

## Chapter 4

# Electron Spin Resonance Spectroscopy

### 4.1 Electron Spins

Unpaired electrons possess a spin  $m_s = \pm\frac{1}{2}$  and, if bound, an orbital angular momentum. The observation of electron spins is possible in an external magnetic field in experiments very similar to those described for nuclear magnetic resonance spectroscopy.

The energy of an electron with spin  $m_s$  can be expressed as function of the magnetogyric ratio  $\gamma = 9.274 \cdot 10^{-24} JT^{-1}$  and the g-factor of the electron (close to 2, but depending on the electron angular momentum), or as function of the Bohr magneton as shown in equation 4.1. Please note that the Bohr magneton is about three orders of magnitude larger than the nuclear magneton, therefore the energy splitting of electron spin states in an external magnetic field is much larger than that of nuclei.

$E_{m_s}$	Spin state energy		$\gamma = -\frac{e}{2m_e}$	(4.1)
$B_0$	Magnetic field		$\mu_b = \frac{e\hbar}{2m_e}$	
$m_s$	electron spin projection		$E_{m_s} = -g_e\gamma\hbar B_0 m_s$	
$\gamma$	electron magnetogyric ratio		$E_{m_s} = g_e\mu_B B_0 m_s$	
$\mu_b$	Bohr magneton			
$g_e$	electron g-value			

Stable organic compounds usually have a closed electronic shell, i.e. no unpaired electrons and therefore no observable electron spin. Electron spin spectroscopy (ESR) in organic compounds is therefore largely limited to the investigation of reactive intermediates (free radicals and triplet states). Metal centers in metal-ligand complexes often have unpaired electrons and detectable electron spins. In the biological sciences, ESR is therefore a common tool for the investigation of metal centers in proteins or prosthetic groups, and to a minor degree for the investigation of radical enzymes. Common metal centers found in proteins and investigated by ESR are iron and copper (see in table 4.1).

Redox reactions are at the core of many biochemical processes, in particular metabolic pathways and catalytic reactions. By probing the electron spins, ESR allows to directly probe the reaction centers, their oxidation states and some aspects about the local geometry.

### 4.2 Electron Spin Spectroscopy

Electron spin spectroscopy is also known as electron paramagnetic resonance (EPR) or electron magnetic resonance (EMR) and measures the transition frequency between different electron spin states. The energy difference between an electron spin state  $m_s = -\frac{1}{2}$  and  $m_s = \frac{1}{2}$  in a reasonably strong magnetic field of 1 Tesla is  $\Delta E = 1.86 \cdot 10^{-23} J$  corresponding to a frequency of 28 GHz. As opposed to the MHz frequencies

Metal <sup>Oxidationstate</sup>	Valence orbital occupancy	Spin
Cu <sup>I</sup>	3d <sup>10</sup>	spin 0 (diamagnetic)
Cu <sup>II</sup>	3d <sup>9</sup>	spin $\frac{1}{2}$
Fe <sup>I</sup>	3d <sup>7</sup>	spin $\frac{3}{2}$
Fe <sup>II</sup>	3d <sup>6</sup>	spin 2 or 0
Fe <sup>III</sup>	3d <sup>5</sup>	spin $\frac{5}{2}$

Table 4.1: Typical metals, oxidation states, and spin properties of metals in proteins and prosthetic groups.

encountered in NMR, the generation of such GHz frequencies is a major challenge and it is not easy to go to the highest possible magnetic fields and RF frequencies. (Note that common electronics, e.g. computer chips, only reach frequencies of few to few tens of GHz.) This is obvious when we consider the discharge time  $\tau = R \cdot C$  of a capacity  $C$  for realistic device capacities and circuit resistances. With a 1 pF capacity (unrealistically small) and a  $50\Omega$  resistance (typical for HF signal transduction), we find a decay time of  $\tau \approx 50ps$  corresponding to 200 GHz.

ESR is therefore performed in a wide variety of magnetic field strength and with a correspondingly diverse set of RF radiation sources, e.g. "L-band" spectrometers in the 1 GHz range and "W-band" spectrometers in the 100 GHz range. Some spectrometers sweep the RF field frequency, others the magnetic field strength, and an increasing number of experiments is performed with pulsed fields and using fourier transform methods as discussed for NMR spectroscopy.

The excitation frequencies in ESR spectra depend on the total magnetic moment. The energy levels of a bound, unpaired electron are therefore different from that of a free electron primarily due to the electron orbital angular momentum. The corresponding g-factors then differ from that of the free electron ( $g_e = 2.0023$  as given in equation 4.1) and can be expressed as a function of the orbital angular momentum  $L$  and the total angular momentum  $J$ :  $g = 1 + \frac{S(S+1) - L(L+1) + J(J+1)}{2J(J+1)}$ . Please note, that the the orbital angular momentum is rather small for main group elements (S and P orbitals), but can be very large for transition metals (D-Orbitals). In the later case, the transition energies are strongly affected by the surrounding field of the ligands. For example, 6 identical ligands bound symmetrically by the d-orbitals in a transition metal lead to a single transition. A Jahn-Teller splitting (distortion of the structure) or the replacement of one or more ligands can give rise to distinguishable transitions from the  $d_{xy}$ ,  $d_{yz}$ ,  $d_{xz}$ ,  $d_{x^2-y^2}$  and  $d_{z^2}$  orbitals. ESR is therefore a sensitive probe for the local environment of a transition metal and can be used to determine the oxidation state and coordination of metal centers in proteins. An example is the oxidation of poisonous compounds by the iron-porphyrin protein P-450, an enzyme found in the liver. A proposed reaction mechanism, partially based on the result of ESR is shown in figure 4.1.

ESR also observes the coupling of electron to nuclear spins ("hyperfine coupling"), carrying information about the local environment of the electronic spin probe. The information content in the hyperfine coupling is quasi identical to that in NMR spectra, but the electron acts as a local probe only for nuclear spins in close vicinity.

### 4.3 Sources for GHz and THz pulses

ESR spectroscopy in the high frequency bands requires high-frequency high-energy electromagnetic radiation beyond the scope of ordinary electronics. Similar radiation sources are required for Radar operation and for the direct excitation of molecular rotations in small molecules. 1 GHz radiation has a wavelength of approx. 30 cm, we therefore discuss radiation in the cm to  $\mu m$  wavelength regime.

If electronic devices have too high capacities and resistances, the obvious solution is to return to vacuum tubes and use freely propagating electrons. The technology of electron tubes of course predates that of semiconductor electronics.

In a Klystron, the velocity of a beam of electrons in vacuum is modulated by an electromagnetic field.

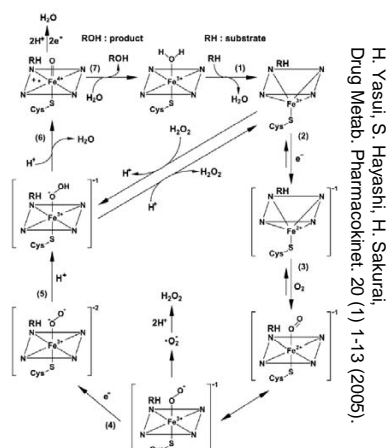


Figure 4.1: Proposed singlet oxidation mechanism of very stable organic compounds (e.g. drugs) by the iron-porphyrin active center in a P-450 enzyme.

The modulated electron beam strikes a catcher cavity from which the signal is extracted. A Klystron can be used to amplify a signal as shown in fig. 4.2. In a different Klystron setup, the lateral electron velocity is modulated, e.g. by deflection of the electron beam. This can be used to bunch the electrons and change the frequency, e.g. doubling the frequency by compressing the electron bunch by a factor of two. Many variations of the Klystron have been developed, but most are now redundant due to advances in semiconductor electronics.

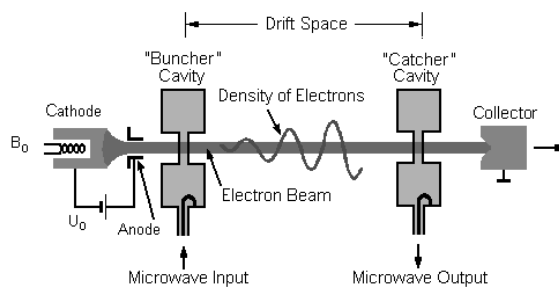


Figure 4.2: Two cavity Klystron amplifier: Electrons travelling through a vacuum tube are deflected in a buncher cavity. During the further propagation of the electrons, the deflection amplitude increases and when the electrons impact on the deflector they induce a strongly amplified signal into the catcher cavity. Graphic reproduced from Wikipedia.

Another very common device creating microwaves is found in commercial microwave ovens and is called magnetron. In the magnetron, electron travel from a wire cathode to the anode walls of an evacuated chamber. A magnetic field forces the electrons into circular trajectories. To obtain RF fields, the electrons must fly past in bunches to induce image charges into an antenna. This is achieved by creating hollow resonators in the anode wall: the electrons flying past the resonator cavity induce currents into the resonator, which in turn help to modulate the electron current into bunches. The frequency is determined by the dimensions of the resonator cavities, and in a common microwave the frequency is tuned to a rotational absorption band of water and therefore heats water and water containing samples.

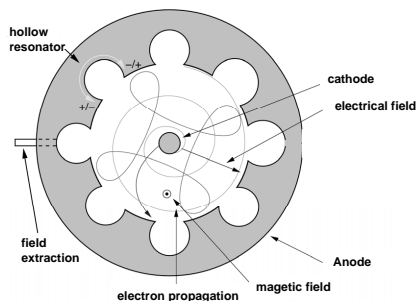


Figure 4.3: Schematic depiction of a magnetron. Electrons travelling from the anode to the cathode are forced on circular orbits by a magnetic field. Interaction with the image charges in the cathode wall lead to a bunching of the electrons. The propagation time of the image charges around the hollow resonators define the resonant frequency of the magnetron and the corresponding frequency can be extracted with an antenna. Graphic adapted from Wikipedia.

## 4.4 Bioinorganic Chemistry

The important role of metal centers in biological systems led to the development of a new field "bioinorganic chemistry". Metal centers are predominantly found in the active centers of catalytic proteins (e.g. iron in P-450) or in crucial structural elements responsible for specific binding (e.g. the "zinc finger" for DNA binding). A number of metals and their catalytic function in biology is listed in ??.

The term bioinorganic is an oxymoron, because the term inorganic chemistry was created specifically to distinguish the non-organic chemistry from that found in organic matter. So a short trip back in time is in order – to amuse ourselves about the chemical specification, and to wonder at the astonishing development of the chemical sciences in the human history.

Since some 100 years, chemists distinguish organic chemistry from biochemistry. This distinction has its roots in the discovery of biological macromolecules, namely proteins, DNA and RNA, which for a considerable time could not be synthesized. The discovery of the DNA structure by Watson and Crick in 1953 may be considered as a key event in biochemistry and opened the way towards a chemical understanding and the synthesis of corresponding molecules. Nowadays, de novo synthesis of a complete virus has been demonstrated and the synthesis of large DNA molecules is routine - hence the distinction between organic and biochemistry is no longer obvious.

Both, organic and inorganic chemistry grew out of alchemy in the 18th and 19th century. The study of metals and salts allowed many chemical transformations and led to the isolation of an increasing number of elements and the recognition of quantitative laws governing chemical transformations. But this "inorganic chemistry" did not reproduce the organic matter of everyday life. It seemed therefore obvious, that the god-given organic chemistry would be fundamentally different from the inorganic chemistry which man controlled. With the urea synthesis in 1828 this distinction was shown to be false, but the nomenclature remained.

A good part of the alchemists in medieval times tried to create gold from lesser metals. We now know that this effort had to fail, but only due to technological limitations of the time. Humans now make gold from many metals, e.g. from mercury via the nuclear reaction  $^{198}\text{Hg} + h\nu (6.8 \text{ MeV}) \rightarrow ^{197}\text{Hg} + \text{neutron}$   
 $\xrightarrow{\tau_{1/2}=2.7d} ^{197}\text{Au}$ .

Chemistry goes back much further, of course, and if you are so inclined you might see the heyday of chemistry in the iron or bronze age when the smelting of metals and alloys revolutionized human life.

Metal ion	Catalytic function
Mg <sup>2+</sup> (Ca <sup>2+</sup> )	Easy hydrolyses, phosphate transfer, (light capture, chlorophyll)
Zn <sup>2+</sup>	Difficult hydrolyses; hydride transfer
Mn <sup>2+</sup>	O <sub>2</sub> -generation; some hydrolyses
Ni <sup>n+</sup>	H <sub>2</sub> activation; urea hydrolysis; F-430 enzymes
Co <sup>n+</sup>	B <sub>12</sub> -enzymes for transformation of diols and other simple saccharides (ribose to deoxyribose)
Cu <sup>n+</sup>	Oxidation of phenols, amino acids, sugars; e-transfer (outside cytoplasm)
Fe <sup>n+</sup>	e-transfer; oxidation (hydroxylation); H-transfer (inside cytoplasm) N.B. haem units
Mo <sup>n+</sup>	Oxygen atom transfer (pterin cofactor); N <sub>2</sub> activation

Table 4.2: Major catalytic functions of metal ions in enzymes (according to R.J.P. Williams, Chem. Commun. 1109 (2003)).



# Bibliography

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