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Comparative Analysis of Long-lived Free Radicals in Medical Grade Ultra High Molecular Weight Polyethylene (UHMWPE) in Absence and Presence of Vitamin E

Afsana Sharmin

COMPARATIVE ANALYSIS OF LONG-LIVED FREE RADICALS IN MEDICAL
GRADE ULTRA HIGH MOLECULAR WEIGHT POLYETHYLENE (UHMWPE) IN
ABSENCE AND PRESENCE OF VITAMIN E

by

Afsana Sharmin

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Abstract

In this research, free radical analyses were performed using electron spin resonance (ESR) technique for two groups of orthopedic ultra-high molecular weight polyethylene (UHMWPE). In the first group, Group A, two UHMWPE resins, GUR 4150 and Hylamer (Himont 1900) were investigated. These materials were provided by the Medical-Device Industry members of the NSF-Industry/University Center for Biosurfaces (IUCB), a partnership program between the State University of New York at Buffalo (SUNY Buffalo) and the University of Memphis (1997-2011). The present study is a part of the ongoing research project which was undertaken in 1998. The long-term effects of temperature (23°C, 37°C and 75°C) upon free radicals in inert environments and in the presence of oxygen (open air) were investigated. ESR spectra recorded in the present study (t = 18 years) indicate the presence of oxygen-induced polyenyle radicals in all samples aged at 23°C or 37°C in open air, with a greater concentration at 23°C. At 75°C in open air, there were no detectable radicals. While the total concentration of radicals in all samples decreased by two orders of magnitude in 18 years, the present data showed that, at 23°C, it decreased less in Hylamer than in GUR 4150, by a factor 2/3. In inert environments, both GUR 4150 and Hylamer showed similar concentration (slightly higher concentration at 23°C than at 37°C). Compared to GUR 4150, Hylamer showed the presence of 0.6, 0.9, 1.0, 1.1 and 1.3 times more radicals at 23°C (air), 37°C (air), 23°C (inert), 37°C (inert) and 75°C (inert), respectively. In terms of radical type (alkyl, allyl, polyenyle, dienyle and trienyle), only a minor difference was found between GUR 4150 and Hylamer.

For the second group, Group B, free radical analyses were performed on vitamin E-containing UHMWPE (GUR 1020) following post-gamma (30 kGy) shelf aging for 10 years at

23°C (room temperature) in open air. Vitamin E (α-tocopherol) was consolidated with GUR

1020 at a concentration level varying between 0% (no vitamin E) and 20% in 2006. The ESR

measurements conducted immediately after gamma irradiation in 2006 showed a decrease in free

radical concentration by an order of magnitude due to vitamin E. In 10 years (2016), the radical

concentration was reduced by another order of magnitude; i. e. from $\sim 10^{17}$ to $\sim 10^{15}$ radicals per

gram in all samples, with or without vitamin E. No measurable difference between radical

concentrations was found as a function of vitamin E .

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Chapter 1

Introduction and Background

1.1 Background

Ultra-high molecular weight polyethylene (UHMWPE) is a frequently used biomaterial of bearing components in total-joint arthroplasty. It has extremely high molecular weight, low degree of crystallinity, high tensile strength, high impact resistance and chemical inertness [1]. While some drawbacks such as creep may limit the application of UHMWPE, many research studies have been carried out to improve the mechanical properties of medical grade UHMWPE [2,3].

Gamma irradiation has been proven to be a reliable and effective method for crosslinking and sterilization of UHMWPE [4,5]. The crosslinking of polyethylene (PE) is achieved by the recombination of free radicals created by the gamma-irradiation, primarily in the amorphous phase. Some free radicals may become trapped in the crystalline phase [6], and may initiate oxidation within the UHMWPE components by reacting with diffused oxygen, and therefore lead to degradation [7]. However, vitamin E (α-tocopherol) can be added to the UHMWPE, and has been found to improve oxidation resistance of UHMWPE [8-10] by scavenging free radicals.

The primary reason for failure of implants due to UHMWPE is the wear of the UHWMPE components. This wear is primarily due to oxidation, with occurs slowly over time, and may be limited by the addition of vitamin E to the UHMWPE components. As free radical content can be considered as indicators to potential oxidation, it is important to study free radicals in UHMWPE and how they change over time in the long run, as well as effects that vitamin E may have on such free radical activity.

1.2 Biomaterials

Biomaterials are natural or synthetic in origin, used to replace any tissue or organ, and treat any function of the body. They need an optimal combination of mechanical properties with appropriate design and manufacturability according to application, and possess biocompatibility such that the interaction between implant and host biological cell tissue should not induce any inflammatory response or formation of unusual tissues [11]. The two main group of biomaterials are metal and non-metal. Metal materials are mainly used to build load bearing implants, wheras nonmetals are primarily comprised of three categories: Polymer, ceramic and composite. Although the use of ceramics [12] and composite materials are aiding the improved properties of orthopaedic or dental implant, polymeric biomaterials are used when complex forms and high flexibility are required.

A polymer is a large molecule composed of many repeating monomeric subunits. It is generally classified into three different categories depending on the source: natural, synthetic and bio-inspired polymers. Natural polymers are obtained from natural sources including both plants and animals, whereas synthetic polymers are made by chemical process in laboratories, and bio-inspired polymers are synthesized to mimic a naturally occurring polymer. Among all three types, synthetic polymers have achieved great attention for the production of medical implants because of their ease of production, availability and versatility of manipulation [13, 14]. Polymers are also classified into two categories depending on the nature of biodegradability: Biodegradable and non-biodegradable polymers. Biodegradable polymers are brittle in nature and decompose over time, reacting with microorganisms or enzymes [15]. Non-biodegradable polymers are made of long molecular chains of carbon and hydrogen atoms with strong

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interatomic bonding, which makes it very difficult for microorganisms to break bonds and decompose.

Polymeric biomaterials are rapidly replacing other materials such as metals, alloys or ceramics because of their versatile range of application. The flexibility of polymers is an important factor to provide ease of manufacturability that is required to produce various shapes of medical devices. Moreover, polymeric materials have strong biodegradability which allow implants to degrade at a rate matching new tissue formation, eliminating the need for second surgical event for removal [16].

1.3 UHMWPE

Polyethylene is one of the synthetic non-biodegradable polymer which is formed from ethylene (C_2H_4) gas of molecular weight 28. The generic chemical formula for polyethylene is $-(C_2H_4)_n-$, where n is the degree of polymerization. Polyethylene is grouped into several categories, depending on molecular weights and chain architectures: Low Density Polyethylene (LDPE), Linear Low Density Polyethylene (LLDPE), High Density Polyethylene (HDPE) and Ultra High Molecular Weight Polyethylene (UHMWPE).

UHMWPE is very high density polyethylene, consisting of >200,000 ethylene repeated units, with average molecular weight of up to 6 million g/mol [17]. It is significantly more abrasion resistant and wear resistant than HDPE. UHMWPE has been widely used as the acetabular cup component of artificial hip joints [18], and in knee replacements [19], because of its excellent wear resistance and biocompatibility. Approximately one million UHMWPE components are implanted yearly worldwide.

GUR Resin (Two types: GUR 4150 and GUR 4120) and 1900 Resin (Designated as Himont 1900 or Hylamer) are two major types of medical grade UHMWPE which are commonly consolidated to form UHMWPE components of artificial hip and knee joint replacements.

1.3.1 Commercial production of UHMWPE resin

The commercial production of UHMWPE was started with the production of two major grades: GUR Resin and 1900 Resin. The company 'Ruhrchemie AG' (Oberhausen, Germany) first produced GUR Resin in 1950s. The first commercial products were distributed as 'GUR 415', 'GUR 412', and 'CHIRULEN P', and distributed across the USA and Europe in for both general and orthopedic applications. In 1992, the company 'Ruhrchemie AG' was renamed to 'Hoechst', where the products were redesignated as 'GUR 4150' and 'GUR 4120', through adding a zero as a last digit. Later, the products were converted again into 'GUR 4150 HP' and '4120 HP', where HP indicates 'High Purity'. In 1998, all the resin products were regrouped into the naming conventions of 'GUR 1150, 1050, 1120 and 1020'. During 2002, the redesignated producer 'Ticona' discontinued the production of GUR1120 and 1150.

On the other hand, the company Hercules Powder (Wilmington, DE) produced 'Hi-Fax 1900' (one of the grades of 1900 Resin) with molecular weights ranging from 2 million to 4 million during the 1960s for the worldwide orthopedic market. The 'Hi-Fax 1900' had been redesignated as 'Himont 1900' during 1983 under the joint venture of the company Hercules and the Montedison (Italy). The 'Himont 1900' was continued on production by the joint company Montell Polyolefins, which was later merged into Basell Polyolefins and continued until 2002. The 'Himont 1900' resin is also known as Hylamer.

Currently, only two grades of GUR Resins, GUR 1050 and 1020, are in commercial production by the company Ticona. [17]

1.3.2 Significant of resin nomenclature

The nomenclature of UHMWPE resin has significant meanings. As an example, the significance of nomenclature of GUR 1050 resin is mentioned below [17]:

'GUR': "Granular" "UHMWPE" "Ruhrchemie"

1st digit: Loose bulk density of the resin in g/l, so "1" corresponded to a bulk density of more than 100g/l.

2nd digit: Presence (1) or absence (0) of calcium stearate

3rd digit: Related to the average molecular weight of the resin

4th digit: Hoechst internal code designation

1.3.3 Polymerization: ethylene gas to UHMWPE powder

The company Ruhrchemie (Oberhausen, Germany), which is currently known as Ticona, started the first commercial polymerization of UHMWPE in 1955 using the Ziegler process. The main ingredients for producing UHMWPE powder are ethylene gas (a reactive gas), hydrogen, titanium tetra chloride (the catalyst), and calcium stearate (additive act as scavenger for residual catalyst). During polymerization, the catalyst produces trace impurities of titanium, aluminum and chlorine, which are minimized by additive calcium stearate. The trace levels of calcium stearate and the ash content depend on the storage and handling of the powder after polymerization. The polymerization takes place in a solvent used for mass and heat transfer. Figure 1 shows the polymerized powder of UHMWPE. [17]

Figure 1: Polymerized UHMWPE powder

1.3.4 Polymerized powder to orthopedic implant

Since UHMWPE does not flow like lower molecular weight polyethylene when raised above its melt temperature, the consolidation process of UHMWPE requires the proper combination of temperature, pressure and time. Compression molding, ram extrusion, hot isostatic pressing, are typical conversion methods to produce commercially available consolidated UHMWPE. A machining step is required to shape the consolidated UHMWPE into orthopedic implant components, except for the direct compression molding technique. The UHMWPE components for total joint replacements are then packaged and sterilized before distribution to the external market [17]. An example of an UHMWPE component (acetabular cup) is shown in Figure 2.

Figure 2: Acetabular cup fabricated from UHMWPE
comprises the articulating surface of a total artificial hip.

1.3.5 Structure and properties of UHMWPE

UHMWPE is an extremely high density polyethylene, consisting of 200,000 ethylene monomeric units. The UHMWPE molecules are made of very long hydro-carbon polymeric chains. Although the intermolecular bonds are relatively weak due to van der waals interactions, overlaps can exist between the long molecular chains, which allow transfer of the large shear forces from molecule to molecule [17]. The polymeric and molecular structures of UHMWPE are shown in Figure 3 (a) and 3 (b).
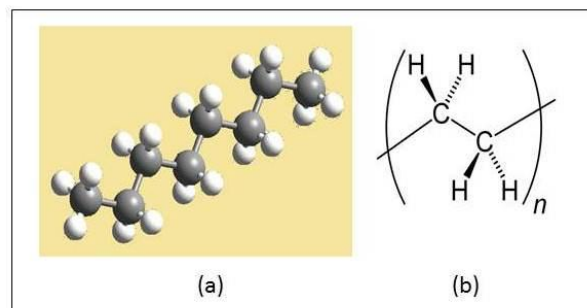
Figure 3: (a) Polymeric structure (b) Molecular structure of UHMWPE

The average physical properties of UHMWPE are shown in Table 1 in comparison with HDPE (High Density Polyethylene). [17]

Table 1: Physical properties of UHMWPE compared to HDPE

Property	High Density Polyethylene (HDPE)	Ultra High Molecular Weight Polyethylene (UHMWPE)
Molecular Weight (10^6 g/mol)	0.05-0.25	2-6
Melting temperature (^{o}C)	130-137	125-138
Poisson's ration	0.40	0.46
Specific gravity	0.952-0.965	0.932-0.945
Tensile modulus of elasticity* (GPa)	0.4-4.0	0.8-1.6
Tensile yield strength* (MPa)	26-33	21-28
Tensile ultimate strength* (MPa)	22-31	39-48
Tensile ultimate elongation* (%)	10-1200	350-525
Impact strength (J/m of notch; 3.175 mm thick specimen)	21-214	>1070
Degree of crystallinity (%)	60-80	39-75
*Testing conducted at 23°C		

1.3.6 Comparison between GUR resin and 1900 resin

The two major types of orthopedic UHMWPE (GUR Resin and 1900 Resin) can be distinguished depending on the trace level of residuals and ash contents during polymerization. The product requirements for both type of resin specified by American Society for Testing and Materials (ASTM) standard F648 and ISO standard 5834-1 are shown in Table 2. [17]

Table 2: GUR resin and 1900 resin product's requirement

Property	Requirements	
Resin Trade name	GUR 1020 & 1050	1900H or Hylamer
Producer	Ticona. Inc.	Basell polyolefins (Now discontinued)
Ash, mg/kg, (max.)	150	300
Titanium, ppm, (max.)	40	150
Aluminum, ppm, (max.)	40	100
Calcium, ppm, (max.)	50	50
Chlorine, ppm, (max.)	20	90

GUR Resin and 1900 Resin can also be distinguished based on the molecular weight and particle morphology. Some reported physical characteristics of two different resin are shown in Table 3. [17]

Table 3: Physical difference between GUR resin and 1900 resin

Resin Type	Average Molecular Weight ($10^6 \ g/mol$)	Mean Particle Size (μm)	Morphology
GUR Resin	3.5-6	140	Lamellar crystalline
1900 Resin	>4.9	300	Spherulitic crystalline

The differences mentioned above between GUR Resin and 1900 Resin can influence the formation of primary radicals upon gamma irradiation, the recombination of radicals, and the long-term oxidation reaction of residual radicals.

1.4   Gamma sterilization

Since medical devices and tools must be sterilized before use, various sterilization techniques have been brought under consideration. The sterilization techniques vary depending on the material of device and their application purpose in medical industry. Some sterilization techniques may not be as effective as others. Autoclave is one sterilization technique, which is mainly used for metal instruments. However, many of the polyethylene components are unable to withstand the high temperature and pressure used in this technique. [20] Ethylene oxide (EtO) is another low temperature sterilization method. Initially, it was thought that EtO was so volatile that it was incapable of leaving a residue on treated products. However, it was discovered that EtO reacts with moisture and chloride ions to form ethylene glycol and 2-cholorethanol, a non-volatile toxic residue. [20]

Therefore, in comparison with other two techniques (Autoclave and EtO), gamma radiation has been widely accepted as a means of effective and reliable sterilization method in the medical device industries due to its penetrating ability during sterilization, single variable to control exposure time, and overall lower cost. Moreover, weight loss measurements revealed that the gamma-sterilized UHMWPE components exhibited lower wear rate than that by EtO-sterilized [21]. Inspite of several advantages, gamma radiation sterilization has also its drawbacks. The strong penetrating gamma radiation breaks the bond in polymer chains and creates the free radicals during the sterilization process [20].

1.5   Free radicals

The stable atoms or molecules contain even number of electrons, where the electrons remain in pair of two. The ionizing radiation can knock out electrons from their pairs, which leaves unpaired electrons. The atoms or molecules with unpaired electrons are known as "free

radicals," which may be very unstable and highly reactive. Once the free radicals get produced, they always tend to become stable by taking off the electrons from other stable molecules.

Free radicals usually attack neighboring molecules, causing the affected molecules to then become free radicals, which then attack the next stable molecules and a chemical chain reaction of radical production occurs [22]. Normally, the body can withstand a modest quantity of free radicals, but if antioxidants are unavailable in the body, or if the free-radical production becomes excessive, damage can occur. These free radicals can fuel oxidation inside the human body and cause a range of disorders including cancer, arthritis, or diabetes [23].

In case of medical grade UHMWPE, the production, as well as the chain reaction of free radicals, increases the potential for degradation of the UHMWPE components. Since gamma sterilization creates free radicals, a wide range of research has been conducted to minimize the number of free radicals. Many researchers have reported that gamma radiation in air provides environment conducive to oxidation of UHMWPE which affects the material properties [21, 24]. So, gamma sterilization in inert environments was then carried out to limit oxidation. It is reported that, although the gamma radiation makes the UHMWPE more susceptible to oxidation, the inert environment provided by Argon or Nitrogen may counter this effect of the gamma radiation [21].

1.5.1 Free radicals in UHMWPE

The free radicals which are produced in UHMWPE during gamma irradiation are known as primary radicals. The reported primary radicals are: [25]

Alkyl: $—CH_2 —•CH —CH_2—$,

Allyl: $—CH_2—CH = CH— •CH—CH_2—$

Polyenyl: $—CH_2—HC• — (CH = CH)n— CH_2—$

For UHMWPE stored in air, the primary radicals can be recombined over time. However, some trapped radicals in the crystalline region convert to short-lived secondary or tertiary radicals, which are precursors to the oxidation process in UHMWPE. [25]

The secondary or tertiary radicals are:

Peroxy: —CHO_2 •—,

Alkoxy: —CHO•—

Vinyl: >CH=•CH_2—.

In addition, in presence of oxygen, two types of radicals (R1 and R2), shown below, can remain trapped in the irradiated UHMWPE for a very long time [25].

R1: — •CH— [CH = CH—]$_m$— , polyenyl with m > 3

R2: —·OCH— [CH = CH—]$_m$— ,Oxygen induced radicals (OIR) with m =2 or 3 (dienyle or trienyle).

The ESR spectra of different radicals which exist in gamma irradiated UHMWPE are shown in Figure 4.
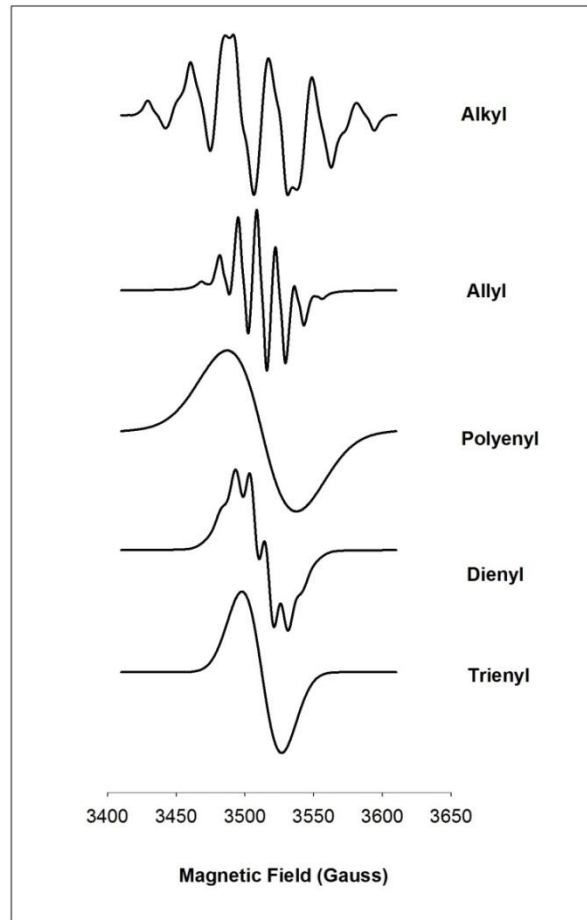
Figure 4: ESR spectra of primary radicals in UHMWPE

1.6 Vitamin E (α-tocopherol)

Vitamin E (α-tocopherol) is an antioxidant which has been proved to improve the oxidation resistance and fatigue strength of irradiated UHMWPE, as compared to post-irradiation melting [26]. When vitamin E is added to UHMWPE, it donates hydrogen to free radicals of UHMWPE in order to stabilize them. However, in this process, vitamin E becomes a free radical itself (tocopheryl radical) - although more stable, relative to radicals of UHMWPE. The structure of the vitamin E (α-tocopherol) molecule and its radical are shown in Figure 5.
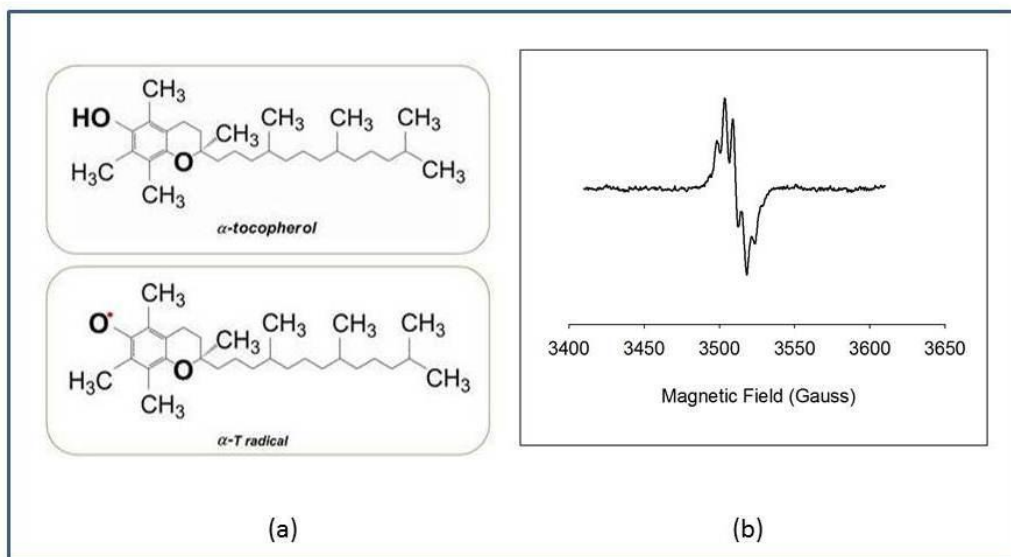
Figure 5: (a) Molecular structure of the α-tocopherol molecule and the α-tocopheroxyl radical (αTO•). (b) ESR spectrum of the gamma irradiated αTO• radical.

1.7   Electron Spin Resonance (ESR)

Electron Spin Resonance (ESR) is the only technique for the direct detection of free radicals [27-29]. The ESR technique is also known as Electron Paramagnetic Resonance (EPR). The theory behind ESR states that each unpaired electron has a magnetic moment associated to its spin. The magnetic moments align either parallel or antiparallel with specific energy in response to the external magnetic field due to the Zeeman Effect [30]. So, the energy states split and electronic transition occurs between the states by absorbing the supplied microwave energy. The resulting absorption spectra are described as electron spin resonance (ESR) spectra. ESR is further explained in Chapter 2.

1.8 Research review

The research review covers the information UHMWPE related to radical activities, the effect of antioxidants in elimination of free radicals, and effects of reinforced particles in improving performance.

Ebru Oral et al. at 2004, showed improved effects of vitamin E (α-tocopherol, α-T) in increasing the oxidative stability and fatigue strength of radiation crosslinked UHMWPE, as compared to that combined with post-irradiation melting [8]. The test confirmed the fatigue strength to be improved by 58% for α-T doped 100-kGy irradiated UHMWPE compared to irradiated and melted UHMWPE. This result was encouraging to produce low-wear crosslinked UHMWPE in high stress orthopedic applications.

Yanning Zhao et al. at 2010, detected and observed the nature of primary radicals in gamma irradiated UHMWPE fiber as a function of radiation dose, annealing temperature, and storage environment [31]. This work described the primary alkyl radical ($-CH_2 -\bullet CH - CH_2-$) as the major product of UHMWPE-fiber after irradiation in vacuum and in air at room temperature, and that some of the alkyl radicals were converted to allyl ($-CH_2-CH = CH-\bullet CH-CH_2$) and polyenyl ($-CH_2-HC\bullet - (CH = CH)n- CH_2-$) radicals during storage in vacuum. The alkyl radicals decayed, reacting with oxygen during storage in air. The low decay rate of alkyl radical was observed indicating the large size of crystallites. The stability of primary alkyl radicals is of importance for the surface modification of UHMWPE fiber.

Ebru Oral et al. at 2011, highlighted the importance of post-irradiation thermal treatment with radiation crosslinking to ensure the oxidative stability of medical grade UHMWPE [32]. Two different methods of thermal treatments were evaluated: (1) annealing below the melting point and (2) annealing above the melting point. These two treatments were compared based on

the resulting mechanical properties. This work hypothesized that the free radicals could be reduced by annealing below the melting point under pressure, effectively keeping the mechanical properties preserved. The test of this hypothesis was positive for radiation crosslinked virgin UHMWPE and vitamin E blended UHMWPE.

In 2016, Honglong Wang et al., studied the effect of high-dose radiation, and further treatment by annealing crosslinked UHMWPE sheet [33]. Improved creep resistance and tensile property of the crosslinked UHMWPE sheet was observed using radiation doses of up to 300 kGy at a dose rate 5 kGy/h and post irradiation annealing at 120°C for 4h. The chemical structure, thermo-stability, crystallinity, creep resistance, and tensile properties of UHMWPE sheet were also investigated. The yield strength was found to be improved by 14% through crosslinking for 300 kGy irradiated UHMWPE, whereas the subsequent post-irradiation annealing further improved the creep resistance and young's modulus.

Tian Liu et al. at 2016, improved the wear resistance and mechanical properties of UHMWPE nanocomposites reinforced with graphite nanoplatelets (GNPs) [34]. The conventional fabrication technique was replaced by their new approach because of extremely high viscosity of UHMWPE and poor filler-matrix interaction. In this work, the new fabrication method used organosilane to modify the GNP surface. The morphological analysis showed the effective dispersion of modified GNP into the polymer matrix, with improved interfacial interactions between phases. The wear resistance and storage modulus of UHMWPE nanocomposites with silanized GNPs was improved by 980% and 170%, respectively.

From this research review, it is clearly seen that many research studies are being conducted to improve the mechanical properties of UHMWPE upon quenching free radicals through

radiation crosslinking and annealing. Since free radicals are mainly responsible for material degradation, the present work is focused to determine the long-term oxidation reaction of free radicals in orthopedic UHMWPE.

1.9 Rationalization of present research

UHMWPE contains different types of radicals; some are primary radicals of amorphous region which are mostly recombined by radiation crosslinking over time [27], and some are trapped in the crystalline region and are more long-lived [35]. The long-live radicals are thoght to be major contributors to the brittleness of the material through oxidation [36]. Although many researchers and medical device industries claim the best way to stabilize free radicals is via post-irradiation thermal treatment [1, 37], there is still debate about effective annealing temperatures and times.

So, the present study is mainly focused to determine the free radical contents remaining and their changing status in orthopedic UHMWPE components after long term storage in air or in oxygen-free environment, and at different temperatures.

1.10 Objectives

In this research, free radical analyses were performed using electron spin resonance (ESR) technique for two groups of orthopedic ultra-high molecular weight polyethylene (UHMWPE).

1.10.1 Group A

In the first group, Group A, two gamma irradiated (30 kGy) UHMWPE resins, GUR 4150 and Hylamer (Himont 1900) were investigated. These materials were provided by the NSF-Industry/University Center for Biosurfaces (IUCB), a partnership program between the State University of New York at Buffalo (SUNY Buffalo) and the University of Memphis. The two

grades of resin samples were stored in open air and inert-sealed conditions at 23°C, 37°C and 75°C for 18 years.

The primary objective of the IUCB program at the University of Memphis Site was to determine free radical reaction in UHMWPE components over the lifetime of their use in medical devices such as total hip and knee replacements. The second objective was to find if the long-term oxidation reaction due to free radicals would be different in the two most commonly used UHMWPE resins at the time of the project's initiation in 1997: GUR4150 and Hylamer.

The third objective was to determine if free radicals could be quenched by annealing the post-gamma samples in their respective sealed (inert) environment at 75°C.

1.10.2 Group B

For the second group, Group B, free radical analyses were performed on vitamin E-containing UHMWPE (GUR 1020) following post-gamma (30 kGy) shelf-aging for 10 years at 23°C (room temperature) in open air.

The first objective of this project was to determine the effect of vitamin E upon the production of free radicals during gamma irradiation. The second objective was to determine whether or not vitamin E had any anti-oxidation effect on the free-radical induced oxidation of gamma-sterilized UHMWPE during shelf-storage in a room environment.

Chapter 2

Electron Spin Resonance (ESR) Theory

2.1 Introduction of Electron Spin Resonance (ESR)

Electron spin resonance (ESR) spectroscopy, also referred to as electron paramagnetic resonance (EPR) spectroscopy, is a versatile, technique which can be used for a variety of applications in biology, medicine and physics. ERS technique is mainly applied to detect atoms or molecules with one or more unpaired electrons which are termed 'free radicals'.

In general, spectroscopy is the measurement and interpretation of the energy difference between atomic or molecular states. According to Plank's law, electromagnetic radiation will be absorbed if:

$$\Delta E = h\nu$$

Where ΔE is the energy difference between two states, h is the Plank's constant and ν is the frequency of electromagnetic radiation. The absorption of this energy causes a transition of an electron from the lower energy state to the higher energy state.

The basis of ESR spectroscopy lies in the spin of an electron and its associated magnetic moment. When an electron is placed within an applied magnetic field, B the magnetic moments of electrons align either parallel or antiparallel to the applied field with specific energy causing an energy difference between the two states. This energy difference is a result of the Zeeman Effect. The lower energy state occurs when the magnetic moment of the electron, μ, is aligned with the magnetic field and a higher energy state occurs where μ is aligned against the magnetic field.

The two states are labeled by the projection of the electron spin, M_S, on the direction of the magnetic field, where $M_S = -1/2$ is the parallel state, and $M_S = +1/2$ is the antiparallel state. Figure 6 shows the splitting of energy state in presence of applied magnetic field.
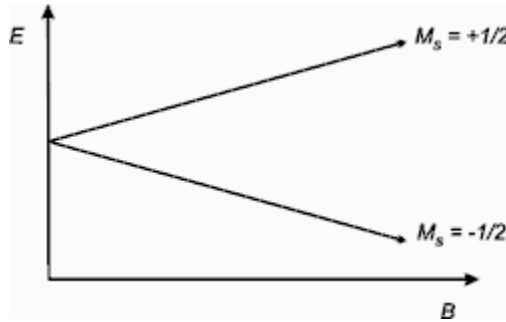
Figure 6: Splitting of energy states in presence of applied magnetic field

The energy of each electronic state is defined as:

$$E = g\,\mu_B B\ M_S = \pm \frac{1}{2} g\,\mu_B B \quad \ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

Where g is the proportionality factor (constant, called g-value, explained in details in section 2.5), μ_B is the Bohr magneton, B is the magnetic field and M_S is the electron spin quantum number. The sign (+/-) is related to the direction of magnetic moments alignment.

So, in presence of applied magnetic field, the energy difference between two states is defined as,

$$\Delta E = h\nu = g\,\mu_B B \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (2)$$

From the equation (1) and (2), it can be stated:

"The two spin states have the same energy when there is no applied magnetic field and the energy difference between the two spin states increases linearly with increasing magnetic field strength."

Unlike other conventional absorption test (where frequency is varied) for ESR, the magnetic field (Gauss) is varied during scanning and the frequency of microwave source radiation is held constant in gigahertz range. This is possible due to the relationship shown in equation (2) where, B is the magnetic field provided by ESR magnet. When the energy difference between electronic states (which is varied as a function of varied magnetic field) matches with frequency of source radiation, transition occurs through absorption of source radiation by unpaired electron.

2.2 Working procedure of ESR spectrometer
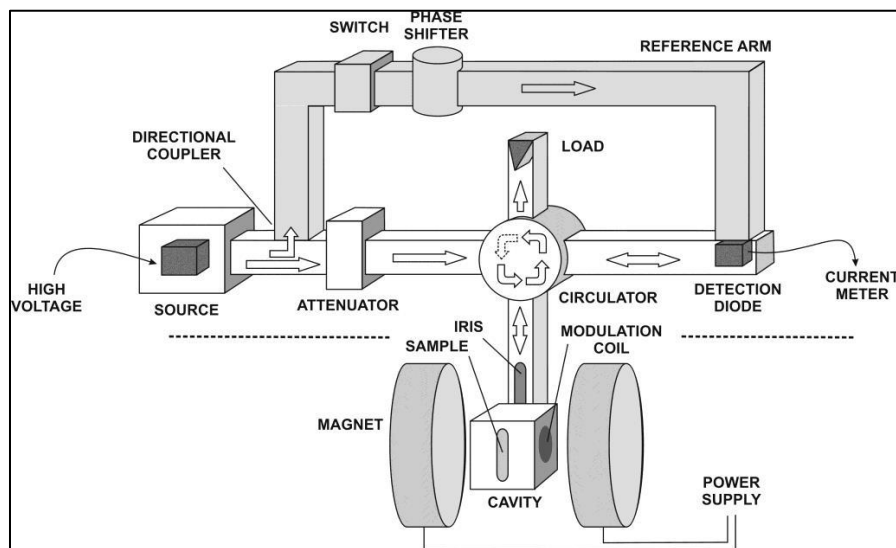
Figure 7: Block diagram of ESR spectrometer with microwave flow direction

In the ESR spectrometer, the microwave radiation is generated from the source and directed to the cavity through attenuator. The cavity is a metal box with a rectangular or cylindrical shape which is designed in such a way that, the electric field components of trapped microwave standing waves is minimum and magnetic field component is maximum. The sample under study

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is kept inside the cavity to ensure maximum magnetic field. The cavity and sample, both are subjected to the varied magnetic field generated by ESR magnet during scanning. The entrance of the cavity is set to tune the amount of radiation reflected back out of the cavity. The reflected radiation returns to the circulator and is directed to the diode for the detection of microwave intensity. Any remaining radiation that reflects back from the detector is forced by the circulator into the upward waveguide that ends in a wedge to convert the radiation into heat. At resonance when the energy difference between electronic states matches with frequency ($\Delta E = h\nu$), cavity stores the microwave energy and no radiation is reflected back out of cavity causing complete absorption. The schematic diagram of ESR spectrometer is shown in Figure 7.

2.3 Observation of ESR spectra

In ESR measurement, the signal is recorded as the first derivative of absorption because of the weak strength and poor signal-to-noise ratio of absorption spectra. Figure 8 shows a typical ESR absorption signal and its first derivative.
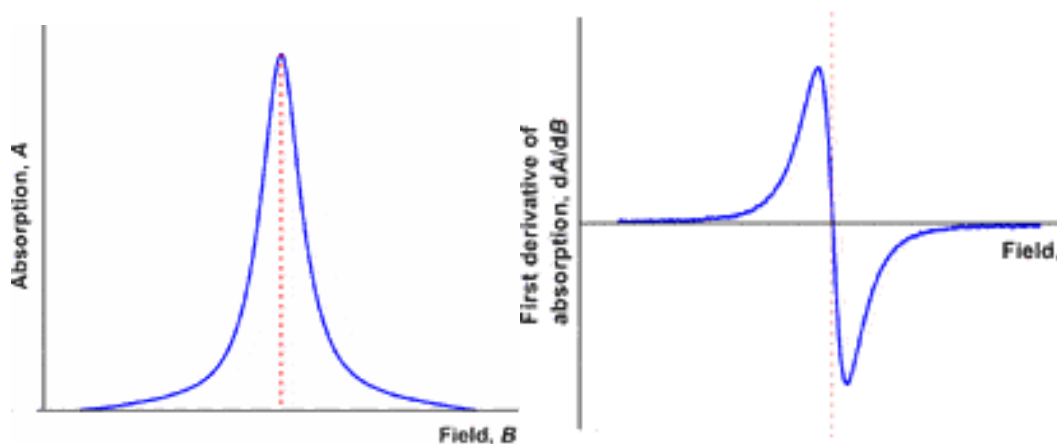

Figure 8: ESR absorption signal and its first derivative

The power (milliwatt) and modulation amplitude (gauss) are two important parameters for ESR spectra associated with phase sensitive detection.

Since the two energy states of electron are nearly equally populated, an intense radiation absorption tends to equalize the population; this effect is called 'saturation'. The another process at which electron returns to the lower energy state via energy transfer to the surroundings, is called spin-lattice relaxation. So, when the rate of relaxation is less than rate of absorption, the system gets saturated easily leading to a decrease in net absorption and the ESR signal is lost. In that case, more power is applied to the sample the higher the chance that an energy quantum is absorbed. So, the appropriate setting of power level is important to find desirable signal. The power setting is also important to detect the radical types in specimens because the saturation occurs in different rate of time for different organic radicals.

The modulation amplitude is set to find the strong ESR signal with high signal-to-noise ratio. The intensity of detected ESR signal increases with increased modulation amplitude; however, if the modulation amplitude is too large, the detected ESR signal broadens and becomes distorted.

2.4 Quantification analysis of ESR spectra

The ESR spectra are generally plotted as intensity versus magnetic field. It can be also plotted in terms of g-value. The strong ESR spectra are usually analyzed by measuring the peak to peak intensity which is roughly proportional to the number of radicals. But, for accurate measurement of number of radicals, the first derivative spectra are integrated twice with baseline correction. The area under the integrated spectra is proportional to the number of radicals, which is indicated in Figure 9.
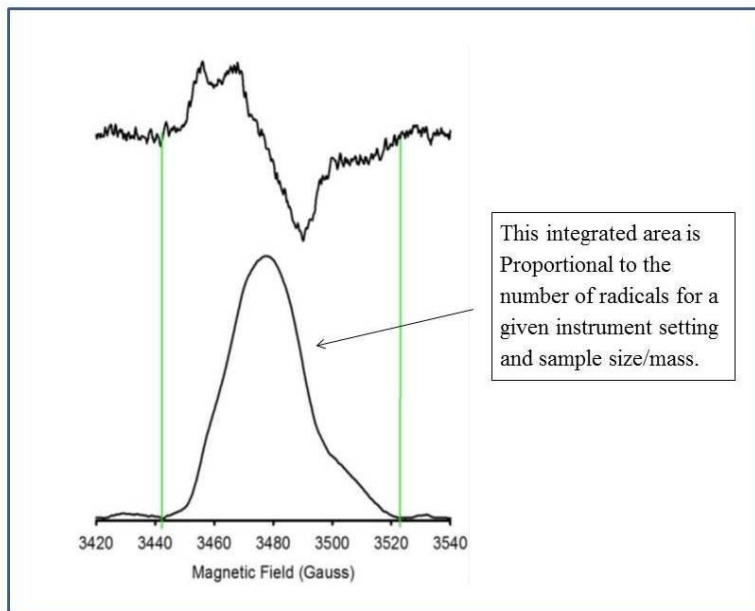
Figure 9: Integration of an ESR spectrum. A default ESR signal (top) is the derivative of the absorption signal below.

2.5 g-value

In ESR technique, during scanning magnetic field (B) is varied and the frequency (ν) is kept constant. When the magnetic field B equals $(h\nu)/g\mu_B$ (that is, $B= (h\nu)/g\mu_B$), 'resonance' occurs and the ESR signal is seen. The constant 'g' is called 'g-factor' or 'g-value' and can be used to describe particular ESR spectra and types of radicals. For free electron g-value is 2.0023 and for organic radicals it is ranged from 1.99-2.01. The g-value varies for different radicals. Since each radical has different molecular structure, the local magnetic fields induced by the molecular framework are different which affects the 'g-value' of radicals. [38] ESR spectra are typically plotted as intensity versus magnetic field (in units of Gauss, or Tesla) but sometimes the location of a specific feature of a spectrum can be better described in intensity versus g-value plot.
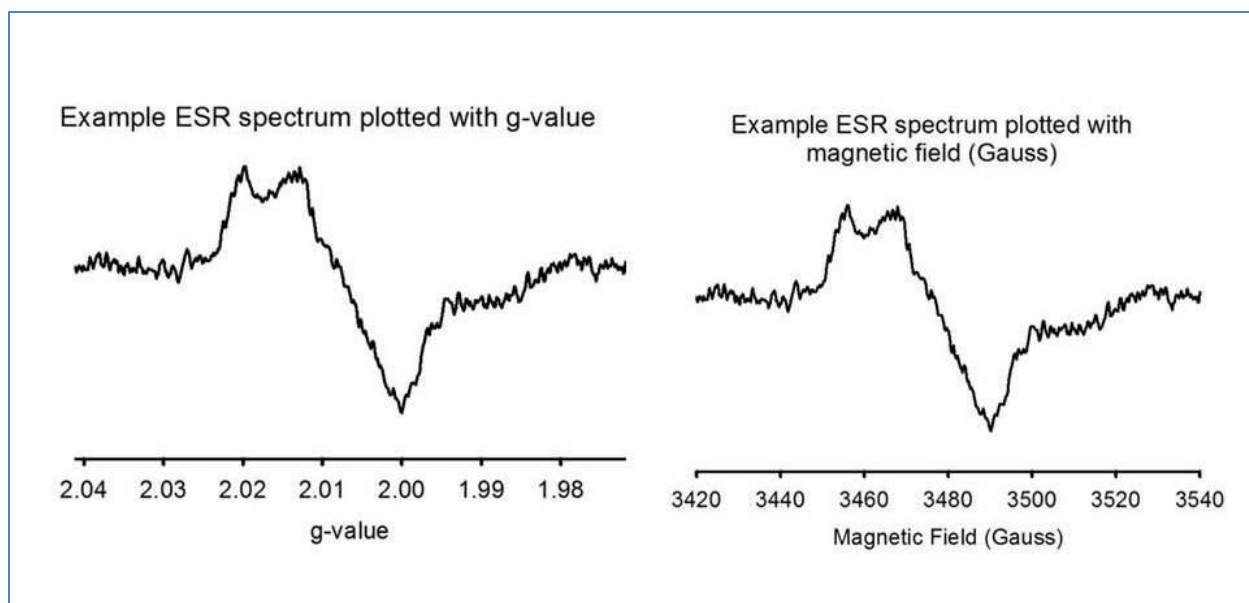
Figure 10: ESR spectra in terms of g-value and magnetic field

Figure 10 shows ESR spectra as function of g-value and magnetic field. The spectra are the same; just the units are called differently. Two spectra of the same radical type may occur at different Gauss locations due to other factors such as tuning frequency, but they will still occur at the same g-value location.

Chapter 3

Materials and Methods

3.1 Materials

3.1.1 Group A

This group of samples was provided by Industry/University Center for Biosurfaces (IUCB). The samples, 10 mm in length and 3 mm in diameter, were machined from ram extruded rod and sealed in ESR sample tubes, one sample per tube, in vacuum or partially-filled inert gas (now labeled as inert sealed). The sample tubes (3.5 mm id x 4.0 mm od, and 200 mm in length) were made out of suprasil quartz (Wilmad Glass). Before sealing, the tubes were evacuated to 1 mTorr or better, and then they were back-filled with an inert gas, argon or nitrogen. After gamma irradiation (30 kGy at room temperature), the sample end (bottom) of each tube was thermally annealed to remove any gamma-induced paramagnetic sites. A group of samples of each kind was also irradiated in open air (in the presence of oxygen). The ESR spectrum of each sample was recorded and labeled as initial (time t=0) spectrum, which provided information about the initial or the primary radicals. Following the initial measurements in 1998, the samples were divided into subgroups and placed at room temperature (23°C), body temperature (37°C) and at elevated temperature (75°C), respectively.

3.1.2 Group B

The shelf storage gamma irradiated UHMWPE samples doped with vitamin E were in this group. The samples were prepared using GUR 1020 resin powder (provided by Ticona) and vitamin E (α-Tocopherol, α-T) with 95% purity (provided by Sigma Aldrich). Vitamin E (α-tocopherol) was consolidated with GUR 1020 at a concentration level varying between 0% (no vitamin E) and 20% in 2006. The α-T doped samples were then irradiated by 30 kGy in air

followed by 10 years of shelf storage. The length of the samples was maintained into 8 mm before test via ESR. The mass of the samples was used during data analysis to ensure the consistency among the compared sample. The UHMWPE samples tested in this work are shown in Figure 11.
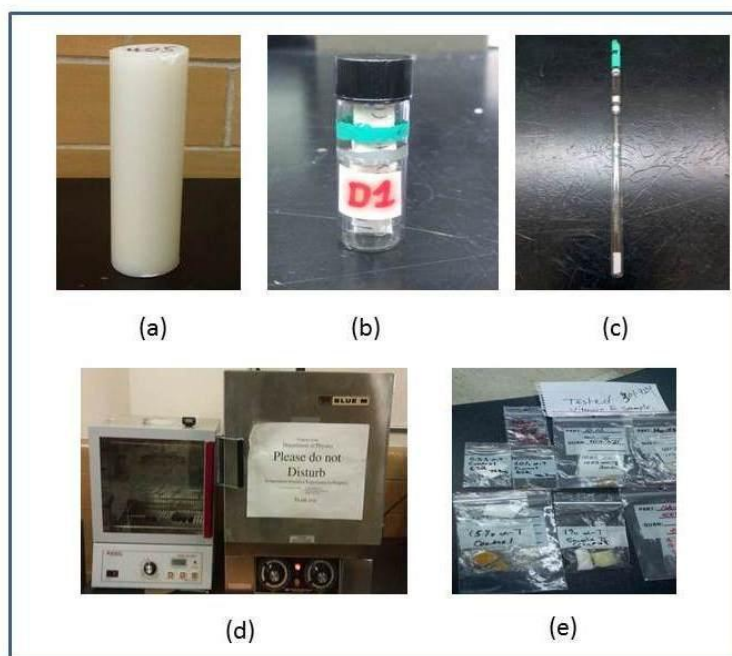
Figure 11: (a) Solid UHMWPE (b) UHMWPE sample stored in air at room temperature (c) UHMWPE sample sealed in inert environment at room temperature (d) UHMWPE samples stored in oven at 37°C and 75°C (e) Shelf storage vitamin E doped UHMWPE samples

3.2 Sample test using ESR technique

Electron Spin Resonance (ESR) technique was used to analyzed the free radical activities in all the samples using an X-band Bruker EMX 300 spectrometer operating at 9.8 GHz microwave frequency and 100 kHz magnetic field modulation frequency. The samples were tested over 200 Gauss sweep width keeping the constant center magnetic field at 3510 Gauss, which provided

1024 data point in ESR spectra. The sweep time for 200 Gauss sweep width was 168 seconds

with time constant 328 milliseconds. The power and modulation amplitude were varied to

generate cleaned signal and to detect the different types of free radicals. The horizontal number

of scan was also increased up to 5 to reduce the noise level. Each ESR spectrum was baseline

corrected using 'WINEPR' software. The ESR spectra were analyzed using 'SigmaPlot-11'

software. The more details on ESR technique are explained in Appendix. Figure 12 shows the

ESR spectrometer which was used in this work.
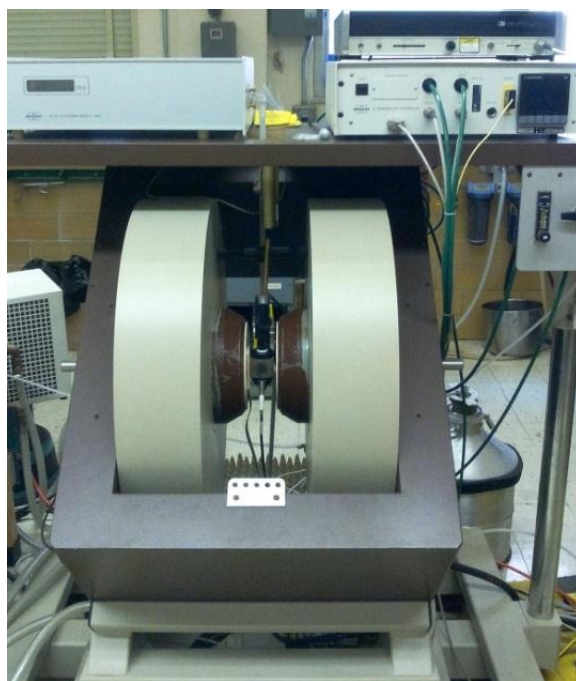

Figure 12: ESR Instruments

3.3 Experimental summary

Two groups of samples were tested using the Electron Spin Resonance (ESR) technique for

the detection of free radicals. During testing, the ESR parameters varied depending on the

purpose of analysis. Each test was repeated at least three times to get the average result within the experimental error. The entire experimental procedure and analysis can be explained in to two parts based on two groups of samples.

3.3.1   Group A

The irradiated GUR 4150 and Hylamer (Himont 1900) samples stored in different temperatures (23^oC, 37^oC and 75^oC) and conditions (air and inert) were tested to detect and analyze the free radicals content remaining after 18 years. The free radical concentrations (spins per gram) were measured by integrating the ESR spectra. The area under the integrated curve is proportional to the number of free radicals. This analysis was mainly focused to quantify the radical contents remaining after 18 years. The comparative analyses was also performed in terms of radical concentration between GUR 4150 and Hylamer (Himont 1900) as a function of temperature and condition. The samples were tested with different power (milliwatt) and modulation amplitude (Gauss). The ESR spectra associated with 1 mW power and 5 Gauss modulation amplitude were taken under this part of analyses.

The ESR spectra of GUR 4150 and Hylamer irradiated in inert-sealed conditions at three different temperatures (23^oC, 37^oC and 75^oc) were simulated using excel software to detect and extract the percent composition of primary radicals present after 18 years. The samples for this analysis were tested using using 1 mW power and 5 Gauss modulation amplitude.

Irradiated GUR 4150 and Hylamer samples stored at room temperature (23^oC) in air were tested separately with varying power ranged 0.01 mW to 160 mW and 1 Gauss modulation amplitude to detect and compare the concentrations of radical type R1 (carbon-centered polyenyle) and R2 (oxygen-centered polyenyle).

3.3.2 Group B

The irradiated GUR 1020 resins doped with various percentage of vitamin E (α-Tocopherol) were tested using 1 mW power and 5 Gauss modulation amplitude. The samples blended with 0.5%, 10%, 15% and 20% vitamin E were taken under analysis.

In this part of analysis, the ESR spectra of each composition were compared to the measurement conducted 10 years back in 2006 by M.D. Ridley (Department of Physics, Biomaterials Laboratory, The University of Memphis) [39].

The same aged GUR 1020 samples without vitamin E and with 0.5% vitamin E were also tested using varying power ranged from 0.01 mW to 100 mW and 3 Gauss modulation amplitude to detect and quantify the radicals R1(carbon-centered polyenyle) and R2 (oxygen-centered polyenyle).

Chapter 4

Experimental Results

4.1 Overall guideline

In the present study, three specimens were tested for each type of sample and the average result was taken for analyses.

The entire experimental results can be explained into two sections for two different research projects. The results are presented using ESR spectra (line graphs), bar graphs with error bar and numerical value in tabulated form. The ESR spectra used in the graphs indicates the intensity of free radicals. The structure of ESR spectra also is an indication of the presence of different types of radicals. The free radical concentration (spins per gram) is presented by color-coded bar graphs for different groups of samples.

4.2 Results for Group A

GUR 4150 and Hylamer samples followed by 18 years of aging in air and in inert condition at different temperatures (23°C, 37°C and 75°C) were brought under analysis. Although many reports have been published on the effect of thermal annealing in increasing the oxidative stability of polymer, [40-42] we have observed the considerable amount of free radicals in GUR 4150 and Hylamer samples even after 18 years of storage at higher temperature and inert environment.

4.2.1 Open air versus inert-sealed storage

In this study, the long-term effects of temperature (23°C, 37°C and 75°C) upon polyethylene (PE) free radicals in inert environments and in the presence of oxygen (open air)

were investigated. Figure 13(a), 13(b) and 13(c) presents the ESR spectra of GUR 4150 resin stored in air and in inert condition at 23°C, 37°C and 75°C respectively.
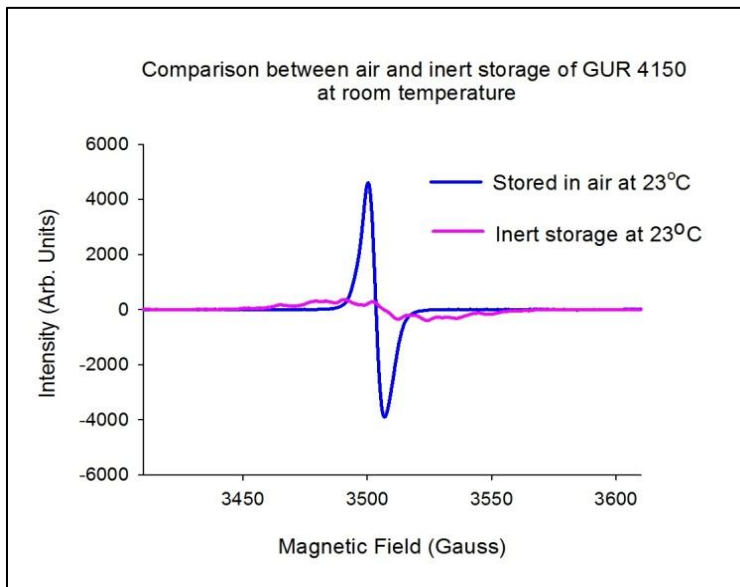

Figure 13(a): ESR spectra of GUR 4150 stored in air and inert condition at room temperature for 18 years
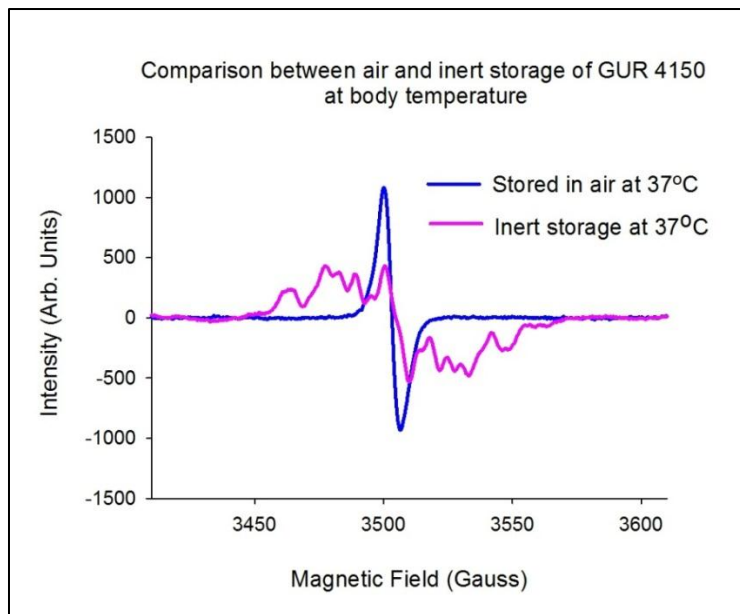

Figure 13(b): ESR spectra of GUR 4150 stored in air and inert condition at body temperature for 18 years
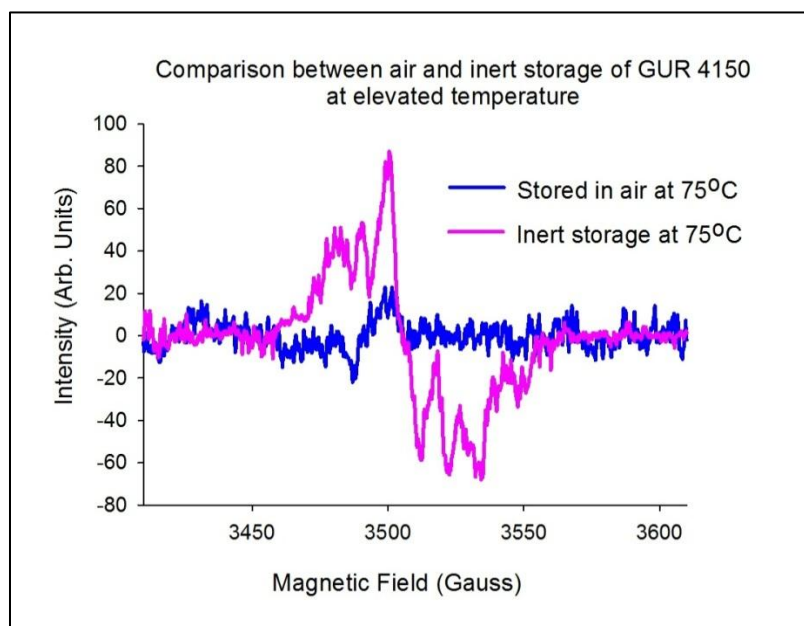
Figure 13(c): ESR spectra of GUR 4150 stored in air and inert condition at elevated temperature for 18 years

ESR spectra recorded in the present study (t = 18 years) indicate the presence of oxygen-induced polyenyle radicals in all samples aged at 23°C or 37°C in open air, with a greater concentration at 23°C. This result shows that, at room temperature most of the primary radicals of GUR 4150 resin converted to the oxygen induced radicals in presence of air. This conversion of radicals is less in 37°C. At 75°C, there was no detectable radicals in open air sample. The ESR spectra for all inert storage sample, showed the presence of primary radicals indicating the oxidation prevention.

Similar ESR spectra were found for Hylamer samples stored in air and inert condition at 23°C, 37°C and 75°C which are shown in Figures 14(a), 14(b) and 14(c). There was no significant difference in ESR spectra between GUR 4150 and Hylamer samples.
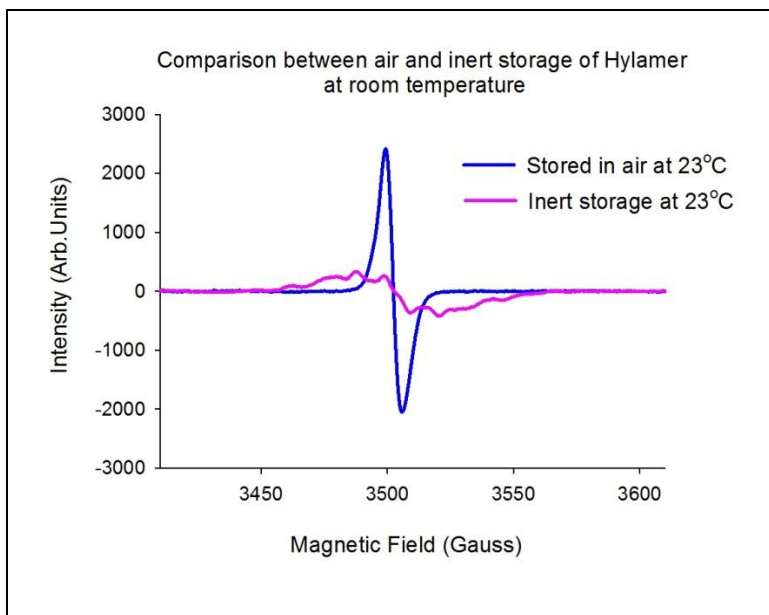
33

Figure 14(a): ESR spectra of Hylamer stored in air and inert condition at room temperature for 18 years
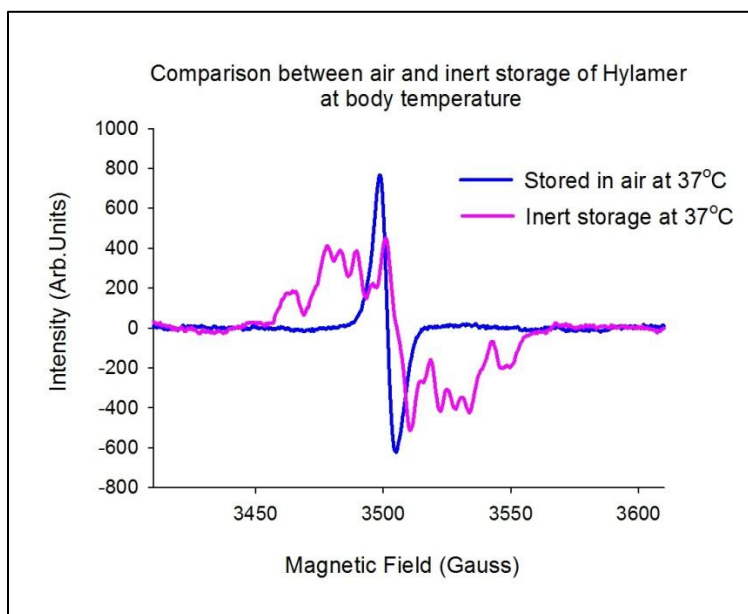

Figure 14(b): ESR spectra of Hylamer stored in air and inert condition at body temperature for 18 years
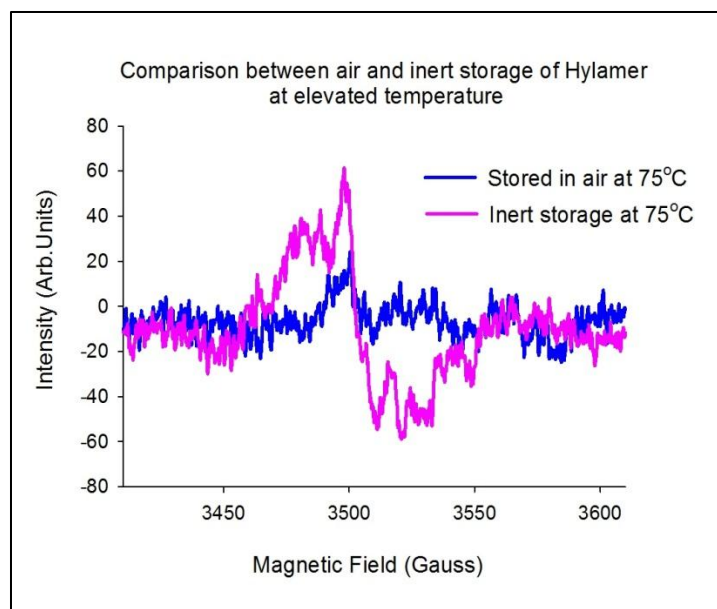
Figure 14(c): ESR spectra of Hylamer stored in air and inert
condition at elevated temperature for 18 years

4.2.2 Quantification of free radicals

The bar graph of figure 15, represents the decrease in free radical concentration (FRC)

(spins per gram) in GUR 4150 and Hylamer over 18 years compared to the initial concentration

of air-and inert-storage samples stored at room temperature. Figure 16 shows the relative free

radical concentration between 18 years aged GUR 4150 and Hylamer samples stored in air and

in inert condition at 23°C, 37°C and 75°C temperatures. The results on figure 15 showed that, the

total concentration of radicals in all samples decreased by two orders of magnitude in 18 years.

Additionally, the present data in Figure 16 showed that, at 23°C, it decreased less in Hylamer

than in GUR 4150, by a factor 2/3. In inert environments, both GUR 4150 and Hylamer showed

similar concentration (slightly higher concentration at 23°C than at 37°C). Compared to GUR

4150, Hylamer showed the presence of 0.6, 0.9, 1.0, 1.1 and 1.3 times more radicals at 23°C (air)

37°C (air), 23°C (inert), 37°C (inert) and 75°C (inert), respectively. However, all the samples

except those stored in air at 75°C, had quantifiable radical contents remaining after 18 years.
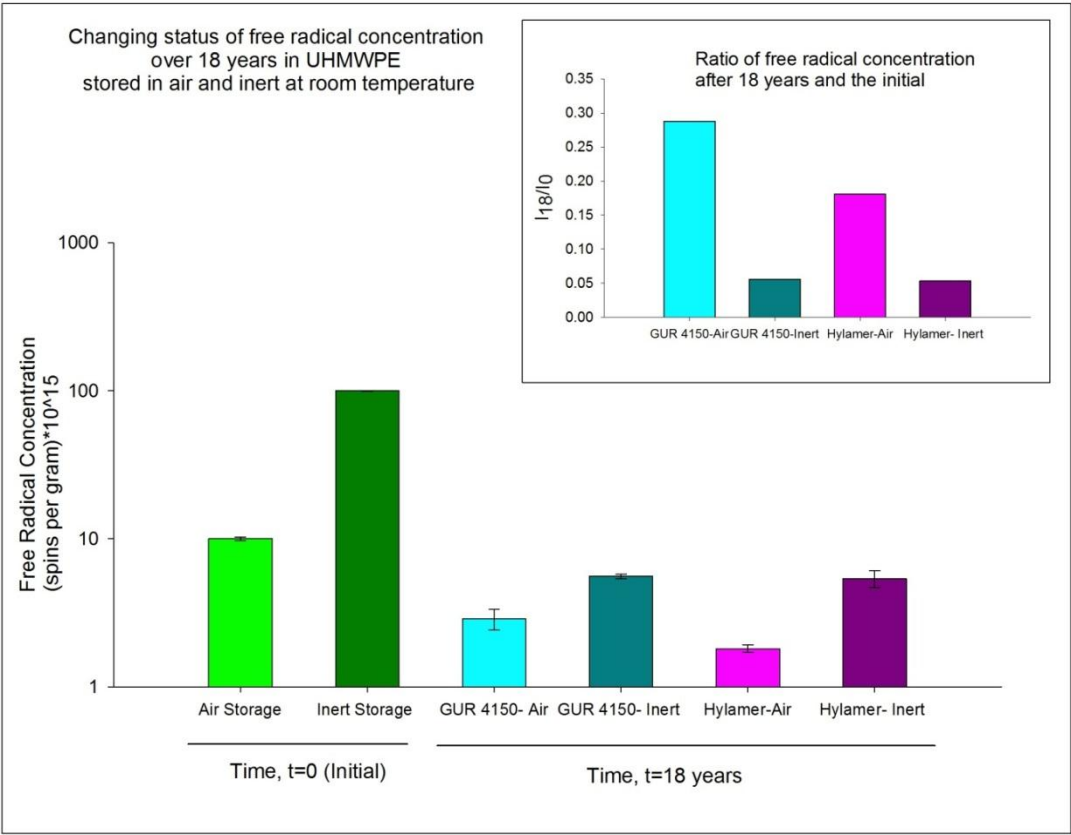
Figure 15: Change of free radical concentration of UHMWPE over 18 years
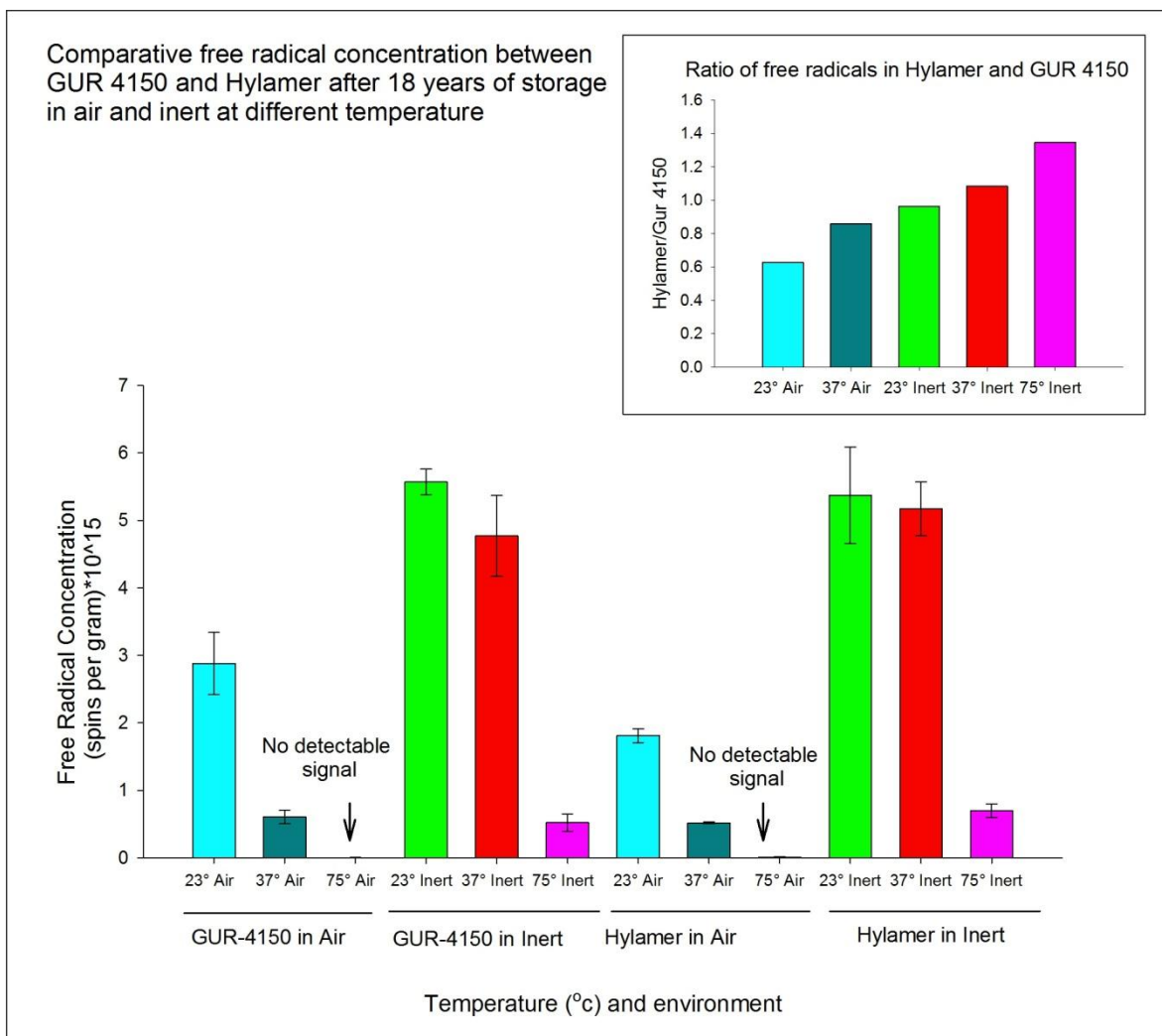
Figure 16: Relative free radical concentration of GUR 4150 and Hylamer after 18 years of aging

The decrease in free radicals of Hylamer (stored in open air at 23°C) compared to GUR 4150 might be influenced by the difference in molecular weight, surface morphology or the polymerization process in between these two samples. The oxidation process leads to convert the primary free radicals to the longer-lived non-free radical species (oxygen centered polyenyl). So, the less number of free radicals in Hylamer compared to GUR 4150 might be an effect of free radical conversion to the non-radical species. In inert storage at 37°C, Hylamer might have less

recombination of free radicals which causes slightly more radical concentration compared to GUR 4150 under the same condition.

4.2.3   Simulation of ESR spectra

The inert storage UHMWPE resin samples of all temperature showed the primary radical structure in ESR spectra, which can be clearly observed in Figure 13 and 14. So, the analysis was aimed to extract the percent composition of radical types observed in GUR 4150 and Hylamer sealed in inert condition at room temperature, body temperature and elevated temperature for 18 years. Figure 17 shows an example of combination of radical types within the GUR 4150 samples sealed in inert condition at room temperature. The bottom two graphs are simulated and experimental ESR spectra.
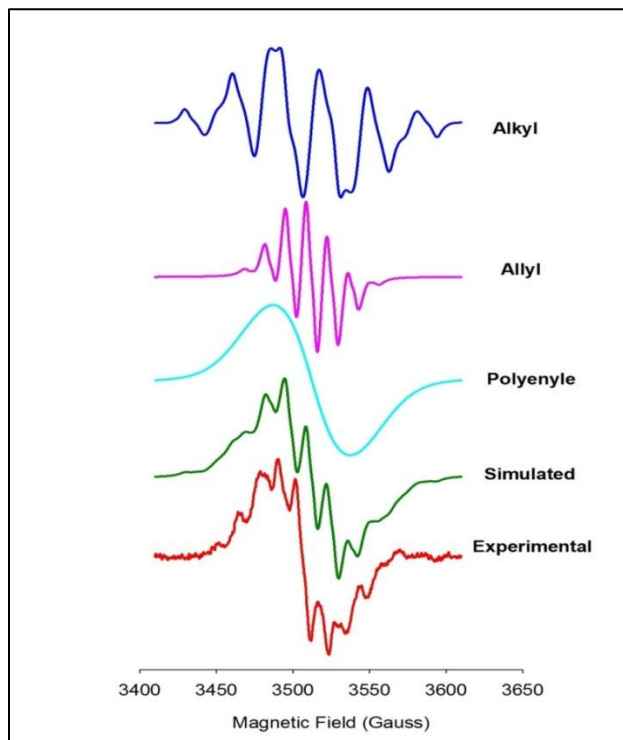

Figure 17: An example of combination of radical types in UHMWPE

Table 4 shows comparative percent (%) composition of different type radicals within GUR 4150 and Hylamer stored in inert environment at different temperature. This analysis was performed using 'simulation tool' in excel software.

Table 4: Radical types detected via ESR in this study

Percent (%) composition of radical types observed in GUR 4150 and Hylamer UHMWPE stored in inert at room temperature, body temperature and elevated temperature						
Types of radical	23°C		37°C		75°C	
	GUR 4150	Hylamer	GUR 4150	Hylamer	GUR 4150	Hylamer
Alkyl	10	16	13	20	15	6
Allyl	40	42	40	51	10	24
Polyenyle	50	42	46	28	0	0
Dienyle	0	0	0	0	74	68
Trienyle	0	0	0	0	0	0

In terms of radical type (alkyl, allyl, polyenyle, dienyle and trienyle), no significant difference was found between GUR 4150 and Hylamer at 23°C (inert), but at 37°C (inert) Hylamer showed the presence of more alkyl (20/13), allyl (51/40) and less polyenyle (28/46). No dienyle or trienyle was observed at 23°C (inert) or 37°C (inert). At 75°C (inert), Hylamer (compared to GUR 4150) showed the presence of lower concentration of alkyl (6/15), higher concentration of allyl (24/10), no polyenyle (0/0), approximately equal number of dienyle (68/74) and no trienyle (0/0). At high temperature, conversion of the primary radicals to the Dienyle (oxygen centerd polyenyle) components might occur due to the thermal motion of the polyethylene molecules.

4.2.4 R1 and R2 type radicals in GUR 4150 and Hylamer

The existence of radical R1 (carbon centered polyenyle) and R2 (Oxygen centered polyenyle) in UHMWPE was already reported in previous work of biomaterials research laboratory, Department of Physics, The University of Memphis. The present study was aimed for further detection of radical R1 and R2 in irradiated UHMWPE after 18 years. The comparative quantification of R1 and R2 was also performed between GUR 4150 and Hylamer followed by 18 years of aging. Figure 18 (a) and 18 (b) show the existence of R1 and R2 radical in GUR 4150 and Hylamer stored in air at room temperature for 18 years.
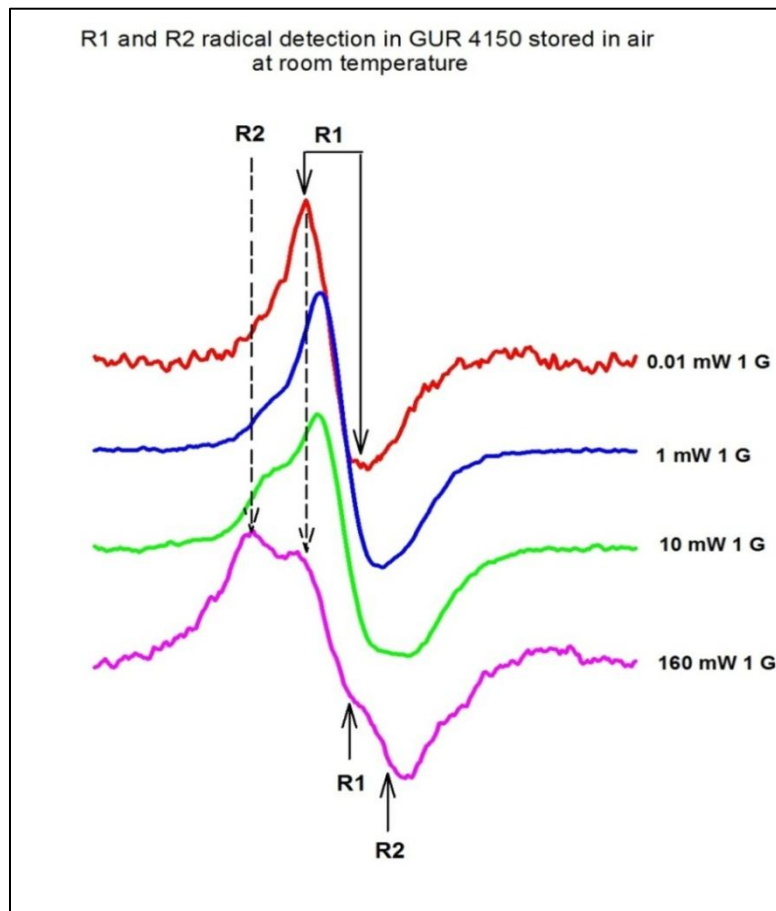
Figure 18 (a): The existence of radical R1 and R2 in GUR 4150
after 18 years of aging
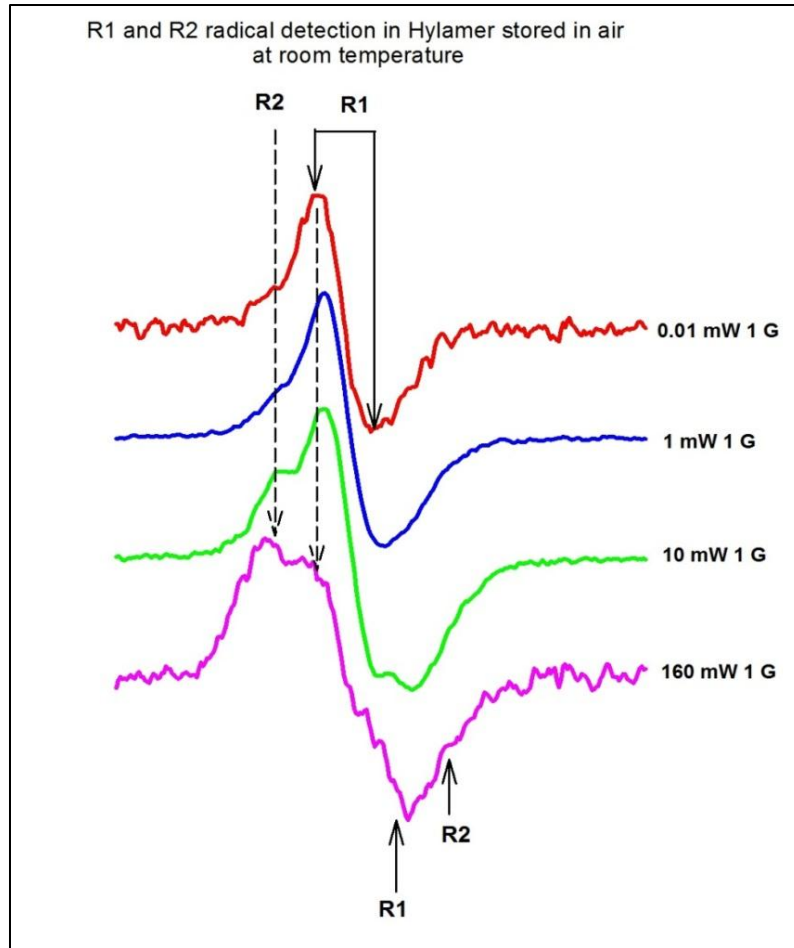
Figure 18 (b): The existence of radical R1 and R2 in Hylamer after 18 years of aging

The bar graph in Figure 19 represents the relative free radical concentration of type R1 and R2 between open air GUR 4150 and Hylamer resin stored at room temperature. Analyses of the ESR data revealed that Hylamer contained lower concentration, 0.6x and 0.5x, of R1 and , R2 respectively, compared to GUR 4150. This results indicate a possible increased oxidation protection in these samples, as fewer oxygen induced radicals were generated.
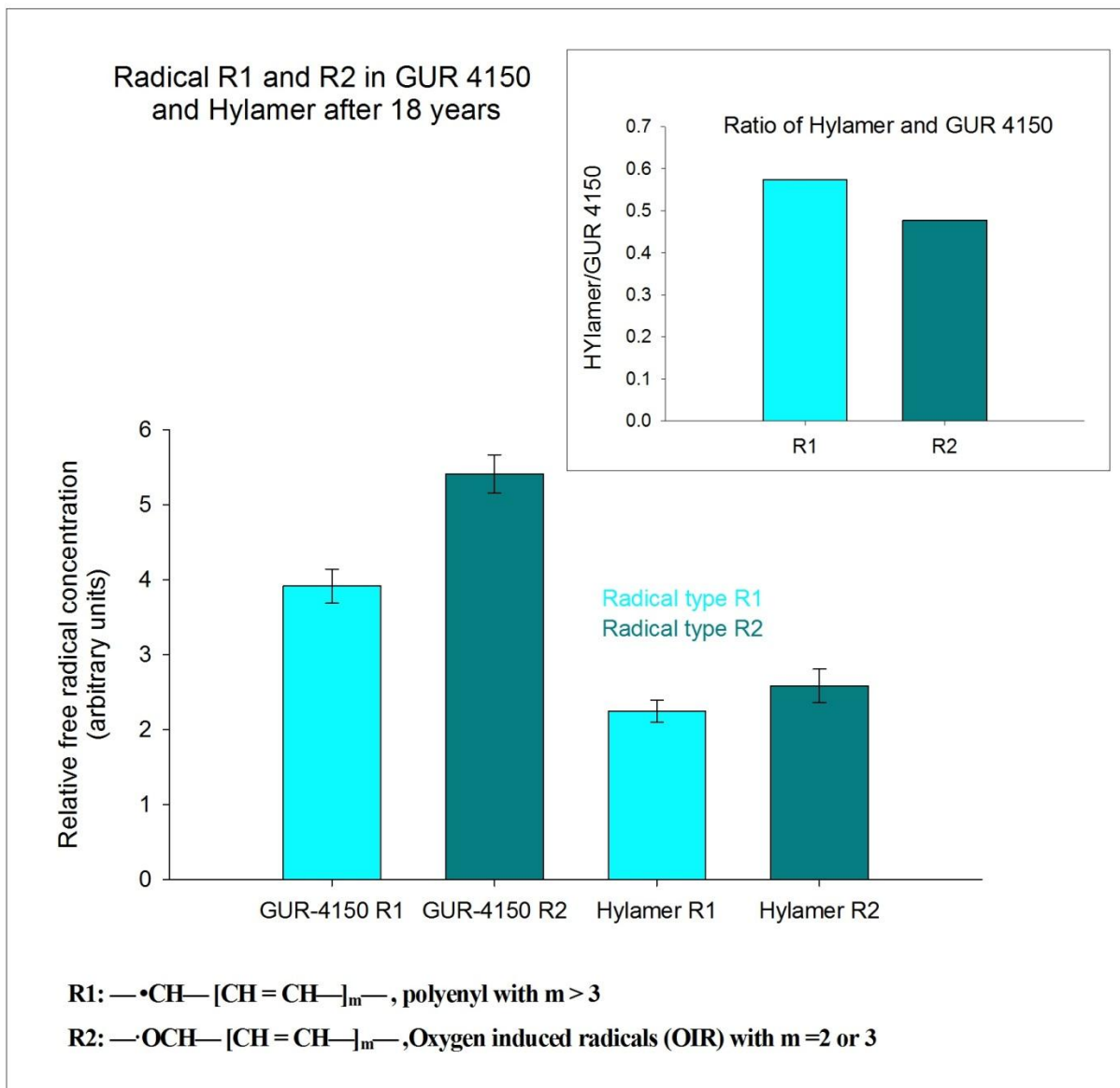
Figure 19: Relative R1 and R2 radical concentration in between GUR 4150 and Hylamer after18 years

4.3 Results for Group B

In this part of results, the long-term effect of vitamin E (α-Tocopherol) in quenching the free radicals of UHMWPE, was investigated.

4.3.1 ESR spectra of UHMWPE without and with vitamin E
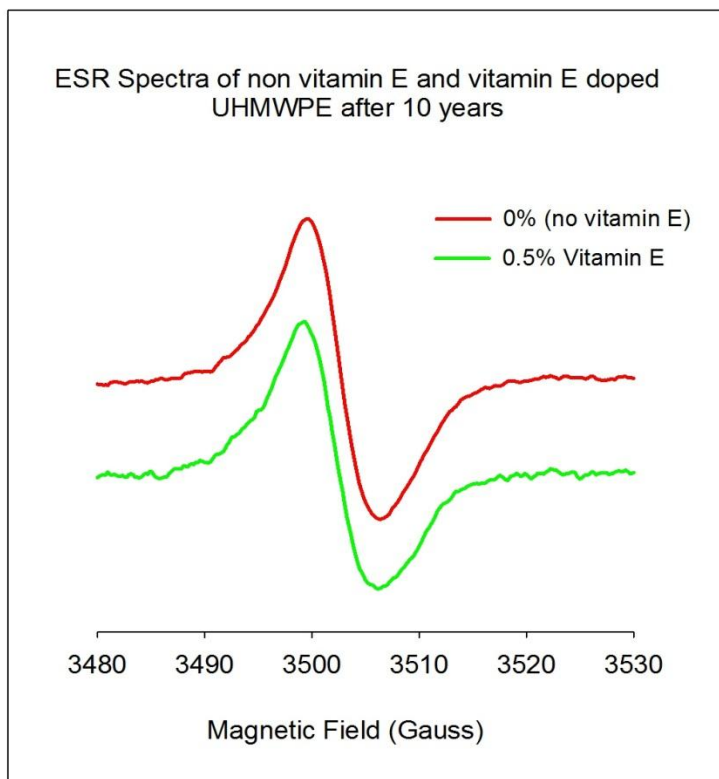
Figure 20: ESR spectra of 10 years aged UHMWPE

The ESR spectra of shelf storage 10 years aged non-vitamin E and 0.5% vitamin E doped GUR 1020 resin sample were shown in Figure 20. Although vitamin E radicals were present in the ESR spectra of immediate post gamma-irradiated vitamin E mixed polyethylene sample (tested at 2006), no vitamin E resonance line was observed in the present ESR spectra. This result suggests that, vitamin E radicals might be stable for short period, but not for the long

period, it gets stabilized either recombining with vitamin E radical itself or any polyethylene radicals.

4.3.2 Free radical concentrations over 10 years

The bar graph shown in Figure 21 presents the free radical concentration (FRC) (spins per gram) of UHMWPE samples mixed with various percentage of vitamin E (α-Tocopherol). The ESR measurements conducted immediately after gamma irradiation in 2006 showed a decrease in free radical concentration by an order of magnitude due to vitamin E [39]. In 10 years (2016), the radical concentration was reduced by another order of magnitude; i. e. from $\sim 10^{17}$ to $\sim 10^{15}$ radicals per gram in all samples, with or without vitamin E. No measurable difference between radical concentrations was found as a function of vitamin E.
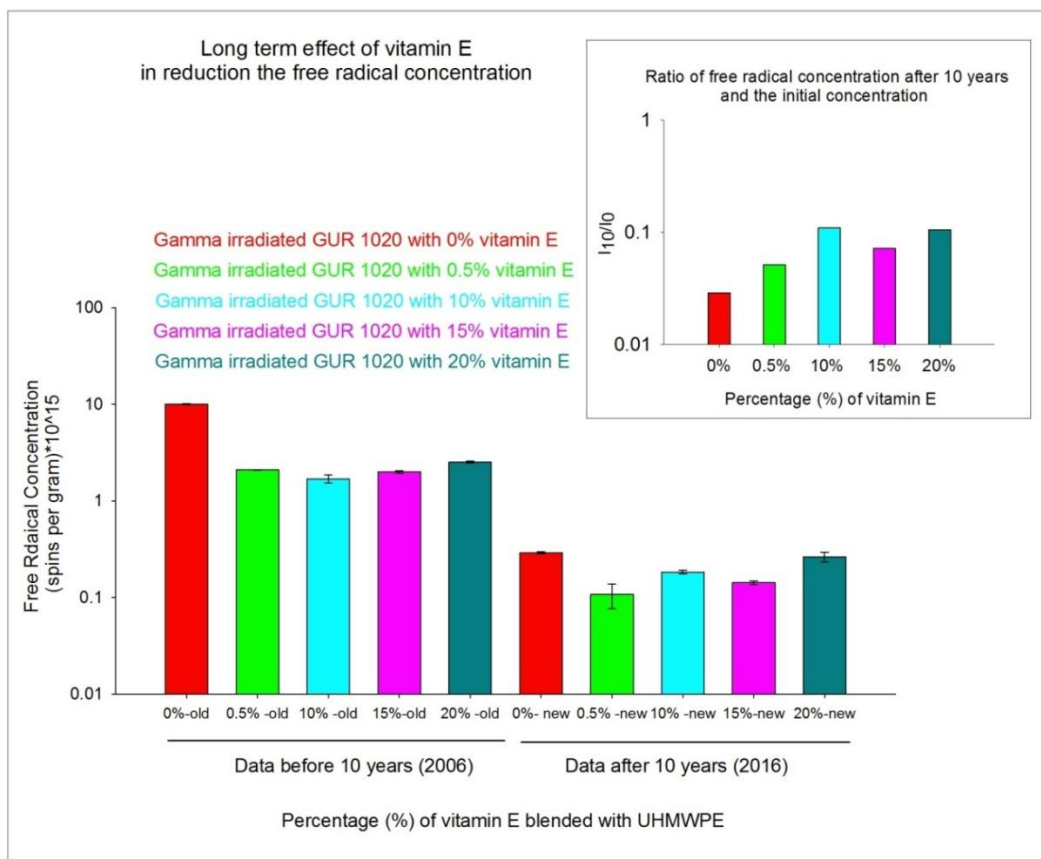
Figure 21: Change of free radical concentration of vitamin E doped UHMWPE over 10 years

4.3.3 R1 and R2 type radicals in absence and presence of vitamin E

The effect of vitamin E in stabilizing the free radicals of UHMWPE was clearly studied by detecting and quantifying the radical R1 (carbon centered polynyle) and R2 (Oxygen centered Polynyle) in similar aged non vitamin E and vitamin E doped UHMWPE samples. Figure 22(a) and 22(b) indicate the presence of R1 and R2 radicals in non-vitamin E and 0.5% vitamin E doped GUR 1020 UHMWPE samples.
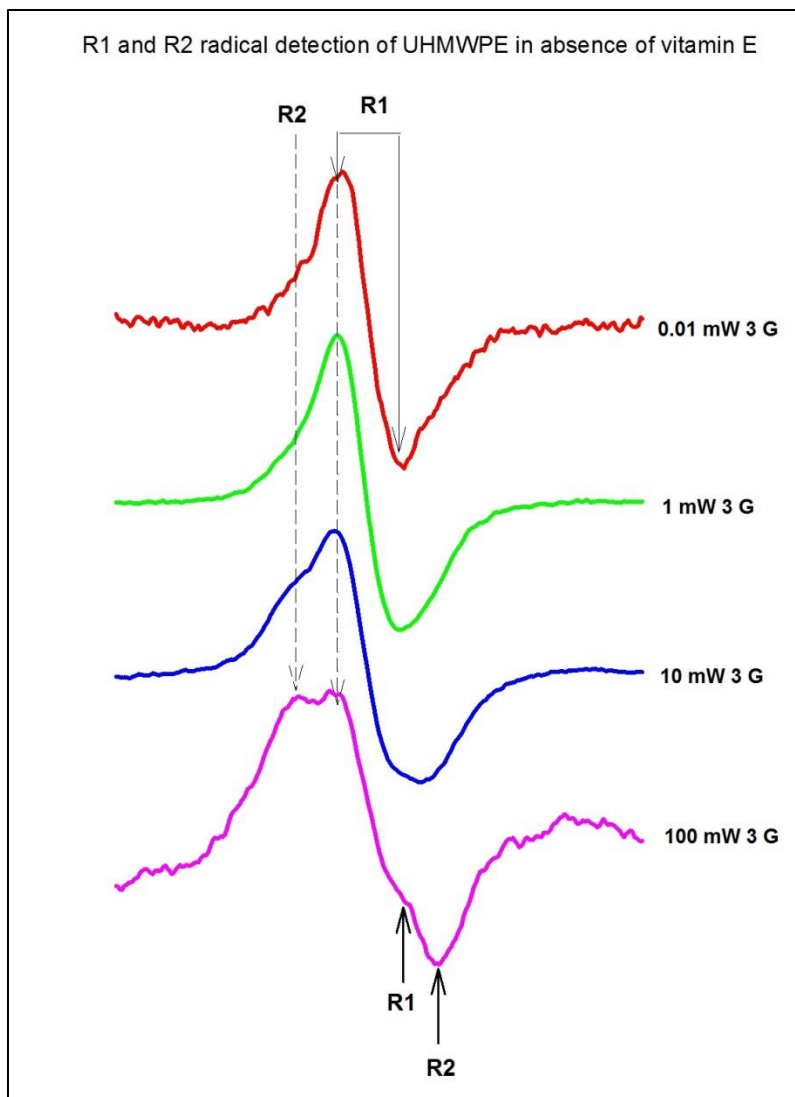
Figure 22 (a): Detection of radical R1 and R2 in non-vitamin UHMWPE after 10 years
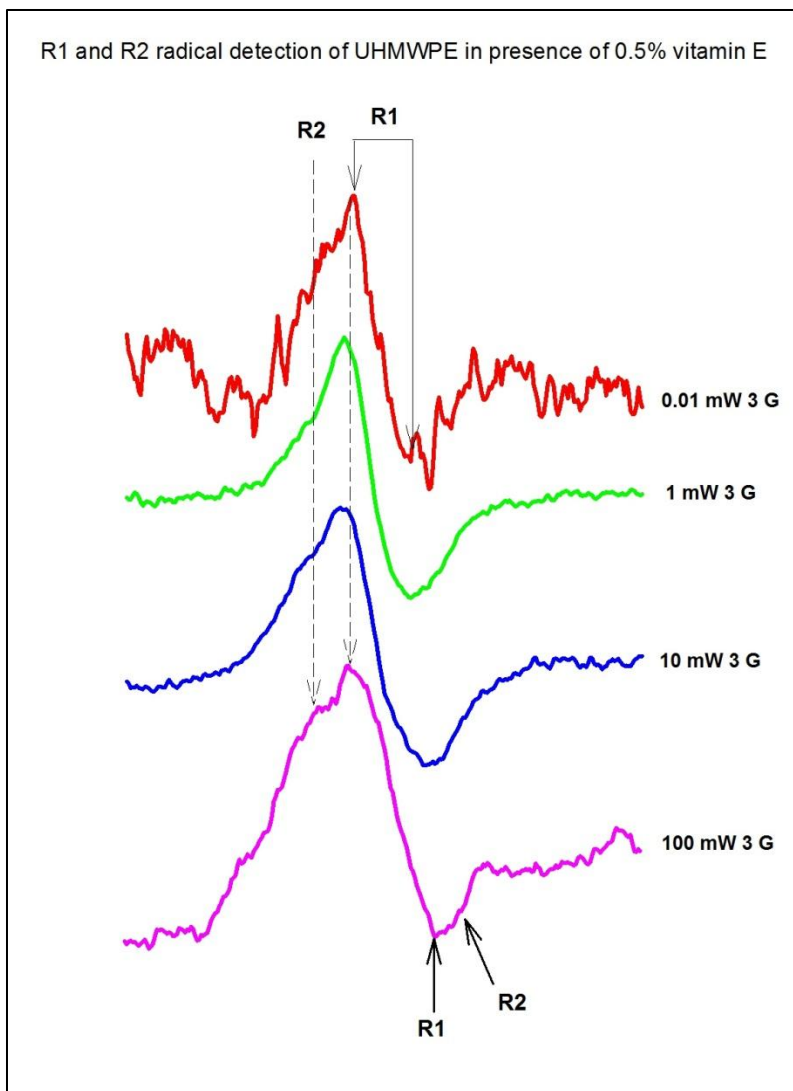
Figure 22 (b): Detection of radical R1 and R2 in vitamin E doped UHMWPE after 10 years
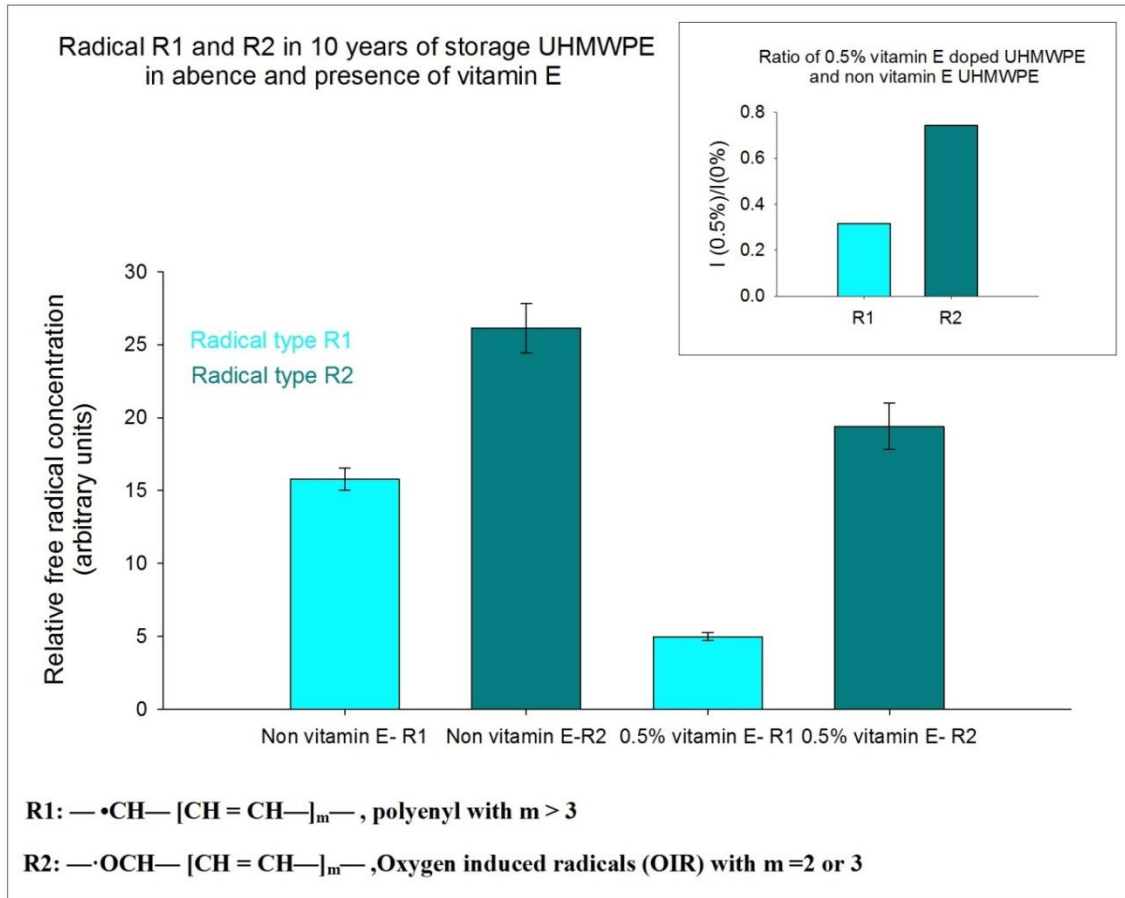
Figure 23: Relative R1 and R2 radical concentration of non-vitamin and vitamin E doped UHMWPE after 10 years

The bar graph shown in Figure 23 revealed that, 0.5% vitamin E decreased the carbon centered polynyle (R1) as well as oxygen centered polyenyle (R2) radical in UHMWPE over 10 years. Analyses of ESR data showed that, vitamin E doped GUR 1020 resin contained lower concentration, 0.3x and 0.7x of R1 (carbon-centered polyenyle) and R2 (oxygen-centered polyenyle), respectively, compared to GUR 4150. This reduction of free radicals confirms about the effect of vitamin E in stabilizing the free radicals of polyethylene. This reduction could result from the reduced amount of radicals present in the vitamin E containing samples at initial time (in 2006).

Chapter 5

Conclusion

The current study has outlined the comparative analysis of long lived free radical in orthopedic UHMWPE resin in absence and in presence of vitamin E. The experimental findings can be discussed separately for two groups of samples.

5.1 Group A

In this group, 18 years aged GUR 4150 and Hylamer (Himont 1900) samples in open air and in inert condition at 23°C, 37°C and 75°C, were investigated. Although the total concentration of radicals in all samples decreased by two orders of magnitude in 18 years, still there is quantifiable radical contents remaining in all the samples except those stored in air at 75°C.

The comparison between GUR 4150 and Hylamer showed some difference in radical behavior, which are explained below in different section.

The radical concentration of Hylamer samples stored in air at room temperature decreased by a factor of 2/3 compared to GUR 4150 over 18 years. This reduction of free radicals in Hylamer might be due to the more conversion of primary polyethylene radicals to the long-lived non-radical species compared to GUR 4150. However, the inert storage of both GUR 4150 and Hylamer showed similar concentration (slightly higher concentration at 23°C than at 37°C).

The inert-storage GUR 4150 and Hylamer, were also compared in terms of radical types (alkyl, allyl, polyenyle, dienyle and trienyle) present after 18 years. In this analysis, there was no significant difference was found between GUR 4150 and Hylamer at 23°C. At 37°C, Hylamer showed the presence of more alkyl (20/13), allyl (51/40) and less polyenyle (28/46). No conversion to dienyle or trienyle was observed at 23°C or 37°C. At 75°C, Hylamer (compared to

GUR 4150) showed the presence of lower concentration of alkyl (6/15), higher concentration of allyl (24/10), no polyenyle (0/0), approximately equal number of dienyle (68/74) and no trienyle (0/0). The large conversion of primary radicals to the dienyle components at 75°C might be due to the thermal motion of poyehtylene molecules at elevated temperature.

Analyses of the ESR data of the open-air samples further revealed that Hylamer contained lower concentration, 0.6x and 0.5x, of carbon-centered polyenyle (R1: — •CH— [CH = CH—]$_m$— , polyenyl with m > 3) and oxygen-centered-polyenyle (R2: —·OCH— [CH = CH—]$_m$— ,Oxygen induced radicals (OIR) with m =2 or 3), respectively, compared to GUR 4150. This result suggests that, Hylamer might have increased oxidation protection compared to GUR 4150.

The difference in radical behavior in Hylamer compared to GUR 4150, might be due to molecular weight, particle morphology or particle size. As mentioned earlier, the characteristics properties of Hylamer resulted from its polymerization process.

After 18 years, the ESR spectra were analyzed for all samples stored in air and inert condition. The structures of ESR spectra were consistent with the storage environment. The samples aged at 23°C or 37°C in open air showed the presence of oxygen-induced polyenyle radicals indicating the strong oxidation in presence of air. However, the ESR spectra of inert storage showed the presence of primary polyethylene radicals indicating the oxidation protection environment.

5.2 Group B

In this group, 10 years-aged shelf storage GUR 1020 samples doped with percent vitamin E (α-Tocopherol) were investigated. The ESR measurements conducted immediately after gamma irradiation in 2006 showed a decrease in free radical concentration by an order of magnitude due to vitamin E [39].

In 10 years (2016), the radical concentration was reduced by another order of magnitude; i. e. from ~10^{17} to ~10^{15} radicals per gram in all samples, with or without vitamin E. No measurable difference between radical concentrations was found as a function of vitamin E.

In ESR spectra analyses, there was no significant difference observed between 10 years-aged non-vitamin and 0.5% vitamin E doped GUR 1020 sample. Although vitamin E resonance lines were present in the ESR spectra of vitamin E doped GUR 1020 resin tested immediately after gamma-irradiation in 2006, there is no indication of the presence of vitamin E radicals in the present ESR spectra. This result suggests that, vitamin E radicals might not be stable over long period.

The effect of vitamin E in stabilizing the free radicals of UHMWPE was also studied by detecting and quantifying the carbon centered polynyle radical (R1) and oxygen centered polynyle radical (R2) in similar aged non vitamin E and vitamin E doped UHMWPE samples. The analyses of ESR data showed that, vitamin E doped GUR 1020 resin contained lower concentration 0.3x and 0.7x, of R1 and R2 respectively, compared to GUR 4150. This reduction of free radicals further confirms about the effect of vitamin E in stabilizing the free radicals of polyethylene. However, this reduction could be the result of initial quenching of radicals at the time of irradiation.

The comparison between the two groups of samples, Group A and Group B, may not provide any meaningful information because the materials, experimental conditions and measurement parameters were different. One piece of information, however is important that is a very slow free radical reaction prevails in all materials through the course of these studies.

References

[1] S. M. Kurtz, editor, "Ultra high molecular weight polyethylene in Total joint replacement and medical devices", *UHMWPE Biomaterials Handbook 2nd edition,* Elsevier Academic Press, 2009.

[2] X. Liu, S. Zhang, X.j. Xu, Z. Zhang, L. Zhou and G. Zhang, "Study on the creep and recovery behaviors of UHMWPE/CNTs composite fiber", *Fibers and Polymers*, vol.14, pp. 1635–1640, 2013.

[3] F.S. Senatov, M.V. Gorshenkov, S.D. Kaloshkin, V.V. Tcherdyntsev, N.Y. Anisimova, A.N. Kopylov and M.V. Kiselevsky, "Biocompatible polymer composites based on ultra-high molecular weight polyethylene perspective for cartilage defects replacement", *Journal of Alloys and Compounds*, vol. 586, pp. 544–547, 2014.

[4] A. George, H.D. Ngo and A. Bellare, "Influence of crystallization conditions on the tensile properties of radiation crosslinked, vitamin E stabilized UHMWPE", *Journal of the Mechanical Behavior of Biomedical Materials*, vol. 40, pp. 406–412, 2014.

[5] N. Hope and A. Bellare, "A comparison of the efficacy of various antioxidants on the oxidative stability of irradiated polyethylene", *Clinical Orthopaedics and Related Research*, vol. 473, pp. 936–941, 2015.

[6] S.-I. Ohnishi, Y. Ikeda, M. Kashiwagi and I. Nitta, "Electron spin resonance studies of irradiated polymers I. Factors affecting the electron spin resonance spectra of irradiated polymers", *Polymer*, vol. 2, pp. 119–141, 1961.

[7] A.A. Edidin, CW Jewett, A. Kalinowski, K. Kwarteng and S. M. Kurtz, "Degradation of mechanical behavior in UHMWPE after natural and accelerated aging", *Biomaterials*, vol. 21, pp. 1451–60, 2000.

[8] E. Oral, K.K. Wannomae, N. Hawkins, W.H. Harris and O.K Muratoglu, "α- Tocopherol-doped irradiated UHMWPE for high fatigue resistance and low wear", *Biomaterials*, vol. 25, pp. 5515–22, 2004.

[9] N. Shibata and N. Tomita, "The anti-oxidative properties of α-tocopherol in ϒ-irradiated UHMWPE with respect to fatigue and oxidation resistance", *Biomaterials*, vol. 26, pp. 5755–62, 2005.

[10] N. Tomita, T. Kitakura, N. Onmori, Y. Ikada and E. Aoyama, "Prevention of fatigue cracks in ultra-high molecular weight polyethylene joint components by the addition of vitamin E",

Journal of Biomedical Material Research: Applied Biomaterials, vol. 48, pp. 474–8, 1999.

[11] Introduction to Biomaterials in Orthopaedic Surgery, *Biomaterials in Orthopaedic Surgery (#05233G)*, ASM International, 2009.

[12] A. Sáenz, E. Rivera-Muñoz, W. Brostow and V. M. Castaño, "Ceramic Biomaterials: An Introductory Overview", *Journal of Materials Education*, vol. 21 (5-6), pp. 297 – 306, 1999.

[13] S. Venkatraman, F. Boey and L. L. Lao, "Implanted cardiovascular polymers: natural, synthetic and bio-inspired". *Progress in Polymer Science*, vol. 33, pp. 853–874, 2008.

[14] W. Khan , E. Muntimadugu , M. Jaffe , and A. J. Domb, "Implantable Medical Devices", *Advances in Delivery Science and Technology*, 2014.

[15] J. R. Joshi and R. P. Patel, "Role of Biodegradable Polymers in Drug deliver", *International Journal of Current Pharmaceutical Research*, vol. 4 (4), pp. 74-81, 2012.

[16] H. Park, J. S. Temenoff and A. G. Mikos, "Biodegradable Orthopedic Implants, Engineering of Functional Skeletal Tissues", Springer, vol. 3, pp. 55-68, 2007.

[17] S. M. Kurtz, editor, "The UHMWPE Handbook", Elsevier, 2004.

[18] E.M. Brach del Prever, A. Bistolfi, P. Bracco and L. Costa, "UHMWPE for arthroplasty: past or future"? *Journal of Orthopaedics and Traumatology*, vol.10, pp. 1–8, 2009.

[19] G.L. Blunn, E.M. Brach del Preva, L. Costa, J. Fisher and M.A. Freeman, "Ultra high molecular-weight polyethylene (UHMWPE) in total knee replacement: fabrication, sterilisation and wear", *The journal of bone and joint surgery*, vol. 84(7), pp. 946, 2002.

[20] R. Kiok, S. Fernandes, S.Gupta and A. James, "Sterilization Methods of Artificial Joint Prostheses", 1997.

[21] S. Affatato, B. Bordini, C. Fagnano, P. Taddei, A. Tinti and A. Toni, "Effects of the sterilisation method on the wear of UHMWPE acetabular cups tested in a hip joint simulator", *Biomaterials*, vol. 23(6), pp. 1439-46, 2002.

[22] H. Cui, Y. Kong and H. Zhang, "Oxidative Stress, Mitochondrial Dysfunction, and Aging", *Journal of Signal Transduction*, 2011.

[23] D. Clancy and J. Birdsall, "Flies, worms and the Free Radical Theory of ageing", *Ageing Research Reviews*, vol. 12(1), pp. 404-12, 2013.

[24] S. H. Naidu, B.L. Bixler and M.J.R. Moulton, "Radiation Induced Physical Changes in UHMWPE Implant Components", *Orthopedics*, vol. 20 (2), pp. 137- 142, 1997.

[25] M.S. Jahan, "ESR Insights into Macroradicals in UHMWPE", *UHMWPE Biomaterials* Handbook, Elsevier, 2009.

[26] P. Bracco and E. Oral, "Vitamin E-stabilized UHMWPE for Total Joint Implants: A Review", *Clinical Orthopaedics and Related Research*, vol. 469(8), pp. 2286–2293, 2011.

[27] E.Oral, Y. Ikeda and O. K.Muratoglu, "Radiation cross-linking in ultra-high molecular weight polyethylene for orthopaedic applications", *Nuclear Instruments and Methods in Physics ResearchSectionB: BeamInteractions withMaterials andAtoms*, vol. 265 (1), pp. 18–22, 2007.

[28] E. J. Lawton, R.S. Powell and J.S.Balwit, "Effect of physical state during the electron irradiation of hydrocarbon polymers. Part II.Additional experiments and discussion pertaining to trapped radicals in hydrocarbon polymers", *Journal of Polymer Science*, vol. 32(125), pp. 277–290, 1958.

[29] P. O'Neill, C. Birkinshaw, J. J. Leahy and R. Barklie, "The role of long lived free radicals in the ageing of irradiated ultra high molecular weight polyethylene", *Polymer Degradation and Stability*, vol. 63(1), pp. 31–39, 1999.

[30] B.M. Weckhuysen, R. Heidler and R. A. Schoonheydt, "Electron Spin Resonance Spectroscopy", *Molecular Sieves*, vol. 4, pp. 295–335, 2004.

[31] Y. Zhao, M. Wang, Z. Tang and G. Wu, "ESR study of free radicals in UHMW-PE fiber irradiated by gamma rays", *Radiation Physics and Chemistry*, vol.79 (4), pp. 429–433, 2010.

[32] E. Oral, B. W. Ghali and O. K. Muratoglu, "The elimination of free radicals in irradiated UHMWPEs with and without vitamin E stabilization by annealing under pressure", *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, vol. 97B (1), pp. 167–174, 2011.

[33] H. Wang, L. Xu, R. Li, J. Hu, M. Wang and G. Wu, "Improving the creep resistance and tensile property of UHMWPE sheet by radiation cross-linking and annealing", *Radiation Physics and Chemistry*, vol.125, pp. 41–49, 2016.

[34] T. Liu, A. Eyler and W.H. Zhong, "Simultaneous improvements in wear resistance and mechanical properties of UHMWPE nanocomposite fabricated via a facile approach", *Materials Letters*, vol.177, pp. 17–20, 2016.

[35] M.S. Jahan, M.C.King, W.O. Haggard, K.L. Sevo and J.E. Parr, "A study of long-lived free radicals in gamma-irradiated medical grade polyethylene", *Radiation Physics and Chemistry*, vol. 62, pp. 141–144, 2001.

[36] L. Costa, M.P. Luda, L. Trossarelli, E.M. Brach del Prever, M. Crova and P. Gallinaro, "Oxidation in orthopaedic UHMWPE sterilized by gamma radiation and ethylene oxide", *Biomaterials*, vol. 19, pp. 659–668, 1998.

[37] D.C. Sun, A. Wang and C. Stark, "The concept of stabilization in UHMWPE", *Transactions of the Fifth World Biomaterial Congress*, vol. 2, pp. 195, 1996.

[38], Peter Atkins and Julio De Paula, "Atkins' Physical Chemistry", *Ninth Edition, Oxford University Press*, 2009.

[39] M. D. Ridley and M.S. Jahan, "Effects of packaging environments on free radicals in ϒ-irradiated UHMWPE resin powder blend with vitamin E", *Journal of Biomedical Materials Research Part A*, vol. 88A (4), pp. 1097–1103, 2009.

[40] S. Kurtz , M. Manley, A. Wang, S.J. Taylor and J.H. Dumbleton, "Comparison of the properties of annealed crosslinked (Crossfire) and conventional polyethylene as hip bearing materials", *Bulletin of the Hospital for Joint Disease*, vol. 61, pp. 17–26, 2002.

[41] A. Wang, H. Zeng, S.S Yau, A. Essner, M. Manely and J. Dumbleton, "Wear, oxidation and mechanical properties of a sequentially irradiated and annealed UHMWPE in total joint replacement", *Journal of Phys D: Applied Physics*, vol. 39, pp. 3213–3219, 2006.

[42] F. J. Medel, P. Pena, J. Cegonino, E. Gomez-Barrena and J.A. Puertolas, "Comparative fatigue behavior and toughness of remelted and annealed highly crosslinked polyethylenes", *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, vol. 83, pp. 380–390, 2007.