

Effects of light in everyday life monitored by electron paramagnetic resonance (EPR) spectroscopy

Kalina Ranguelova, Ph.D., EPR Application Scientist at Bruker BioSpin Corp.

Photochemical reactions are initiated by absorption of energy in the form of light and involve formation of free radicals and transient excited states whose chemical and physical properties differ greatly from the original molecules. For example, one of the most well-known flavor defects in beer across the world is to become skunky or 'lightstruck' after exposure to UV light. The reason for this 'lightstruck flavor' is the photodecomposition of isohumulones, the major flavor components of hops, via free radical photodegradation mechanism. Free radicals and excited states are often short-lived but play crucial roles in significant photochemical processes such as photooxidation, photodegradation, photostability, photocatalysis, photosynthesis, and polymerization.

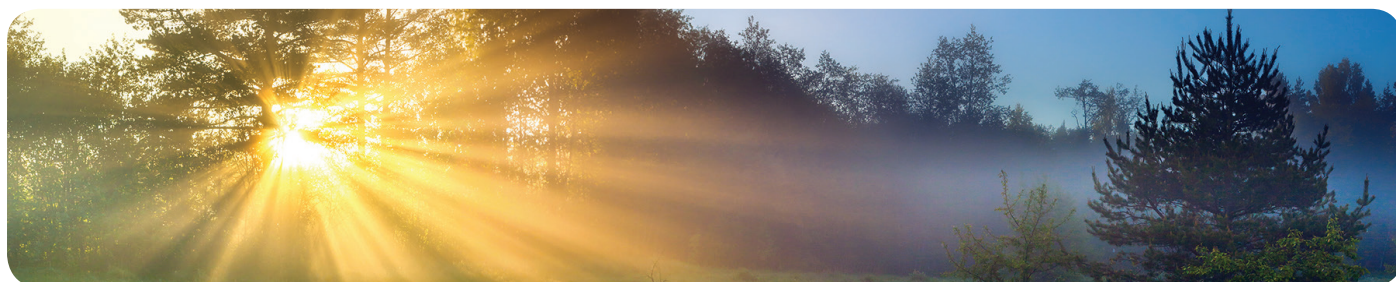
Electron paramagnetic resonance (EPR) spectroscopy is the only analytical technique capable of detecting species with free radicals and transient excited states, in a direct and non-invasive manner. The technique is very versatile as it can be applied to gaseous, liquid, or solid samples over a large range of temperatures. It can be used to detect, quantify and monitor the intrinsic photogeneration of short-lived species and is therefore extremely useful for detecting free radicals in photochemical reactions in polymer science, pharmaceuticals, environment, etc. The aim of this paper is to present a general overview of the diversity of EPR applications when it comes to photochemical reactions.

I. Photodegradation studies using EPR

Light is an important factor in considering the stability of materials and is important to study and evaluate it if light exposure does result in unacceptable changes in the final product or compromise its shelf-life. For example, degradation

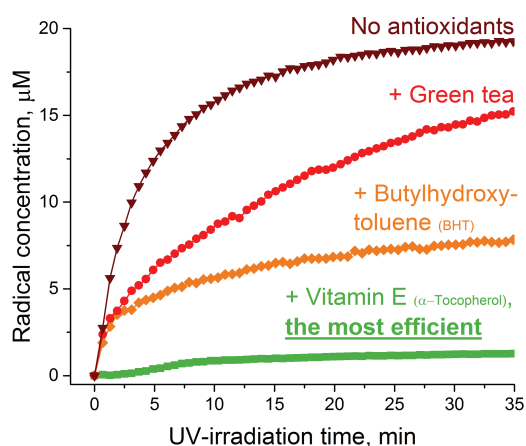
of polymers due to light exposure leads to discoloration and a decrease in the mechanical properties (elasticity, toughness, etc.). To prevent this decomposition, hindered amine light stabilizers (HALS) are added to the polymer. When the polymer is exposed to UV-irradiation HALS suppresses radical damage in the polymer by forming a HALS-based nitroxyl radical, i.e. HALS sacrifices itself to protect the polymer molecules. EPR detects the HALS radicals and by monitoring and quantifying the EPR signal, the effectiveness of HALS can be evaluated. This EPR application is successfully used to determine polymer photodegradation in both academic and industrial settings for research and quality control. For example, Ford Motor Company published an EPR study where concentrations of HALS radicals during the early stages of weather exposure provide direct insight into the long-term weathering performance of clearcoat/basecoat automotive paint systems (1).

Skin is a very susceptible target organ to photodegradation (photo-aging). UV-light is the main cause of skin disorders including sunburn, premature aging, and photocarcinogenesis, resulting from UV-induced oxidative stress and mediated by short-lived radicals named reactive oxygen species (ROS). Thus, photo-protection is crucial for preventing the undesired effects of sun exposure and is mainly achieved using sunscreens and other skin care products containing UV-filters, and antioxidants. Their radical scavenging activity can be measured by EPR spectroscopy as well as screening of their efficacy and safety. The product is typically tested under accelerated conditions (UV-irradiation) to increase the rate of photodegradation and the data generated from these stability studies (stress testing) can be transferred to real time stability data.



The efficacy of antioxidants in skin care products depends on their penetration kinetics, reactivity with non-radical components, and photo-stability. For example, when three commonly used antioxidants (green tea, butylhydroxytoluene, and vitamin E) were studied for their effectiveness in a skin care product via accelerated UV-irradiation, the EPR data showed almost ~90% decrease of the radical yield in the case of vitamin E followed by ~60% and ~25% decrease for butylhydroxytoluene and green tea, respectively (Figure 1). The total EPR acquisition time for all three antioxidants and the control sample was approximately 2 hours which demonstrates the capability of EPR as a quick method to evaluate the skin care product quality.

Figure 1



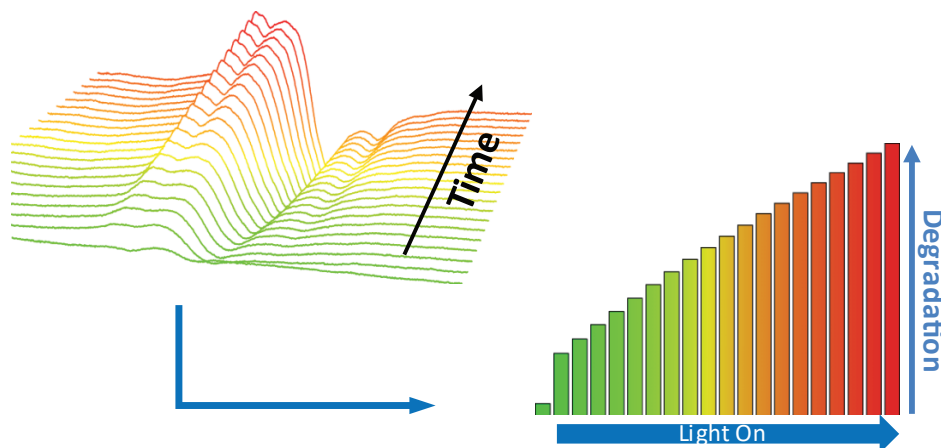
Effectiveness of antioxidants (green tea, BHT, and vitamin E) on skin care product aging upon UV-irradiation evaluated by EPR method.

EPR spectroscopy is also successfully used to monitor free radical formation during photodegradation of pharmaceutical products, identify the radical intermediates and help determine the reaction mechanism. Light can compromise drug

product stability and may cause degradation of the active pharmaceutical ingredients (APIs), inert materials (excipients), or the final medicinal products (formulations) resulting in loss of product potency or toxic by-product generation. Photodegradation processes quite often involve free radicals and transition metals that are responsible for most of the damage that occurs in drug products. The International Conference of Harmonization (ICH) guidelines state that the intrinsic photo-stability characteristics of all new APIs and products should be evaluated to demonstrate that light exposure does not result in unacceptable change. These guidelines have been implemented in Europe, the United States, and Japan for over 20 years. EPR spectroscopy can be used to determine the root cause of degradation, measure the extent of degradation, and predict long-term stability characteristics of the APIs, excipients and formulations and because of EPR's inherent high sensitivity the measurements are very quick and only use a small quantity of the API or drug formulation, so the technique could be used during the early drug development phase (2). For example, photodegradation of the hypertension drug Nifedipine after exposure to light shows the formation of a free radical (Figure 2). The time evolution of the free radicals is followed by EPR and the signal is identified as N-based free radical formed due to the photodegradation of the API. The increasing amount of radicals quantified by EPR shows the level of API degradation and can be used to predict the product photostability.

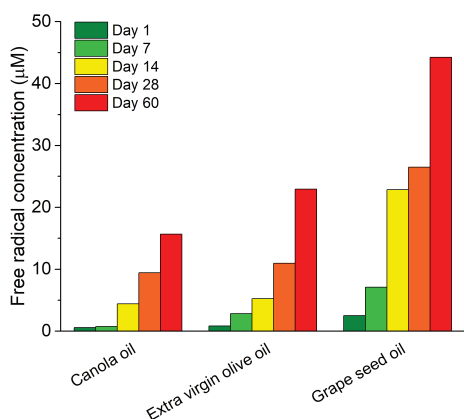
Free radical photochemical reactions are very common in food and beverages and they play a major role in food photodegradation. In addition, there is strong evidence for the fundamental role free radicals play in many diseases. However, there are also studies showing that free radicals generated during food processing may play key functional roles in texturization, flavor formation, and other reactions contributing to food properties and characteristic qualities. Therefore, identifying and understanding photochemical reactions that are free radical-medi-

Figure 2



Photodegradation of Nifedipine – time evolution of N-based radical formed in the API and monitored by EPR.

Figure 3



Quantitative EPR radical analysis after window exposure of edible oils for 60 days.

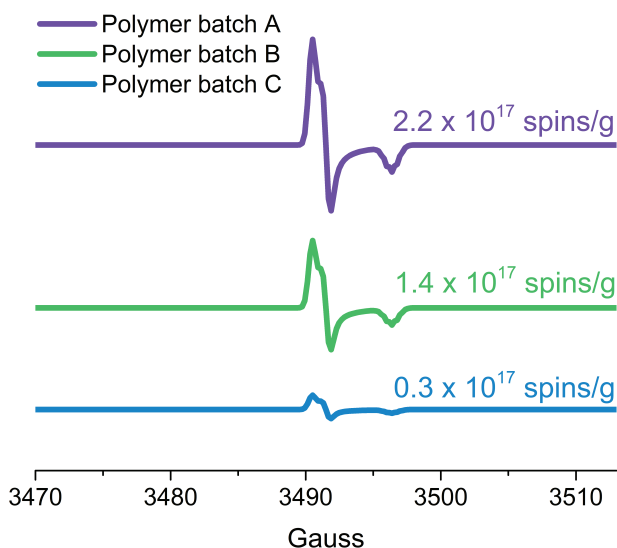
ated in foods and beverages, and learning to control these reactions associated with food qualities and shelf-life stability give us sufficient reason to be interested in EPR detecting and studying radicals in foods and beverages. We mentioned in the beginning of this article about the effects of UV-light on beer associated with beer 'skunking'. Another example of food photodegradation is rancidity of edible oils. It arises during storage or transport and is due to the free-radical mediated photooxidation of unsaturated fatty acids that affects quality and determines, in many cases, the shelf-life of the oil. Fatty acid photodegradation occurs in three well-documented phases: (i) initiation, (ii) propagation, and (iii) termination. The oxidized compounds that result at the end of the process give the oil an undesirable odor and taste. With the help of EPR stress testing, oxidation profiles and accurate calculation of

free radical concentrations provide a measure for oxidative resistance at each stage of the production process (Figure 3). This enables manufacturers to make rapid and informed process control decisions to optimize product shelf-life.

EPR spectroscopy has been successfully applied in studies of photodegradation reactions in material science with respect to products such as solar cells, fuel cells, batteries, etc. As a result, paramagnetic imperfections such as defects, vacancies, and free radicals affect the performance and properties of these materials. Therefore, it is crucial and mandatory to detect and characterize those species and with the EPR method one can gain insight into the stability 'soft spots' of the materials which is important for developing robust products. For example, Susarova et al. used EPR as a highly sensitive analytical technique for quality assessment of conjugated polymers and their performance in organic solar cells (3). It has been shown that different batches of the same polymer reveal different photovoltaic performances correlating with the respective free radical concentrations. EPR data show that this is related to structural defects or impurities possessing unpaired electrons (Figure 4). These free radicals are believed to behave as deep traps for mobile charge carriers thus affecting the performance of the conjugated polymers in solar cells.

Photodegradation can be a two-edged sword. Photodegradation is gaining importance in the area of wastewater treatment especially for wastewater containing small amounts of refractory organic substances such as pharmaceutical residues. A vast number of pharmaceuticals have been detected in surface water and drinking water around the world, which indicates their ineffective removal from water and wastewater using conventional treatment technologies. Concerns have

Figure 4



Polymer batch	Spin density Spins/g	Power Conversion Efficiency, %	Half Lifetime, hrs
A	2.2e17	4.5	30
B	1.4e17	6.2	50
C	0.3e17	7.1	150

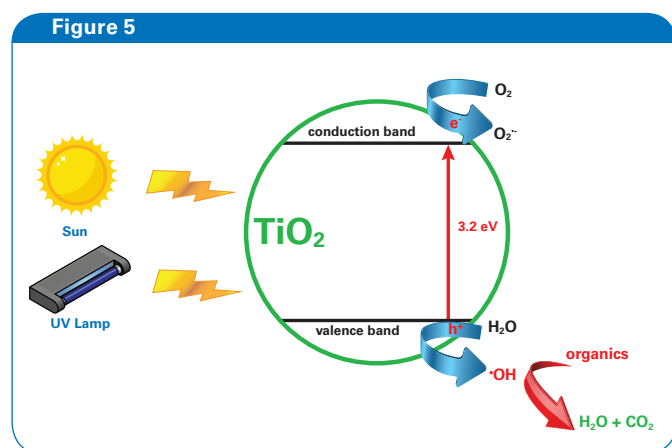
Quantitative EPR analysis of free radical defects presented as spins/g in conjugated polymers (used in solar cells). The number of defects correlates with the solar cell efficiency and stability.

been raised over potential adverse effects of pharmaceuticals on public health and aquatic environments. Among the different treatment options, photochemical advanced oxidation processes (light AOPs) are very promising for efficient degradation of pharmaceuticals and other organic pollutants in water and wastewater. In general, AOPs are based on the chemistry of hydroxyl radicals ($\bullet\text{OH}$), which are non-selective reactive oxygen species, able to oxidize water pollutants into inactivated end-products. Examples of light AOPs include ozone/UV-radiation, H_2O_2 /UV-radiation, photo-Fenton chemistry, and heterogeneous photodegradation using TiO_2 as a photocatalyst. There are articles comparing different AOPs on the basis of their effectiveness to remove the organic pollutants, even measured by cumulative concentrations such as natural organic matter and total organic carbon, but the $\bullet\text{OH}$ concentration, and consequently the oxidation efficiency, strictly depends on the AOP conditions so that comparison of the method performance is not related to a single variable, but depends on a large number of parameters that do not allow a rationalization of the results. The only technique to detect, identify, and quantify hydroxyl radicals produced in light AOPs is EPR and there-

fore it can be used to develop design and optimization of the AOP of interest to achieve the most effective treatment and reduce operating costs (4).

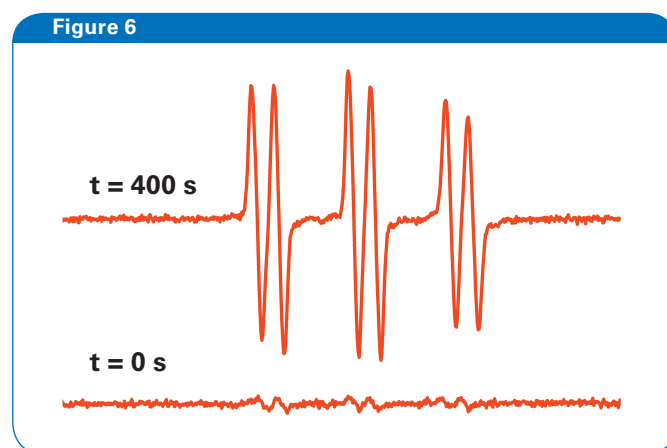
II. Photocatalysis studies using EPR

Stability, low cost, non-toxicity and appropriate photocatalytic activeness are some of the criteria for a good photocatalyst. Among many these, titanium dioxide meets these criteria for industrial-scale utilization and is predisposed to a wide range of applications in various areas (photocatalysts, solar cells, thin film capacitors, gas sensors, self-cleaning surfaces, etc.) In general, the photoactivity of TiO_2 is determined by the process of electron/hole pair generation, recombination, interfacial transfer, and by the surface reactions of these charge carriers with the species adsorbed on the surface of the photocatalyst. TiO_2 derives its activity from the fact that when photons of a certain wavelength are incident upon its surface, electrons are promoted from the valence band and transferred to the conduction band. This leaves positive holes in the valence band, which react with the hydroxylated surface to produce $\bullet\text{OH}$ radicals which as we discussed above are the most potent oxidizing agents (Figure 5).



Scheme of photocatalysis (taken from Ref. (5)).

Another EPR application in photocatalysis is related to detection of paramagnetic reactive oxygen species (ROS) during photocatalytic activities of nanomaterials. For example, Vankayala et al. reported that singlet oxygen is generated via photo-irradiation of gold nanoparticles which can be used as photothermal agents in treating cancerous tumors (6). Many micro- and nanomaterials are capable of initiating ROS generation under varying experimental conditions which can be considered as an intrinsic parameter of a given nanomaterial, like other physiochemical properties such as particle size, morphology, etc. EPR is used to develop appropriate ROS-active nanomaterials for specific applications since their photocatalytic and photobiological activity correlates with the ROS concentration monitored by the resonance technique (7).

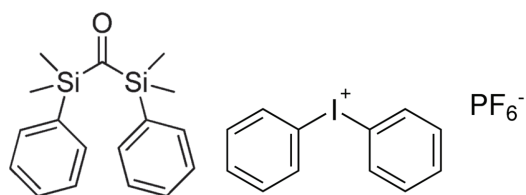


Hydroxyl radical detection by EPR in UV-irradiated TiO_2 after 0 and 400 sec. The spin trapping technique was used to capture the radicals.

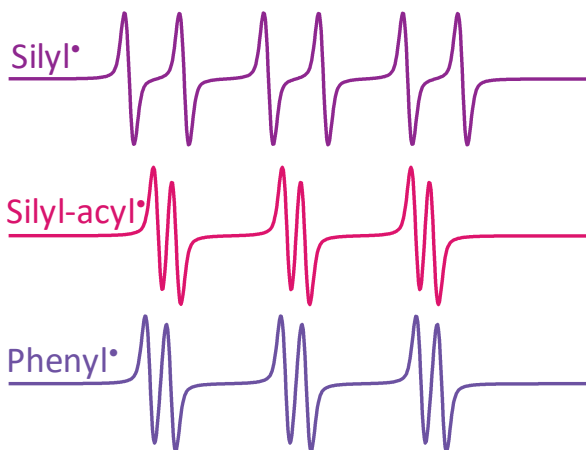
III. Study of photopolymerization using EPR

Free radical photopolymerization is the most widespread application so far, and modern technologies are constantly looking for new and efficient radical photoinitiators. On absorption of light, a photoinitiator induces free radical chemistry reactions that result in significant changes in the solubility and physical properties of suitable formulations. EPR spectroscopy is used to detect and monitor free radical intermediates shedding light on the underlying mechanisms. For example, EPR methods were successfully applied in development of a novel class of silyl radical generating photoinitiators used for photopolymerization of methacrylates, e.g., in dental materials (8). Three different radical intermediates were detected and identified by EPR (Figure 7).

Figure 7



Photoinitiators



EPR spectra of radical species generated in photoinitiator bis-silylketone used for photopolymerization of methacrylates, e.g., in dental materials.

Furthermore, quantitative EPR analysis provided information about the radical reactivity and photopolymerization efficiency. The data appeared to be considered as a starting idea for the development of other photoinitiating systems in this series.

In summary, understanding photochemical reactions is crucial for many applications in science and technology. EPR provides the unique capability to directly measure the radicals that play a part in these reactions. By detecting, identifying, monitoring, and quantifying radical intermediates, EPR is the solution for better understanding free radical photochemistry reactions and thereby helps to improve products' performance and their shelf-life.



References

- [1] Gerlock J.L. et al., Determination of active HALS in automotive paint systems II: HALS distribution in weathered clearcoat/basecoat paint systems, *Polym. Degrad. Stab.* (2001) 73 201
- [2] Williams H. et. al., Predicting the photostability characteristics of active pharmaceutical ingredients using electron paramagnetic resonance spectroscopy, *Drug Dev. Ind. Pharm.* (2012) 38(2) 200
- [3] Susarova D. et al., ESR spectroscopy as a powerful tool for probing the quality of conjugated polymers designed for photovoltaic applications, *Chem Commun.* (2015) 51 2239
- [4] Brame J. et al., Trading oxidation power for efficiency: Differential inhibition of photo-generated hydroxyl radicals versus singlet oxygen, *Water Res.* (2014) 60 259
- [5] Saracino M. et al., Water remediation 2.0: Advanced oxidation processes, *La Chimica & l'Industria*, October (2015)
- [6] Vankayala R. et al., Morphology dependent photosensitization and formation of singlet oxygen ($^1\Delta_g$) by gold and silver nanoparticles and its application in cancer treatment, *J. Mater. Chem. B* (2013) 1 4379
- [7] He W. et al., Composition directed generation of reactive oxygen species in irradiated mixed metal sulfides correlated with their photocatalytic activities, *ACS Appl. Mater. Interfaces* (2015) 7 16440
- [8] (8) Graff B. et al., Development of novel photoinitiators as substitutes of camphorquinone for the LED induced polymerization of methacrylates: a bis-silyl ketone, *Macromol. Rapid Commun.* (2017) 38 1600470

About Bruker Corporation (NASDAQ: BRKR)

For more than 55 years, Bruker has enabled scientists to make breakthrough discoveries and develop new applications that improve the quality of human life. Bruker's high-performance scientific instruments and high-value analytical and diagnostic solutions enable scientists to explore life and materials at molecular, cellular and microscopic levels.

In close cooperation with our customers, Bruker is enabling innovation, productivity and customer success in life science molecular research, in applied and pharma applications, in microscopy, nanoanalysis and industrial applications, as well as in cell biology, preclinical imaging, clinical phenomics and proteomics research, clinical microbiology and molecular pathology research.

For more information, please visit:

www.bruker.com/epr



info@bruker.com
www.bruker.com