Summary
Electron Spin Resonance (ESR) or Electron Paramagnetic Resonance (EPR) are increasingly common techniques for determining the presence of free radicals in materials. In this application note we discuss the basic theory and background behind these techniques, and in particular their usage in quantifying residual free-radicals in biomedical polyethylene.

Background
The orthopaedic industry has recently substantially converted their use of ultra-high molecular weight polyethylene (UMWPE) from a conventional consolidated resin to a highly crosslinked resin. This strategy trades some toughness for greatly enhanced wear properties and opens the potential for devices made from this material to expect an implant life well in excess of 20 years. However, the most common crosslinking process is through ionizing irradiation, either electron beam or gamma ray, which forms crosslinks through the recombination of temporarily generated unpaired free electrons Figure 1. These unpaired electrons generally safely recombine to form crosslinks, terminal groups or other inert structures. However, in some cases these free electrons do not recombine and are free to react in the future. Frequently this reaction is with oxygen, causing premature oxidation of the polymer and loss of mechanical properties.
Luckily the industry has a number of techniques for minimizing the risk of these radicals including thermal treatments (annealing and remelting) and the addition of antioxidants (Vitamin E and AOX®). However, determining the presence of these unpaired electrons is an important quality control step indicating that these minimization steps have worked, and a valid indictor of potential future oxidation issues with the materials.

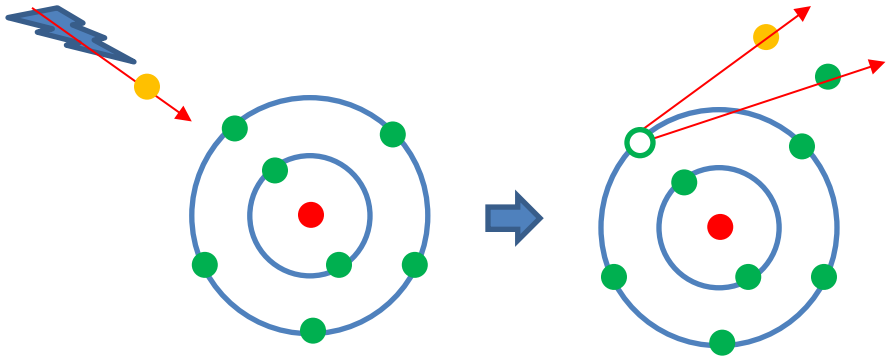

Figure 1: Formation of unpaired electron after impingement by a high energy electron.

Theory
An unpaired electron can form in virtually any material and need not be the result of irradiation. However, in the case of polyethylene, the incoming high energy electron cleaves a bond (carbon-carbon, or carbon-hydrogen) and forms two radicals (see Figure 1). During this process the incoming electron dislodges an electron from the bond, and loses energy at the same time, while both electrons carry on deeper in to the material. The number (flux) of electrons therefore increases, but as they scatter and lose energy they eventually reach a threshold energy beyond which they are insufficiently energetic to cleave any more bonds. In polyethylene, if this occurs at the hydrogen, the resulting radical can recombine, or interact with an adjacent chain to form a crosslink. If it is the carbon-carbon bond that is split, these can either benignly recombine or stay permanently disconnected, resulting in chain scission. It is the balance between scission and crosslinking that influences how radiation resistance polymers are. These radicals can be mobile, essentially hopping along the polymer chain, and this allows them to either recombine with a neighboring atom on the backbone, reverting the chain back to “normal” or with an adjacent chain, generating a crosslink, or not reacting with anything and therefore living indefinitely. It is these long-lived electrons that are the source of potential oxidation in the future, usually through a complex cascade of reactions known as the Bolland cycle.

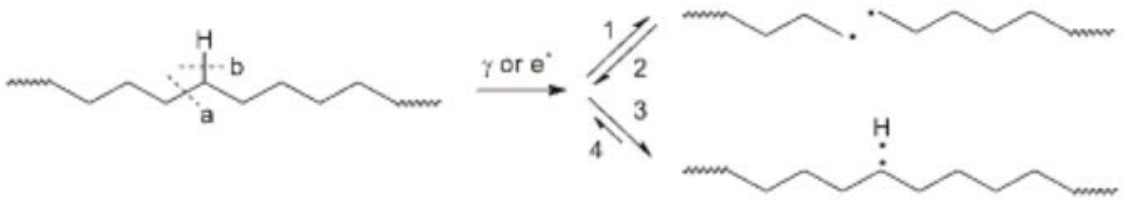

Figure 2: Primary radical generation mechanism in UHMWPE (from Kurtz, UHMWPE Biomaterials Handbook)

Basics of EPR

A free electron moves when a voltage or magnetic field is applied. The unpaired electrons on atoms are no exception, although because they reside within the structure of an atom, they have limited mobility. In reality, they are free to sit within the orbital of the atom at specific quantum energies. However every electron has a "spin" that can be either an "up" or "down" spin value of a half. Normally, with no magnetic field present, electrons of either spin have the same energy, but when a magnetic field is applied, the electron aligns with the field and there is now an energy difference between the two spin states. The difference in energy between these states is shown schematically in Figure 3.

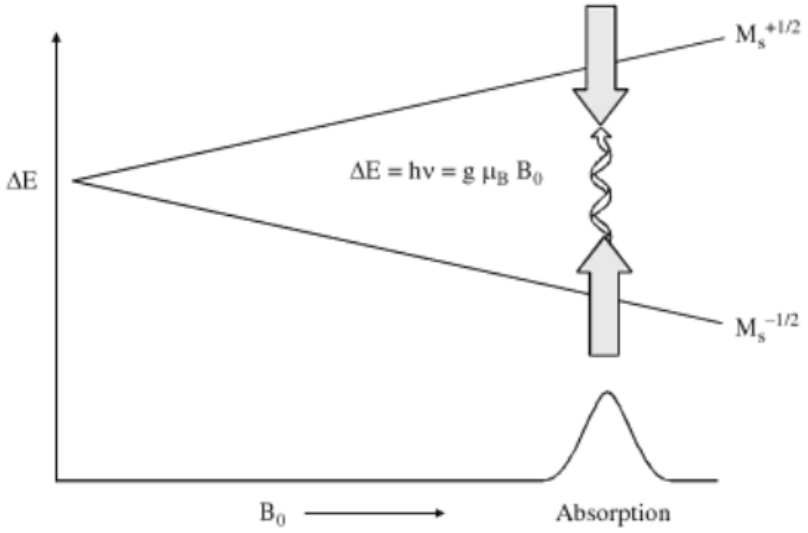

Figure 3: Energy differences due to magnetic field of opposed spin electrons (from Quantitative EPR, Eaton et al.).

This energy difference is known as the Zeeman effect. Thus, at any particular magnetic field a specific atomic electron has a distinct energy resonance at a specific frequency. An analogy would be a children's play park swing where the length of the swing ropes is the magnetic field, and the "pumping" of the child's legs is the energy input. It is only when the pumping frequency coincides with the swing length that the child is effective and makes the swing move. Conventionally (due to technical limits) the pumping frequency is held constant, and the magnetic field is scanned to find distinctive resonances indicative of specific atoms.

Hyperfine interactions

An atom with an unpaired electron in space has a simple, single, distinctive resonance that is indicative of the atom examined. However, neighboring atoms in a molecule often have magnetic moments that act to induce a magnetic field on the atom. Since this can be aligned, or mis-aligned with the imposed magnetic field it acts to "split" available energies. Adding more atoms with magnetic moments further splits these energies. This splitting is known as hyperfine interactions and results in distinctive EPR spectra that "fingerprint" the atom and adjacent atoms. Thus EPR can resolve the difference between different radical structures and can tell to some extent reaction pathways. Irrespective, the total energy absorbed under specific EPR conditions is related linearly to the number of

unpaired electrons present, in other words the number of free radicals in the material. Thus the EPR spectrum is proportional to the number of radicals present in a material.

Quantitative EPR

One of the great challenges in using EPR as a routine quantitative tool is the question of how to calibrate the instrument. Since the resonance recorded by the EPR is dependent on the instrument conditions, not least the selected magnetic field, a suitable reference material has to be used to calibrate the reported data against a known standard. A further complication is that whatever material is selected has to be reproducible and stable, a particular challenge for free radicals. In the 1970s a standard was generated by NIST composed of Ruby (SRM-2601) with known radical concentrations but this was discontinued. Aside from that, the Chromium ions present in the NIST standard do not have the same center field as polymer-based free electrons and the standard was not produced at a concentration (~4×10^{18} ions/g) of value to the orthopaedic industry where generally free radical concentrations are less than 10^{16} spins/g. A number of materials have been used for reference purposes, such as DPPH (diphenyl-β-pictryl hydrazil), nitroxide radicals, and pitch (tar). Of the many compounds tried, a stable nitroxide radical (TEMPOL – 4-hydroxy tempo) is a useful material for developing a calibration curve since it is cheap, and readily dissolve in organic solvents.

Calculation

EPR spectrometers generally report a distinctive spectrum (see Figure 4) that can be regarded as a "fingerprint" for the radicals present in the material. In reality this spectrum can be complex, since it represents the superposition of all the spectra resulting from the radicals present. With simple materials, where perhaps only one radical is present, the peak-to-peak height of the spectrum can be used as a quantification of the number of radicals present. However, in more complex systems this approach can be deceptive since the radical spectrum tends to be distributed across a broader magnetic field (see figure including different radicals). In this case a more absolute calculation method is required. The EPR spectrum is actually the derivative of the resonance peak of the radical. Therefore if one performs a double integral on the EPR spectrum would arrives at the area under the resonance peak, or the total energy absorbed in the resonance. This value is then proportional to the number of radicals present, irrespective of their type or concentration.

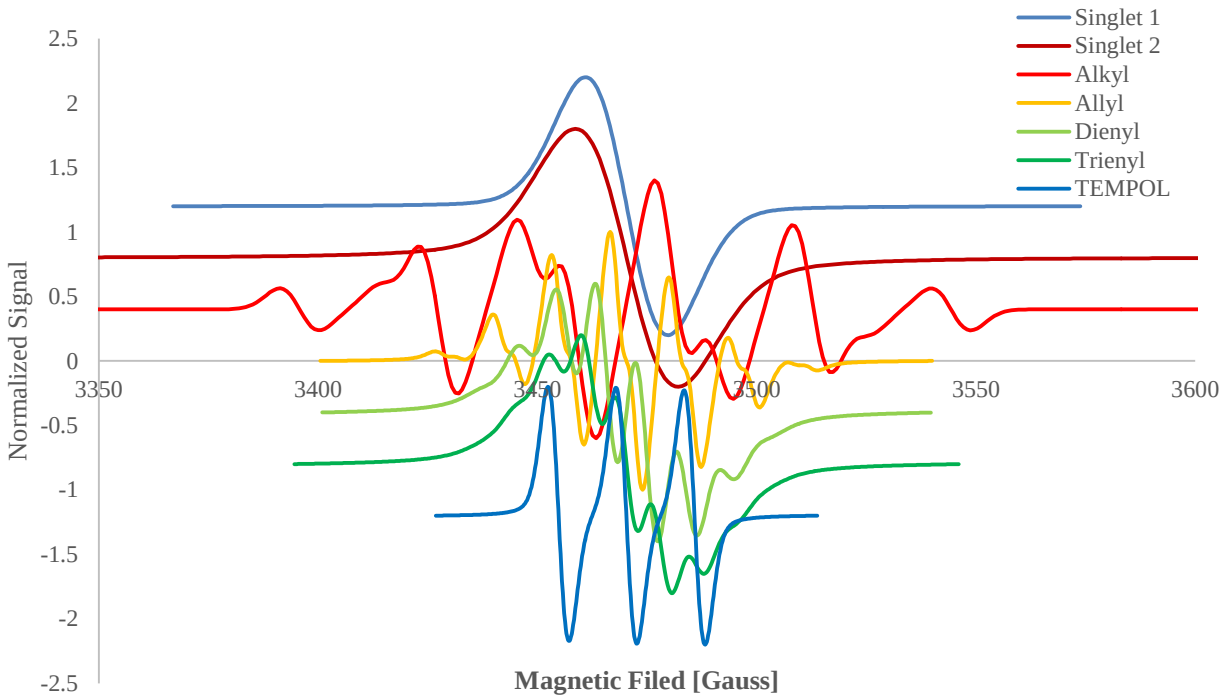

Figure 4: Representative ESR spectra for different radicals in irradiated polyethylene. Scales normalized and offset for clarity

Calibration

There are a number of materials that may be suitable for calibration of an EPR system, but in this document we discuss only one, TEMPOL. This material (see figure) has one stable nitroxide radical per molecule. Thus a 1 μM solution has 6.023×10^{17} radicals, or unpaired electrons (spins). TEMPOL dissolve readily in toluene and as a result, 100 μL of solution has 6.023×10^{13} spins. It is therefore relatively easy to build a calibration curve using fresh solutions in the range anticipated for orthopaedic implants. At the same time, weak pitch is frequently used as a signal to noise standard in EPR spectrometers, and using this material allows a fixed reference point to be used to validate the constructed calibration curve. An example of a calibration curve is shown in Figure 5. Since the TEMPOL radical is nitroxide based, it is centered at a slightly different magnetic field location to radicals present in polyethylene, but this does not change the general calculation method.

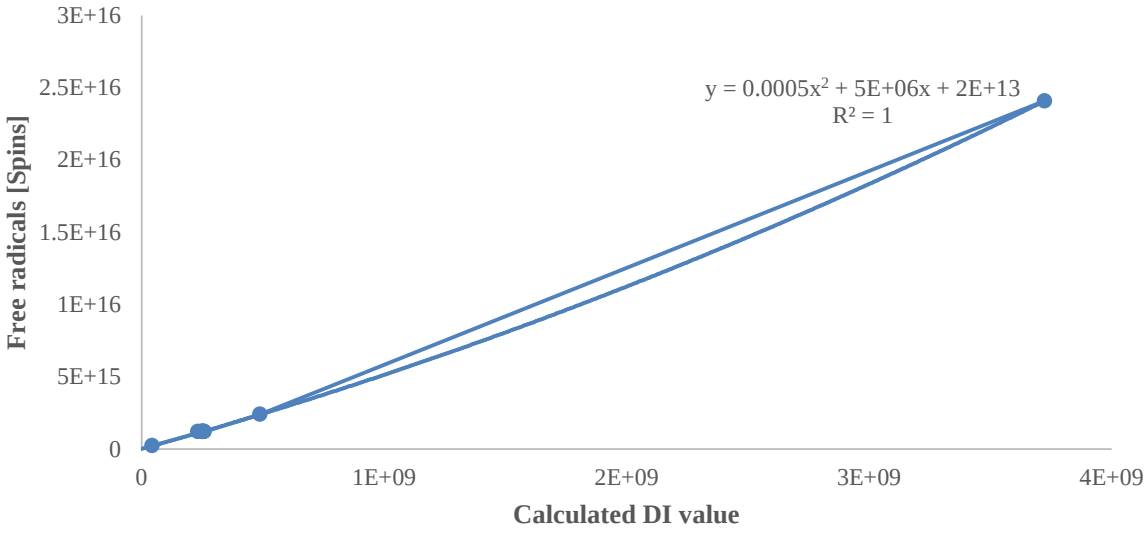

Figure 5: Calibration curve for ESR using TEMPOL.

Results

Four different samples were analyzed using ESR to determine free-radical concentrations (see Figure 6). In each case the double integral under the curve was determined and the effective total spins calculated from the calibration curve. The determined spins/g are provided in Figure 7.

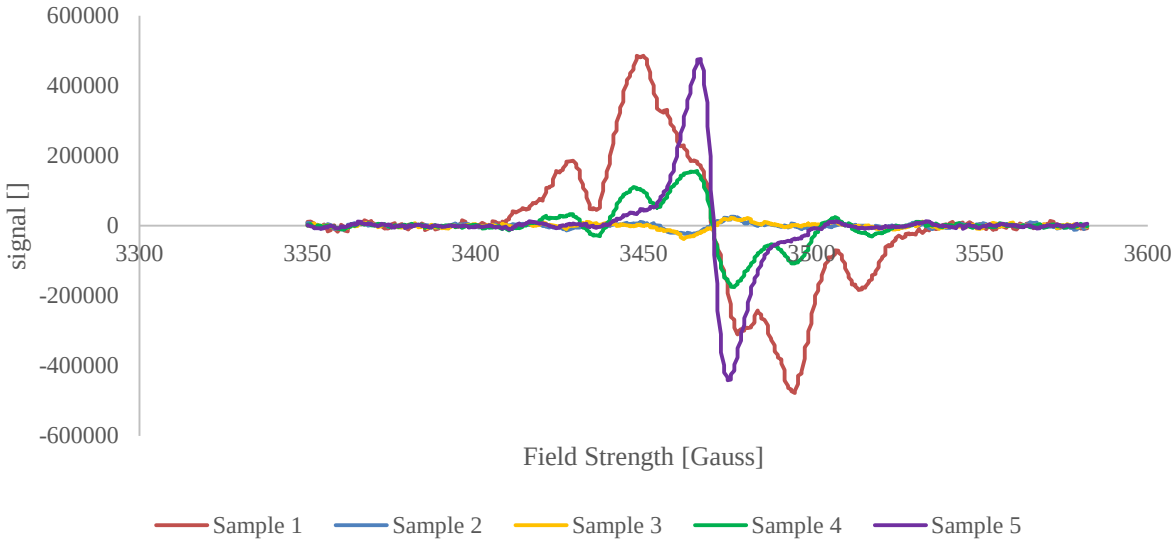

Figure 6: ESR spectra for four different specimens with different radiation histories. Exact conditions proprietary.

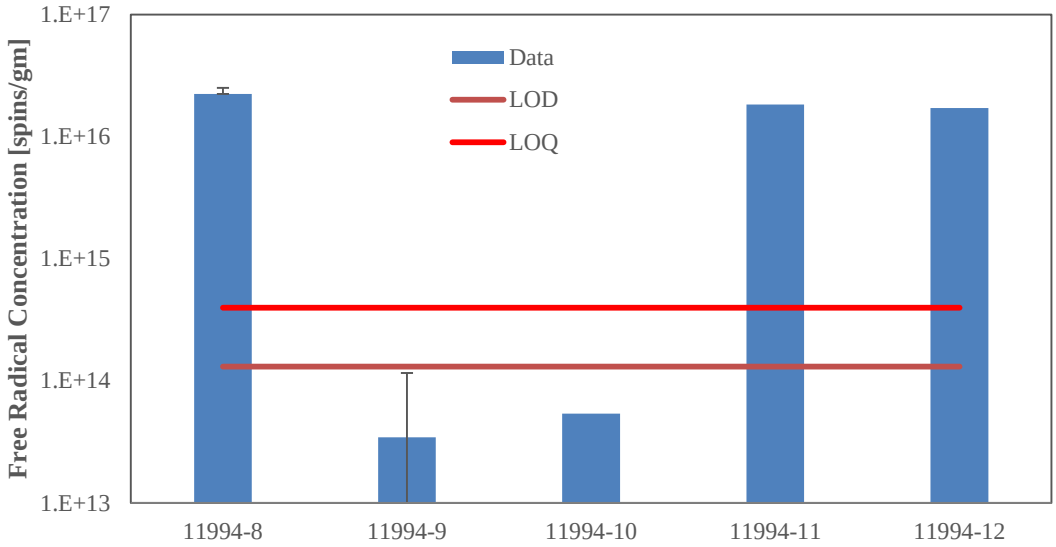

Figure 7: Determined free radical concentration from the samples presented in Figure 6. Error bars (on sample -8 and sample -9) are one standard deviation.

Other uses

Although the emphasis in this document is in the use of EPR in the orthopaedic industry, which are primarily carbon centered, the technique has substantial application in a number of different industries. For example, because it is sensitive to unpaired electrons, it can be used to quantify the presence of transition metals, such as vanadium or molybdenum in engine oils, or in sea water. It has also been used as an assay in drug research, to examine radicals in cigarette smoke, protein redox or even beer stability. It is frequently used, in conjunction with Alanine as a radiation dosimeter, and it can be used on foods or physiological fluids as an *in vivo* marker for received dose. Thus the selectivity of EPR to only unpaired electrons frequently gives it sensitivity to specific species in complex systems that cannot be obtained by other techniques.