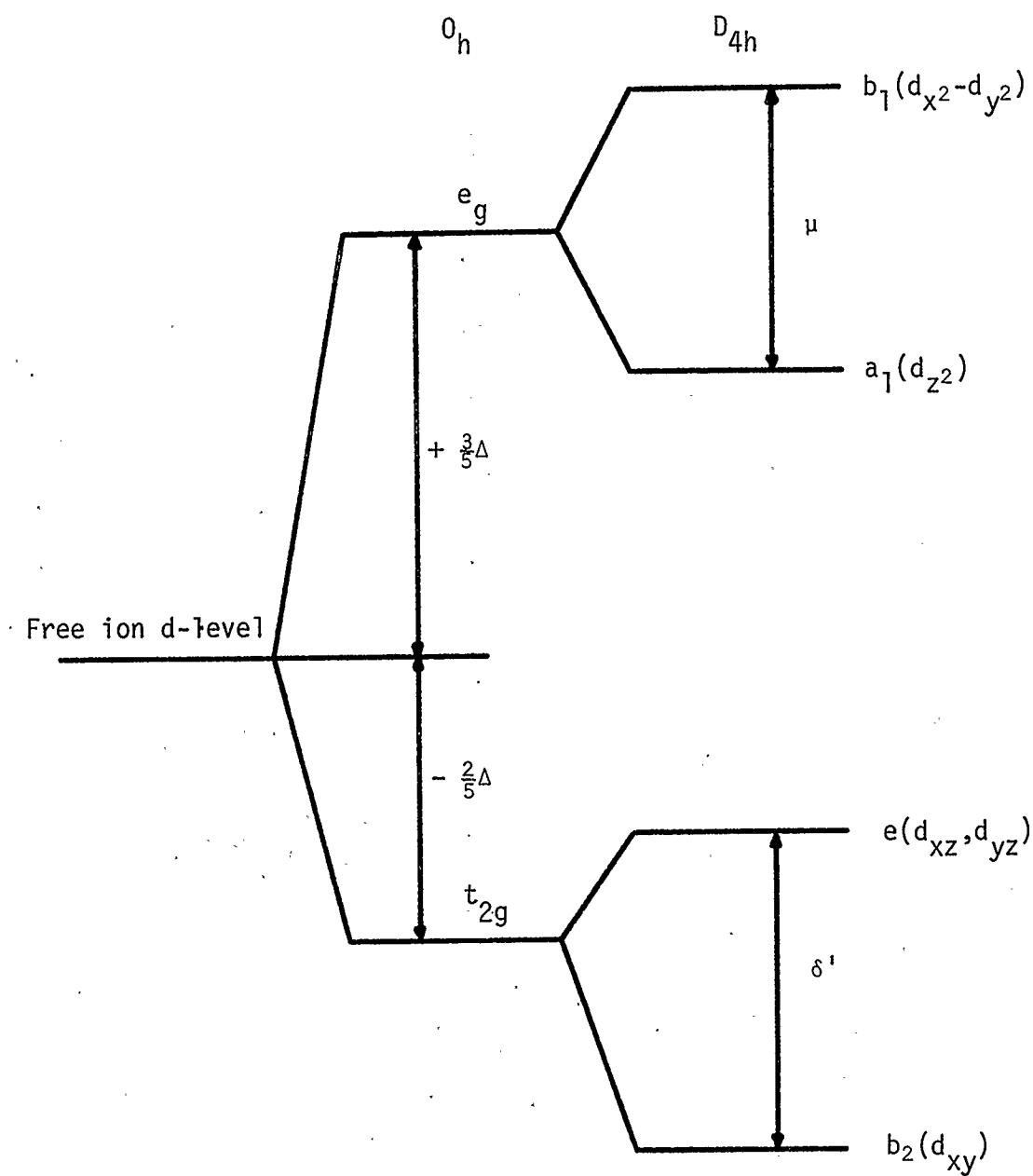


Figure (6.4).

Splitting of the d-electron energies in a strong crystal-line electric field of tetragonal symmetry.

three crystalline field parameters Δ , μ and δ' . These parameters are related to the A coefficients by

$$\begin{aligned}\Delta &= \frac{5}{14\sqrt{\pi}} A_4^0 + \frac{5}{6}\sqrt{\frac{5}{14\pi}} A_4^4 \\ \mu &= -4 \frac{\sqrt{5}}{14\sqrt{\pi}} A_2^0 - \frac{5}{14\sqrt{\pi}} A_4^0 + \sqrt{\frac{5}{14\pi}} A_4^4 \\ \delta' &= 3 \frac{\sqrt{5}}{14\sqrt{\pi}} A_2^0 - \frac{5}{14\sqrt{\pi}} A_4^0 + \sqrt{\frac{5}{14\pi}} A_4^4\end{aligned}\quad (6.3.7)$$

It is convenient to define the three crystalline electric field parameters in a form that is more appropriate to the description of iron porphyrin compounds. Harris (1968b) defined three additional parameters Δ_{0h} , C' and u' given by

$$\begin{aligned}\Delta_{0h} &= \Delta + \frac{1}{2}u + \frac{2}{3}\delta' \\ C' &= \frac{3}{7}u + \frac{4}{7}\delta' \\ u' &= \frac{4}{7}u - \frac{4}{7}\delta'\end{aligned}\quad (6.3.8)$$

so that the crystalline electric field can then be described by the two tetragonal parameters u' and C' , which are zero in O_h symmetry, and a cubic parameter Δ_{0h} which depends on the average of the six-ligand interaction. Further insight into the physical significance of variation in the parameters Δ_{0h} , C' and u' can be obtained from the crystalline field expressions for these parameters in terms of ligand-ionic distances and ligand electric charges. Harris (1968b) has shown that

$$\begin{aligned}
 \Delta_{Oh} &= \frac{5}{3} \cdot \left(\frac{q}{a^5} \right) \langle r^4 \rangle \\
 u' &= \frac{8}{7} \left(\frac{q}{a^3} - \frac{p}{b^3} \right) \langle r^2 \rangle \\
 c' &= \frac{10}{21} \left(\frac{q}{a^5} - \frac{p}{b^5} \right) \langle r^4 \rangle
 \end{aligned} \tag{6.3.9}$$

Harris (1968b) used Eq. (6.3.9) in a qualitative manner to relate changes in the properties of a series of heme complexes with changes in the parameters Δ_{Oh} , u' and c' . The parameters u' and c' are related to differences between the axial field strength and the in-plane field strength. The parameters u' and c' have the same sign and it is expected that they are both positive for heme complexes because the in-plane bonding interaction is likely to be stronger than the axial bonding interaction. Harris (1968) made the assumption that $c' = fu'$ where f is a measure of the relative magnitude of the t_{2g} and e_g splittings δ' and u . Consequently, if the in-plane interaction is kept constant and the axial interaction is increased then Δ_{Oh} increases but c' and u' decrease. The three parameters Δ_{Oh} , c' and u' were varied extensively in computer programs in a manner consistent with the crystalline electric field model.

6.3.2 The Five-Electron States

In the strong field limit there are five configurations or possible assignments of the electrons in the t_{2g} and e_g orbitals. These configurations (t_{2g}^n, e_g^{5-n}) lead to forty-three energy states which are equivalent to the free ion 'terms.' The electrostatic

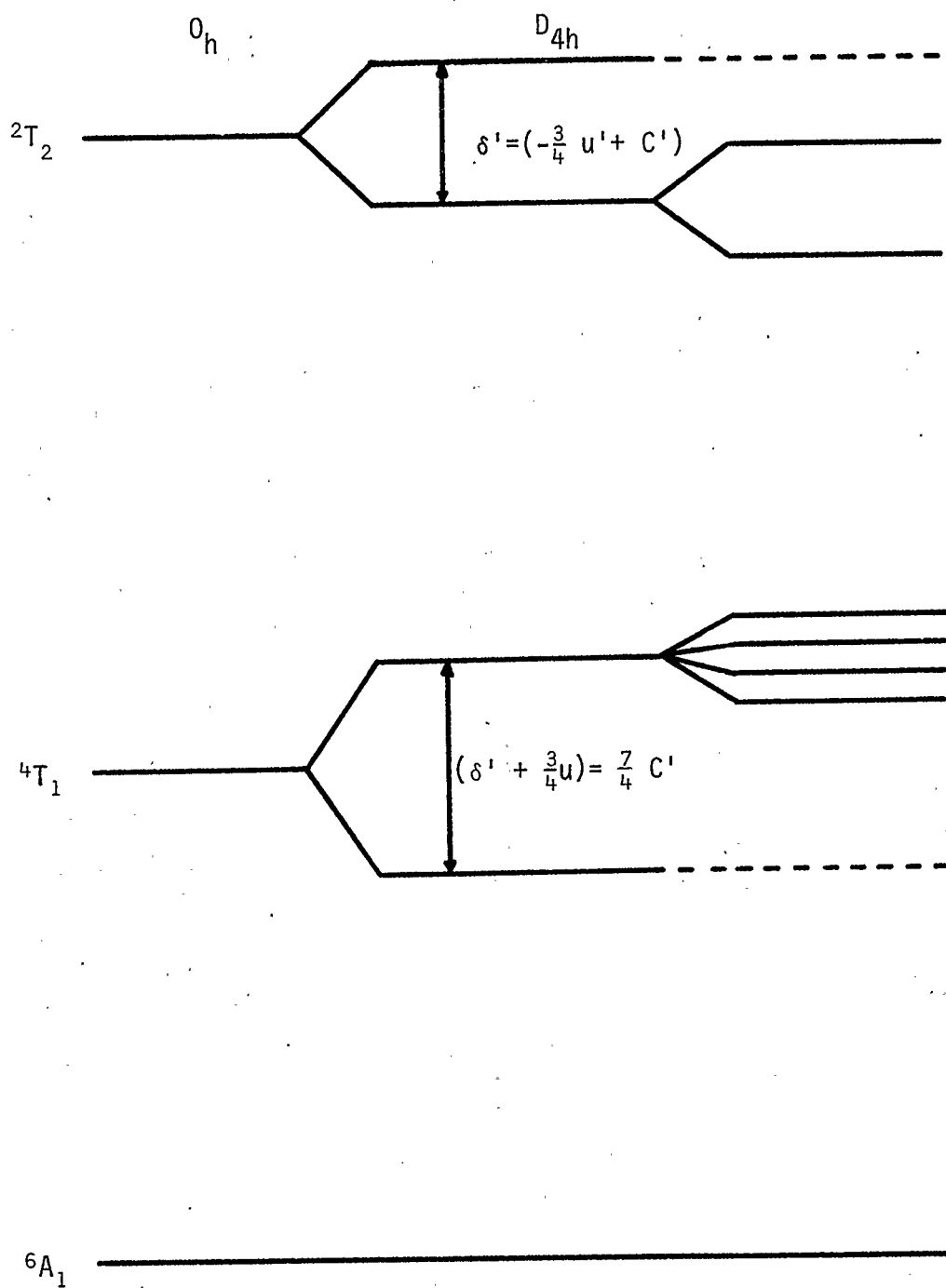


Figure (6.5).

Illustration of the energy level scheme for the low-lying energy states of the d^5 configuration in a strong crystalline electric field of tetragonal symmetry.

interaction between the electrons in these states has been calculated by Tanabe and Sugano (1954) and Griffith (1961).

Experiments on the magnetic properties of heme compounds are concerned primarily with the ground state of the iron. For this reason Harris (1966) did a detailed calculation of the effects of spin-orbit coupling on the ground state for high spin heme compounds to determine which of the excited states contributed significantly to the ground state properties. She found that only the $2T_2 \left(t_{2g}^5 \right)$ and $4T_1 \left(t_{2g}^4 e_g^1 \right)$ excited states could contribute appreciably to the ground state properties. These are the two lowest excited states in Fig. (6.2) for values of Δ less than the critical field strength. It is assumed that the same conclusion applies when the O_h symmetry is distorted to D_{4h} symmetry.

6.3.3 Spin-Orbit Coupling

The first-order electrostatic, crystalline electric field and spin-orbit coupling energies in D_{4h} have been calculated by Harris (1968b) using the irreducible tensor method described by Griffith (1962). The energy level scheme for a high spin derivative is given in Fig. (6.5). The splitting of the quartet state by the tetragonal crystalline electric field is a function of only c' . Consequently the zero field splitting in high spin compounds will depend primarily on the values of c' and Δ_{O_h} because spin-orbit coupling between the quartet states and the ground state appears to be the dominant mechanism producing the zero field splitting. The third column in Fig. (6.5) gives the additional splitting, to first order, among the twelve doublets due to spin-orbit coupling. The spin-orbit matrix diagonalization was performed

by the computer for a range of parameter variation, in particular the crystalline electric field parameters C' and Δ_{Oh} .

6.4 The Spin-Hamiltonian

The spin-Hamiltonian was introduced by Abragam and Pryce (1951) to describe and interpret the results of EPR experiments. It has been shown in Sec. (6.3) that the ground state eigenfunction solution of the Hamiltonian $\mathcal{H} = \mathcal{H}_F + V$ is a complicated admixture of free ion spin and orbital eigenfunctions. Abragam and Pryce (1951) showed that the complete Hamiltonian need not be employed for the low-lying energy levels involved in EPR experiments and that a simpler Hamiltonian using only spin operators could be used. A single fictitious spin quantum number S' is used to describe the EPR spectra because it is determined by equating the observed number of lines to $(2S' + 1)$ and it need not correspond to the total spin of the paramagnetic ion. The energy separation of the low lying energy levels can be obtained from the eigenvalues of the equation

$$\mathcal{H}_S |\psi\rangle = E |\psi\rangle \quad (6.4.1)$$

where $\mathcal{H}_S$ is the Spin-Hamiltonian operator

E represents the possible eigenvalues

and $|\psi\rangle$ is the eigenfunction describing the fictitious spin state.

The effects of excited states on the ground state and the contribution to the paramagnetism of the ion from orbital angular momentum is included implicitly in the various spin parameters of the spin-Hamiltonian. These parameters are normally determined by experiment.

The symmetry of the crystalline electric field is reflected in the angular variation of the spectroscopic splitting factor g as a function of the orientation of the crystalline symmetry axes with the direction of the applied magnetic field. In some cases, the point group symmetry of the crystalline electric field at the paramagnetic ion can be determined experimentally.

The various terms in a simple spin-Hamiltonian are

$$\mathcal{H}_s = \beta \vec{S} \cdot \vec{g} \cdot \vec{B} + \vec{S} \cdot \vec{A} \cdot \vec{I} + \vec{S} \cdot \vec{D} \cdot \vec{S} - g_N \mu_N \vec{I} \cdot \vec{B} + \vec{I} \cdot \vec{Q} \cdot \vec{I} \quad (6.4.2)$$

The first term represents the Zeeman interaction of the "fictitious" spin with the magnetic field. The second rank tensor g is referred to as the spectroscopic splitting factor and it can be diagonalized to give the principal g -values g_1 , g_2 and g_3 . In this frame of reference the Zeeman term simplifies to

$$\beta \vec{S} \cdot \vec{g} \cdot \vec{B} = \beta (g_1 S_x B_x + g_2 S_y B_y + g_3 S_z B_z) \quad (6.4.3)$$

The principal g -values are determined experimentally. When the g -value variation has axial symmetry it is conventional to use the notation

$$g_1 = g_2 = g_{\perp} \quad \text{and} \quad g_3 = g_{\parallel}$$

where $g_{\parallel}$ is then the g -value when the direction of the magnetic field is parallel to the symmetry axis of an axial crystalline electric field.

and $g_{\perp}$ is the g -value when the direction of the magnetic field is perpendicular to the symmetry axis of the axial crystalline electric field.

The "hyperfine" interaction of the nuclear magnetic moment and the electronic magnetic moment is given by the term $\vec{S} \cdot \mathbf{A} \cdot \vec{I}$. At typical EPR spectrometer frequencies the electronic Zeeman interaction effect is much greater than the hyperfine interaction which then produces a splitting of the Zeeman levels. The second rank tensor $\mathbf{A}$ contains an isotropic contribution arising from a Fermi contact interaction and an anisotropic contribution due to the magnetic interaction of the nuclear and electronic spins.

The third term in the spin-Hamiltonian describes the zero field ground state splitting. It is appropriate to include this term only for a ground state with $s > 1$. The frame of reference in which $\mathbf{D}$ is diagonal gives

$$\vec{S} \cdot \mathbf{D} \cdot \vec{S} = D_1 S_x^2 + D_2 S_y^2 + D_3 S_z^2 \quad (6.4.4)$$

Normally, Eq. (6.4.4) is expressed in the form

$$\vec{S} \cdot \mathbf{D} \cdot \vec{S} = D \left[S_z^2 - \frac{1}{3} S(S+1) \right] + E \left(S_x^2 - S_y^2 \right) \quad (6.4.5)$$

Equating Eq. (6.4.4) and Eq. (6.4.5) and setting $S_x^2 + S_y^2 + S_z^2 = S(S+1)$ gives

$$D = \frac{3}{2} D_3 \quad \text{and} \quad E = \frac{1}{2} (D_1 - D_2)$$

For axial symmetry $E = 0$ and the zero field splitting can be described by the first term in Eq. (6.4.5). It is sometimes necessary to include fourth-order terms in the spin operators to describe the observed zero field splitting of $s = \frac{5}{2}$ s-state ions. The derivation of the spin-Hamiltonian given by Abragam and Pryce (1951) is invalid for

an s-state ion. Consequently the spin-Hamiltonian formalism required to describe the zero field splitting of an s-state ion provides only a phenomenological description of the EPR spectrum.

The fourth-term in the spin-Hamiltonian given in Eq. (6.4.2) is the nuclear Zeeman effect. This term, as well as the nuclear quadrupole interaction, is normally small in relation to the other terms. These interactions can be studied by nuclear magnetic resonance, ENDOR, and by Mössbauer spectroscopy for certain substances, e.g. Fe^{57} .

6.5 The Spin-Hamiltonian Required to Describe the EPR of Ferric Iron in High Spin Heme Compounds

The derivation of the energy states for the high-spin ferric ion in a strong tetragonal crystalline electric field, given in Sec. (6.3), has shown that the ground state is predominantly in a spherically symmetrical 6A_1 state equivalent to the free ion 6s state. The presence of low lying quartet states leads to some mixing of these states with the ground state via spin-orbit coupling. In a crystalline electric field of tetragonal symmetry the quartet states are split so that the spin-orbit coupling with the ground state leads to a zero field splitting in the ground state. Consequently zero field splitting terms must be included in the spin-Hamiltonian used to describe high-spin heme compounds. The spin-Hamiltonian parameters can be determined experimentally.

Koster and Statz (1959), Ray (1964) and Grant and Strandberg (1964) have described how to derive a generalized spin-Hamiltonian for a paramagnetic ion in a crystalline electric field with a particular symmetry. The number of adjustable parameters that arise in generalized spin-Hamiltonians is the maximum number necessary consistent with the

symmetry of the crystalline electric field. These parameters must be determined experimentally. The generalized spin-Hamiltonian cannot be used to describe the EPR of ferric iron in metmyoglobin because only the transition between the Zeeman splitting of the $(\pm\frac{1}{2})$ doublets has been observed and this information is inadequate to assign values to all the adjustable parameters that arise in the derivation of the generalized spin-Hamiltonian for $s = \frac{5}{2}$ in a tetragonal crystalline field.

Ingram et al (1956) demonstrated the existence of a single anisotropic EPR resonance with experimental g -values, $g_{||} = 2$ and $g_{\perp} = 6$ from MbH_2O and HbH_2O . These results differ considerably from the expected EPR spectra for an $s = \frac{5}{2}$ state assuming even a small degree of zero field splitting between the three Kramers' doublets of the sextet state. Griffith (1956) interpreted the EPR results from metmyoglobin as arising from the Zeeman splitting of the lowest doublet of the $s = \frac{5}{2}$ state. The early experiments established therefore that large zero field splitting must exist between the various Kramers doublets.

If the ground state is assumed to be an isolated doublet with effective spin $s' = \frac{1}{2}$ in an axial crystalline electric field then

$$\mathcal{H}_B = g_{||} \beta B_z S_z + g_{\perp} \beta (B_x S_x + B_y S_y) \quad (6.5.1)$$

The eigenvalue solution of Eq. (6.5.1) for the lowest doublet when the magnetic field is parallel to the crystalline symmetry axis is

$$E(+\frac{1}{2})|+\frac{1}{2}\rangle = \mathcal{H}_B|+\frac{1}{2}\rangle = \frac{1}{2} g_{||} \beta B_z |+\frac{1}{2}\rangle \quad (6.5.2a)$$

and

$$E\left(-\frac{1}{2}\right) \left| -\frac{1}{2} \right\rangle = \cancel{\mathcal{H}}_B \left| -\frac{1}{2} \right\rangle = -\frac{1}{2} g_{\parallel} \beta B_z \left| -\frac{1}{2} \right\rangle \quad (6.5.2b)$$

so that

$$E\left(\frac{1}{2}\right) - E\left(-\frac{1}{2}\right) = g_{\parallel} \beta B_z \quad (6.5.3)$$

When the magnetic field is in the x direction then the eigenvalue solution of Eq. (6.5.1) is

$$\begin{aligned} \cancel{\mathcal{H}}_B \left| +\frac{1}{2} \right\rangle &= g_{\perp} \beta B_x S_x \left| +\frac{1}{2} \right\rangle \\ &= g_{\perp} \beta B_x \frac{1}{2} (S_+ - S_-) \left| +\frac{1}{2} \right\rangle \end{aligned} \quad (6.5.4)$$

where

$$S_{\pm} |M\rangle = \sqrt{S(S+1) - M(M\pm 1)} |M\pm 1\rangle$$

so that

$$\cancel{\mathcal{H}}_B \left| +\frac{1}{2} \right\rangle = g_{\perp} \beta B_x \frac{3}{2} \left| -\frac{1}{2} \right\rangle \quad (6.5.5a)$$

and

$$\cancel{\mathcal{H}}_B \left| -\frac{1}{2} \right\rangle = g_{\perp} \beta B_x \frac{3}{2} \left| +\frac{1}{2} \right\rangle \quad (6.5.5b)$$

The Zeeman splitting of the doublet is given by the difference in the eigenvalues of the secular determinant.

$\left +\frac{1}{2} \right\rangle$	$\left +\frac{1}{2} \right\rangle$	$\left -\frac{1}{2} \right\rangle$
$\left -\frac{1}{2} \right\rangle$	E	$\frac{3}{2} g_{\perp} \beta B_x$
$\left -\frac{1}{2} \right\rangle$	$\frac{3}{2} g_{\perp} \beta B_x$	E

(6.5.6)

The solution of the secular determinant in Eq. (6.5.6) is

$$E(\pm\frac{1}{2}) = \pm\frac{3}{2}g_{\perp}\beta B_x \quad (6.5.7)$$

so that the Zeeman splitting is

$$E(\frac{1}{2}) - E(-\frac{1}{2}) = 3g_{\perp}\beta B_x \quad (6.5.8)$$

The orbital contribution to the paramagnetism is negligible because the ion is in a 6A_1 ground state. Consequently the spin-Hamiltonian parameters $g_{||}$ and $g_{\perp}$ will be close to the free spin value $g_o = 2.00$. From Eq. (6.5.3) and Eq. (6.5.8) the experimental g -values should be $g_{||}(\text{exp}) = 2.00$ and $g_{\perp}(\text{exp}) = 6$. These predicted values are in good agreement with those obtained experimentally by Bennett *et al* (1957). Griffith (1956) estimated the zero field splitting to be 10cm^{-1} on the basis of the field dependence of $g_{\perp}(\text{exp})$.

No EPR results have been obtained from transitions between the different Kramers doublets in MnH_2O so that no direct information is available on the exact nature of the zero field splitting terms that should be included in the complete spin-Hamiltonian. It has been assumed by Griffith (1964), Kotani (1964) and Weissbluth (1967) that the dominant term in the zero field spin-Hamiltonian of MnH_2O and heme compounds must be $D\left[S_z^2 - \frac{1}{3}S(S+1)\right]$. This assumption has been substantiated by the far-infra-red experiments of Richards, *et al.* (1967) on heme compounds.

Helcké (1963), Kotani (1967) and Helcké *et al.* (1968) showed that $g_{\perp}(\text{exp})$ varied when the magnetic field was rotated in the plane of the heme group. Consequently the crystalline electric field symmetry is

not purely axial and must contain a rhombic component. This can be described by the additional term in the spin-Hamiltonian $E(s_x^2 - s_y^2)$. Therefore, the zero field splitting spin-Hamiltonian is

$$\mathcal{H}_{zF} = D(S_z^2 - \frac{1}{3}S(S+1)) + E(s_x^2 - s_y^2) \quad (6.5.9)$$

where Kotani (1967) has estimated that $\frac{E}{D} \approx \frac{1}{400}$.

The possibility of a small fourth-order term in the zero field spin-Hamiltonian was considered by Uenoyama, et al. (1968) in discussing magnetic susceptibility results. At present, there appears to be insufficient experimental evidence on MbH_2O from any source to justify the estimation of the relative magnitude of D and a fourth order term.

The nuclear spin of the most abundant isotope of iron, Fe^{56} , is zero and consequently, no hyperfine or quadrupole splitting is observed. A good approximation to the spin-Hamiltonian for high-spin heme compounds is

$$\mathcal{H}_S = \mathcal{H}_{zF} + \mathcal{H}_B$$

where $\mathcal{H}_{zF}$ is given by Eq. (6.5.9)

and $\mathcal{H}_B$ is given by Eq. (6.5.1)

6.6 The Variation in $g_{\perp}(\text{exp})$ with Magnetic Flux Density

Kirkpatrick, Müller and Rubins (1964) showed that $g_{\perp}(\text{exp})$ from an $s = \frac{5}{2}$ ion will be magnetic field dependent. The variation in $g_{\perp}(\text{exp})$ with magnetic field is a function of the magnitude of the parameter D in the zero-field spin-Hamiltonian, Eq. (6.5.9). This theory applies when $DS_z^2 \gg g\beta BS_z$ because the magnetic field term can then be treated

as a perturbation. A third-order perturbation calculation of the effect of the magnetic field on the value of $g_{\perp}(\text{exp})$ yields

$$g_{\perp}(\text{exp}) = 3g_{\perp} \left[1 - \frac{2(g_{\perp}\beta B)^2}{(2D)^2} \right] \quad (6.6.1)$$

Kirkpatrick, et al. (1964) interpreted the EPR spectrum of an Fe^{3+} ion in strontium titanate in terms of an oxygen vacancy in the normal $(\text{Fe}^{3+})(\text{O}^{2-})_6$ octahedral symmetry. The oxygen vacancy created the tetragonal crystalline electric field which lead to the large zero field splitting of the $S = \frac{5}{2}$ state. Using Eq.(6.6.1) on EPR results obtained at 11GHz and 23GHz, Kirkpatrick, et al. (1964) estimated that $2D = (2.85 \pm .15)\text{cm}^{-1}$. Subsequent studies by Baer, Wessel and Rubins (1958) at 35, 65, 70 and 75GHz showed that $2D = (2.707 \pm .008)\text{cm}^{-1}$. They also showed that fourth order terms were much less than $2D$. This work demonstrated the validity of Eq. (6.6.1) for estimating the zero field splitting in terms of the field dependence of $g_{\perp}(\text{exp})$. Equation (6.6.1) is used to estimate the zero field splitting of the ferric ion in metmyoglobin from the measurements described in Chapter VIII.

TABLE II

Properties of Four Oxygen Transporting Molecules

	Hemoglobin	Chlorocruorin	Hemocyanin	Hemerythrin
Colour	red	green	blue	red
Metal	Fe	Fe	Ca	Fe
Prosthetic group	heme	heme		
Molecules of oxygen per atom of metal	1.1	1.1	1.2	1.3
Occurrence	In the cor- puscles of mammals, birds, reptiles and fishes and in the plasma of annelids and molluscs.	In the plasma of annelids.	In the plasma of molluscs.	In the cor- puscles of annelids.

CHAPTER VII

HEMOGLOBIN AND MYOGLOBIN

This chapter commences with a brief description of the structure and function of hemoglobin and myoglobin in living systems. The crystallization procedures adopted in this study and the crystal structure of myoglobin and hemoglobin are described in Sec. (7.2). A review of the EPR research on MbH₂O and HbH₂O is given in Sec. (7.3). The review emphasizes the single crystal studies and does not include reference to all the work on polycrystalline or liquid samples. A brief account of the relevant magnetic susceptibility and Mössbauer experiments is given in Sec. (7.4).

7.1 Structure and Function

Hemoglobin and myoglobin belong to a class of proteins referred to as hemoproteins because they are formed from a protein to which is bonded the nonprotein substance or prosthetic group heme. This class of proteins includes a large number which are extremely important in living organisms. The cytochromes play a central role in the oxidation reduction sequences in the "respiratory chain." Molecular oxygen from air is bound by hemoglobin in the lungs and is transported in the blood circulation system throughout the body. Hemoglobin is present in all vertebrates, and in some invertebrates, and it is the most common of the four known oxygen transporting molecules. These molecules and some of their properties are listed in Table II. It has been found that each animal species has a unique oxygen transporting protein although the oxygen molecule always bonds to the iron atom in the heme group. The sequence of amino acids

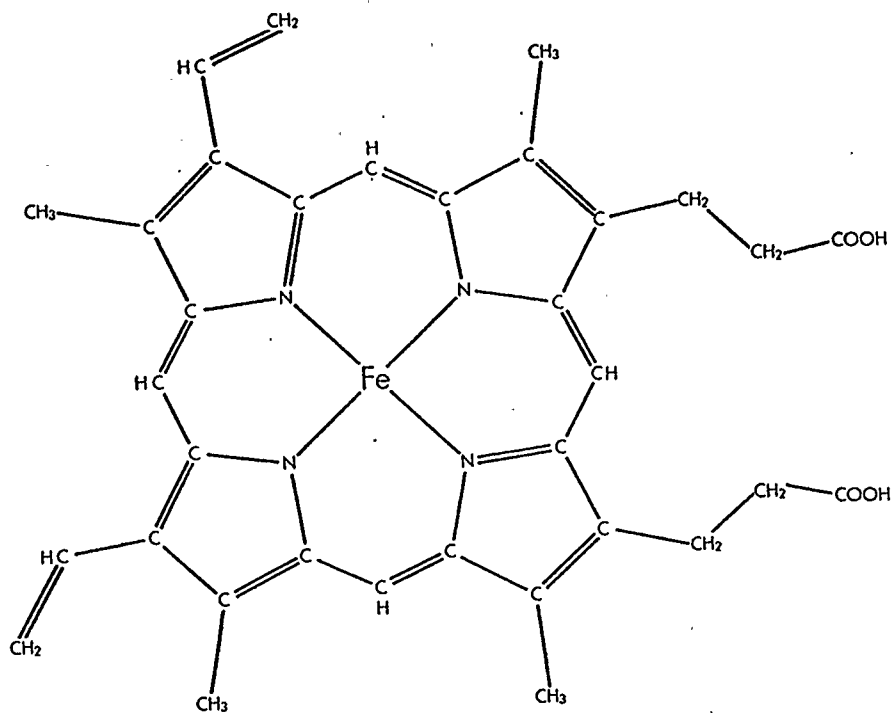


Figure (7.1).

Two-dimensional diagram showing the structure of the heme group.

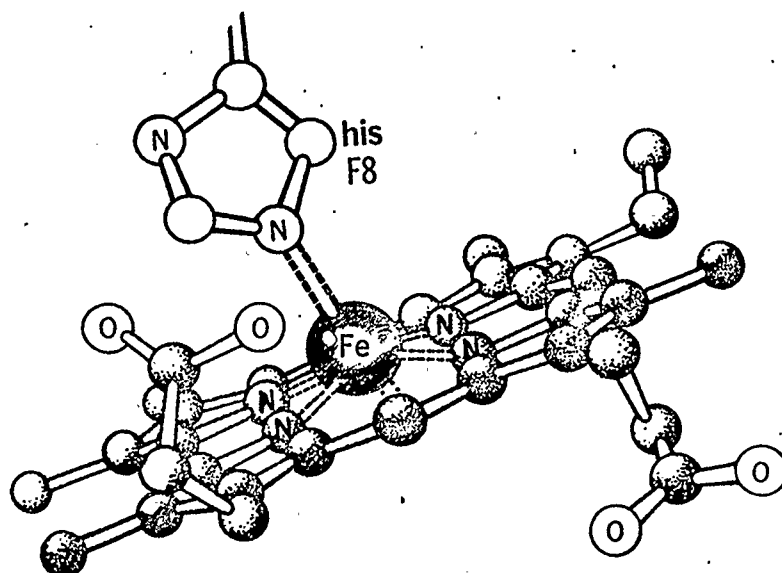


Figure (7.2).

Three-dimensional diagram showing the bonding of the heme group to the protein.

in these proteins is the unique feature for each species.

Myoglobin binds and stores oxygen in muscle tissue until it is required. The muscle tissue of the large aquatic mammals, such as whales, is particularly rich in myoglobin.

The heme groups comprise only about 1-2% of the molecular weight of a hemoprotein, however they play a very important role in its function. Oxygen binds reversibly to the heme group in hemoglobin and myoglobin, while in the cytochromes the heme iron changes its oxidation state during the electron transport sequence in the respiratory mechanism. The role of the polypeptide chain in hemoproteins is not fully understood, however the reactivity and role of the iron in different hemoproteins is altered by different heme-polypeptide interactions. For example, these interactions are necessary in hemoglobin since the isolated heme group does not bind molecular oxygen.

The chemical structure of the heme group is shown in Fig. (7.1). The iron atom is coordinated to the four nitrogen atoms of the four linked pyrrole rings in the porphyrin structure. The heme group is approximately planar. The iron can form two other bonds along the four-fold symmetry axis perpendicular to the heme plane which are labelled the fifth and sixth coordination positions. In myoglobin and hemoglobin, the main bond between the heme group and the protein is at the fifth coordination position to a nitrogen atom of the imidazole ring of the amino acid histidine as shown in Fig. (7.2). The sixth ligand in myoglobin can be any one of a number of small molecules such as H_2O , O_2 , CO , F , OH or CN . These derivatives of myoglobin have widely different magnetic properties corresponding to different spin and oxidation states.

The three dimensional structure of both myoglobin and hemoglobin has been studied extensively by x-ray crystallography. The resolution in the structure of sperm whale myoglobin has been refined to $1.4\text{\AA}$ by Kendrew (1961). Bolton and Perutz (1970) have studied horse hemoglobin at $2.8\text{\AA}$ resolution. Hemoglobin has four heme groups each of which is associated with one of four polypeptide chains. Two pairs of polypeptide chains called the α and β chains, have a common molecular two-fold symmetry axis. The molecular weight of hemoglobin is approximately 65,000 which is four times that of myoglobin. The structure of myoglobin resembles one of the chains of the hemoglobin molecule, however there are significant differences in their amino acid composition.

The iron atom in physiologically normal myoglobin and hemoglobin remains in the ferrous state irrespective of whether oxygen is bound or not. On the uptake of oxygen by hemoglobin the iron atom position changes with respect to the heme plane. Perutz (1970) has shown that this change in position can be linked with further conformational changes in the protein structure. The position of the iron atom is approximately the same in oxyhemoglobin and in methemoglobin. In metmyoglobin and methemoglobin the iron atom is displaced out of the heme plane by $0.3\text{\AA}$ towards the histidine amino acid, whereas the displacement in deoxyhemoglobin is $0.75\text{\AA}$. The study of the met, or ferric form of the hemoproteins myoglobin and hemoglobin is of interest because many details of the model required to describe the met forms must be retained in the description of biologically active ferrous hemoproteins.

7.2 Crystallization and Crystal Structure

Freeze-dried salt free sperm whale myoglobin was obtained from

Koch-Light Biochemical Company. Crystallization was performed from $(\text{NH}_4)_2\text{SO}_4$ using the method of Kendrew and Parrish (1956): 0.2 gm of the freeze dried myoglobin was dissolved in 2cc of $1\text{M}(\text{NH}_4)_2\text{SO}_4$ buffered to pH 6.2. The resulting solution was centrifuged and five drops were added to tubes containing between 15 and 25 drops of $4\text{M}(\text{NH}_4)_2\text{SO}_4$. These tubes were left undisturbed at room temperature. The ionic strength in some of the tubes was usually correct for the growth of some good crystals. When grown from acidic ammonium sulphate solution the myoglobin crystals are known as type A. Single, type A crystals have been used almost exclusively in the investigation of the magnetic properties of the ground state of the ferric ion in crystalline metmyoglobin. The $\{001\}$ face in a type A crystal of myoglobin is well developed and the crystal can be mounted accurately on this face.

The crystalline space group of type A myoglobin crystals is $P2_1$, which means that the crystalline b axis of symmetry is present if a rotation of π radians about the b axis and a translation of one half the unit cell dimension b along the b axis leaves the molecular arrangement in the unit cell apparently undisturbed. Helcké (1963) has calculated that the nearest neighbour iron atom separation in this crystal structure is $30.9\text{\AA}$ and next nearest neighbour separation is $34.7\text{\AA}$.

The hemoglobin crystals were grown from freeze dried horse hemoglobin obtained from the Nutritional Biochemical Company. These crystals were more difficult to grow than the myoglobin crystals and took much longer to attain a usable size. The crystallization method was based on the work of Perutz (1967). The protein was dissolved in 0.01M phosphate buffer pH 7 to a concentration of about 2%. This was

centrifuged and the hemoglobin solution was mixed in 10cc tubes with a concentrated salt solution consisting of two volumes of $4M (NH_4)_2SO_4$ and one volume of $2M (NH_4)HPO_4$ in varying proportions to a total volume of approximately 3cc as given by Perutz (1967). The tubes were sealed and left undisturbed for a few months. Usable crystals were obtained after a minimum of six months but in some cases continued to grow for well over a year. It is possible that hemoglobin crystals will grow more readily if the protein solution was prepared from fresh blood rather than commercially available freeze dried material. Perutz (1967) has obtained good crystals in about one month using the much more involved biochemical preparation beginning from fresh horse blood.

The space group of the hemoglobin crystals grown in this way is $C2$ with two molecules per unit cell. The orientation of each molecule is the same and the two-fold dyad axis is coincident with the molecular two-fold axis. The two molecules in each unit cell will therefore give identical spectra and there will be a maximum of four spectra from the four different heme planes.

7.3 EPR Measurements

The pioneer EPR research on hemoglobin derivatives by Bennett, Ingram, George and Griffith (1955) was performed using polycrystalline samples of HbH_2O , HbF and MbH_2O . Bennett, Gibson and Ingram (1957) then studied single crystals of MbH_2O and HbH_2O . These experiments established that each ferric ion gave a single anisotropic resonance with $g_{\perp} = 6$ and $g_{\parallel} = 2$. The single crystal studies also determined the orientations of the heme normals relative to the crystalline axes and were a significant contribution to the x-ray crystallographic structural

research of both hemoglobin and myoglobin. EPR results were also obtained by Gibson, Ingram and Schonland (1958) on single crystals of the azide complex of metmyoglobin, MbN_3 , and on the hydroxide complex, MbOH . The results of research on MbH_2O from a number of animals and in different crystallographic forms was reported by Bennett *et al.* (1961).

Eisenberger and Pershan (1966) studied the EPR spectra of single crystals of MbH_2O at 13 GHz and 35 GHz. Using the perturbation result for the field dependence of $g_{\perp}(\text{exp})$, given by Kirkpatrick *et al.* (1964), they estimated the zero field splitting to be $2D = (8.76 \pm 1.2)\text{cm}^{-1}$. Unfortunately, they appear to have neglected the anisotropy in $g_{\perp}(\text{exp})$ and give no details of the crystal orientation used to obtain the experimental values. The difference in the values given by Eisenberger *et al.* (1964) for $g_{\perp}(\text{exp})$ at the two different frequencies is less than the possible variation in $g_{\perp}(\text{exp})$ in the heme plane observed by Kotani (1967), Helcké (1963) and Helcké, Ingram and Slade (1968). Kotani (1967) demonstrated that the anisotropy in $g_{\perp}(\text{exp})$ in MbH_2O could be fitted to the spin-Hamiltonian given by Eq. (6.5.9). The ratio E/D was determined to be 0.0025 by careful measurement of the angular variation in the EPR spectrum at 9 GHz. This result is in agreement with the work of Helcké, *et al.* (1968) at 35 GHz.

Estimation of the zero field splitting in MbH_2O , using the method of Kirkpatrick, *et al.* (1964), was also attempted by Slade and Ingram (1969). They estimated ($2D$) from an analysis of the polycrystalline lineshape at 24 GHz, 34 GHz and 70 GHz. This analysis is very difficult because of the variation in linewidth with frequency, which changes the shape of the polycrystalline lineshape. The variation in linewidth with heme plane orientation relative to that of the applied magnetic field is also

complicated and incompletely understood. Consequently, the error in the value of the average value of $g_{\perp}(\text{exp})$ obtained from a polycrystalline sample will be larger than that obtained from an analysis of a single crystal spectra. On the basis of the polycrystalline experiments Slade and Ingram, (1968) have estimated $2D = (8.8 \pm .9)\text{cm}^{-1}$. There appears to be a typographical error since their published g -values at 26 GHz, 34 GHz and 70 GHz are consistent with $D = 8.8\text{ cm}^{-1}$. This error has been confirmed in a private communication by Slade. Some comparative experiments which have been performed on polycrystalline samples of MbH_2O and HbH_2O will be described in Chapter VIII.

The angular variation of the linewidth in single crystals of MbH_2O was studied originally by Helcké (1963). His results showed that there was a small random misorientation in the direction of the heme normal in a crystal. The random misorientation may arise from imperfections in the crystal structure or from a statistical variation in the orientation of the heme plane in the protein. Eisenberger and Pershan (1967) have also studied the variation in linewidth from single crystals of MbH_2O and MbN_3 and confirmed the presence of the misorientation effect. They showed that the contribution to the EPR linewidth ΔB_{θ} from a misorientation effect is given by

$$\Delta B_{\theta} = k[g_{\theta}(\text{exp})]^{-3} \sin 2\theta \, d\theta \quad (7.3.1)$$

where θ is the angle between the direction of the magnetic field and of the heme normal

$g_{\theta}(\text{exp})$ is the experimental g -value at the angle θ

and k is a constant for any particular microwave frequency

Eisenberger and Pershan obtained $d\theta = 1.5^\circ$ for the standard deviation of θ in good agreement with the value $d\theta = 1.6^\circ$ given by Helcké et al (1968). The latter study showed that the linewidth at 35 GHz varied as a function of the orientation of the magnetic field in the heme plane. The magnitude of this effect was greater than could be explained by either misorientation effect or other common line broadening mechanisms. Slade and Ingram (1968) also showed that this linewidth was microwave frequency dependent.

The misorientation effect in θ is insufficient to explain the line broadening observed from the EPR spectra of crystals of the low spin derivative, MbN_3 . Helcké et al. (1968) attributed the additional broadening mechanism to a statistical variation in the azimuthal angle ϕ . They estimated that a value $d\phi = 4^\circ$ for the standard deviation in ϕ was required to explain the observed linewidth. Helcké et al. (1968) suggested that this result implied a statistical variation of 4° in the orientation of the histidine ring with respect to the protein.

Eisenberger and Pershan (1967) proposed an alternative mechanism in which the observed linewidths in crystals of MbN_3 were explained by the misorientation effect combined with a statistical variation in the strength of the rhombic component of the crystalline electric field. The line broadening when the magnetic field is parallel to the heme plane will be discussed in Chapter IX.

7.4 Magnetic Susceptibility and Mössbauer Measurements

The original classification of myoglobin and hemoglobin derivatives as 'high spin' and 'low spin' was based on magnetic susceptibility experiments of Pauling and Coryell (1936) and Hartree (1946). The analysis of the magnetic susceptibility experiments can be performed

TABLE III

Theoretical Values for the Effective Bohr Magneton
Number n for Different Values of the Spin Quantum Number s

$s =$	0	$\frac{1}{2}$	1	$\frac{3}{2}$	2	$\frac{5}{2}$
$n =$	0	1.73	2.83	3.87	4.90	5.92

TABLE IV

Experimental Values for the Effective Bohr Magneton
Number n for some Myoglobin and Hemoglobin Derivatives

Myoglobin Derivatives

Ligand	N_3^-	CN^-	OH^-	CH_3COO^-	$HCOO^-$	H_2O	F^-
	3.30	1.96	5.04	5.70	5.67	5.73	5.77

Hemoglobin Derivatives

Ligand	N_3^-	CN^-	OH^-	CH_3COO^-	$HCOO^-$	H_2O	F^-
	2.35	2.50	4.66	5.44	5.44	5.65	5.76

using Curie's law:

$$\chi = \frac{N\mu^2}{3kT} + \chi_{\text{dia}} \quad (7.4.1)$$

where N is the number of magnetic ions in the volume of the sample
for which χ is defined

μ is the effective magnetic dipole moment

k is the Boltzmann's constant

and χ_{dia} is the diamagnetic susceptibility.

The effective magnetic dipole moment is

$$\mu = n\beta \quad (7.4.2)$$

where β is the Bohr magneton

and n is the number of Bohr magnetons making up the magnetic moment.

If the paramagnetism arises from only the spin angular momentum
then

$$\begin{aligned} n &= 2[S(S+1)]^{1/2} \\ &= [\mathfrak{f}(\mathfrak{f}+2)]^{1/2} \end{aligned} \quad (7.4.3)$$

where $\mathfrak{f}$ is the number of electrons with parallel spins.

The determination of the effective Bohr magneton number n has been used to classify M_b and H_b derivatives. The theoretical values of n , for different values of the spin quantum number S are given in Table III. Table IV gives some experimental values for n measured at room temperature. It can be seen that there are a number of derivatives which do not have the appropriate value for an integral number of electron spins contributing to the paramagnetism.

Theoretical studies by Griffith (1956) indicate that a $S = \frac{3}{2}$ ground state is unlikely and George, Beetlestone and Griffith (1964)

TABLE V

Experimental Results for the Determination of the D-parameter on the
Basis of the Temperature Dependence of Magnetic Susceptibility

Author	Sample	Result (cm^{-1})
McKim (1961)	single crystal MnH_2O	$(2.5 < D < 7.5)\text{cm}^{-1}$
George (1964)	polycrystalline MnF	$D \approx 28\text{cm}^{-1}$
Morimoto et al (1965)	single crystals MnH_2O	$D \approx 10\text{cm}^{-1}$
Tasaki et al (1967)	polycrystalline MnH_2O	$D \approx 10\text{cm}^{-1}$
Uenoyama et al (1968)	single crystals MnH_2O	$D = (10.5 \pm 0.5)\text{cm}^{-1}$
Uenoyama et al (1968)	single crystals MnF	$D = (6.5 \pm 0.25)\text{cm}^{-1}$

have proposed that intermediate values for n arise because the high spin and low spin forms are in thermal equilibrium. On the basis of the crystalline electric field model outlined in Chapter VI, Harris (1968) has shown that intermediate values for n can arise for particular values of the tetragonal distortion parameter c' in a strong crystalline electric field. She fitted the effective Bohr magneton numbers for the derivatives given in Table 3 with theoretical curves for slightly different values of c' .

Equation (7.4.1) is invalid for the high spin derivatives of M_B and M_H at low temperatures because the large zero field splitting affects the populations of the different levels and at liquid helium temperatures ($4K^\circ$) only the lowest doublet of the $s = \frac{5}{2}$ sextet is appreciably populated. This situation has been treated theoretically by Kotani (1961) and Griffith (1964). Their results were used to estimate the zero field splitting by George (1964) and Tasaki, et al. (1965). Further experiments, using type A single crystals of MbH_2O , on the anisotropy of the susceptibility tensor were performed by McKim (1961), Morimoto, et al. (1965) and Uenoyama et al. (1968). The temperature dependence of the results was fitted to theoretical curves for different values of D . The D value results of these and the polycrystalline magnetic susceptibility experiments are summarized in Table V.

The Mössbauer spectra of Fe^{57} in frozen solutions of hemoproteins and heme compounds have been studied by Lang and Marshall (1966) and Moss, Bearden and Caughey (1969), amongst others. These results are of interest to the present work because it is possible to infer some details of the bonding character and spin state of the iron atoms by studying the local electric and magnetic fields at the nucleus through the

observed Mössbauer spectra.

Lang and Marshall (1966) studied various hemoglobin derivatives obtained from Fe^{57} enriched rat blood. Results were obtained for a wide range of derivatives but only those relevant to the EPR research will be reviewed. The zero field splitting (2D) in the ground state of HbF was estimated to be $\sim 14 \text{ cm}^{-1}$ on the basis of the variation in the hyperfine structure of the Mössbauer spectra with an applied magnetic field. The Mössbauer spectra of HbH_2O differed from that of HbF . This was an unexpected result because the EPR results on the two compounds reported by Bennett, et al. (1955) were comparable. The Mössbauer spectra of HbH_2O was interpreted as showing that the spin lattice relaxation time was faster in HbH_2O than HbF and faster than expected from a $L = 0$ state. This result implies that the zero field splitting in MbH_2O is greater than in MbF .

CHAPTER VIII

RESULTS

This chapter describes the results obtained from EPR experiments at 34 GHz and 55 GHz on metmyoglobin and methemoglobin derivatives. The zero splittings in the ground state of the iron atom in MbH_2O and MbF are predicted using accurate g-value data from single crystal samples at the two microwave frequencies. The EPR linewidth variation of single crystal samples of MbH_2O and MbF is also described. EPR g-values and linewidth results are given for single crystals of HbH_2O . Results from polycrystalline EPR spectra of MbH_2O and HbH_2O are also given.

8.1 Sample Mounting

Single crystals of myoglobin or hemoglobin are dark red to black in colour. Water and salt molecules in the crystalline lattice, particularly between the protein molecules, make the crystal extremely fragile and difficult to handle. Ions can diffuse through the crystal because of the loose water-filled structure of the crystals. Following Uenoyama *et al.* (1968) some MbH_2O crystals were converted to the MbF form by the addition of 20 mg/ml of sodium fluoride to the ammonium sulphate solution in which the crystals were stored. Dehydration and shrinkage occurs if the crystals are removed from the salt solution in which they were grown. For this reason, an eye dropper was used to transfer the crystals from the salt solution to the polarising microscope for examination, or to the EPR sample cavity. Some of the salt solution was allowed to surround the crystals when they were mounted

in the sample cavity. This solution was frozen and did not interfere with the EPR measurement since all experiments were performed at 77°K.

Both the myoglobin and hemoglobin crystals varied considerably in volume and usually only a small number of crystals in any batch were large enough for EPR study. The crystals used had an average volume of $\sim 0.1 \text{ mm}^3$ although some smaller crystals were studied. Type A myoglobin and hemoglobin crystals were used in the work reported in this thesis.

A cylindrical TE_{111} cavity was used in the 54-60 GHz spectrometer. The optimum position for the EPR sample in this mode is on the inside end surface of the cylinder away from the coupling hole. This surface is the face of a tuning plunger so that the sample crystal could be mounted easily before the cavity was assembled. Some experiments were performed with the sample crystals mounted on tiny teflon wedges glued to the tuning plunger. The faces on the wedges were cut to provide particular orientations of the crystallographic axes with respect to the plane of rotation of the applied magnetic field. These wedges were not totally successful at 55 GHz because the angles could be determined with only limited accuracy. The most accurate and reproducible measurements were obtained with the crystals mounted with the ab-plane on the tuning plunger.

Cylindrical, tunable TE_{111} and TE_{011} cavity modes were used at 34 GHz. Most of the measurements were performed using the TE_{011} cavity with the cavity axis perpendicular to the plane of rotation of the applied magnetic field. Consequently the direction of the microwave magnetic field is always perpendicular to that of the applied magnetic field and the EPR signal is optimized for any orientation of the applied magnetic field. The crystals were mounted on a thin piece

of mica supported by a quartz tube either with the crystalline ab-plane parallel or perpendicular to the plane of the cavity tuning plunger. The quartz tube was mounted in a hole in the tuning plunger to support the crystal in the position of maximum microwave magnetic field half-way along the TE_{011} cavity axis.

Bennett, et al. (1957) showed that the two heme normals in MbH_2O make equal angles with the a-, b- and c-axes. Consequently, the EPR spectra from the two heme groups will coincide when the applied magnetic field is directed along either the a- or b-axis. The cross-over points in the EPR spectra of a crystal mounted in the ab-plane should then be $\frac{1}{2}\pi$ radians apart if the crystal is mounted correctly. Bennett, et al. (1957) also showed that the four heme normals in hemoglobin could be separated into two pairs and that the heme normals in each pair made equal angles with the a-, b- and c-axes. When the direction of the applied magnetic field is rotated in the ab-plane there will be two cross-over points for each pair of heme normals. In practice, there are only two cross-over points because of the large linewidth and the small angle between the two pairs of heme normals. These cross-over points should be $\frac{1}{2}\pi$ radians apart if the crystal is mounted correctly in the ab-plane.

When the crystal or sample was satisfactorily mounted the tuning plunger was screwed into the cavity body to an extent determined by preset lock rings on the plunger. These lock rings ensured that the cavity resonant frequency was within the tuning range of the microwave power sources. The lock rings were particularly useful in the 34 GHz spectrometer because the tuning range of the VA 313K klystron

is limited to (34.0 ± 0.35) GHz. The contraction of the cavity on cooling from room temperature to 77°K was taken into account in setting the lock rings.

8.2 g -Values for MbH_2O and MbF

The estimation of the zero-field splitting in MbH_2O and MbF was based on accurate g -value measurements at 34 GHz and 55 GHz. The maximum g -values in the ab -plane were measured carefully because in this orientation possible errors due to sample misorientation are minimized. The results for $g_{\perp}$ (ab -plane) are summarized in Table VI. Only the results obtained from those crystals whose EPR spectra were both symmetrical about the b -axis and whose cross-over points were $\frac{1}{2}\pi$ radians apart have been used in this table. The minimum linewidth in the ab -plane and Helcke's Q band results for MbH_2O have been included for comparison.

The major errors in the experimental values given in Table VI arise from two sources, both connected with the relatively large linewidth. If the spectrometer is imperfectly adjusted and a small fraction of d_I is present in the signal then the zero crossing point in the output spectrum is displaced slightly from the true centre of the EPR line. The lineshape is symmetrical about the zero output level* and the response to oscillator f.m. noise is minimized when the pure absorption component a_I is being measured. Normally both these conditions

* This assumes that the absorption lineshape a_0 is symmetrical about the peak value of a_0 .

TABLE VI

Experimental Results for the Value of $g_{\perp}$ (ab-plane) and the Linewidth at $g_{\perp}$ (ab-plane) for Mb crystals at Different Microwave Frequencies.

Sample	Microwave frequency	$g_{\perp}$ (ab-plane)	Linewidth at $g_{\perp}$ (ab-plane)
MbH ₂ O	34 GHz	5.971 ± 0.004	$6.4 \pm 0.4\text{mT}$
MbH ₂ O	54 GHz	5.958 ± 0.005	$9.5 \pm 0.6\text{mT}$
MbF	34 GHz	6.008 ± 0.003	$5.0 \pm 0.3\text{mT}$
MbF	54 GHz	5.967 ± 0.004	$5.8 \pm 0.3\text{mT}$
MbH ₂ O (Helcké, 1968)	35 GHz	5.98 ± 0.01	$\sim 6\text{mT}$
MbD ₂ O	54 GHz	5.96 ± 0.01	$10.0 \pm 0.6\text{mT}$

could be readily satisfied ensuring that this possible source of error was minimized. The second source of error in the g -values arises from the determination of the centre of the line. The magnetic field was adjusted until it corresponded to the zero crossing of the a_I spectrum and was then measured by proton magnetic resonance. The error in determining this magnetic field value was found to be approximately $(\pm \frac{1}{25})$ of the linewidth measured between the extrema of the a_I line-shape. The microwave frequency for the 34 GHz results was measured to an accuracy of $1:10^8$. The error in this value is insignificant in the g -value determinations. The g -value results at 55 GHz were calculated using

$$g = \frac{g(\text{DPPH}) \cdot B(\text{DPPH})}{B} \quad (8.2.1)$$

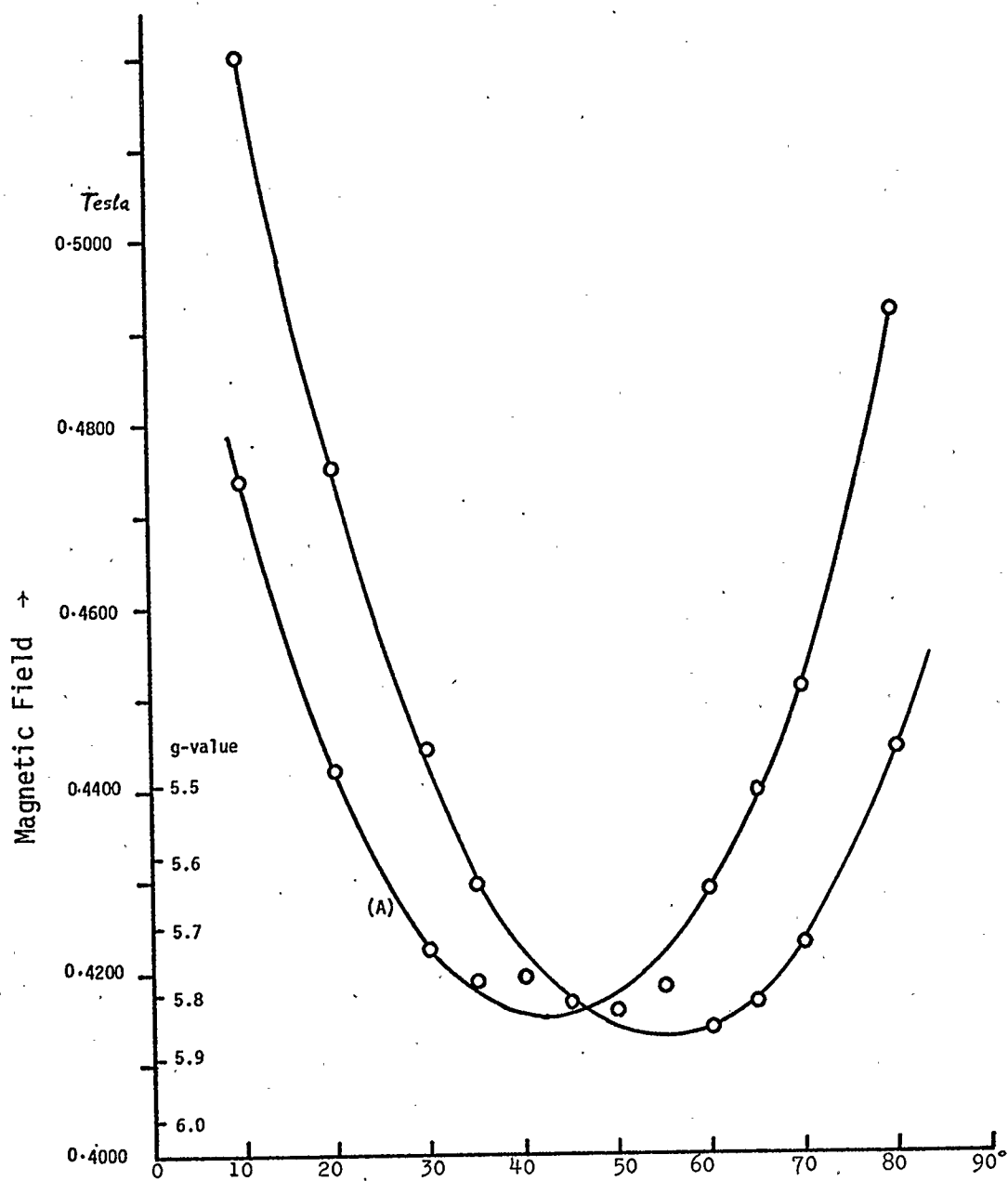
where $g(\text{DPPH}) = 2.0036 \pm 0.0002$ [Weidner and Whitmer (1953)]

$B(\text{DPPH})$ is the measured field at the centre of the polycrystalline DPPH line

and B is the measured field at the centre of the EPR line being studied.

Because the EPR linewidth of the metmyoglobin and methemoglobin samples is much larger than that of polycrystalline DPPH line the dominant error arises in the measurement of the magnetic field corresponding to the centre of the EPR line from the methemoprotein sample.

Single crystals of metmyoglobin which had been grown from heavy water solutions were studied, particularly at 55 GHz. The value of $g_{\perp}$ (ab-plane) and the minimum linewidth are given in Table VI. It can be seen that there is no significant difference in these results compared to those obtained from single crystals of MbH_2O at the same frequency.



Orientation of the Magnetic Field, (arbitrary).

Figure (8.1).

Graphs showing the angular variation of the g-value for MbH₂O when the c*-axis is horizontal.

TABLE VII

Experimental Results for the Maximum and Minimum g-values in the Heme Plane for Mb Crystals.

Sample	Microwave Frequency	g_y	g_x	(E/D)
a) MbH ₂ O	10GHz	5.99 ± 0.01	5.87 ± 0.01	0.0025
b) MbH ₂ O	34GHz	5.98 ± 0.01	5.86 ± 0.01	0.0025 ± 0.0004
c) MbH ₂ O	34GHz	5.971 ± 0.004	5.845 ± 0.015	0.0026 ± 0.0004
d) MbF	10GHz	6.06 ± 0.01	5.88 ± 0.01	0.0038

- a) Kotani (1967)
 b) Helcké (1963), Helcké et al (1968)
 c) Present work
 d) Kotani (1967)

The variation in the g -value observed when the magnetic field is in the heme plane has been studied by Kotani (1967) for single crystals of MnH_2O and MnF . The variation in $g_{\perp}$ was described in terms of the ratio of the spin-Hamiltonian parameters E and D . The relationship between the ratio (E/D) and the variation in g -value in the heme plane is given by Kotani (1967).

$$g_{\phi} = \frac{1}{2} (g_x + g_y) (1 - 4(E/D) \cos 2\phi) \quad (8.2.2)$$

where g_x and g_y are the minimum and maximum g -values in the heme plane and ϕ is the angle in the heme plane relative to $\phi = 0$ at g_x .

The values of the (E/D) ratio obtained from the measurement of the maximum and minimum g -values in the heme plane are given in Table VII. The results a), b) and c) show good agreement in the determination of the (E/D) ratio for MnH_2O . The maximum and minimum g -values in Helcké's (1963) work were taken to be the maximum g -values obtained with the crystal mounted in the ab - and bc^* -plane. Kotani (1967) showed that the minimum g -value in the heme plane was close to, but not exactly that obtained with the crystal mounted in the bc^* -plane. The minimum g -value in the heme plane in the present work was taken to be the maximum g -value from one heme group obtained with the crystal mounted with the c^* -axis horizontal and the b -axis displaced by approximately 30° from the horizontal. The observed spectra is given in Fig. (8.1). The $g_{\perp}$ value from heme group A is taken as being very close to the minimum g -value in the heme plane. The maximum g -value in the heme plane in all cases was taken to be that obtained with the crystal mounted in the ab -plane.

8.3 Zero Field Splitting in the Ground State of MnH_2O and MnF

The value of the zero field splitting (2D) in MnH_2O and MnF has been calculated from the g -value results presented in Sec. (8.2).

Using a computer diagonalization of the spin-Hamiltonian discussed in Chapter VI and given in Eq. (8.3.1), Slade (1968) has shown that g_x and g_y diverge linearly from $g_{\perp}$ with an increasing value of E for $E < (0.005)D$.

$$\mathcal{H} = D(S_2^2 - \frac{1}{3}S(S+1)) + E(S_x^2 - S_y^2) + g_x\beta BS_x + g_z\beta BS_z \quad (8.3.1)$$

If $E = 0$ then g_x and g_y can be replaced by $g_{\perp}$. For $E = (0.0025)D$ the perturbation theory derived by Kirkpatrick *et al.* (1964) can still be used to estimate the value of D in Eq. (8.3.1) provided the calculation is based on g -values measured at different frequencies when the crystal is in exactly the same orientation and $g_{\perp}(\text{exp}) = \frac{1}{2}[g_x(\text{exp}) + g_y(\text{exp})]$ is used. The detailed formula for $g_{\perp}$ and (2D) in terms of the experimental results at two magnetic fields B_1 and B_2 are given by Kirkpatrick *et al.* (1964).

$$g_{\perp} = \frac{g_{\perp}(\text{exp } 1)}{3} \times \frac{1}{\alpha} \times \frac{\alpha - \delta}{1 - \delta} \quad (8.3.2)$$

$$(2D) = g_{\perp} \beta \frac{2\alpha(B_2^2 - B_1^2)}{\alpha - 1}^{\frac{1}{2}} \quad (8.3.3)$$

where $\delta = (B_1/B_2)^2$

$$\alpha = \frac{g_{\perp}(\text{exp } 1)}{g_{\perp}(\text{exp } 2)}$$

and B_1 and B_2 are magnetic fields at $g_{\perp}(\text{exp } 1)$ and $g_{\perp}(\text{exp } 2)$ respectively.

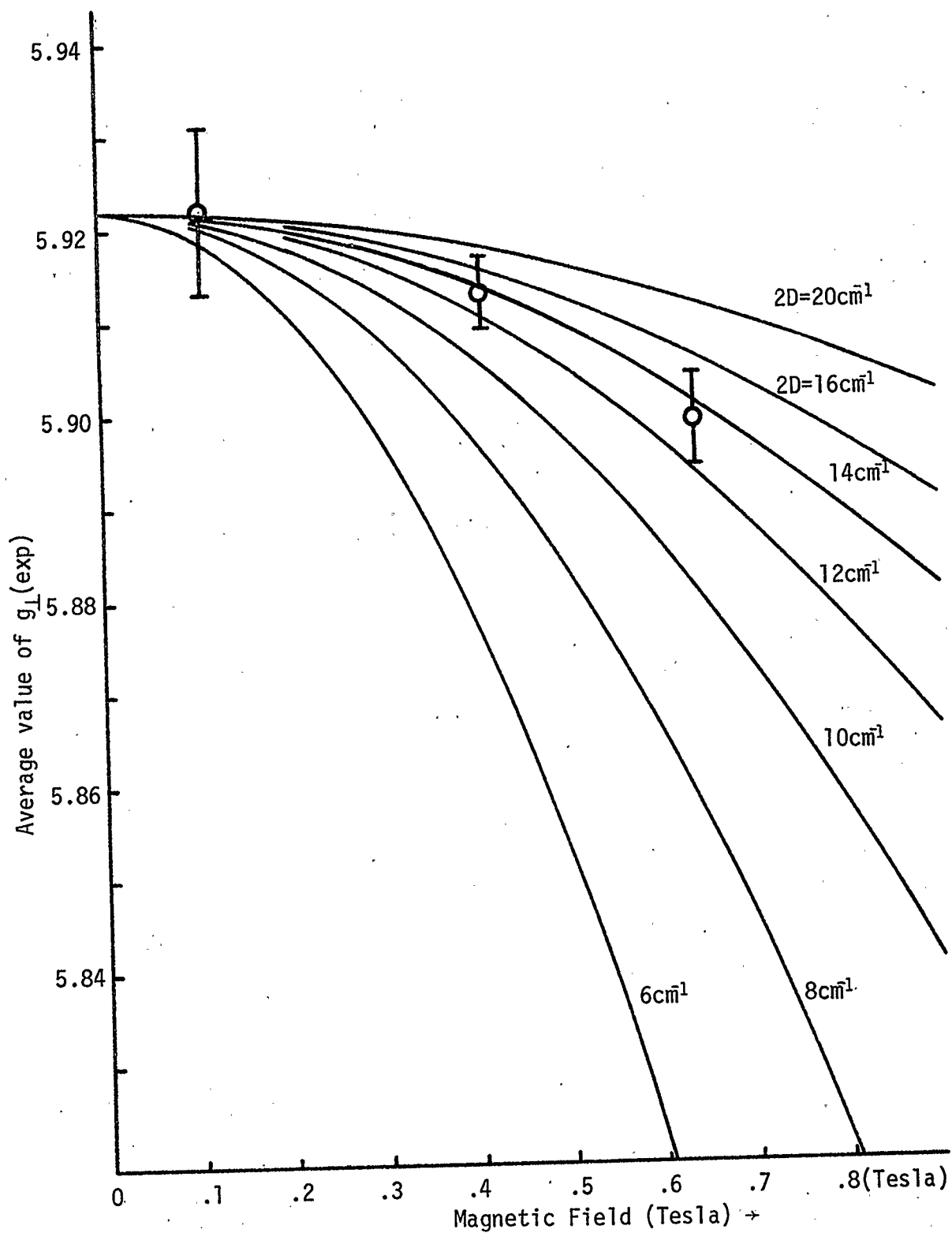


Figure (8.2).

Graph showing the dependence of the average value of $g_{\perp}(\text{exp})$ for MbH_2O in the heme plane on the magnetic field for different values of $(2D)$.

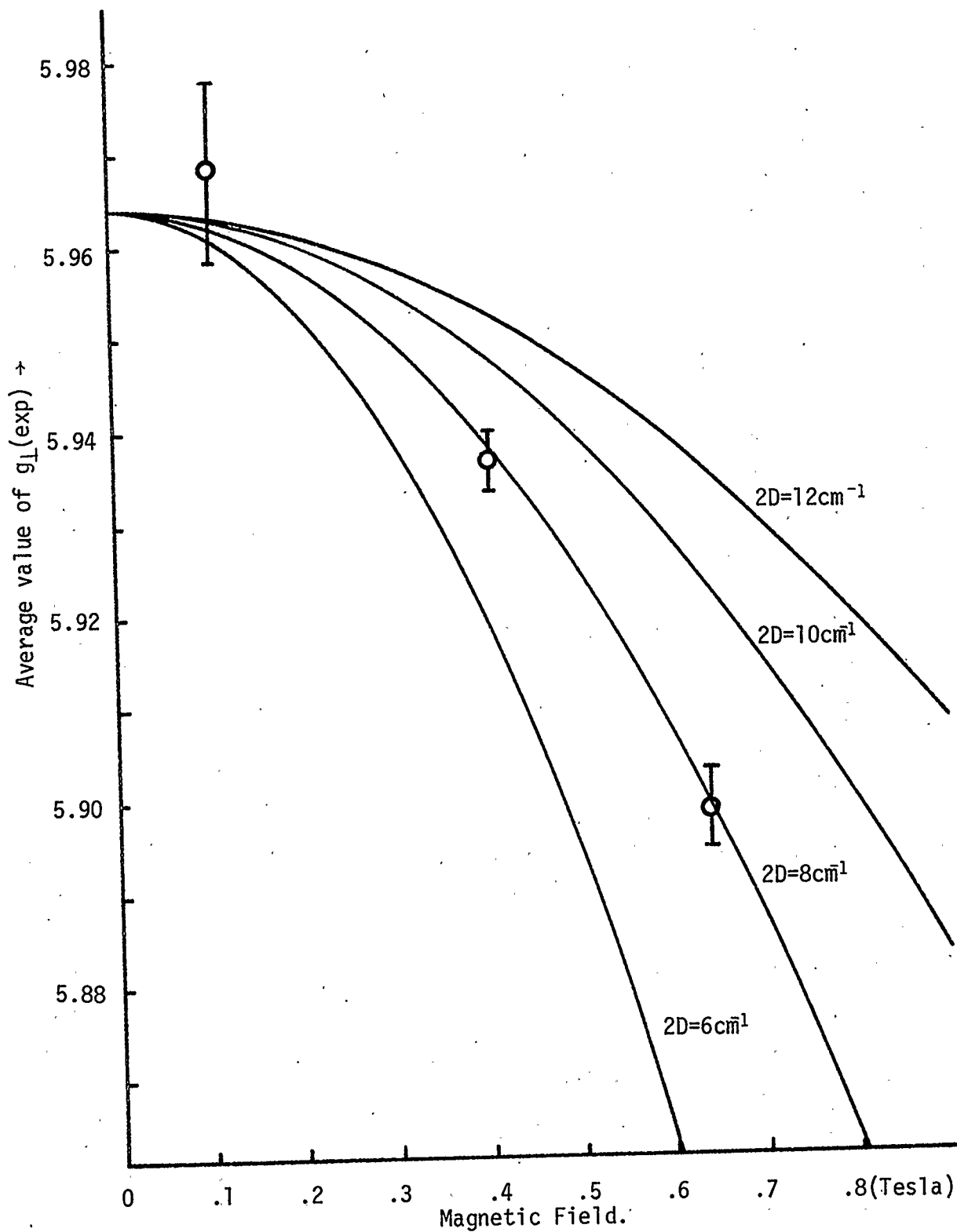


Figure (8.3).

Graph showing the dependence of the average value of $g_{\perp}(\text{exp})$ for MbF in the heme plane on the magnetic field for different values of $(2D)$.

TABLE VIII

Experimental Results for the spin-Hamiltonian Parameters $g_{\perp}$, (2D) and E for Mb Crystals.

Sample	$g = \frac{1}{2}(g_x + g_y)$	(2D)	E
MbF	1.988 ± 0.002	$(8.1 \pm 1.0)\text{cm}^{-1}$	$0.031 \pm .005\text{cm}^{-1}$
MbH ₂ O	1.974 ± 0.0025	$\begin{pmatrix} 13.7 + 3.0 \\ - 2.0 \end{pmatrix} \text{cm}^{-1}$	$0.34 \pm .005\text{cm}^{-1}$

N.B. $g_{\perp} = \frac{1}{2}(g_x + g_y)$

The value of $g_{\perp}$ obtained from Eq. (8.3.2) is the average value of g_x and g_y in the spin-Hamiltonian given in Eq. (8.3.1). Equation (8.3.3) shows that the most important experimental quantity in the calculation of (2D) is the ratio of the experimental $g_{\perp}$ values rather than their absolute values. As a result the relative accuracy of magnetic field measurements is more important than their absolute accuracy. The value of $g_{\perp}(\text{exp})$ can be calculated from the accurate experimental g -value obtained in the ab -plane by substituting the value of (E/D) given in Table VII and $\phi = \frac{1}{4}\pi$ radians into Eq. (8.2.2). The error in the value of $g_{\perp}(\text{exp})$ obtained in this way is not significant to the error in (2D) because of the large uncertainty in the value of $(\alpha - 1)$ in the denominator of Eq. (8.3.3). Results for $g_{\perp}$, 2D and E are given in Table VIII.

Figures (8.2) and (8.3) illustrate the dependence of the average value of $g_{\perp}(\text{exp})$ in the heme plane on the magnetic field. These curves have been drawn to compare the 34 GHz and 55 GHz results with the 10 GHz results estimated from Figures (1) and (2) in Kotani (1967). Figure (8.2) has been calculated for MbF with $g_{\perp} = 1.988$ and Fig. (8.3) has been calculated for MbH_2O with $g_{\perp} = 1.974$. The low field points have been estimated from Kotani's 10 GHz results in both Figures (8.2) and (8.3). It can be seen that Kotani's results are consistent with the results given in Table VI within experimental error.

8.4 Linewidth Variation in MbH_2O and MbF at 34 GHz and 55 GHz

Helcké, et al. (1968) and Eisenberger and Pershan (1967) showed independently that there was a statistical variation in the orientation

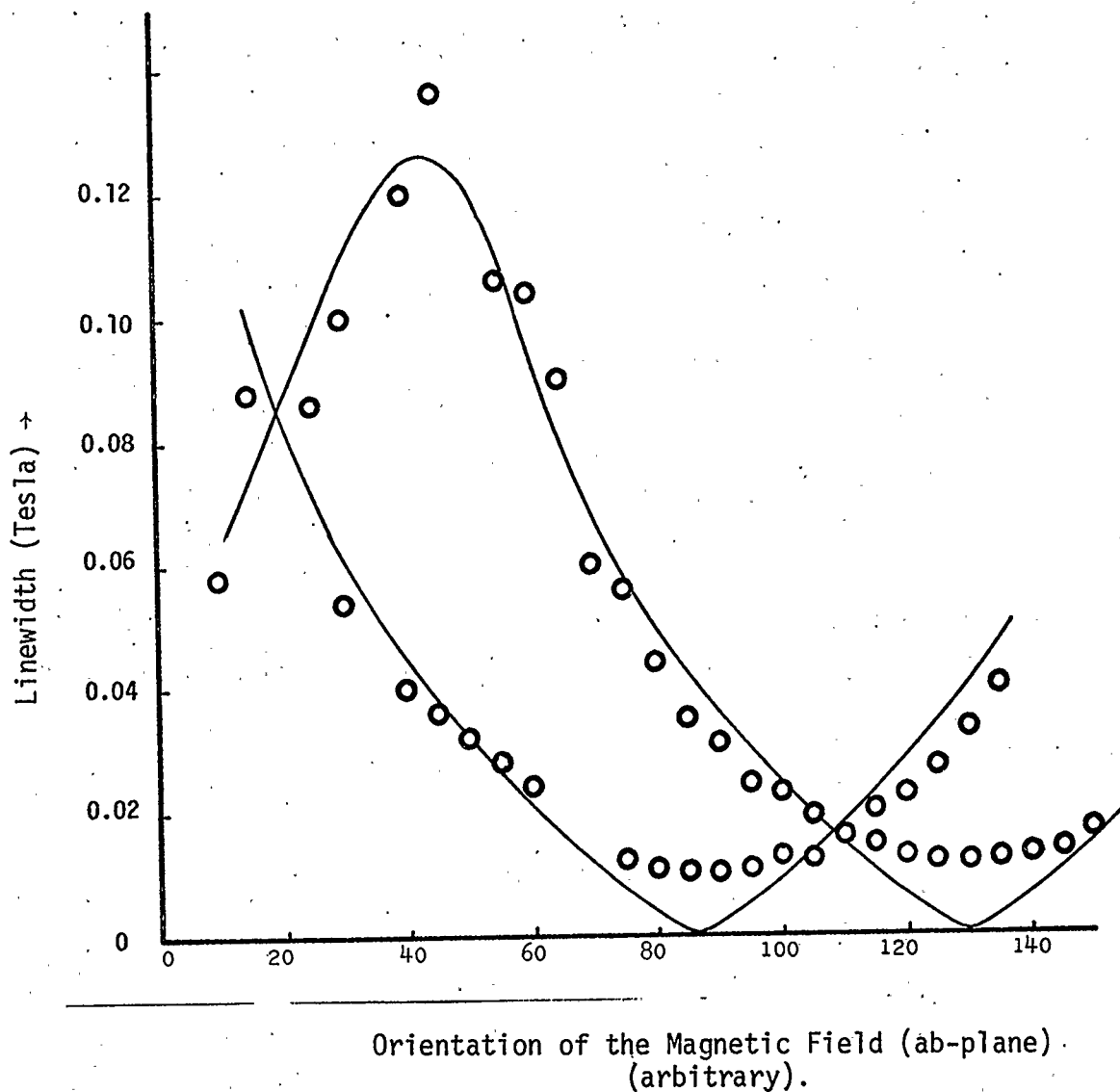


Figure (8.4).

Graphs showing the angular variation of the linewidth in the ab-plane measured at 55GHz.

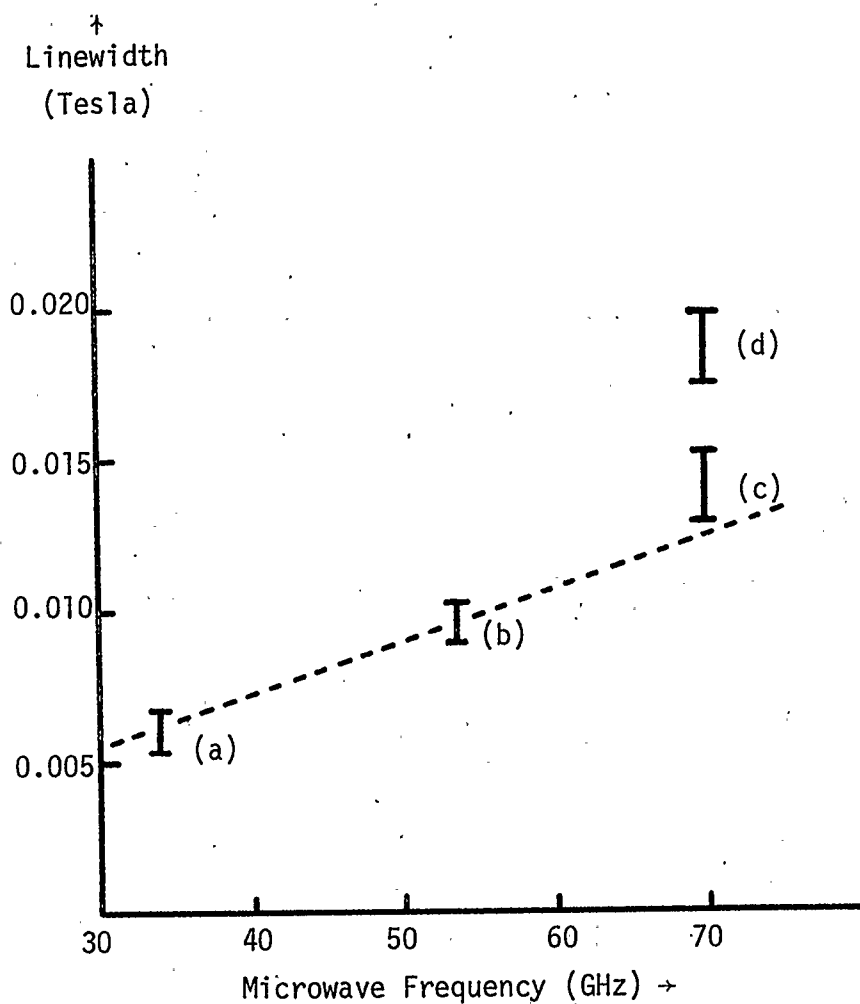


Figure (8.5).

Graph showing the minimum linewidth in the ab-plane for MbH_2O plotted as a function of the measurement frequency. Points (a) and (b) were obtained in the present work and points (c) and (d) were estimated from Fig.1, Slade and Ingram (1968).

of the heme normals in single crystals of MbH_2O . This misorientation effect could arise either in the crystal structure or in the angle adopted by the heme plane with respect to the protein. Helcké et al. (1968) showed that the standard deviation of the angular distribution of the heme normals is $\Delta\theta = 1.6^\circ$ in good agreement with the value $\Delta\theta = 1.5^\circ$ obtained by Eisenberger and Pershan (1967). This misorientation effect does not explain the frequency dependence of the linewidth at $g_{\perp}(\text{exp})$ observed by Slade (1968) and in the present study. Figure (8.4) illustrates the linewidth variation in the ab-plane at 55 GHz for type A MbH_2O crystals. The theoretical linewidth variation given by the solid line is plotted for a statistical variation in the heme normal with a standard deviation $\Delta\theta = 1.6^\circ$. The disagreement between the misorientation theory and experimental results in the region close to the value of $g_{\perp}(\text{ab-plane})$ is apparent. The frequency dependence of the linewidth at (ab-plane) is given in Fig. (8.5). Points (a) and (b) were obtained in the present work and points (c) and (d) have been estimated from Fig. (1), Slade and Ingram (1968). Point (c) is an estimate of the minimum linewidth at 70 GHz in the ab-plane and point (d) is the linewidth at the maximum g-value in the ab-plane. The straight line has been drawn for an increase in linewidth directly proportional to the increase in frequency. The misorientation effect predicts that the minimum linewidth will be at the maximum g-value in the ab-plane. Slade and Ingram (1968) have demonstrated that at 70 GHz there is a difference of approximately 10° in the position of the minimum linewidth and maximum g-value in the ab-plane. A similar but smaller effect has been observed at 55 GHz in the present work. Figure (8.6)

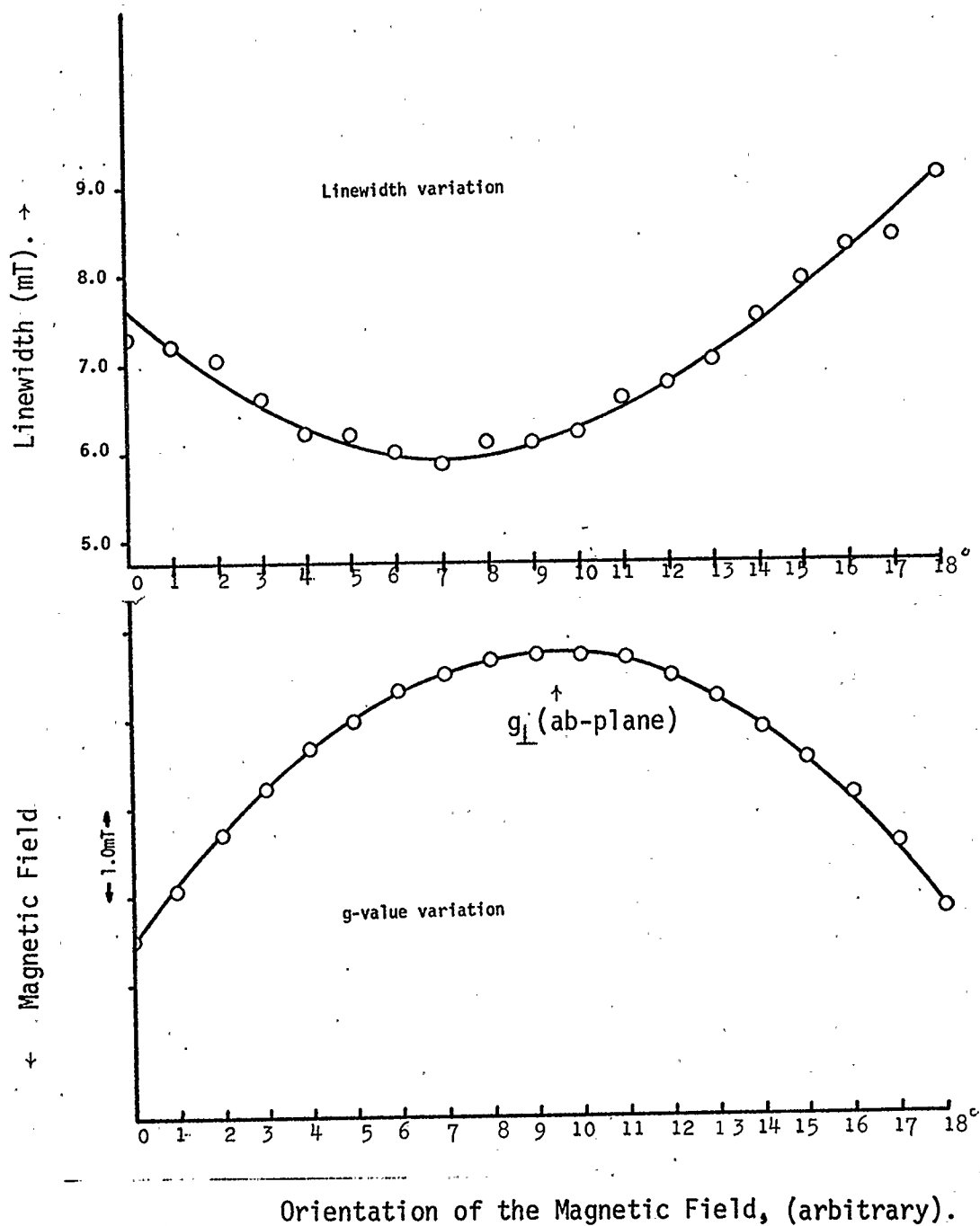


Figure (8.6).

Graphs showing the linewidth and g-value variation of MbF measured at 55GHz in the ab-plane as a function of the magnetic field orientation.

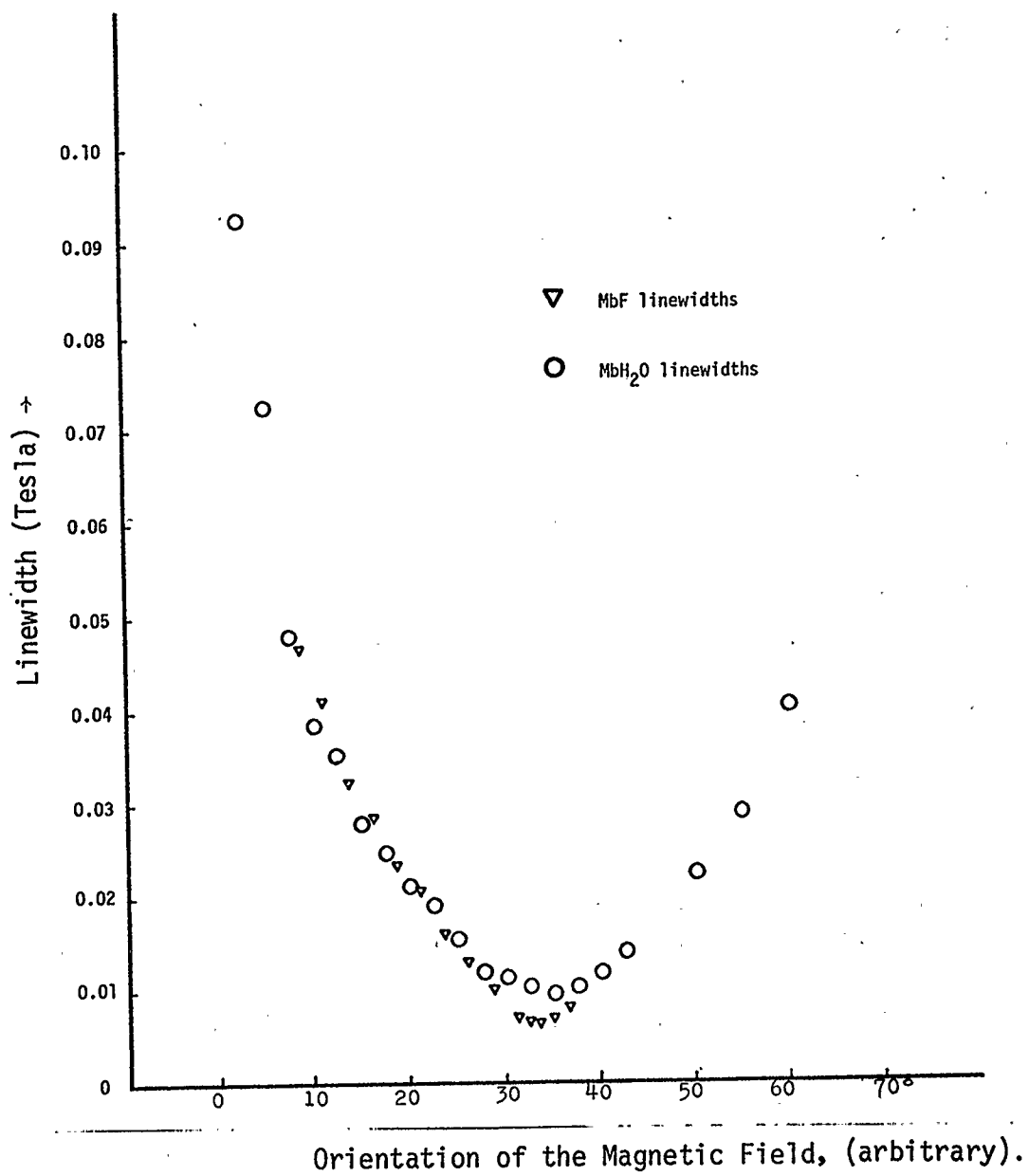


Figure (8.7).

Graphs comparing the measured linewidths of MbH₂O and MbF in the the ab-plane at 55GHz.

illustrates the 55 GHz variation in linewidth in a region of the ab-plane for a single crystal of MbF. The discrepancy between the maximum g-value and the linewidth minimum at 55 GHz is approximately $2\frac{1}{2}^\circ$ in both MbF and in MbH₂O. Clearly this effect, and the frequency dependence of the linewidth in the heme plane, cannot be explained by the misorientation effect.

Figure (8.7) compares part of the 55 GHz variation in linewidth for single crystals of MbH₂O and MbF mounted in the ab-plane. It is evident that the linewidths at equivalent points in the ab-plane for both crystals are equal, within experimental error, except for the region close to the maximum g-value. The minimum linewidth is (5.8 ± 0.3) mT for MbF and (9.5 ± 0.5) mT for MbH₂O as given in Table VI. Similarly the 34 GHz variations in linewidth for MbH₂O and MbF in the ab-plane are identical within experimental error except for the small differences in linewidth close to the maximum g-value given in Table VI. The possible origins of the linebroadening when the applied magnetic field is in the heme plane will be discussed in Chapter IX.

8.5 Results for HbH₂O

Bennett, et al. (1957) pioneered the EPR study of single crystals of type A HbH₂O. This work established the orientation of the heme normals with respect to the crystallographic axes and was very important in complementing the initial x-ray crystallographic structure determination. Accurate g-values and information on the linewidth variation in HbH₂O have not been reported prior to the present study.

The 55 GHz g-value variation in the ab-plane of a crystal of

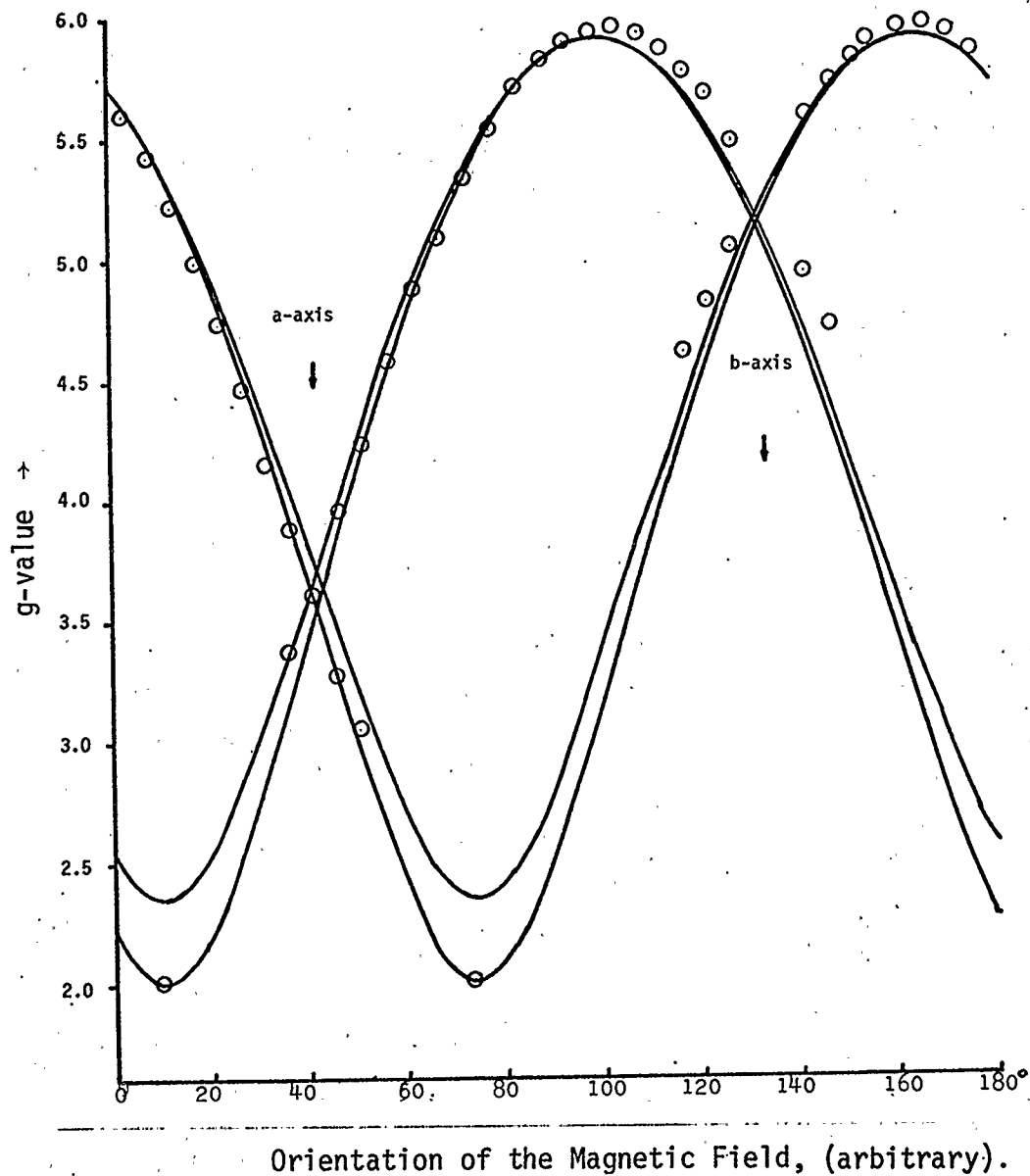


Figure (8.8).

Graphs comparing the measured and theoretical (assuming $\Delta\theta = 3.3^\circ$) g-value variation of HbH_2O in the ab-plane measured at 55GHz.

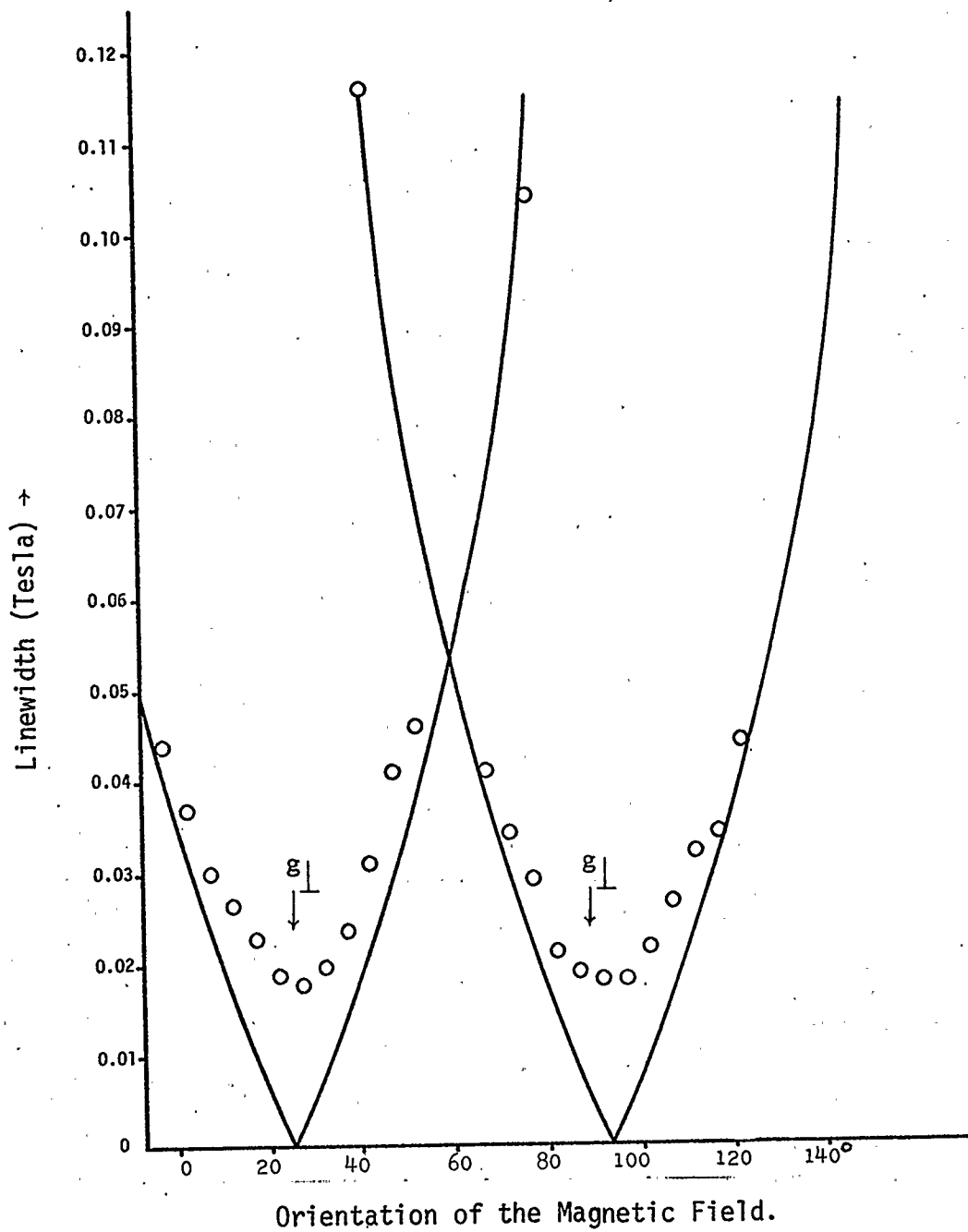


Figure (8.9).

Graphs showing the theoretical variation in linewidth in the ab-plane for a misorientation effect with $\Delta\theta = 3.3^\circ$ and the experimentally obtained points.

HbH_2O is given in Fig. (8.8). The theoretical spectrum has been calculated using the direction cosines of the heme normals given by Bennett et al. (1957). No variation in the g -value in the heme plane has been considered in this diagram or in any of the theoretical spectra given in this chapter. The experimental data is incomplete because of the very broad ($>0.1\text{T}$) linewidth in the region $g = 2$ to $g = 3$. Clearly, a large misorientation effect is responsible for the large linewidth in this region of the angular variation in the ab -plane. The 55 GHz linewidth variation as a function of orientation in the ab -plane is given in Fig. (8.9). The contribution of a misorientation effect for $\Delta\theta = 3.3^\circ$ has been calculated and included in Fig. (8.9). It can be seen that there is a large discrepancy between the linewidth at the maximum g -value and the linewidth predicted by the misorientation effect.

The magnitude of the misorientation effect can also be estimated directly from the results obtained in the ab -plane. Bennett et al. (1957) showed that two of the heme normals lie in the ab -plane. Consequently, the g -value from these heme groups should be very close to the free spin-value at two orientations in the ab -plane. An asymmetric EPR signal was observed at $g = 2$ over a small angular variation ($\sim 5^\circ$) in the ab -plane. The magnitude of the misorientation effect was estimated to be $\Delta\theta = 2.8^\circ \pm 0.5^\circ$ using the angular variation of the $g = 2$ signal and Fig. (8.9).

Perutz (1967) has shown that Hb crystals grow most readily at pH7 but unfortunately often contain a lattice disorder. As the crystals used in the present work were grown at approximately this pH an attempt

TABLE IX

Table of g-values and Linewidths of HbH_2O Crystals Measured in the ab-plane at 34GHz and 55GHz.

Sample	Microwave		Linewidth at	
	frequency	$g_{\perp}$ (ab-plane)	$g_{\perp}$ (ab-plane)	
HbH_2O	34 GHz	5.938 ± 0.005		$(12.0 \pm 1.0)\text{mT}$
HbH_2O	55 GHz	5.94 ± 0.01		$(18.0 \pm 1.5)\text{mT}$

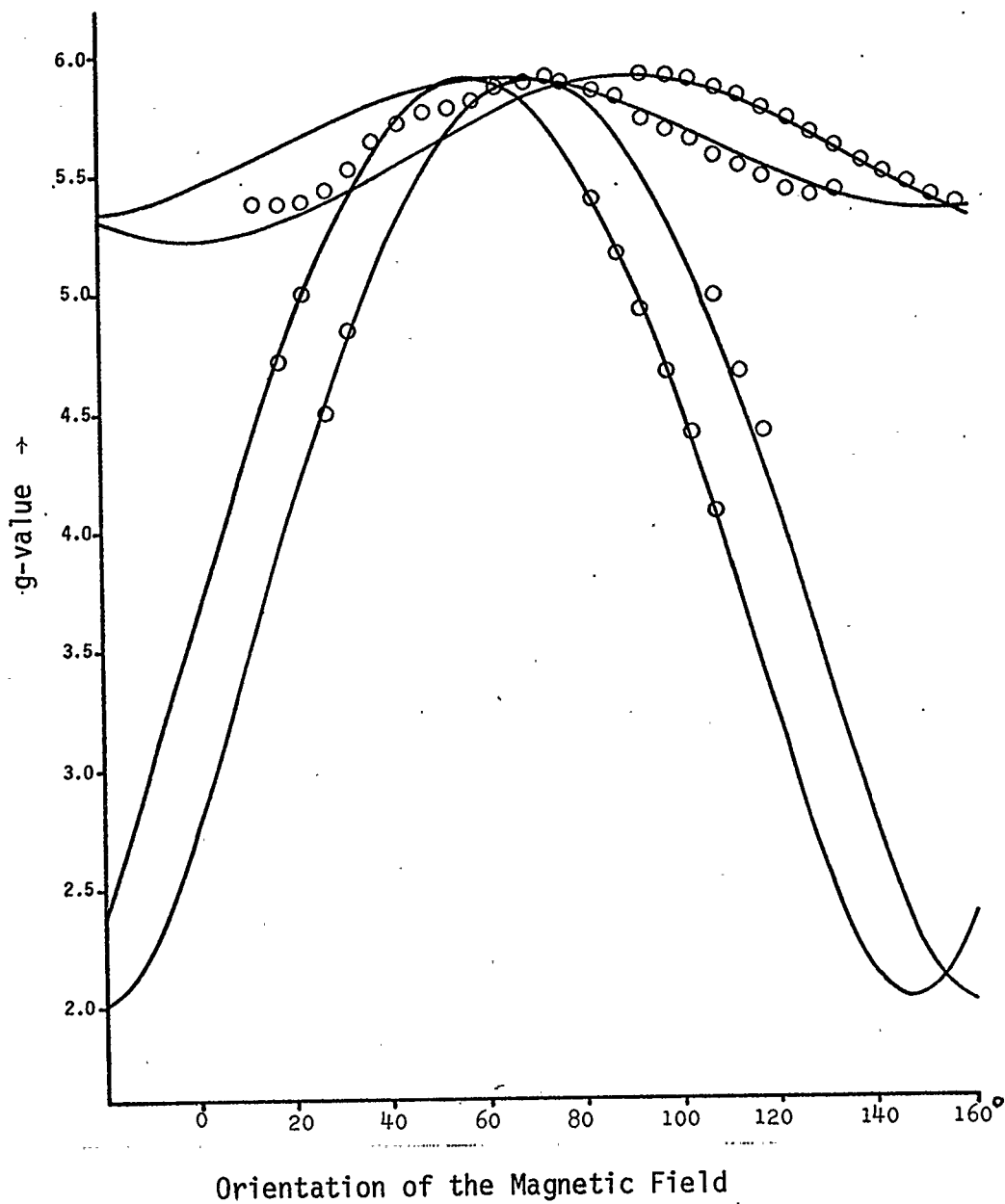


Figure (8,10).

Graphs showing the theoretical and measured g-value variation of HbH_2O in the $\{110\}$ -plane at 55GHz.

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was made to reduce the lattice disorder by increasing the pH according to the method suggested by Perutz (1967). Unfortunately the linewidth measurements from crystals treated in this way were unaltered. Notwithstanding the misorientation effect and the possible existence of a lattice disorder, the results obtained from the Hb crystals are useful because the estimation of D and E is based on the values of $g_{\perp}(\text{exp})$ which are unaffected by the misorientation effect. It can be seen from Fig. (8.9) that the contribution from the misorientation effect to the linewidth is negligible when the direction of the magnetic field is in the heme plane.

The g-values and linewidths for the HbH_2O crystals are summarized in Table IX. It is impossible to estimate the zero field splitting accurately on the basis of the two values of $g_{\perp}(\text{ab-plane})$ because they are almost identical. The results can be used however to estimate a lower limit of approximately 15 cm^{-1} for the zero field splitting.

HbH_2O crystals can be mounted flat on the $\{110\}$ face. The 55 GHz g-value angular variation for a crystal mounted in this orientation is given in Fig. (8.10) superimposed on the theoretical spectra. Poor resolution of the individual EPR resonances from the four different heme groups was obtained because of the relatively large linewidth from each heme group. Comparison of the experimental points with the theoretical spectra indicate that the simple model using $g_{\parallel} = 2$, $g_{\perp} = 5.9$ is inadequate and that there may be a variation in the g-value in the heme plane. Also, the maximum g-value in the $\{110\}$ plane is 5.86 ± 0.02 which is significantly less than the value of 5.94 ± 0.02 obtained in the ab-plane. These g-values give a more direct indication

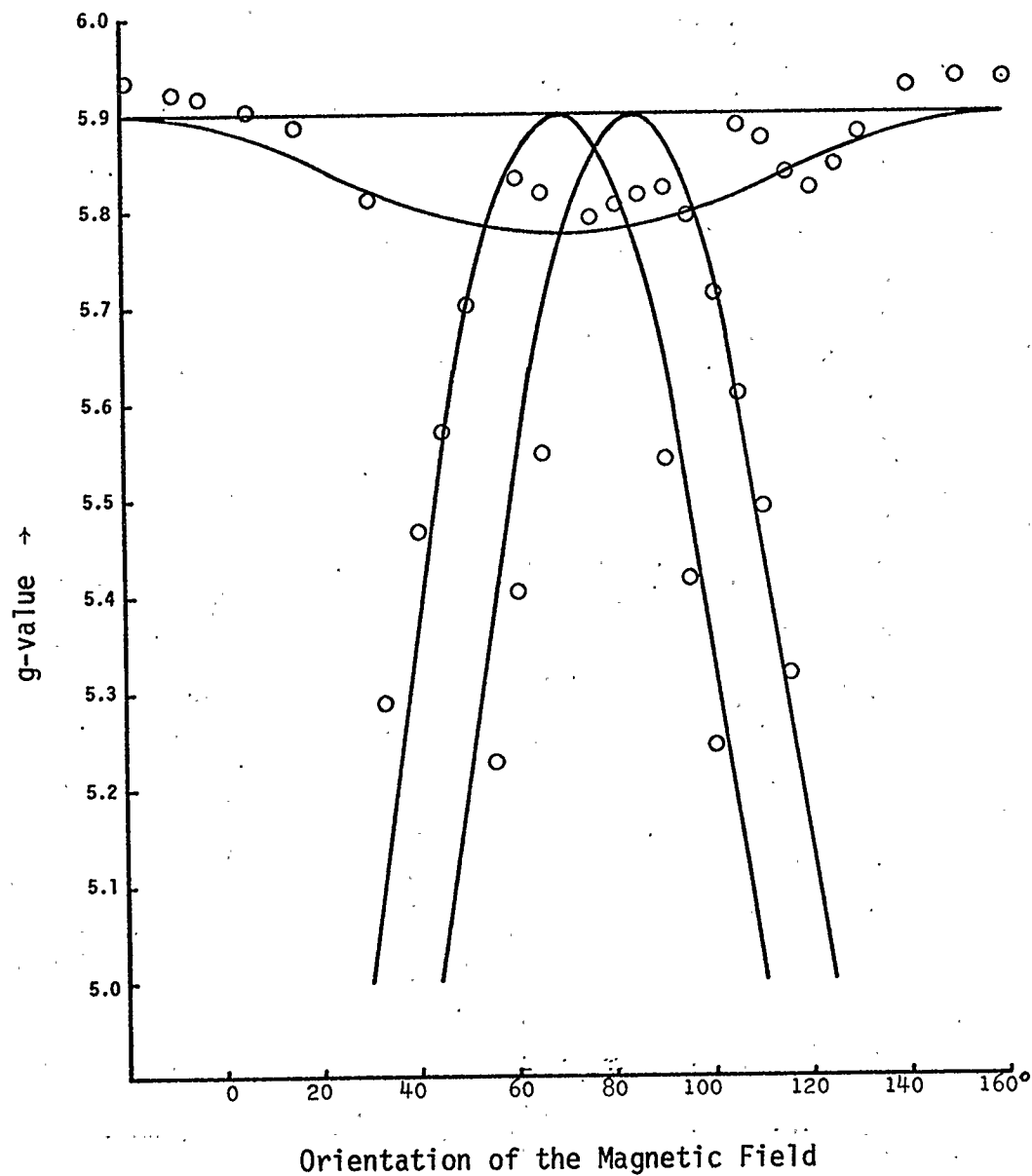


Figure (8.11).

Graphs showing the theoretical and experimental g-value variation for HbH₂O with one heme plane parallel to the plane of rotation of the applied magnetic field.

TABLE X

Results from Polycrystalline EPR Spectra of MnH_2O and NiH_2O

Sample	Microwave frequency	Value of low field extrema of a_{I}	Linewidth between two low field extrema
MnH_2O	34 GHz	5.96 ± 0.02	$(13.5 \pm 1.0)\text{mT}$
MnH_2O	55 GHz	5.95 ± 0.02	$(17.5 \pm 1.5)\text{mT}$
NiH_2O	34 GHz	5.99 ± 0.025	$(22 \pm 2.0)\text{mT}$
NiH_2O	55 GHz	5.98 ± 0.03	$(28.5 \pm 2.5)\text{mT}$

of the presence of a rhombic term in the spin-Hamiltonian for HbH_2O .

Some experiments were performed at 34 GHz with the crystal mounted in a plane such that one heme plane was in the plane of rotation of the applied magnetic field. The crystal was mounted first with the ab-plane flat on a thin piece of mica. The crystal was aligned on the mica so that when it was mounted in the cavity the ab-plane was vertical and the a-axis made an angle of 32° with the vertical. The theoretical spectrum is given in Fig. (8.11). Any variation in the g-value in the heme plane is neglected. The experimental results given in Fig. (8.11) confirm the presence of a variation in the g-value in the heme plane. An estimate of the magnitude of (E/D) can be obtained from Fig. (8.11) and the maximum g-values in the crystal orientations.

$$g_{\text{ (ab-plane) }, 34 \text{ GHz}} = 5.938 \pm 0.005 \quad (\sim g_y)$$

$$g_{\text{ [110] }, 34 \text{ GHz}} = 5.87 \pm 0.02$$

$$g_{\text{ (bc}^*\text{-plane) }, 34 \text{ GHz}} = 5.80 \pm 0.03 \quad (\sim g_x)$$

Although the error in the value of $g_{\perp}$ obtained for the bc^* -plane is large, the ratio of (E/D) is comparable or slightly greater than that obtained from MbH_2O . The average value of $g_{\perp}(\text{exp})$ at 34 GHz is 5.87 ± 0.02 this is significantly less than the value of 5.91 ± 0.01 obtained for MbH_2O .

8.6 Polycrystalline Experiments

EPR experiments on polycrystalline samples of MbH_2O and HbH_2O have been performed at 34 GHz and 55 GHz. The g-values for the low field extrema of the lineshape were measured and the values are given in Table X.

Slade (1968) estimated the zero field splitting in MbH_2O using the g -values of the $a_{\perp}$ low field extrema, however these values do not correspond exactly to the average g -value in the heme plane because the individual paramagnets making up the polycrystalline spectra have a finite linewidth. It is interesting to note that the g -values given for the low field extrema of HbH_2O cannot be equal to the average values of $g_{\perp}(\text{exp})$ at 34 GHz and 55 GHz because the results are larger than the maximum g -values obtained in the ab -plane. It is suggested that the discrepancy between the low field extrema of the $a_{\perp}$ lineshape and the average value of $g_{\perp}$ has arisen because of the effect of the finite linewidth of the individual paramagnets making up the polycrystalline spectra. On the basis of these results it can be concluded that the estimation of $(2D)$ for MbH_2O and HbH_2O from the frequency dependence of these g -values is both difficult and not as accurate as the single crystal method.

The last column in Table X gives the linewidth between the two low-field extrema of the polycrystalline lineshape. The linewidth in the HbH_2O spectra must be due to a larger average value for the linewidth from an individual paramagnet of HbH_2O in relation to MbH_2O because the value of E/D is comparable for MbH_2O and HbH_2O .

CHAPTER IX

DISCUSSION

The disagreement between the D-value results obtained from magnetic susceptibility experiments and from the EPR experiments described in the present work is discussed in Sec. (9.1). The g-value and D-value results for MbH_2O , MbF and HbH_2O are compared in Sec. (9.2) and discussed in relation to the crystalline electric field model. The microwave frequency dependence of the EPR linewidth, when the applied magnetic field is parallel to the heme plane, is discussed in Sec. (9.3). Suggestions for future research are included in Sec (9.4).

9.1 Comparison of the EPR and Magnetic Susceptibility Results for the Zero Field Splitting of MbH_2O and MbF

The spin-Hamiltonian parameter D has been determined in an indirect manner for the temperature dependence of magnetic susceptibility data. The D-values obtained from the single crystal experiments performed by Uenoyama et al (1968) are probably the most accurate of the magnetic susceptibility experiments because a wider temperature variation was employed than in the previous work of McKim (1961). Nevertheless, a wide range of results for (2D) has been given from magnetic susceptibility experiments as shown in Table V.

The EPR experiments performed to estimate D are also indirect in the sense that the zero field splitting is not measured directly from transitions between the lower two doublets, however the EPR results relate directly to the spin-Hamiltonian parameters $g_{\perp}$ and D.

Richards, et al. (1967) have shown that fourth-order terms in the spin-Hamiltonian for ferric porphyrin compounds are small in comparison to the second order DS_z^2 term. Even if fourth-order terms exist in the spin-Hamiltonian they do not couple the levels of the lowest doublet in a third-order perturbation calculation. Consequently the theory of Kirkpatrick et al. (1964) is applicable in the calculation of the zero field splitting for ferric hemoproteins irrespective of the presence of small fourth-order terms in the spin-Hamiltonian. Slade and Ingram (1968) have shown that the values for $g_{\perp}$ and $2D$ obtained using the formulae given by Kirkpatrick et al. (1964) are the same as those obtained from a computer diagonalization of the secular determinant for the spin-Hamiltonian.

It is felt, therefore, that the values for the zero field splitting are likely to be more reliable when calculated from EPR results rather than the magnetic susceptibility results. In general, magnetic susceptibility experiments indicate larger D -values than those estimated on the basis of EPR experiments (private discussion with Dr. Chatterjee). It is hoped that the apparent contradiction in D -values from the two experimental techniques will be resolved in the near future by a high frequency EPR or far-infrared experiment.

9.2. Discussion of the Results for MbH_2O , MbF and HbH_2O and Comparison with the Crystalline Electric Field Theory

The origin of the zero field splitting has been discussed briefly in Chapter VI. The dominant mechanism appears to be spin-orbit coupling among the low lying energy levels, particularly between the lowest quartet states and the ground sextet [Griffith (1961), (1964);

Harris (1966); Kotani (1964)]. The zero field splitting is large because there must be a strong interaction of the iron orbitals with the surrounding ligands. Consequently the lowest excited states are very close to the ground state. Although the crystalline electric field model, used by the above authors, neglects the effect of covalency their calculations have been successful in accounting for a number of the properties of ferric porphyrin compounds. Comprehensive computer calculations have accounted for a number of the properties of low-spin ferric hemoproteins, Harris (1966), high-spin ferric hemoproteins, Harris and Weissbluth (1966) and a series of ten high-spin ferric porphyrin compounds, Harris (1968). The variation in g-value, magnetic susceptibility, zero field splitting and the electric field gradient at the iron nucleus have been explained by consistent variation in a small number of crystalline electric field parameters. In particular the variation in the parameter c' , describing the tetragonal distortion along the heme normal, produces relatively large changes in the zero field splitting. The change in c' is consistent with the known changes in the zero field splitting in the porphyrin compounds studied by Richards et al. (1967) when only the fifth ligand on the tetragonal axis of the porphyrin compound was altered.

Zerner, Gouterman and Kobayashi (1966) performed a molecular orbital calculation for a five-coordinated iron porphyrin compound. Although electron delocalization of the iron and ligand orbitals is likely to some extent, the crystalline field model appears at present to be the most suitable model for discussion of the available data on the ground state properties of the heme compounds. The reasons for this statement are summarized below.

TABLE XI

Comparison of Experimental Results for MnH_2O and MnF with
Theoretical Results of the Crystalline Electric Field Theory.

	Theoretical Results		Experimental Results	
	(i)	(ii)	MnF	MnH_2O
$\Delta \text{ cm}^{-1}$ (octahedral parameter)	24,000	24,000		
$\mu \text{ cm}^{-1}$ (tetragonal parameter)	4,000	5,000		
Zero field splitting (cm^{-1})	7.64	14.28	(8.1 ± 1.0)	$\begin{pmatrix} 13.7 & + 3.0 \\ & - 2.0 \end{pmatrix}$
$g_{ }$	1.996	1.996		
$g_{\perp}$	1.990	1.983	(1.988 ± 0.002)	(1.974 ± 0.0025)
$\Delta g_{\perp} \%$	1.06	0.30	(0.96 ± 0.1)	(0.28 ± 0.06)
$\mu \text{ eff}$ (Bohr magnetons)	5.77	5.65	5.77^*	5.73^*

* Experimental results of Schoffa (1964)

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(i) The molecular orbital theory of Zerner, et al. (1966) and the crystalline field theory of Harris (1968) predict opposite signs for the electric field gradient at the iron nucleus in iron porphyrin complexes. Mössbauer experiments by Moss, Bearden and Caughey (1969) showed that the sign of the electric field gradient is in agreement with that predicted by the crystalline field theory.

(ii) The molecular orbital calculation cannot be changed easily to apply to the six-coordinated ferric hemoproteins from the five-coordinated porphyrin compound for which the calculations were performed. The change from five- to six-coordination can be achieved simply in the crystalline electric field model by adjusting the value of the tetragonal distortion.

(iii) The molecular orbital theory has a relatively large number of adjustable parameters. The application of the theory is difficult because of the inadequate experimental data on the iron in the hemoproteins.

The results of the experiments can be compared semi-quantitatively with the predictions of the crystalline electric field theory. Harris and Weissbluth (1966) have tabulated results for crystalline electric field parameters which lead to zero field splittings in the range 4.3 to 14.3 cm^{-1} . By inspection of Table 1 in Harris and Weissbluth (1966) two columns in the table have values of the zero field splitting between the lower two doublets which are comparable to the results obtained for MbF and MbH_2O . These columns are reproduced in Table XI together with the results for MbF and MbH_2O . The experimental and theoretical values

for $g_{\perp}$ and the field dependence of $g_{\perp}(\text{exp})$ can be compared. The latter is expressed as the percentage change in $g_{\perp}(\text{exp})$ with a change in magnetic flux density from 0 to 0.6T. The $g_{\perp}$ value for MbF is in agreement with the theoretical value quoted in Table XI. The $g_{\perp}$ value for MbH₂O is lower than the theoretically predicted value on the basis of a zero field splitting of 14.28cm^{-1} . This disagreement may be due to a neglect of covalency in the crystalline electric field theory. There is however too little experimental data to draw any conclusions about the limitations of the crystalline electric field model. The agreement between the experimental and theoretical values for the magnetic field dependence of $g_{\perp}(\text{exp})$ cannot be taken as strong support for the crystalline electric field theory. It shows that the crystalline electric field model leads to a spin-Hamiltonian of the correct form since a discrepancy in the experimental and theoretical values for the magnetic field dependence of $g_{\perp}(\text{exp})$ would have indicated an inconsistency in this model. The agreement between the predicted magnetic susceptibility and the experimental values of Schoffa (1964) is good for MbF but not as good for MbH₂O.

The experimental results for MbH₂O and HbH₂O show that the zero field splitting and the $g_{\perp}$ values are different in the two molecules. This result is unexpected because the nearest neighbour atoms are the same in the two molecules. The heme-plane with the water molecule attached at the sixth co-ordination position of the iron can be treated as being essentially rigid. Consequently the bonding from the iron to the protein is the most likely position to consider for a bonding change which could explain the difference between the HbH₂O and MbH₂O results.

The possibility exists that the conformation of the heme group in relation to the histidine amino acid of the protein differs slightly in MbH₂O and HbH₂O. This conformation change could be responsible for the change in bonding to the iron atom. Dr. E.F. Slade (private communication) has shown that the EPR results from different single crystals of MbH₂O vary depending on the history of the protein. For example, the zero field splitting in crystals of MbH₂O depends on whether the crystals were grown from freeze dried material or from non-freeze dried, salted samples. The implication of Slade's observation is that the EPR of the iron ion in high-spin heme proteins must be sensitive to relatively small changes in the bonding between the heme and the protein. These bonding changes may be due to small conformation changes. The freeze drying technique may produce some small degree of molecular damage adjacent to, or even remote from, the heme group which affects the bonding between the heme and the protein.

These suggestions are tentative and definite conclusions on the origin of the different results from the different samples of MbH₂O must await further experiments. It is interesting to speculate, however, that the EPR of the ferric ion may act as a probe to molecular conformation of the heme with respect to the protein and even to molecular damage remote from the ferric ion. The differences in the EPR results between the different samples of MbH₂O and between HbH₂O and MbH₂O are probably connected with the small energy separation of the high-spin and low-spin states. Small changes in the environment of the iron may then produce relatively large changes in the separation of the ground state and the low lying excited states and, as a result, in the properties of the ground state. In the context of the crystalline

electric field theory, the properties of the different heme compounds have been explained by Harris (1968a) primarily on the basis of a variation in the tetragonal distortion of a strong octahedral crystalline electric field. The biological effectiveness of the hemo-proteins may also be dependent on the small energy separation of the high and low-spin states of the iron ion.

9.3 Discussion of the EPR Linewidths

The misorientation effect has been discussed by Helcké (1963), Helcké et al. (1968) and Eisenberger and Pershan (1967). In the present work it has been shown that the misorientation effect is comparable in crystals of MbH_2O and MbF . The misorientation effect in the crystals of HbH_2O was larger than that observed in the Mb crystals but this may be due to the presence of a crystalline lattice disorder. Slade and Ingram (1968) have shown that the observed linewidth, when the magnetic field is parallel to the heme plane, is both larger than predicted by the misorientation theory and microwave frequency dependent. The frequency dependence of the linewidth was confirmed in the present work. It has also been shown that the linewidth in the heme plane is different for MbH_2O and MbF particularly when measured at 55 GHz.

No definite mechanism has been proposed which explains fully the observed linewidth variation in the heme plane and its frequency dependence. Many of the more usual line-broadening mechanisms can be discarded because they are frequency independent. This section discusses briefly the implications of a statistical variation in the g -value in the heme plane. The EPR results given in Chapter VIII show that for

MbH_2O , MbF and HbH_2O the linewidth at 55 GHz in the heme plane increases as the value of $(2D)$ increases. If the frequency dependent linewidth arises because of a statistical variation in the g -value in the heme plane then there should also be a statistical variation in $(2D)$.

Conversely, if there is a statistical variation in $(2D)$ then this might be apparent in the linewidths at $g_{\perp}$. There is some evidence from the far-infra-red experiments of Richards et al. (1967) that suggests there could be a statistical variation in $(2D)$. In this work it was shown that the zero field transition between the lower doublets in hemin chloride occurred at 13.7cm^{-1} with a linewidth at half maximum amplitude of 1.7cm^{-1} . The Zeeman splitting of the lowest doublet is insensitive to changes in the zero field splitting and the observed linewidth from the lowest doublet is narrower than the zero field splitting linewidth between the lower two doublets. Consequently, the observed zero field linewidths in the hemin compounds studied by Richards et al. (1967) may be due to a statistical variation in the zero field splitting. By inspection of Fig. (8.2) and consideration of the experimental variation in $g_{\perp}$ with $(2D)$ given in Table VIII, it can be seen that for a comparable statistical variation in $g_{\perp}$ there would be a larger linewidth for those ferric ions in hemoprotein derivatives with a smaller value of $(2D)$. The opposite has been observed in this work and, as a result, it is felt that this linebroadening mechanism is unlikely though not impossible.

9.4 Suggestions for Future Work

It would be interesting to compare the results for the crystals of HbH_2O with results from crystals which had been grown from non-freeze

dried hemoglobin. Experiments on HbF would also be valuable in order to compare the results with those for MbF.

Further systematic research should be done on single crystals of MbH₂O, possibly in conjunction with x-ray crystallography, in order to try to explain the variation in g-values from the samples of MbH₂O with different purification procedures.

Experimental results on the linewidth variation when the magnetic field direction is parallel to the heme plane at 55 GHz and 70 GHz may help in explaining the peculiar linewidth variation with microwave frequency. These experiments should be attempted for both MbH₂O and MbF.

ADDENDUM

Subsequent to the completion of this thesis the author obtained a reference in Feher (1970) to unpublished far-infrared work by Brackett and Richards on the zero field splitting of MbF. The result was quoted as $(2D) = 12\text{cm}^{-1}$ which is outside the experimental error of the value $(2D) = (8.1 \pm 1.0)\text{cm}^{-1}$ given in this thesis. It is likely, however that the sample used in this work was in the form of a freeze-dried powder similar to the hemin samples used in the work of Richards et al (1967). Considering Dr. Slade's observation of a variation in $(2D)$ from different crystals of MbH_2O , the discrepancy between the value quoted in this thesis for $(2D)$ in crystals of MbF and the value obtained by Brackett and Richards may be due to differences in the samples.

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APPENDIX A

This appendix shows that the locus of the reflected wave from an EPR sample with a Lorentzian lineshape traces out a circle in the Smith chart. The proof applies when the sample is placed in a resonant microwave cavity under conditions of no saturation and when the magnetic field, not the microwave frequency, is varied through the resonance condition.

The components of the change in sample susceptibility due to the magnetic resonance have been given by Eq. (2.3.11) and Eq. (2.3.12).

When there is no saturation of the sample $B_1^2 T_1 T_2 \ll 1$ and Eq. (2.3.11) and Eq. (2.3.12) becomes

$$\chi' = \frac{1}{2} \chi_0 \omega_L T_2 \left(\frac{\Delta \omega T_2}{1 + \Delta \omega^2 T_2^2} \right) \quad (1)$$

$$\text{and } \chi'' = \frac{1}{2} \chi_0 \omega_L T_2 \left(\frac{1}{1 + \Delta \omega^2 T_2^2} \right) \quad (2)$$

where the symbols are as defined in Chapter II.

Rewriting Eq. (1) and Eq. (2) using

$$\hbar \omega_L = g \beta B_L \quad (3)$$

$$\text{and } \hbar \Delta \omega = g \beta \Delta B \text{ yields} \quad (4)$$

$$\chi' = \frac{1}{2} \chi_0 a^2 B_L T_2^2 \left(\frac{\Delta B}{1 + a^2 \Delta B^2 T_2^2} \right) \quad (5)$$

$$\text{and } \chi'' = \frac{\frac{1}{2} \chi_0 a B_L T_2}{1 + a^2 \Delta B^2 T_2^2} \quad (6)$$

$$\text{where } a = \left(\frac{g \beta}{\hbar} \right).$$

It has been shown in Section (3.1) that the fractional changes in resistance and reactance of the cavity equivalent circuit, due to an EPR resonance, are proportional to χ' and χ'' respectively.

Eq. (3.1.4) and Eq. (3.1.5) show that the constant of proportionality is equal for the two cases. Consequently, the components v' and v'' of the microwave voltage arising from the variation in χ' and χ'' , as a function of the magnetic flux density B , will also be proportional to χ' and χ'' . Therefore

$$v' = k\chi' \quad (7)$$

$$\text{and } v'' = k\chi'' \quad (8)$$

where k is a constant for a particular cavity power, cavity Q and EPR sample and v' and v'' are microwave voltages in phase with the r' and r'' components of the cavity reflected wave.

From Eq. (6) and Eq. (8)

$$\Delta B = \frac{1}{aT_2} \left[\frac{k\chi_0 aB_L T_2}{2v''} - 1 \right]^{\frac{1}{2}} \quad (9)$$

Substituting ΔB into Eq. (5) and Eq. (7) yields

$$v' = v'' \left[\frac{k\chi_0 aB_L T_2}{2v''} - 1 \right]^{\frac{1}{2}} \quad (10)$$

Therefore

$$v'^2 = -v''^2 + bv'' \quad (11)$$

$$\text{or } v'^2 + (v'' - \frac{1}{2}b)^2 = \frac{1}{4}b^2 \quad (12)$$

$$\text{where } b = \frac{1}{2}k\chi_0 aB_L T_2$$

Eq. (12) is the equation of a circle of radius $\frac{1}{2}b$ and centre $(\frac{1}{2}b, 0)$.

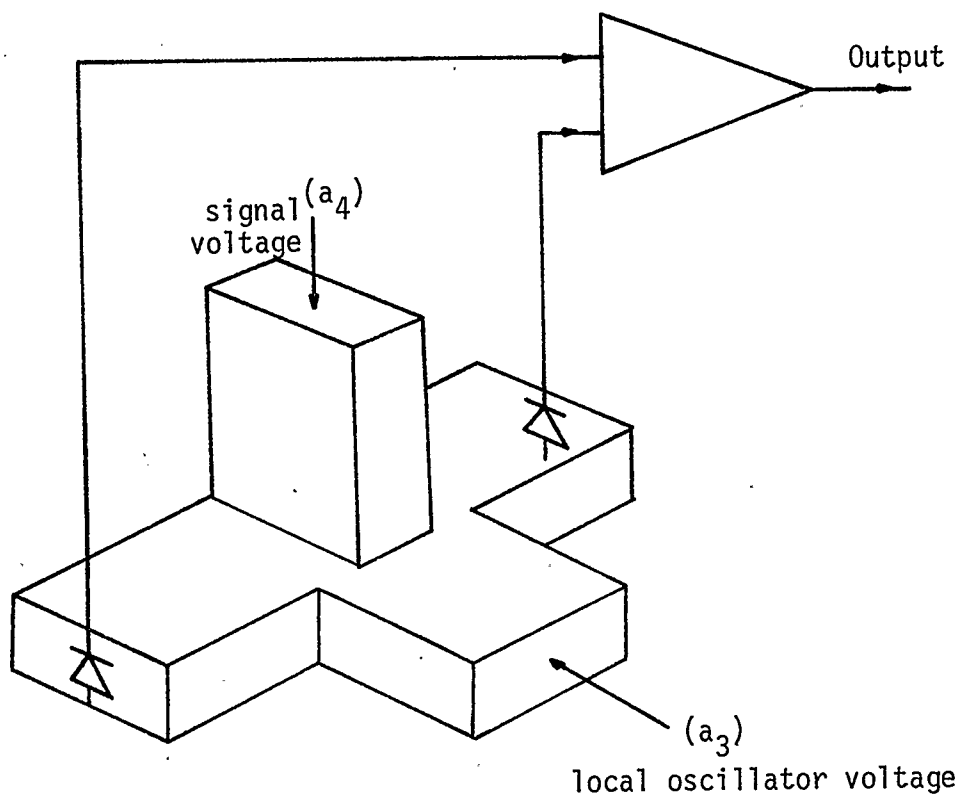


Figure (A.1).

Illustration of the configuration of the magic tee , microwave diodes and differential amplifier used in balanced synchronous demodulation.

APPENDIX B

This appendix gives a derivation of the expression for the output voltage of a balanced microwave synchronous demodulator as used in the EPR homodyne and superheterodyne spectrometer described in this thesis.

Fig. (A.1) shows the configuration of the magic tee, the microwave diodes and the differential amplifier. The scattering matrix for the magic tee can be used to derive an expression for the microwave voltage incident on each of the microwave diodes D_1 and D_2 . The scattering matrix S for a perfect magic tee is given by Altman (1964).

$$S = \frac{1}{\sqrt{2}} \begin{vmatrix} 0 & 0 & 1 & 1 \\ 0 & 0 & 1 & -1 \\ 1 & 1 & 0 & 0 \\ 1 & -1 & 0 & 0 \end{vmatrix}$$

The voltages incident on the diodes D_1 and D_2 can be derived from

$$\vec{b} = S\vec{a} \quad (1)$$

where $\vec{b}$ is a column vector with components b_1 , b_2 , b_3 , and b_4 describing the output voltage in each arm and $\vec{a}$ is a column vector with components a_1 , a_2 , a_3 , and a_4 describing the input voltage in each arm.

The input voltages are

$$\begin{aligned} a_1 &= a_2 = 0 \\ a_3 &= V_0 \cos(\omega_0 t) \\ a_4 &= V_s \cos(\omega_s t + \phi_s) \end{aligned}$$

where a_3 is the local oscillator voltage and a_4 is the signal voltage.

Using Eq. (1) the components of the output voltage in each arm of the magic tee are

$$b_1 = \frac{1}{\sqrt{2}} \left[V_0 \cos(\omega_0 t) + V_s \cos(\omega_s t + \phi_s) \right] \quad (2)$$

$$b_2 = \frac{1}{\sqrt{2}} \left[V_0 \cos(\omega_0 t) - V_s \cos(\omega_s t + \phi_s) \right] \quad (3)$$

$$\text{and } b_3 = b_4 = 0$$

When the EPR spectrometer bridge is balanced the condition $V_0 > \sim 10^3 V_s$ is satisfied.

Altman (1964) has assumed that under this condition, each microwave diode acts as a linear half-wave rectifier. Consequently, the output voltage is

$$V_{\text{out}} \propto V_s \cos[(\omega_0 - \omega_s)t - \phi_s] \quad (4)$$

Thus, for a small signal voltage the output of the i.f. amplifier is independent of the amplitude, or time variations in the amplitude (a.m. noise), in the local oscillator voltage. When the spectrometer is operated as a homodyne then $\omega_0 = \omega_s$ and the output of the amplifier depends on the relative phase of V_s and V_0 . The optimum output is obtained for $\phi_s = 0$.

Even if the microwave demodulators are not biased into their linear region, a change in output voltage from each demodulator, due to a change in V_s , is proportional to the change in V_s provided that the condition $V_0 \gg V_s$ is satisfied. The output voltage V_1 from diode D_1 due to the voltage b_1 , is given by Faulkner (1964).

$$V_1 = \left(\frac{dV_1}{dP} \right) \cdot \left(\frac{\overline{b_1^2}}{R} \right) \quad (5)$$

where R is the characteristic impedance of the waveguide, $\overline{b_1^2}$ is the average of b_1^2 over a period of time greater than $\frac{1}{\omega_0}$ and $\left(\frac{dV_1}{dP} \right)$ defines the operating condition of the diode for an incident microwave power P .

The output of the differential amplifier is

$$V_{out} = G(V_1 - V_2) \quad (6)$$

where G is the amplifier gain.

Using Eq. (5) and Eq. (6) it can be shown that

$$V_{out} \propto G \left(\frac{dV_1}{dV_0} \right) V_s \cos[(\omega_0 - \omega_s)t - \phi_s] \quad (7)$$

This is essentially the same result as given in Eq. (4) because $\left(\frac{dV_1}{dV_0} \right)$ is a constant for a particular value of local oscillator voltage. Eq. (7) also shows that, in the homodyne spectrometer, the operating point of the crystal demodulators is not critical in obtaining balanced synchronous demodulation provided that $V_0 \gg V_s$.

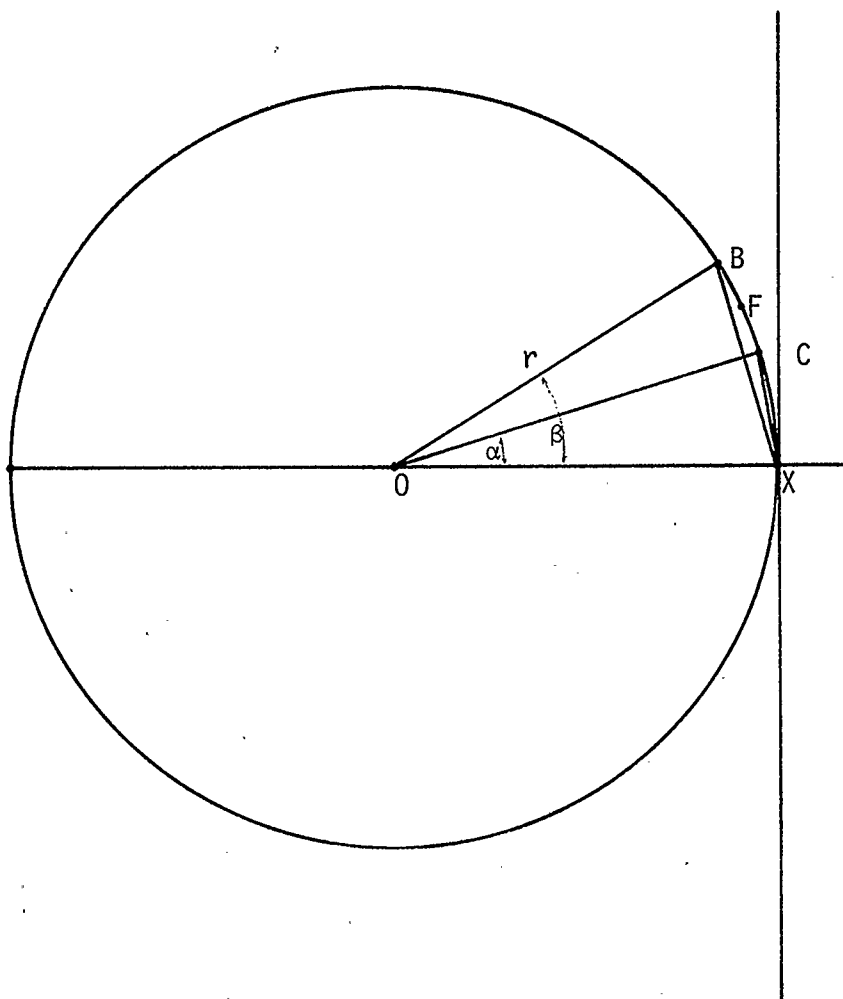


Figure (A.2).

The Smith chart representation of the reflected wave from a balanced EPR bridge.

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APPENDIX C

This appendix gives a derivation of the expressions, quoted in Section (4.4), for the spectrometer sensitivity to f.m. noise. The expressions for s_{fb} (Eq. 4.4.1) and s_{fu} (Eq. 4.4.1,b) give the voltage sensitivity to f.m. noise for balanced and unbalanced synchronous demodulation respectively.

Fig. (A.2) illustrates the Smith chart representation of the reflected wave from a balanced EPR bridge. The circle with centre 0 and the point X on the circle give the extremes of the phasors representing the reflected wave as a function of frequency. When the klystron frequency is tuned exactly to the cavity resonant frequency and the EPR bridge is perfectly balanced, then the point X represents the spectrometer operating point.

Wilmschurst (1968) has considered the situation in which there is an error frequency between the cavity resonant frequency and the klystron frequency which is greater than the peak frequency deviation of the f.m. noise. The point F in Fig. (A.2) gives the extreme of the phasor XF due to the steady error frequency and the points B and C represent the extremes of the frequency modulation about F. The amplitude and phase of the voltage reflection coefficient at the extremes of the frequency modulation are proportional to the lines XB and XC. The points C, F and B are drawn to illustrate the derivation. The angles α and β are normally less than $\sim 5^\circ$.

The frequency modulation at the final demodulation frequency f_m between the points B and C, leads to an amplitude modulated wave in the signal arm. The phasor diagrams Figs. (A.3a and b) show the two possible

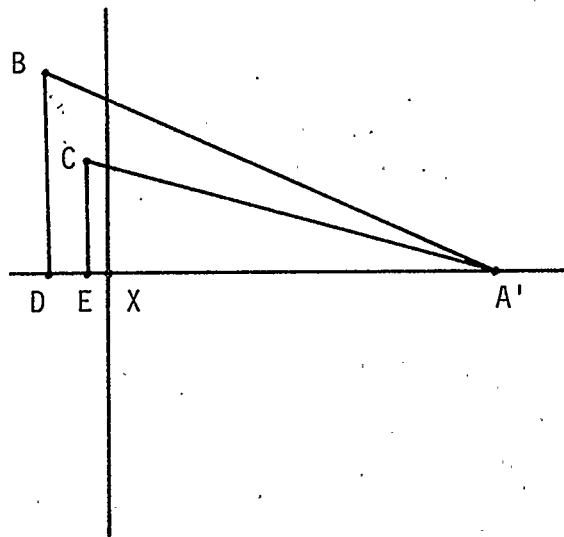
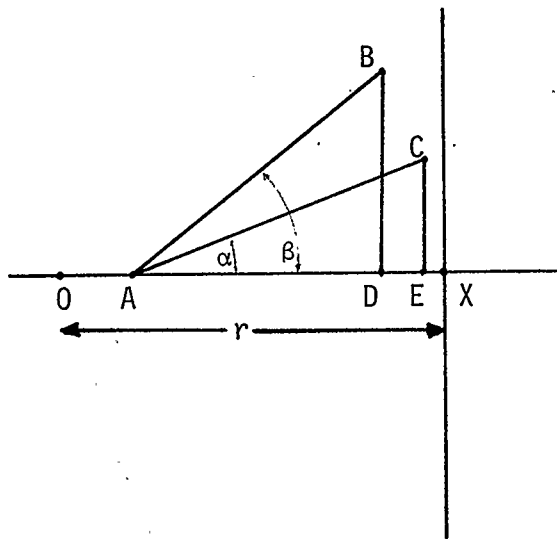


Figure (A.3a and b).

Phasor diagrams for the combination of the a.m. signal due to the f.m. noise with the two possible phasings AX and A'X for the homodyne voltage.

phasor configurations for the spectrometer detection of an absorption signal using an unbalanced microwave demodulator. The lines XA and XA' represent the magnitude and the two possible phases for the homodyne voltage. The a.m. signal at the output of the demodulator will be proportional to $|AB| - |AC|$ in Fig. (A.3).

$$|AB| - |AC| = \left(AD^2 + BD^2 \right)^{\frac{1}{2}} - \left(AE^2 + CE^2 \right)^{\frac{1}{2}} \quad (1)$$

$$= \left[\left(r \cos \beta - AO \right)^2 + r^2 \sin^2 \beta \right]^{\frac{1}{2}} - \left[\left(r \cos \alpha - AO \right)^2 + r^2 \sin^2 \alpha \right]^{\frac{1}{2}} \quad (2)$$

where $r = OX$

and Eq. (2) is applicable to Fig. (A.3a).

Using the expansions

$$\cos \beta = 1 - \frac{1}{2} \beta^2$$

$$\text{and } \cos \alpha = 1 - \frac{1}{2} \alpha^2$$

and substituting in Eq. (2) yields

$$|AB| - |AC| = \frac{AO \cdot r}{(AO - r)} \cdot (\beta^2 - \alpha^2) \quad (3)$$

The radius of the circle is proportional to the microwave voltage incident on the sample cavity. Without loss of generality the distances in Eq. (3) can be written in terms of the voltage transfer to the cavity V_C and the homodyne voltage to the demodulator V_D

according to

$$r = \frac{1}{2} V_C \quad (4)$$

$$AO = V_D$$

therefore

$$OA = V_D - \frac{1}{2} V_C \quad (5)$$

An expression for the angles α and β in Eq. (3) can be calculated using the expression for Γ'' calculated in Section (3.2) and contained in Eq. (3.2.7). For a balanced EPR bridge $\Gamma_0 = 0$ in Eq. (3.2.7).

$$d \approx \sin \alpha = \frac{CE}{r} = \frac{V_C \Gamma_a''}{r}$$

where Γ_a'' is the component of the reflected wave at the frequency $(\delta f - \frac{1}{2}df)$ in phase with the Γ'' axis. Using Eq. (3.2.7)

$$\alpha = \frac{2(\delta f - \frac{1}{2}df)}{\Delta f} \quad (6)$$

where $\Delta f = \left(\frac{\omega r}{Q_u}\right)$ is the unloaded cavity bandwidth

similarly

$$\beta = \frac{2(\delta f + \frac{1}{2}df)}{\Delta f} \quad (7)$$

therefore

$$\beta^2 - \alpha^2 = \frac{8 \delta f \cdot df}{\Delta f^2} \quad (8)$$

substituting Eq. (4) and Eq. (8) into Eq. (3) yields

$$|AB| - |AC| = 2V_C \left(\frac{V_C}{V_D} - 2 \right) \cdot \left(\frac{\delta f}{\Delta f^2} \right) \cdot df. \quad (9)$$

similarly for the alternate homodyne phase given in Fig. (A.3b)

$$|AB| - |AC| = 2V_C \left(\frac{V_C}{V_D} + 2 \right) \cdot \left(\frac{\delta f}{\Delta f^2} \right) \cdot df \quad (10)$$

Consequently the voltage sensitivity to f.m. noise for an EPR spectrometer using an unbalanced detector is

$$S_{fu} = k_d V_C \left(\frac{V_C}{V_D} \pm 2 \right) \cdot \left(\frac{\delta f}{\Delta f^2} \right) \quad (11)$$

where k_d is a constant for the microwave demodulator and the $\pm$ signs in Eq. (11) represent the two possible phases for the homodyne voltage.

The balanced synchronous demodulation case can be calculated by consideration of Fig. (A.3). AX and AX' represent the homodyne voltages to the two microwave demodulators in the balanced mixer. The a.m. noise output of the spectrometer due to the f.m. noise is proportional to

$$\begin{aligned} S_{fb} &\propto \{|AB| - |A'B|\} - \{|AC| - |A'C|\} \\ &= \{|AB| - |AC|\} - \{|A'B| - |A'C|\} \end{aligned} \quad (12)$$

Therefore the voltage sensitivity to f.m. noise for a balanced mixer is simply the difference of the two terms for unbalanced demodulators with the two possible phases.

Therefore

$$S_{fb} = 2k_d V_c \left(\frac{\delta f}{\Delta f^2} \right). \quad (13)$$