

CHAPTER-II

MATERIALS AND METHODS

In this chapter, a brief description of the ESR and DNP measurements using ESR spectrometer and Overhauser-enhanced magnetic resonance imaging technique is presented.

2.1 ESR MEASUREMENTS

In an ESR experiment, the field of the spectrometer magnet is swept linearly to excite the unpaired electrons in the lower energy level to the upper energy level while the sample is exposed to fixed microwave irradiation. The free or the unpaired electrons have a small magnetic field and orient themselves parallel to the larger field produced by the spectrometer's magnet. At a particular magnetic field strength, the microwave irradiation will cause some of the free electrons to "flip" and orient against the spectrometer's magnetic field. ESR spectroscopy can be carried out by either

1. varying the magnetic field and holding the frequency constant or
2. varying the frequency and holding the magnetic field constant

Typically, a commercial spectrometer works by varying the magnetic field and holding the frequency constant. ESR spectrometers are working at frequencies ranging from several hundred MHz to several hundred GHz.

2.2 TYPES OF ESR SPECTROMETERS

There are two types of ESR spectrometer

- i) Continuous wave ESR spectrometer
- ii) Pulsed ESR spectrometer

2.2.1 Continuous Wave ESR spectrometer

Real samples contain a large number of spins distributed in the constant magnetic field between the two allowed energy states according to thermal equilibrium. The relative population of two levels is described by the Boltzmann distribution

$$N_+/N_- = \exp(-\Delta E/kT)$$

where k is the Boltzmann constant and T is the temperature of the sample. For the free electron with $g_e = 2.0023$, the spin population ratio is ~ 1.0015 for a field of 0.34 Tesla (for X-band ESR), or about 1 in 670 excess spins in the lower energy state. The oscillating magnetic field at the resonance frequency ν_0 induces transitions from up to down and from down to up with equal probability. However, the population of lower energy is larger than that of the upper one, leading to a larger number of transitions to the upper level, and as a consequence, to energy absorption by the sample. This effect is the basis for the simplest and most widely used experimental approach, the so-called continuous wave (CW) ESR experiment, in which the sample containing paramagnetic species is irradiated by microwaves with frequency fixed by the source, and the magnetic field is varied to search for the microwave absorption. The ESR absorption spectra can be recorded at resonance condition.

2.2.2 Pulsed ESR Spectrometer

Pulsed ESR spectrometers have many of the major parts similar to those found in a CW ESR instrument including microwave source, resonant cavity, magnet and detection system. As in the CW instrument, the resonance condition is obtained by varying the field at fixed microwave frequency. The key difference in doing pulse ESR is the generation of pulse sequences with large microwave power, and detection of the weak microwave emission of the free induction decay (FID) and echo signals. These differences determine the specific requirement for the sources, cavities, and detectors. In a pulsed ESR

instrument, a klystron or Gunn diode generates CW microwaves in the milliwatt range at a set frequency. The source unit includes also a pulse programmer that controls generation of the high-power microwave pulse, protection of receivers, and triggering of the acquisition devices. The pulse-forming unit usually consists of several channels with individual adjustments for amplitude, phase, and length of the pulses.

2.3 CW ESR SPECTROMETER

Fig. 2.1 shows the typical X-band ESR spectrometer. The details about the various components and functioning are given below [1-11].

2.3.1 Source

The frequency of radiant energy used in the X-band ESR spectrometers is approximately 9.5 GHz (X-band), in the medium-frequency microwave region. This frequency corresponds to a wavelength of about 32 mm. The microwave source is usually a klystron, which is a vacuum tube well known for its low-noise characteristics. The field B_1 is generated by oscillations within its own tunable cavity. In the range of about 1–100 GHz, the mode of energy transmission is either by special coaxial cables or waveguides. In standard instrument, the microwave power is incident on the sample continuously (i.e., continuous wave). Alternatively, in pulse ESR spectrometer, the power is pulsed. In addition to the waveguide-connected klystron, the most important components are resonator, magnet and detector.

2.3.2 Resonator

The resonator cavity admits microwaves through an iris. The frequency of the source is tuned to the appropriate resonant frequency of the cavity. The corresponding resonant wavelengths are related to the dimensions of the cavity. The resonant mode, which maximizes B_1 at the location of the sample. At resonance, the energy density in the resonator is thousands of times that in the

waveguide, which can detect resonant absorption in a sample. A recently developed loop-gap resonator has been advocated as an alternative to the usual resonant cavities for energy dissipative samples.

2.3.3 Magnetic Field

In magnetic resonance experiments, the static magnetic field B usually must be very well controlled and stable. The variations of the magnetic field are translated into corresponding variations in energy separation ΔE . The magnitude of B can be measured and controlled by a Hall-effect detector.

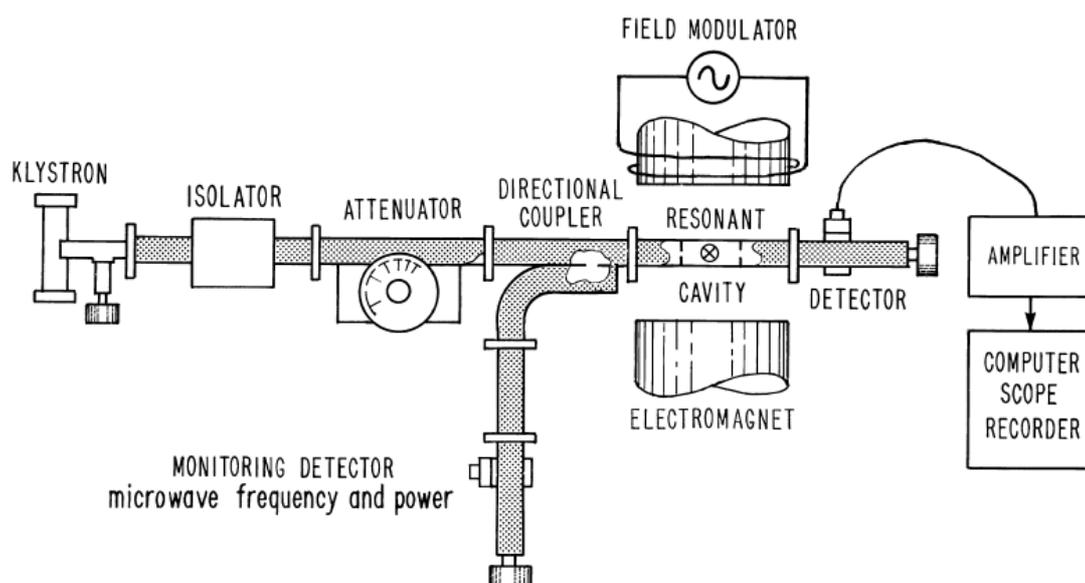


Fig. 2.1 Block diagram of a continuous-wave (cw) electron spin resonance (ESR) spectrometer

2.3.4 Magnetic field modulation

In order to increase the sensitivity and resolution, one uses magnetic field modulation. A coil at each side of the cavity is fed with a current of 100 kHz that can give a modulation with a maximum amplitude of about

2-4 mT. Consequently the detector gives a signal with the frequency 100 kHz, whose amplitude is proportional to the derivative of the absorption with respect to the field. The modulation must be optimized with regard to signal/noise and resolution.

2.3.5 Amplifier system

The signal with the frequency 100 kHz is detected using a phase sensitive amplifier and directed to the Y axis of the recorder. The receiver gain, time constant of the filter and offset can be adjusted at the console.

2.3.6 Detector

Numerous types of solid-state diodes are sensitive to microwave energy. The absorption lines can be observed in the ESR spectrum when the separation of two energy levels is equal to (or very close to) the quantum energy $h\nu$ of an incident microwave photon. The absorption of such photons by the sample is indicated by a change in the detector current. The direct detection of the absorption signal, which is shown in Fig. 2.1. In a typical fixed-frequency magnetic-resonance spectrometer, the role of the light chopper is taken by a field modulator to impose an alternating component on the static magnetic field B . This results in an alternating signal at the microwave detector that can be amplified in a narrow-band amplifier. Typically, the resulting signal is rectified and takes on a B dependence that resembles the first derivative of an absorption line. The shape of the absorption line often is fitted to a functional formula (e.g., Gaussian, Lorentzian) approximating its field or frequency dependence.

2.3.7 Samples

The sample is held in a quartz tube with an inner diameter of 3 mm. Quartz glass is necessary to avoid unwanted signals from impurity. In the

cavity, there is not only an oscillating magnetic field that induces the transitions sought for but also an electric alternating field. In a water solution, one gets a dielectric absorption in the water (compare with a microwave oven) that reduces the Q-value of the cavity and accordingly the strength of the signal. For water solutions, one can use a tube with an internal diameter of about 1mm. Frozen solutions on the other hand absorb much less, and one can use a normal 3mm tube. Crystal rotations are done with the help of a goniometer, with an accuracy of ± 1 degree. The first derivative of the absorption curve is shown in Fig. 2.2. It takes a little practice to get used to looking at first-derivative spectra, but there is a distinct advantage: first-derivative spectra have much better apparent resolution than absorption spectra.

2.3.8 Saturation

In actual practice, the ESR signals have finite width because of the larger relaxation time. In order to improve accuracy, the signals are recorded as the derivative as shown in the absorption curve with respect to the magnetic field. The derivative curve is simply its slope at a given point; the derivative of the spectral trace is $dA/d\nu$, where A is the energy absorbed or emitted. In order to do this, the magnetic field strength is modulated sinusoidally at a certain modulation frequency (usually 100 kHz). The effect of modulation is depicted in Fig. 2.3. This technique allows amplification of ESR signal using AC techniques, elimination of most of the noise-contributing components and enhanced spectral resolution.

The spin-lattice relaxation causes the spins to achieve thermal equilibrium according to the Boltzmann law, so that the population difference between the spin energy levels is re-established. The continuous microwave irradiation is applied, hence the microwave power becomes high enough, so that the relaxation is slow compared with the transition rate, finally an equal population of the spin energy levels will occur, which is known as saturation.

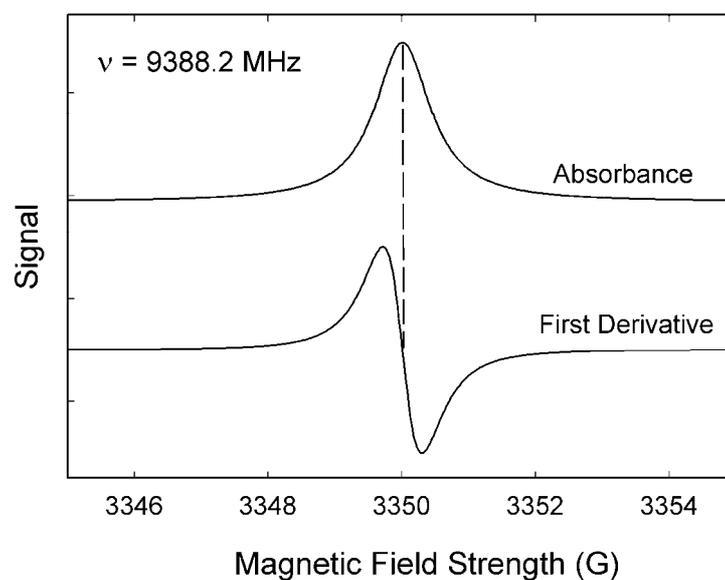


Fig. 2.2 The absorption and first derivative ESR spectrum of a single electron

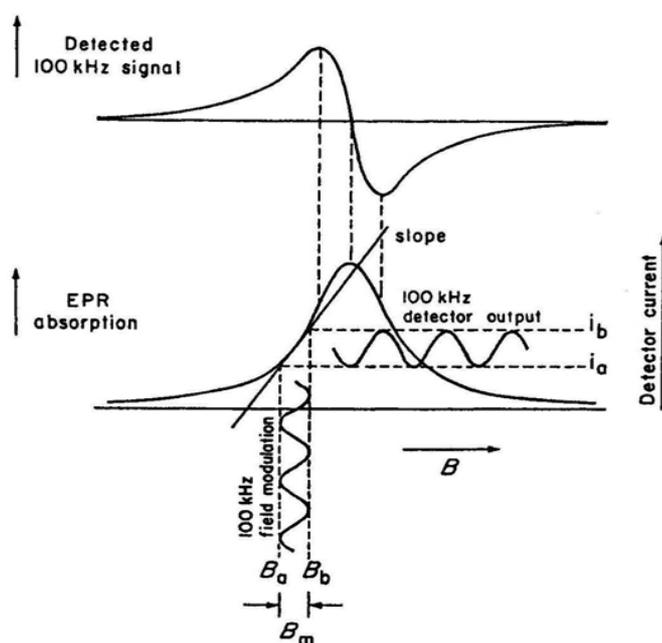


Fig. 2.3 Effect of small amplitude 100 kHz field modulation on the detector output current. The static magnetic field B is modulated between the limits B_a and B_b . The corresponding detector current varies between the limits i_a and i_b . The upper diagram shows the recorded rectified 100 kHz signal as a function of B .

In the non-saturating regime, the square of the double integral over the intensity I of a derivative signal is proportional to the microwave power (P) irradiated. If the line width does not change, the equation is as follows

$$I = \sqrt{P} \quad (1.35)$$

As the line is saturating the signal intensity does not grow as fast as P anymore and finally decreases. The power, where the quotient out of I and P has been reduced to one half of its non-saturating value, which is called half saturation power $P_{1/2}$. The saturation curve usually obeys the empirical law as given below.

$$\frac{I}{\sqrt{P}} = c \cdot \frac{1}{\left(1 + \frac{P}{P_{1/2}}\right)^{b/2}} \quad (1.36)$$

where c is a constant, $b = 1$ in case of an inhomogeneous line broadening and $b = 2$ for a homogeneous line broadening.

Intensities of the spectra obtained are not only dependent on the microwave power but on the concentration of the ESR active species (spins) in the sample. The integrated intensity of an ESR absorption signal is directly proportional to the spin concentration and can therefore be used as estimation for the concentration of the paramagnetic species. Thus, this is a technique to estimate the amount of free radical present; the method is extraordinarily sensitive, in favorable cases some 10^{-13} mol of free radical being detectable.

2.4 ESR MULTI FREQUENCY SPECTROMETERS

The ESR spectrometers are classified into S, X, K, Q and E bands depending upon the irradiating microwave frequency and their classifications are given in Table 2.1. The most commonly used ESR spectrometer is in the

range of 9-10 GHz (X-band). However, advances in electronics have facilitated the development of spectrometers working at frequencies ranging from several hundred MHz to several hundred GHz. ESR spectrometers are working at 1-2 GHz (L-band) and 2-4 GHz (S-band), 8-10 GHz (X-Band).

Table 2.1 Microwave frequency bands and the corresponding magnetic fields for $g = 2$.

Band	Wavelength λ (mm)	Frequency ν (GHz)	Magnetic field B_0 (T)
L	300	1	0.03
S	100	3	0.11
X	30	10	0.33
K	12.5	24	0.86
Q	8.5	35	1.25
E	4.0	75	2.7
W	3.2	95	3.5

Multi frequency ESR provides an experimental route to study the magnetic interactions in paramagnetic materials. The parameters like line width, g -factor, hyperfine coupling constant and rotational correlation time which characterizes these interactions, can lead to an understanding of atomic and molecular structure at magnetic sites. ESR spectroscopy, combined with techniques such as spin trapping, can be used to detect and follow free radical reactions in biological systems. High-frequency (95 GHz) ESR instrumentation provides the high sensitivity and ability to study very small samples and often provides extraordinary resolution and discrimination between similar species. Low-frequency ESR spectrometers and special microwave detectors and circuitry provide the capability to obtain spectra from living animals and

perfuse organs, including the concentration of oxygen in tissues, redox metabolism, distribution of spin-labeled molecules and biophysical measurement. Special materials are developed for sensitive ESR probes of both oxygen and nitric oxide (NO) in tissues.

In this work, the multi frequency ESR spectrometers were used for recording the spectrum

2.4.1 X-band ESR measurements

The ESR spectra of the samples were recorded at room temperature using a Bruker EMS plus X-band spectrometer. The photographic view of X-band ESR spectrometer is shown in Fig. 2.4.

2.4.2 L band and low frequency (300 MHz) ESR measurements

The work described in this report was carried out with an L-band and low frequency ESR spectrometer (JEOL, Tokyo). Fig. 2.5 shows the photographic view of L-band ESR spectrometer and Fig. 2.6 shows the photographic view of 300 MHz ESR spectrometer

2.5 DYNAMIC NUCLEAR POLARISATION (DNP) MEASUREMENTS

The DNP experiments were performed on a custom-built (Philips Research Laboratories, Hamburg, Germany), human whole-body (Magnet bore: 79 cm diameter; 125 cm length), low field (14.53 mT) scanner operating in a field-cycled mode to avoid excess RF power deposition during the ESR cycle [12-16]. DNP spectra were recorded by sweeping the magnetic field (B_0^{ESR}), which is shown in Fig. 2.7.

Imaging experiments were performed by using standard spin warp gradient echo sequence for MRI, except that each phase encoding step was preceded by an ESR saturation pulse to elicit the Overhauser enhancement, which is shown in Fig. 2.8.



Fig. 2.4 Photography view of X-band ESR spectrometer.



Fig. 2.5 Photography view of L-band ESR spectrometer.

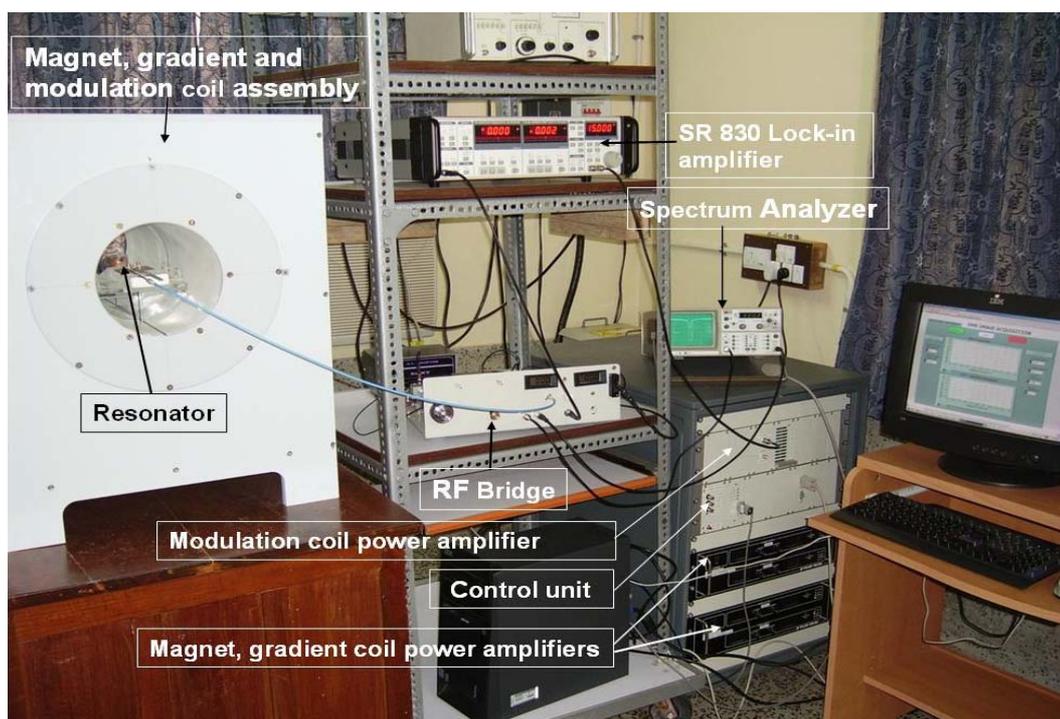


Fig. 2.6 Photography view of 300 MHz ESR spectrometer.

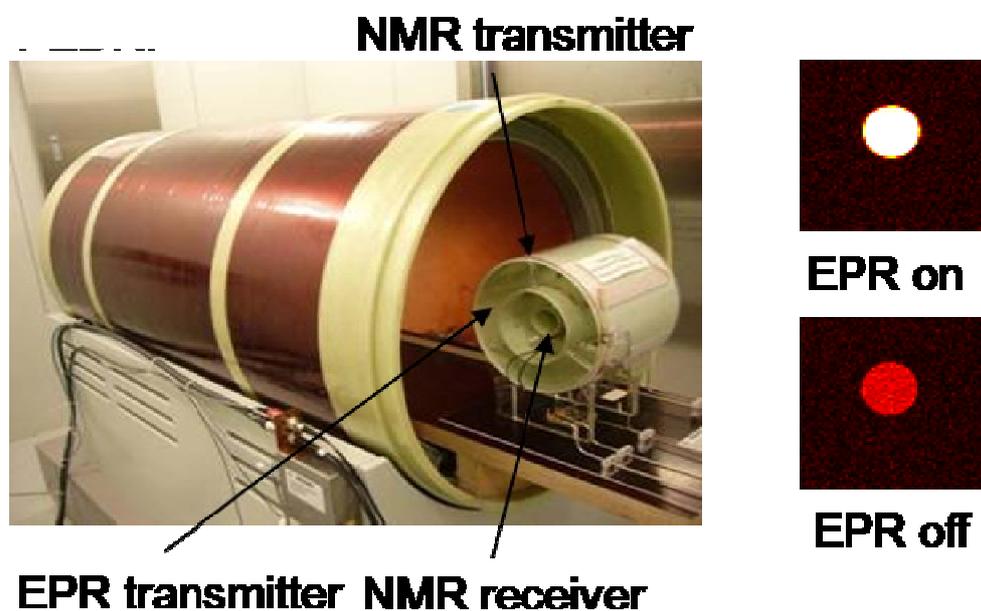


Fig. 2.7 Photographic image of Overhauser-enhanced magnetic resonance imaging setup

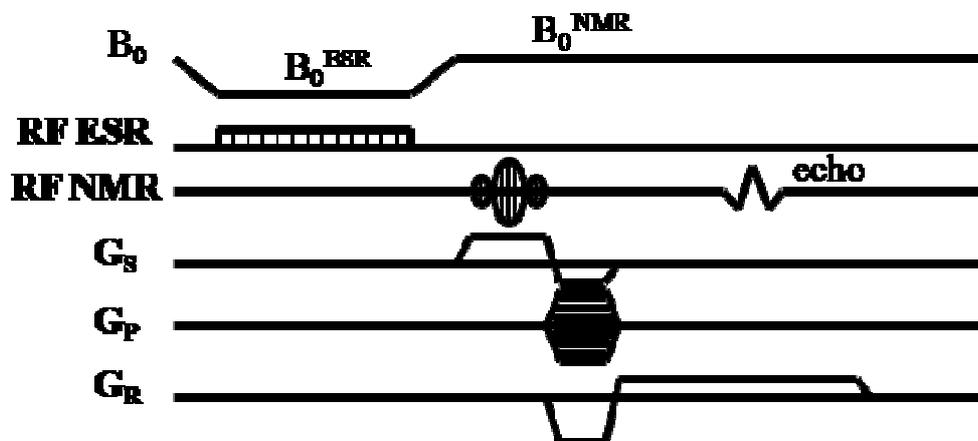


Fig. 2.8 Field-cycled OMRI pulse sequence starts with the ramping of the B_0^{ESR} field, followed by switching on the ESR irradiation. The B_0 is ramped before the NMR pulse and the associated field gradients are turned on.

2.6 CHEMICALS

The spin probes, 4-oxo-2,2,6,6-tetramethyl-piperidine-1-oxyl (TEMPONE), 2,2,6,6-tetramethyl piperidine-1-oxyl (TEMPO), 4-hydroxy-2,2,6,6-tetramethyl-piperidine-1-oxyl (TEMPOL), 3-carbamoyl-2,2,5,5-tetramethyl-pyrrolidin-1-yloxy, (carbamoyl-PROXYL), 3-carboxy-2,2,5,5-tetramethyl-1-pyrrolidinyloxy, (carboxy-PROXYL) and L- α Phosphatidylcholine (Egg PC) were purchased from Sigma Aldrich Chemical Co, St. Louis, MO, USA. The ^{14}N -labeled deuterated 3-methoxycarbonyl-2,2,5,5-tetramethyl-pyrrolidine-1-oxyl (MC-PROXYL) and ^{14}N -labeled deuterated 3-carboxy-2,2,5,5-tetramethyl-pyrrolidin-1-oxyl (carboxy-PROXYL) was synthesized as described earlier [17]. ^{15}N -labeled nitroxyl probes were synthesized by using ^{15}N -ammonium chloride and deuterated acetone (Cambridge Isotope Laboratories, Inc. MA, USA) as per literature [18]. All other chemicals were analytical grade.

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