Effects of light in everyday life monitored by Electron Paramagnetic Resonance (EPR) spectroscopy

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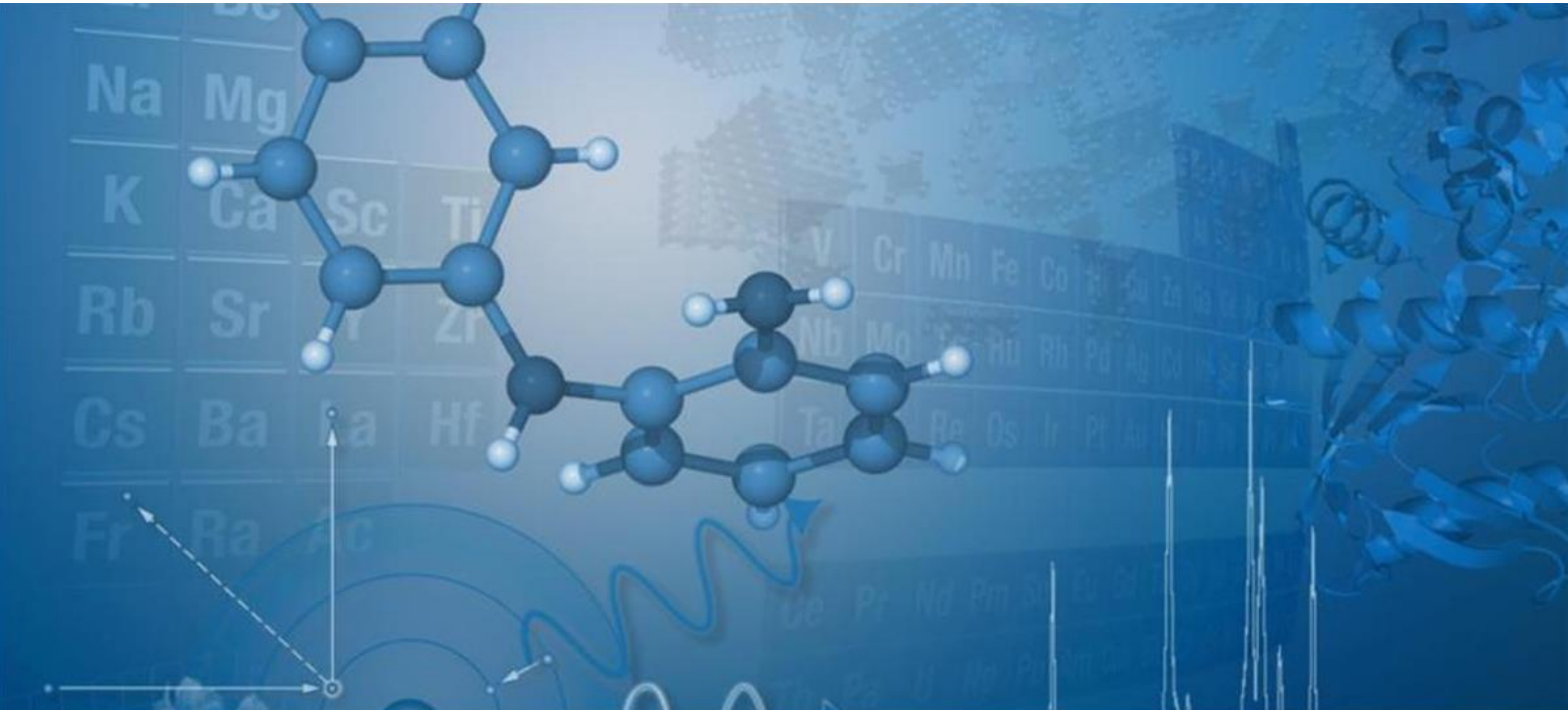
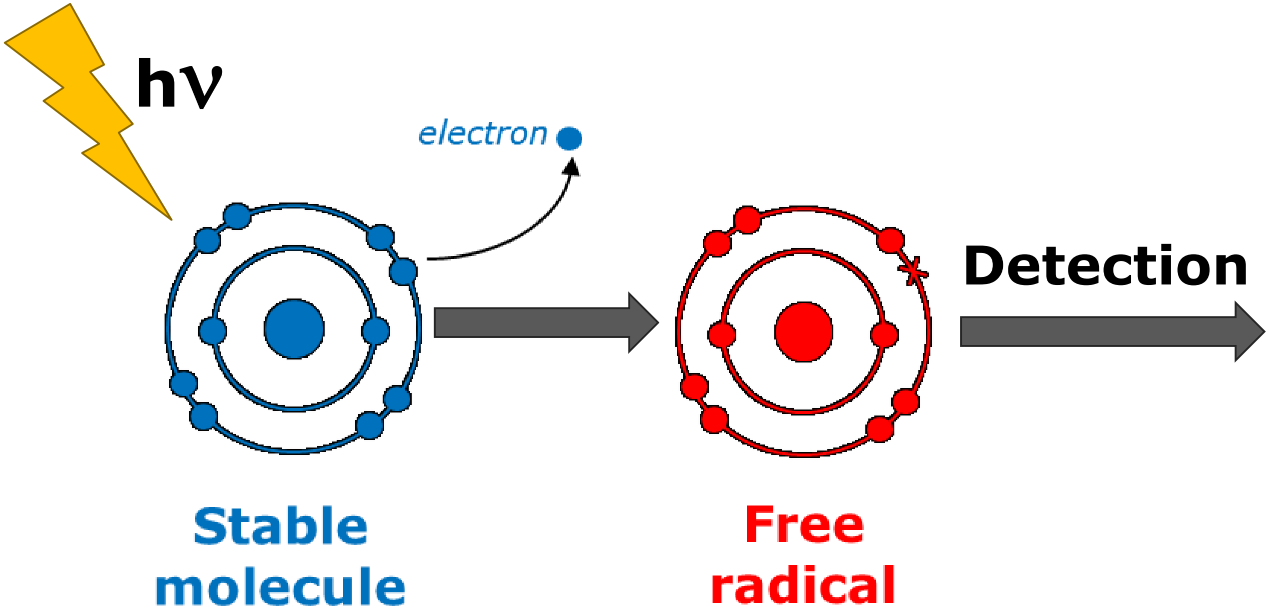


What is EPR?

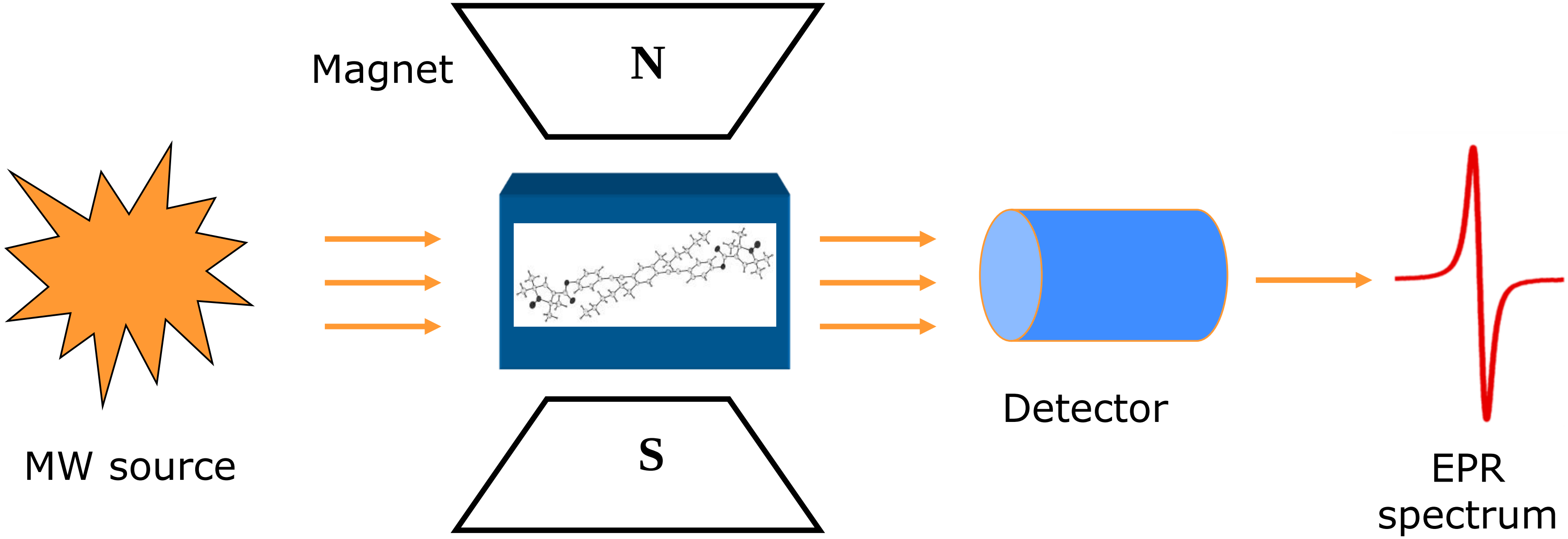
- EPR is a magnetic resonance technique that detects **unpaired electrons**
- **Unpaired electrons** occur in **free radicals** and many **transition metals**
- **Free radicals** and **transition metal ions** often participate in photochemical processes



EPR solutions EMXnano package

How does EPR work?

- External magnetic field
- Microwave irradiation source
- Sample with unpaired electron (**free radical** or **transition metal ion**)
- Detector



The EPR spectrometer

Features at-a-glance

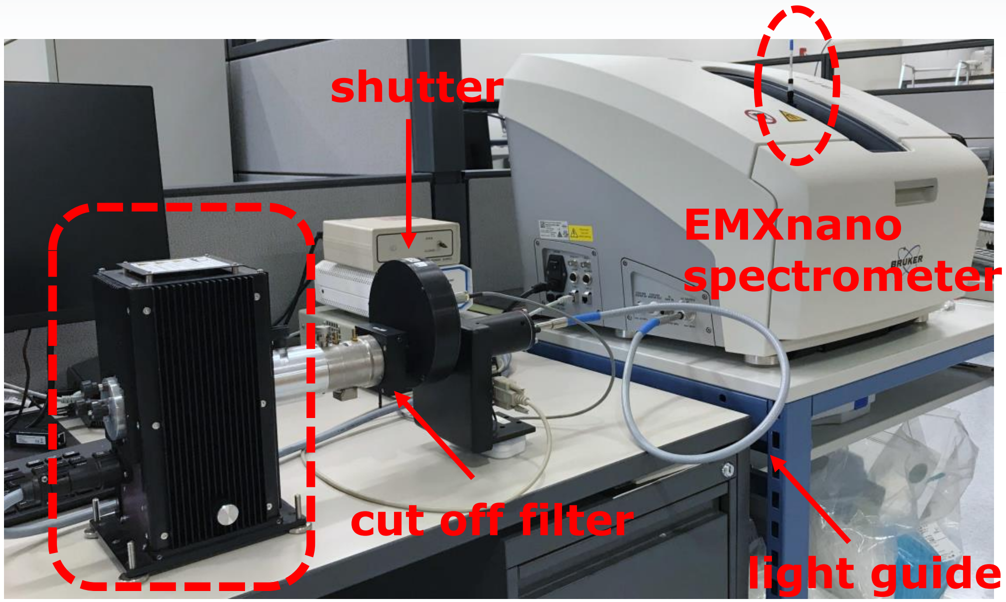
- Automated tuning and measurement
- Integrated, motorized amplitude and field reference standard (marker)
- Fully-calibrated for quantitative analysis with dedicated application workflows. No need of calibration curve
  - **SpinCount**: Reference free quantification of EPR species
  - **SpinFit**: Spectrum fitting and identification of EPR species
  - Spin-trap library of EPR spectra
- Video how-to guide
- Low infrastructure requirement and low cost-of-ownership

EMXnano EPR spectrometer

The UV-Vis irradiation system

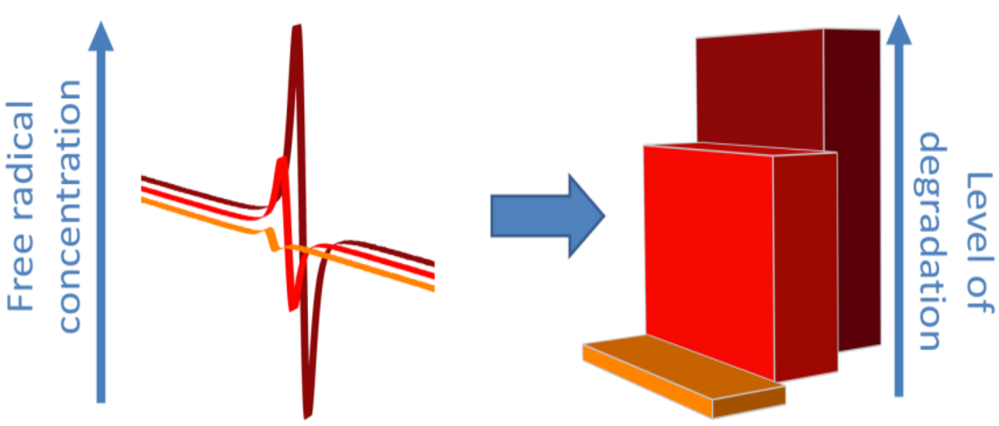
Irradiate sample in situ

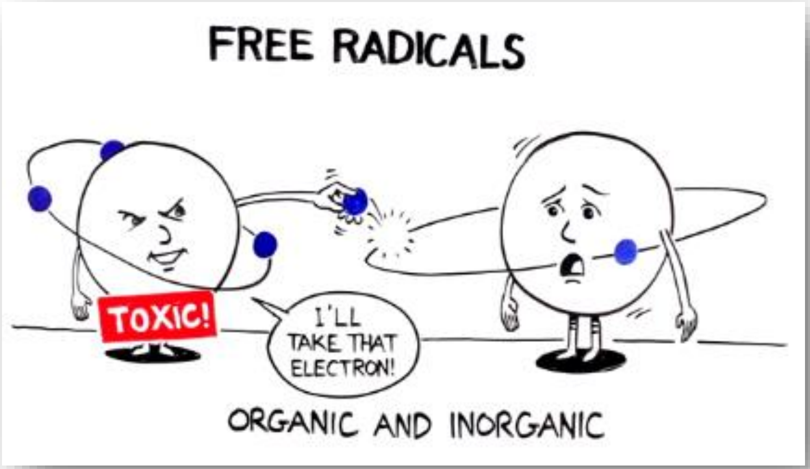
- Irradiation source: a 100 W Hg or Xe lamp for 200 – 2000 nm wavelength range
- Shutter (for triggered exposure)
- Light guide
  - High reproducibility
  - Safety – light path is contained with no leakage
- Wavelength filters for specific wavelengths
- Tubes with OD: 3-, 4-, 5-, and 10-mm OD
- Tube holders ensure precise and reproducible sample positioning
- Capillaries for limited size samples



Why doing EPR?

- **Electron Paramagnetic Resonance (EPR)** spectroscopy is very versatile as it can be used to detect, quantify and monitor the intrinsic photogeneration of short-lived species
- **Free radicals** participate in photochemical reactions in polymer science, pharmaceuticals, environment, and often they are the main source of photo damage in materials, organisms and food products
- By analyzing an EPR signal, one can determine temporal behavior of the **free radicals** involved in product photodegradation.



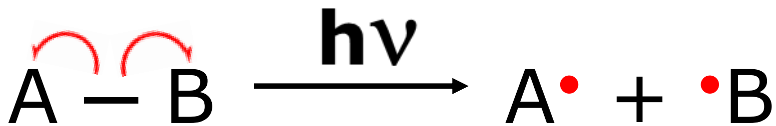


watch the video: https://www.bruker.com/products/mr/epr/what-is-epr.html

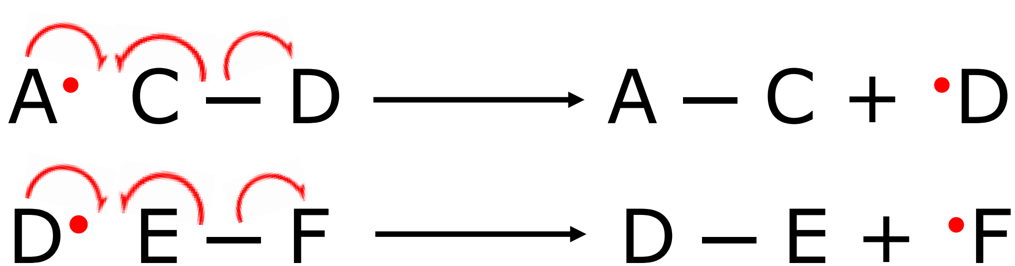
Photochemical free radical chain reactions

Absorption of photons causes cleavage of covalent bonds producing free radicals and sometimes initiates chain reactions. There are three phases of the radical chain reactions:

- **Initiation:** the step that initially creates a radical species. In most cases, this is a homolytic cleavage event and often UV radiation is necessary to overcome the energy barrier



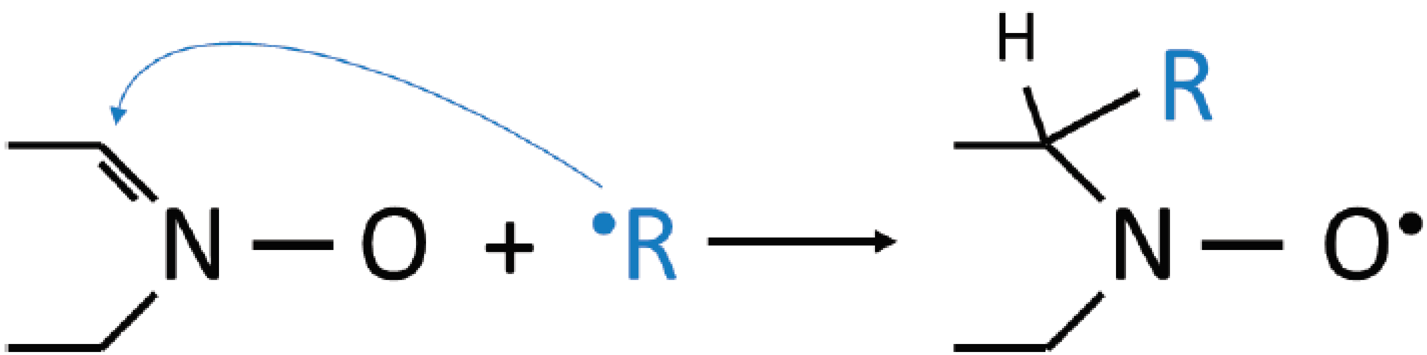
- **Propagation:** describes the 'chain' part of chain reactions. Once a reactive free radical is generated, it can react with stable molecules to form new free radicals



- **Termination:** occurs when two radical species react with each other to form a stable, non-radical adduct $F^{\bullet} + {}^{\bullet}G \longrightarrow F - G$

Strategy to detect short-lived and reactive free radicals

Spin trapping technique



Spin trap

Free radical

- Half-life: msec, sec

Radical adduct

- Very characteristic EPR spectra depending on the •R
- Half-life: min, hrs

EPR and food

Lipid photodegradation

European Journal of Lipid Science and Technology — Euro Fed Lipid

Research Article

Degradation of Edible Oil During Deep-Frying Process by Electron Spin Resonance Spectroscopy and Physicochemical Appreciation

Ying Liu, Yuanpeng Wang, Peirang Cao, Yuanfa Liu

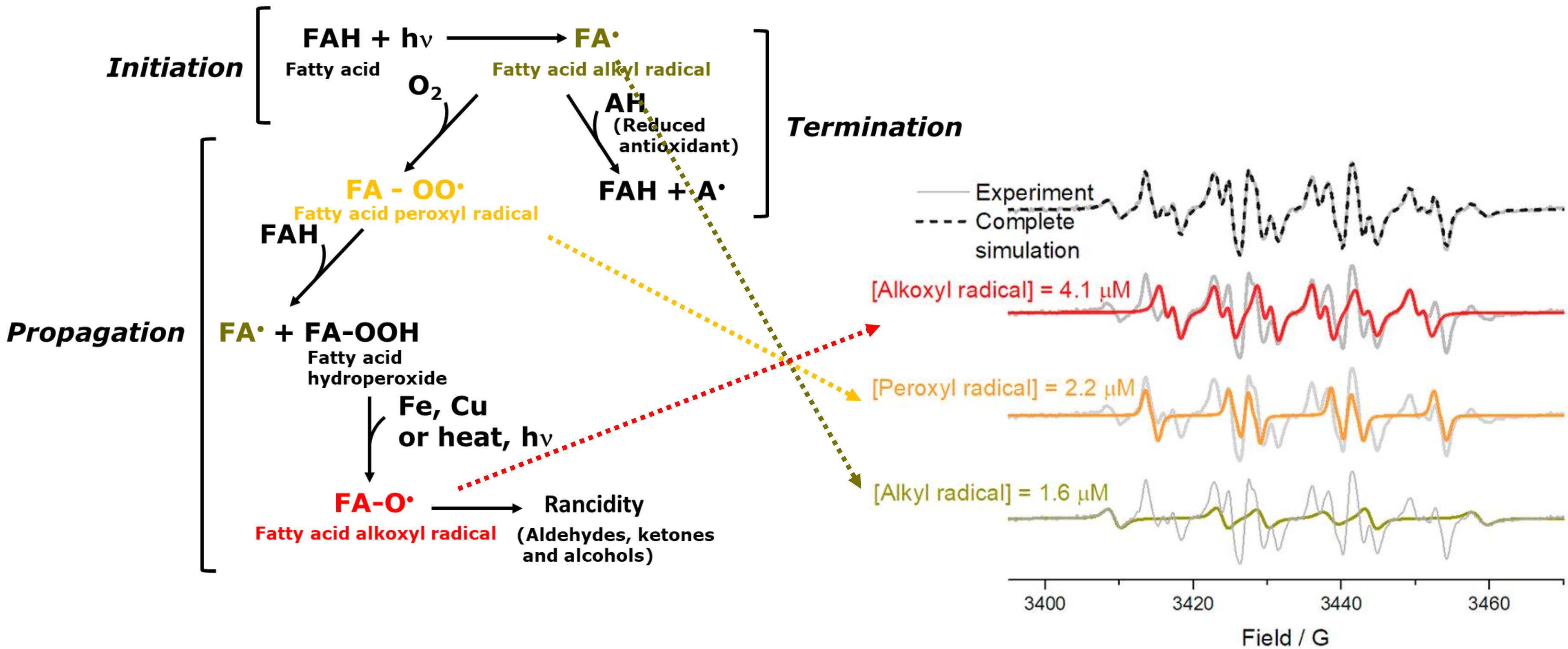
Journal of the American Oil Chemists' Society

January 2017, Volume 94, Issue 1, pp 89–97 | Cite as

Evaluating Electron Paramagnetic Resonance (EPR) to Measure Lipid Oxidation Lag Phase for Shelf-Life Determination of Oils

Authors | Authors and affiliations

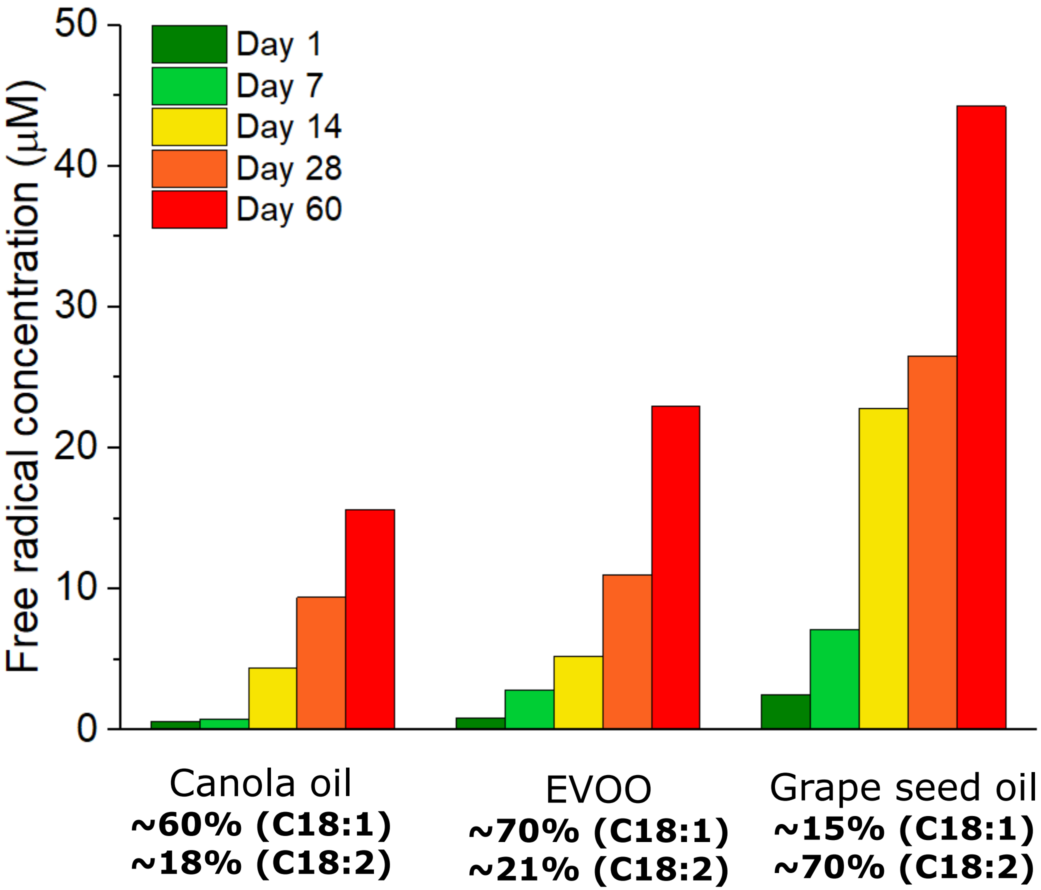
Leqi Cui, Paul M. Lahti, Eric A. Decker



EPR and food

Lipid photodegradation

Window light exposure



- Photooxidation of unsaturated fatty acids results in rancidity of fat containing foods
- Oxidation profiles and accurate free radical concentrations provide a measure for oxidative resistance at each stage of the photodegradation process
- EPR enables manufacturers to make rapid and informed process control decisions to optimize product shelf life

EPR and food

Starch photodegradation

Carbohydrate Polymers
Volume 210, 15 April 2019, Pages 339-349

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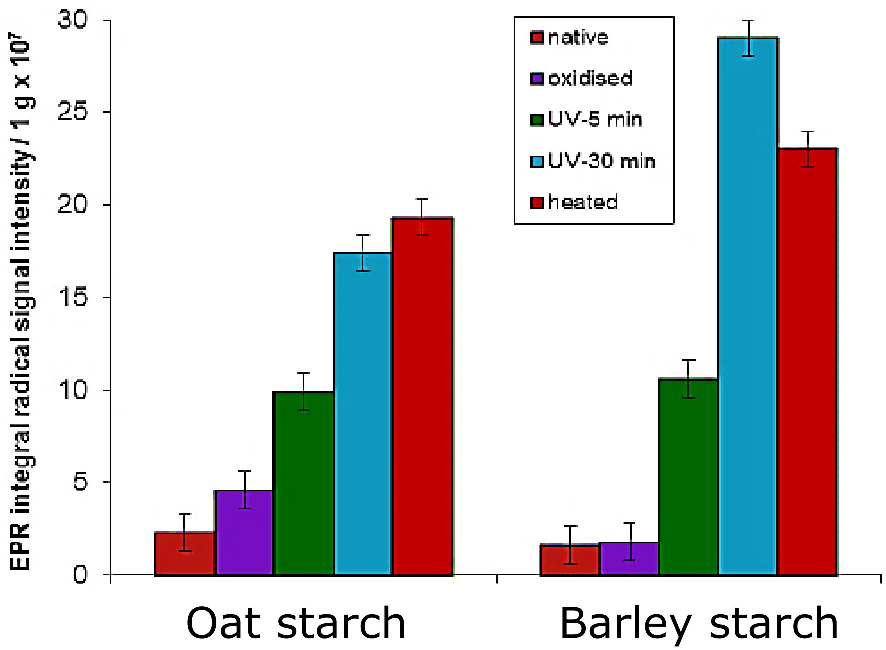
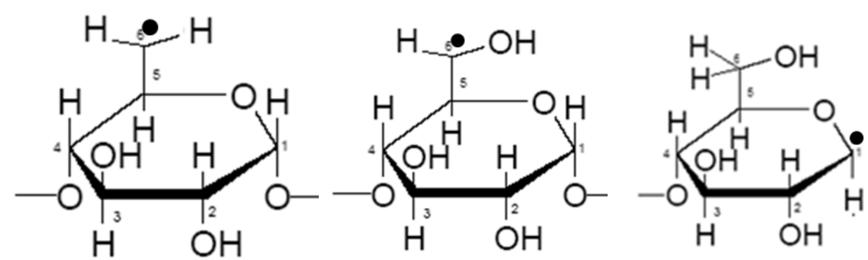
Changes in the physicochemical properties of barley and oat starches upon the use of environmentally friendly oxidation methods

Magdalena Kurdziel [a], Maria Łabanowska [a], Sławomir Pietrzyk [b], Joanna Sobolewska-Zielińska [b], Marek Michalec [a]



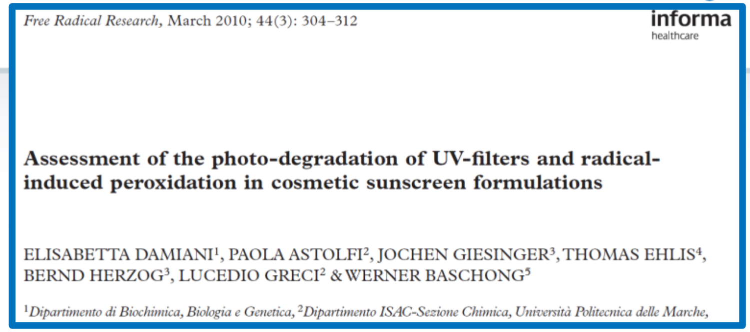
- EPR showed that thermal treatment caused depolymerization of starch chains while the UV-irradiation was effective in oxidation of oat and barley starches
- Barley starch was less stable than oat starch based on the quantitative EPR
- Three C-centered polysaccharide radicals were identified from the EPR spectra:



- The radical formation correlates with decline in starch crystallinity and molecular weight

EPR and antioxidants

Efficiency



Free Radical Research, March 2010; 44(3): 304–312

informa healthcare

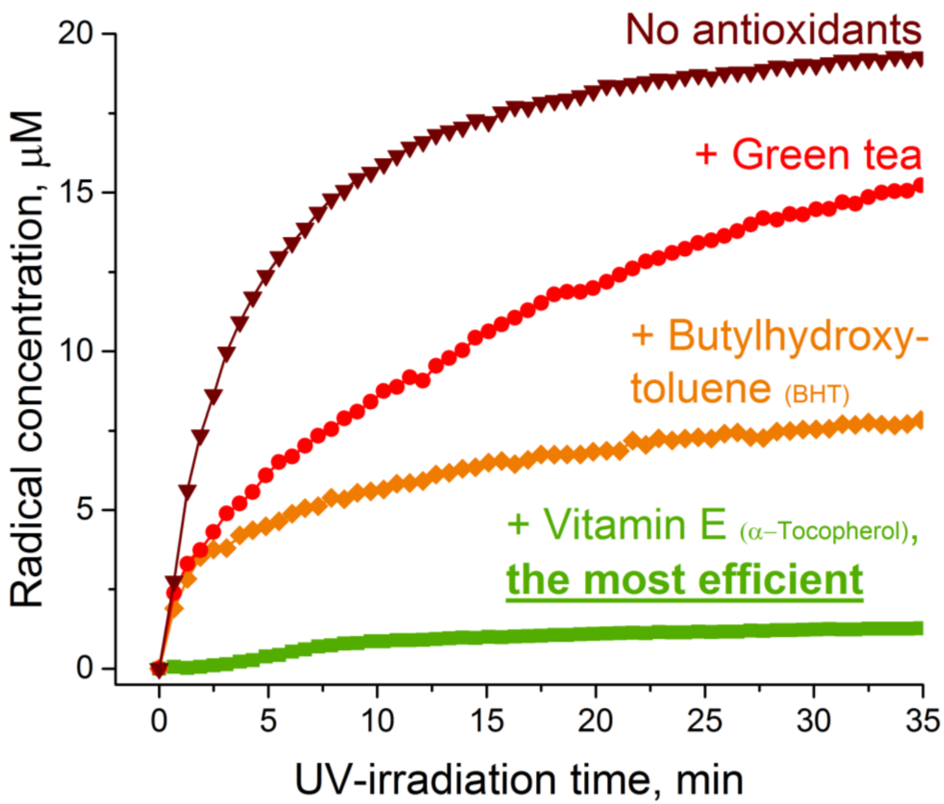
Assessment of the photo-degradation of UV-filters and radical-induced peroxidation in cosmetic sunscreen formulations

ELISABETTA DAMIANI[1], PAOLA ASTOLFI[2], JOCHEN GIESINGER[3], THOMAS EHLIS[4], BERND HERZOG[3], LUCEDIO GRECI[2] & WERNER BASCHONG[5]

[1]*Dipartimento di Biochimica, Biologia e Genetica,* [2]*Dipartimento ISAC-Sezione Chimica, Università Politecnica delle Marche,*

An example: Antioxidants' effect on a skin care product during UV-irradiation:

- UV radiation leads to radical chain reactions in anti-aging and skin care products that can be monitored by EPR
- The efficacy of antioxidants depends on their penetration kinetics, reactivity with non-radical components, and photo-stability
- EPR monitors the time-efficacy profile showing how fast an antioxidant can unfold its full potential in skin and how stable its activity is



EPR and beverages
Photodegradation

ASBC Methods of Analysis

BEER

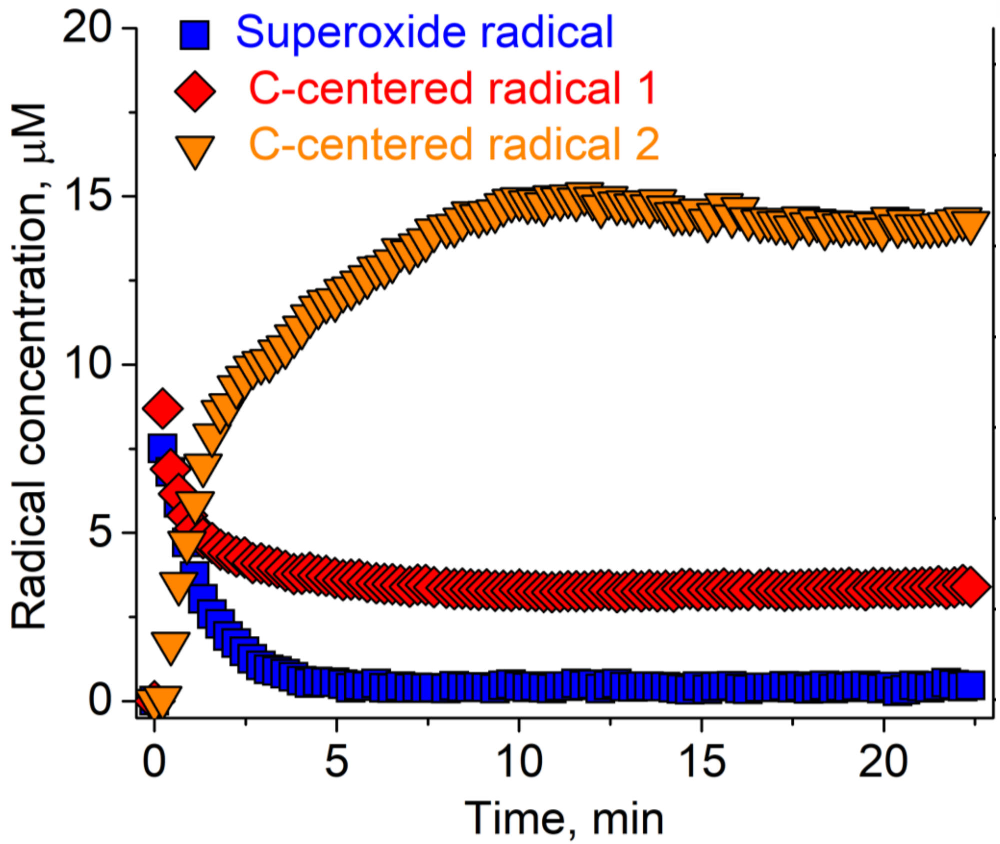
Beer-46

Measurement of Oxidative Resistance in Beer by Electron Paramagnetic Resonance

VIEW METHOD

Summary
This method determines the oxidative resistance of beer. Electron paramagnetic resonance (EPR) spectroscopy is used to directly measure free radical production in the beer during a forced oxidation assay. The assay is based on an EPR-spin trapping experiment in which free radicals form covalently bound adducts with the (-phenyl-*t*-butylnitrone spin trapping reagent. The accumulation of these adducts is detected by EPR during the forced oxidation period and directly reflects the resistance (or lack of resistance) of the beer to oxidation. Two metrics obtained from the assay, lag time and EPR intensity at 150 min (T150) values, can be used to quantitatively assess a beer's oxidative resistance.

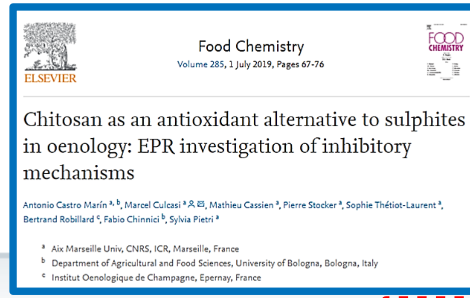
- Flavor quality of beer is compromised by exposure to light resulting in photooxidation, a condition known as 'lightstruck'
- EPR sheds light on the reaction mechanism for the formation of this unpleasant flavor by detecting free radicals upon UV light exposure.
- Three radicals are identified and their reaction kinetics are monitored



Huvaere K. et al., Photochem. Photobiol. ***5*** *961 (2006)*

EPR and beverages

Photodegradation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Food Chemistry

Volume 285, 1 July 2019, Pages 67-76

Chitosan as an antioxidant alternative to sulphites in oenology: EPR investigation of inhibitory mechanisms

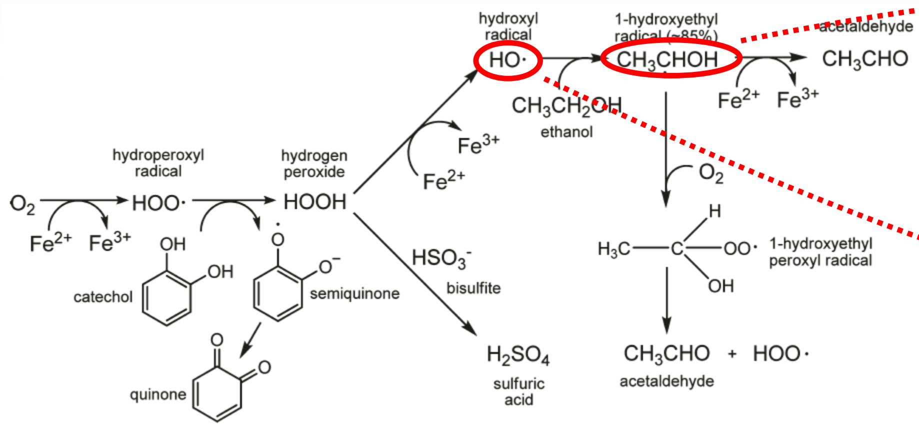
Antonio Castro Marín [a, b], Marcel Culcasi [a], Mathieu Cassien [a], Pierre Stocker [a], Sophie Thétiot-Laurent [a], Bertrand Robillard [c], Fabio Chinnici [b], Sylvia Pietri [a]

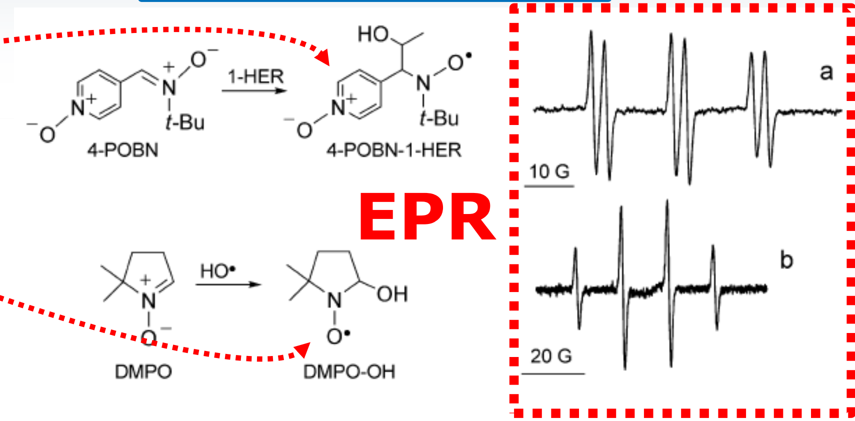
[a] Aix Marseille Univ, CNRS, ICR, Marseille, France

[b] Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy

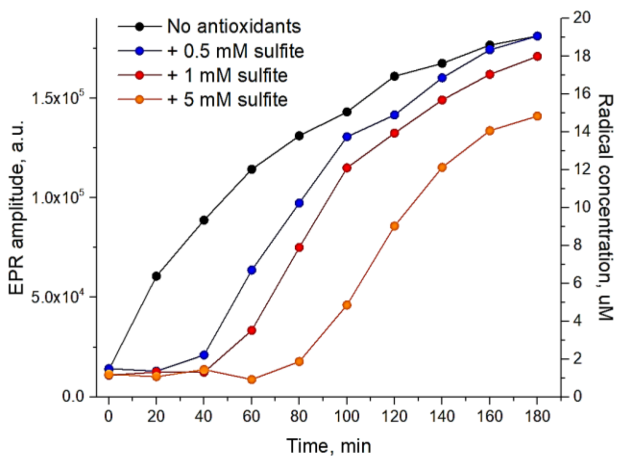
[c] Institut Oenologique de Champagne, Epernay, France

Wine oxidation mechanism





Sulfite effect



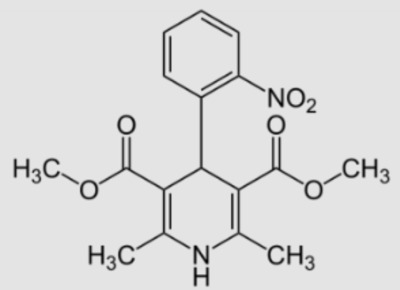
- Winemaking is susceptible to free radical chain reactions and may occur at any stage of the process
- The radical oxidation process involves hydroxyl- and 1-hydroxyethyl radicals and their formation is monitored by EPR
- Sulfite is presumed to lessen overall wine oxidation process by scavenging H_2O_2 to yield sulfate
- EPR helps in searching for new antioxidants (chitosan) due to adverse effects caused by sulfite

Elias. R.J. et al., J. Agric. Food Chem. ***57*** *4359 (2009)*
Marin A. C. et al., Food Chem. ***285*** *67 (2019)*

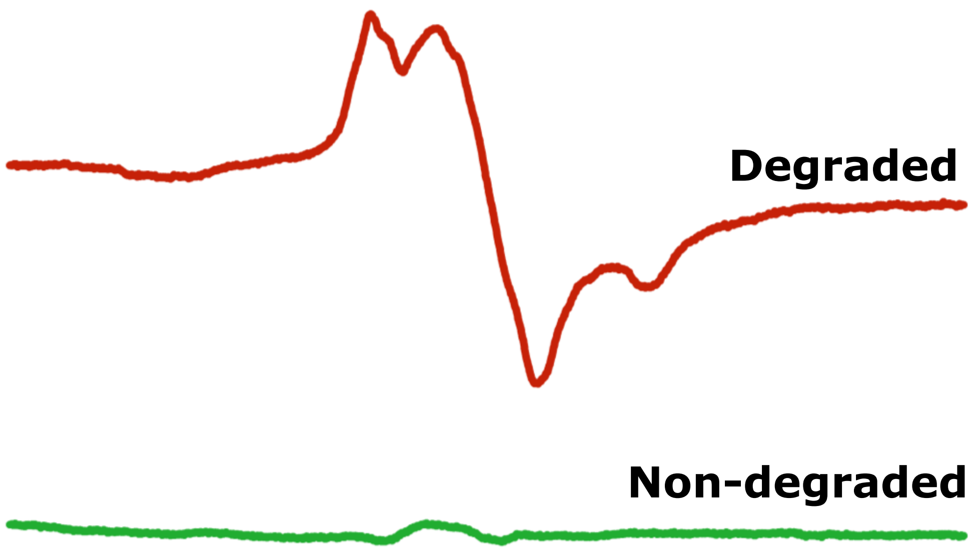
EPR and pharmaceuticals

Drug photodegradation

An example: Photodegradation of Nifedipine (a hypertension drug):



Nifedipine

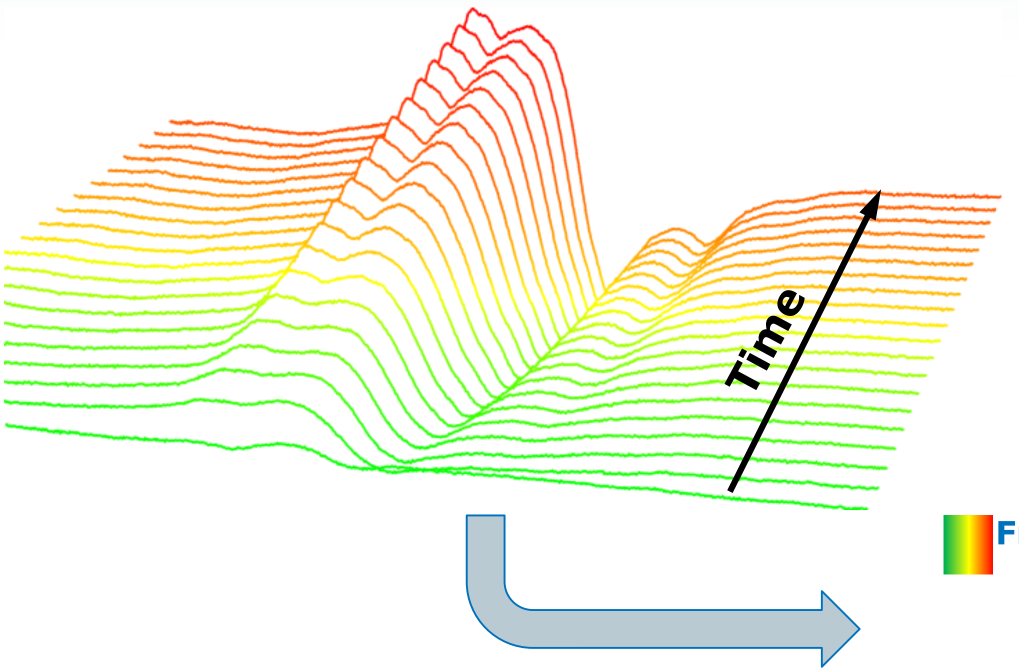


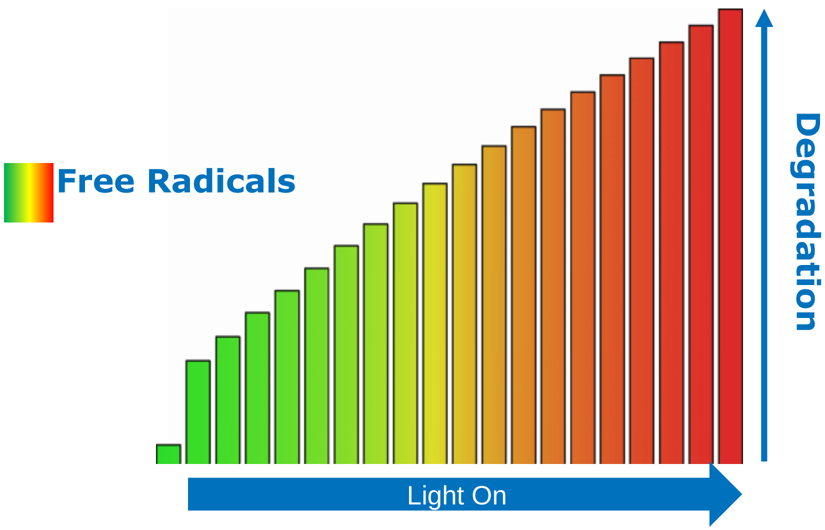
- Photodegradation of Nifedipine after exposure to light shows the formation of N-based free radical
- The amount of free radicals corresponds to the level of degradation

EPR and pharmaceuticals

Drug photodegradation

- The time evolution of the N-based radical can be followed by EPR:



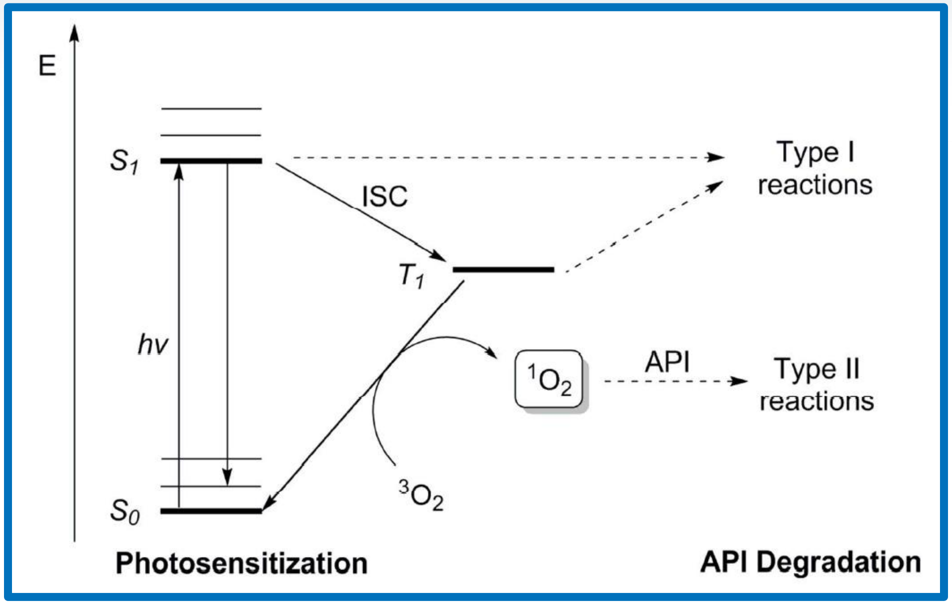


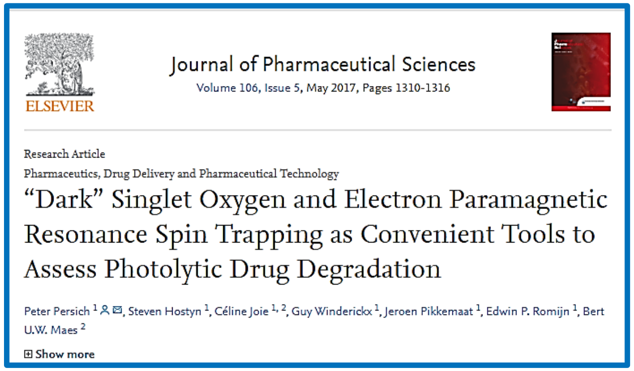
- The increasing amount of radicals shows the level of API degradation and can be used to predict stability of the product

Williams H.E. and Claybourn M., *Drug Dev. Ind. Pharm.* **(2012)** 38(2) 200

EPR and pharmaceuticals

Drug photodegradation





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Journal of Pharmaceutical Sciences

Volume 106, Issue 5, May 2017, Pages 1310-1316

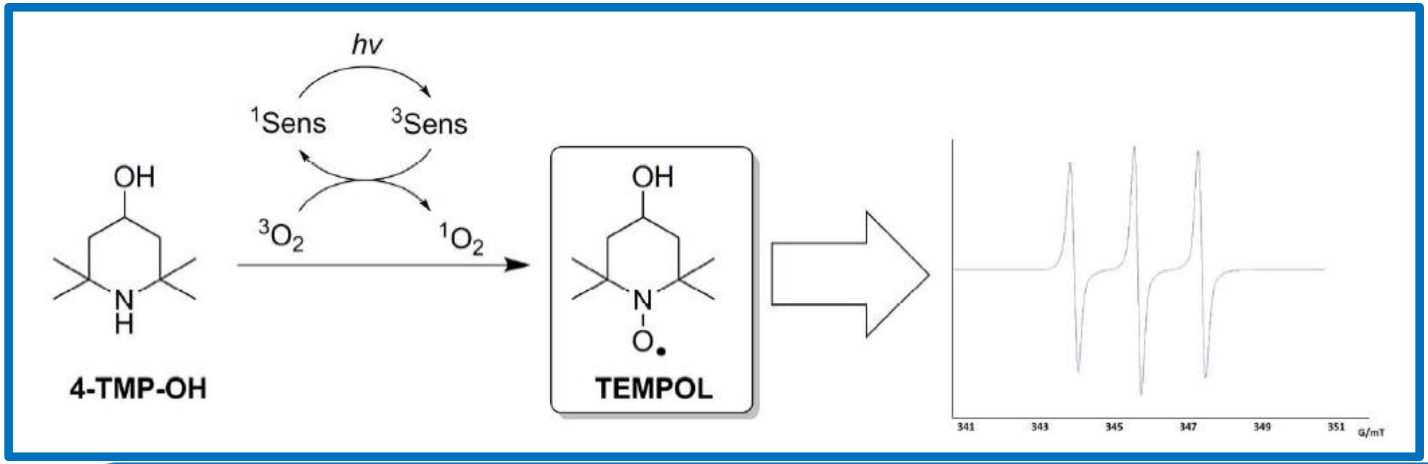
Research Article

Pharmaceutics, Drug Delivery and Pharmaceutical Technology

“Dark” Singlet Oxygen and Electron Paramagnetic Resonance Spin Trapping as Convenient Tools to Assess Photolytic Drug Degradation

Peter Persich [1], Steven Hostyn [1], Céline Joie [1, 2], Guy Winderickx [1], Jeroen Pikkemaat [1], Edwin P. Romijn [1], Bert U.W. Maes [2]

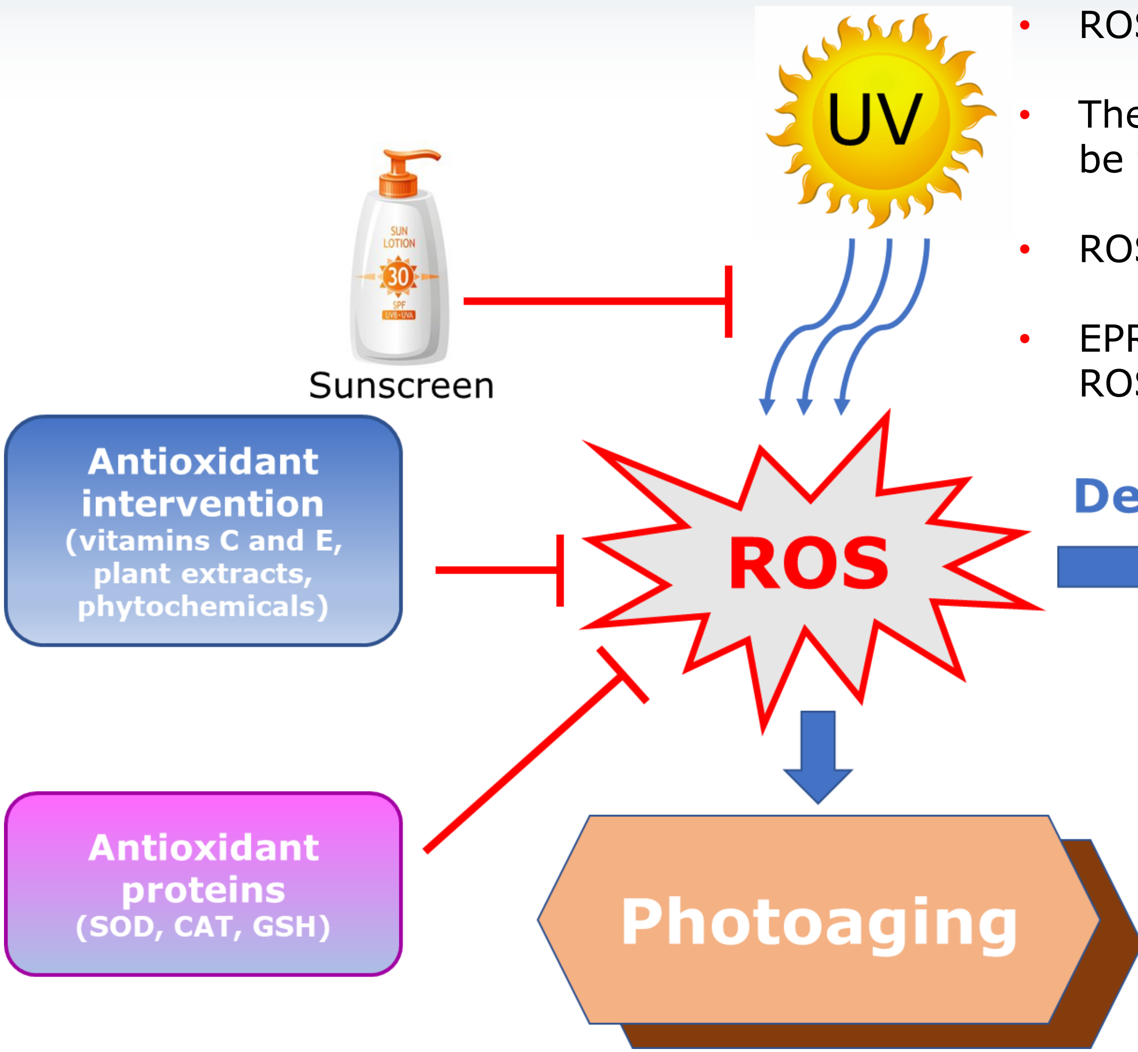
⊞ Show more



EPR and skin care (photoaging)

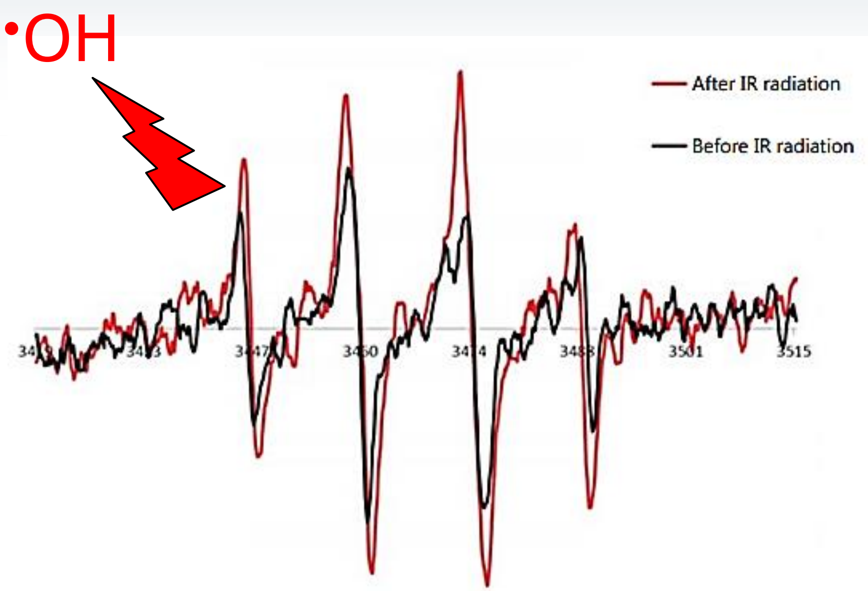
Reactive Oxygen Species (ROS)

- ROS are free radicals and highly reactive
- Their activities are beneficial but can also be toxic
- ROS are detectable by EPR
- EPR can tell us how much and what type of ROS are detected



Skin photodegradation (photoaging)

Reactive Oxygen Species (ROS)



- Infrared radiation penetrates the epidermal and dermal layers of the skin reaching deeper than UV
- EPR results shows an increase in the hydroxyl radical in the IR-irradiated skin compared to the native skin
- Bicosomes and β-carotene (Bcb) reduce ROS in the skin

Original Paper

Skin Pharmacology and Physiology

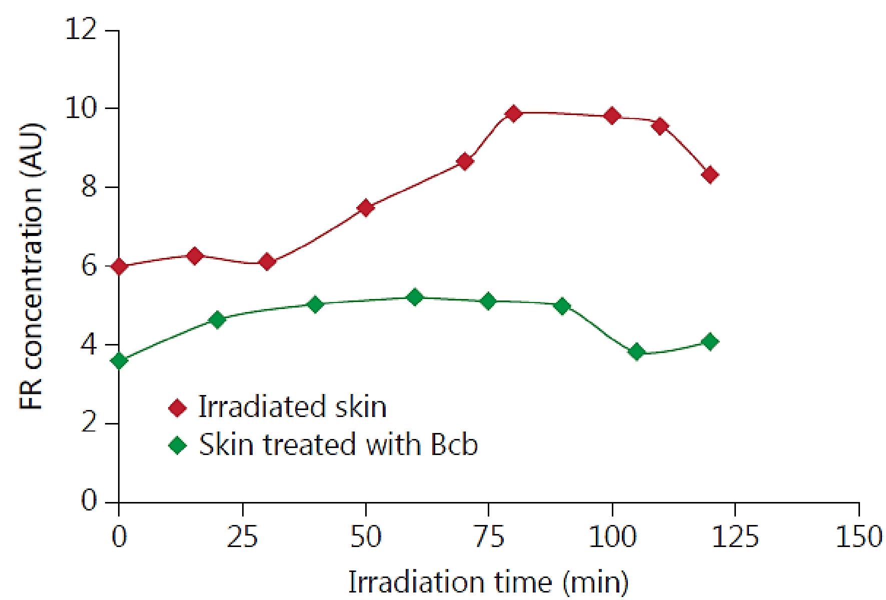
Skin Pharmacol Physiol 2016;29:169–177
DOI: 10.1159/000447015

Received: January 13, 2016
Accepted after revision: May 19, 2016
Published online: July 6, 2016

Reducing the Harmful Effects of Infrared Radiation on the Skin Using Bicosomes Incorporating β-Carotene

Estibalitz Fernández[a] Lluís Fajarí[a] Gelen Rodríguez[b] Mercedes Cócera[b] Verónica Moner[a] Lucyanna Barbosa-Barros[b] Christina S. Kamma-Lorger[c] Alfonso de la Maza[a] Olga López[a]

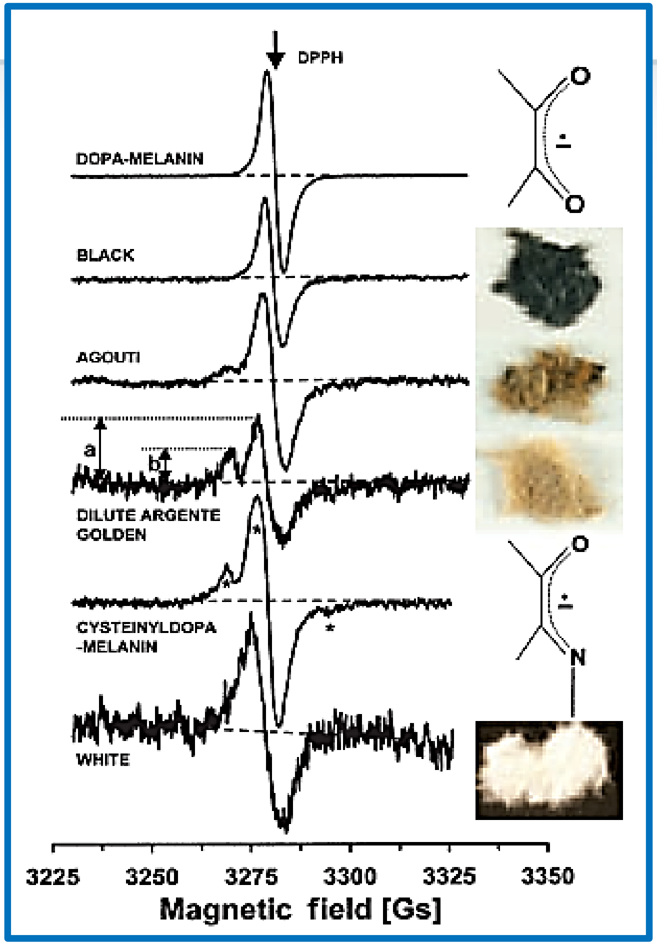
[a]Institute of Advanced Chemistry of Catalonia (IQAC-CSIC), and [b]Bicosome S.L., Barcelona, and [c]ALBA Synchrotron, Cerdanyola del Vallès, Spain



EPR and skin care (photoaging)

Melanin

- Forms a stable semiquinone radical which makes it an excellent marker for oxidative stress on melanin-containing systems
- The EPR signal of melanin is used in studying skin cancer, UV-irradiation mediated damage of skin and iris, age-related macular degeneration (AMD) and other pathologies
- For example, EPR studies have shown that the skin of Afro-Caribbeans and Asians is better protected against UV-induced cancers in comparison to the skin of Caucasians and this was attributed to different melanin levels

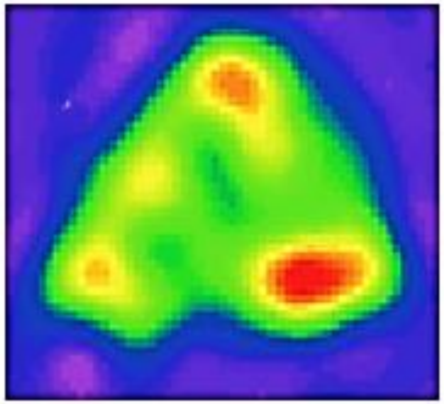


EPR spectra of melanin in hair and skin tumors

*Haywood R. et al., Free Radic. Biol. Med. **44** 990 (2008)*
*Plonka P.M., Exp. Dermatol. **18** 472 (2009)*

EPR and melanoma research

Freeze-dried mouse lung with melanoma B16 metastases



2D EPR image

Review Article

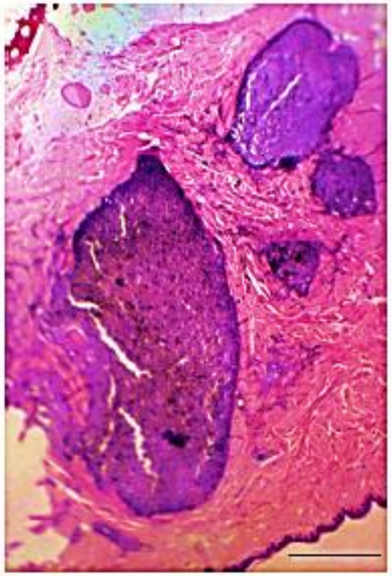
The Contribution of Electron Paramagnetic Resonance to Melanoma Research

Quentin Godechal and Bernard Gallez

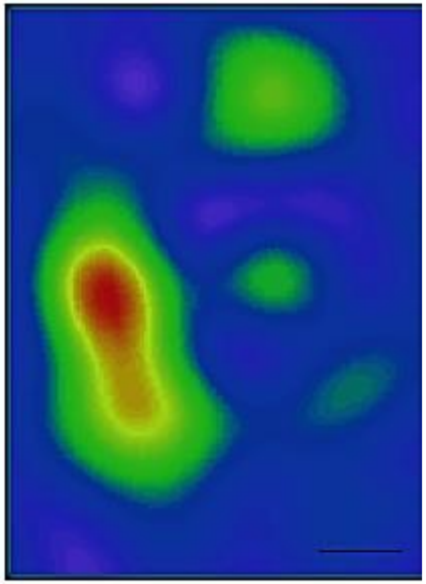
Biomedical Magnetic Resonance Research Group, Louvain Drug Research Institute (LDRI/REMA), Université Catholique de Louvain, Avenue Mounier 73.40, 1200 Brussels, Belgium

Correspondence should be addressed to Bernard Gallez, bernard.gallez@uclouvain.be

Received 15 March 2011; Accepted 22 July 2011



Histological section from human melanoma

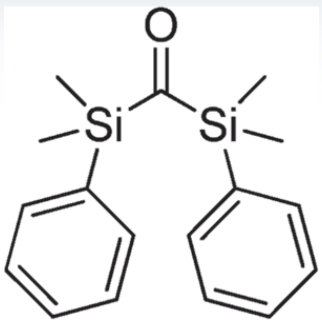


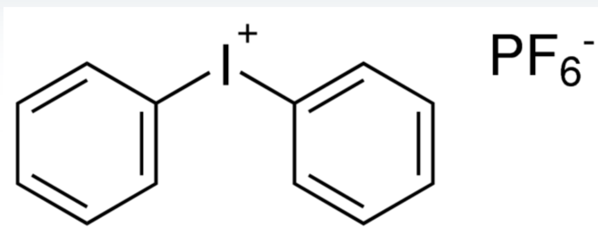
2D EPR image

- EPR measures accurately and sensitively the presence of melanin pigments inside melanoma samples
- There is a robust correlation between the intensity of the EPR signal of melanin and tumor growth

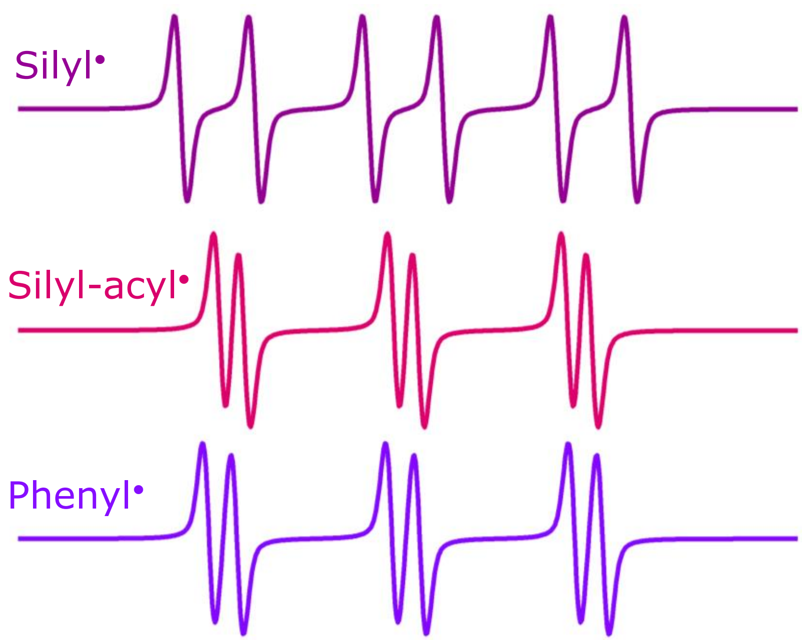
EPR and polymers

Initiation and photopolymerization





Photoinitiators



Communication — Macromolecular Journals — Macromolecular Rapid Communications

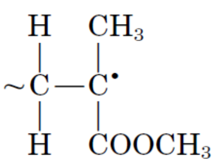
Development of Novel Photoinitiators as Substitutes of Camphorquinone for the LED Induced Polymerization of Methacrylates: A Bis-Silyl Ketone

Bernadette Graff, Joachim E. Klee,* Christoph Fik, Maximilian Maier, Jean Pierre Fouassier, Jacques Lalevée*

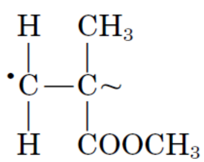
- Photoinitiators are used to generate highly efficient radicals for the polymerization of methacrylates upon exposure to blue light.
- The proposed mechanism is that the photoinitiation process proceeds via several radical species.
- Quantitative EPR analysis of all three intermediates (silyl, acyl, and phenyl radicals) provides information about the photopolymerization efficiency.

EPR and polymers

Polymer photodegradation

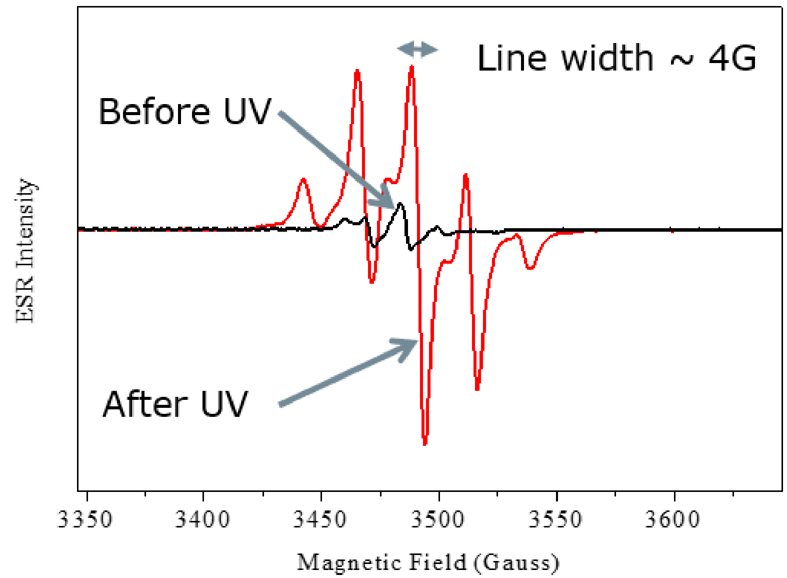


(R1)

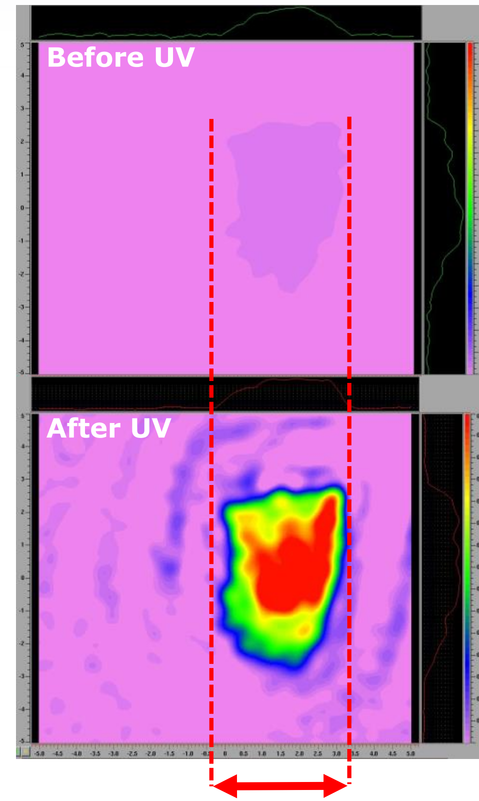


(R2)

CW-EPR



EPR Imaging

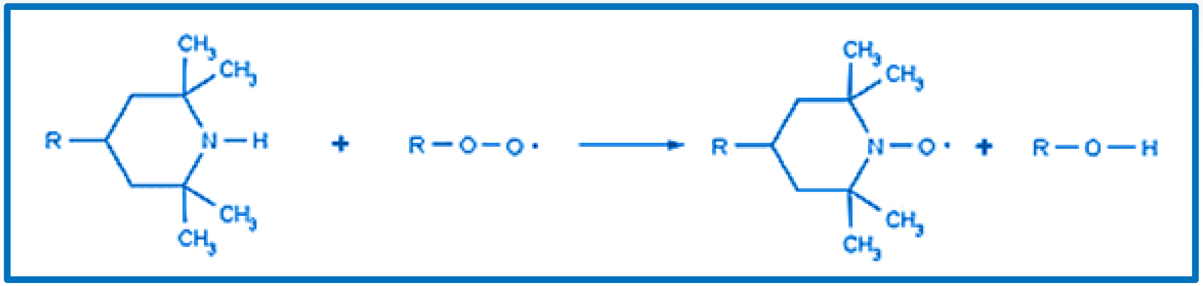


- Poly(methyl methacrylate) (PMMA) degrades due to light exposure forming two radical species (R1 and R2) identified by EPR
- EPR imaging clearly shows the effect of UV irradiation

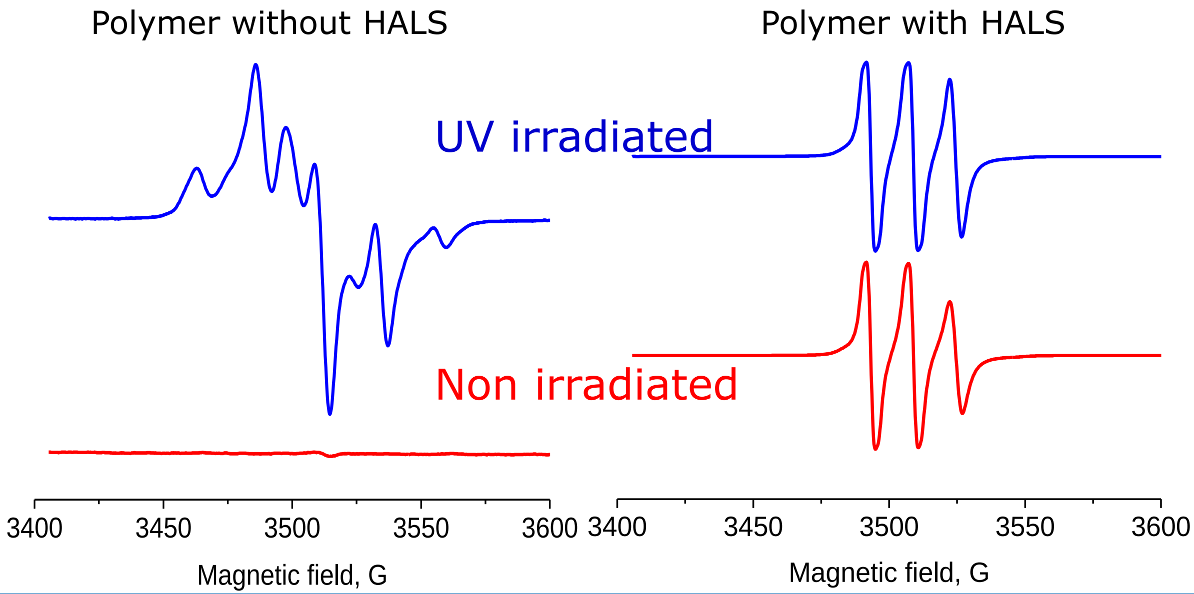
EPR solutions in polymer industry

Polymer photodegradation

- 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 as radical scavengers
- EPR detects HALS nitroxyl radicals generated during paint oxidation upon UV exposure
- Quantitative EPR analysis shows correlation between the free radical concentration and the exposure duration confirming the effectiveness of HALS as an antioxidant



Initial reaction of a HALS with a polymer peroxy radical: this step stabilizes the polymer and converts the HALS to its aminoxyl form

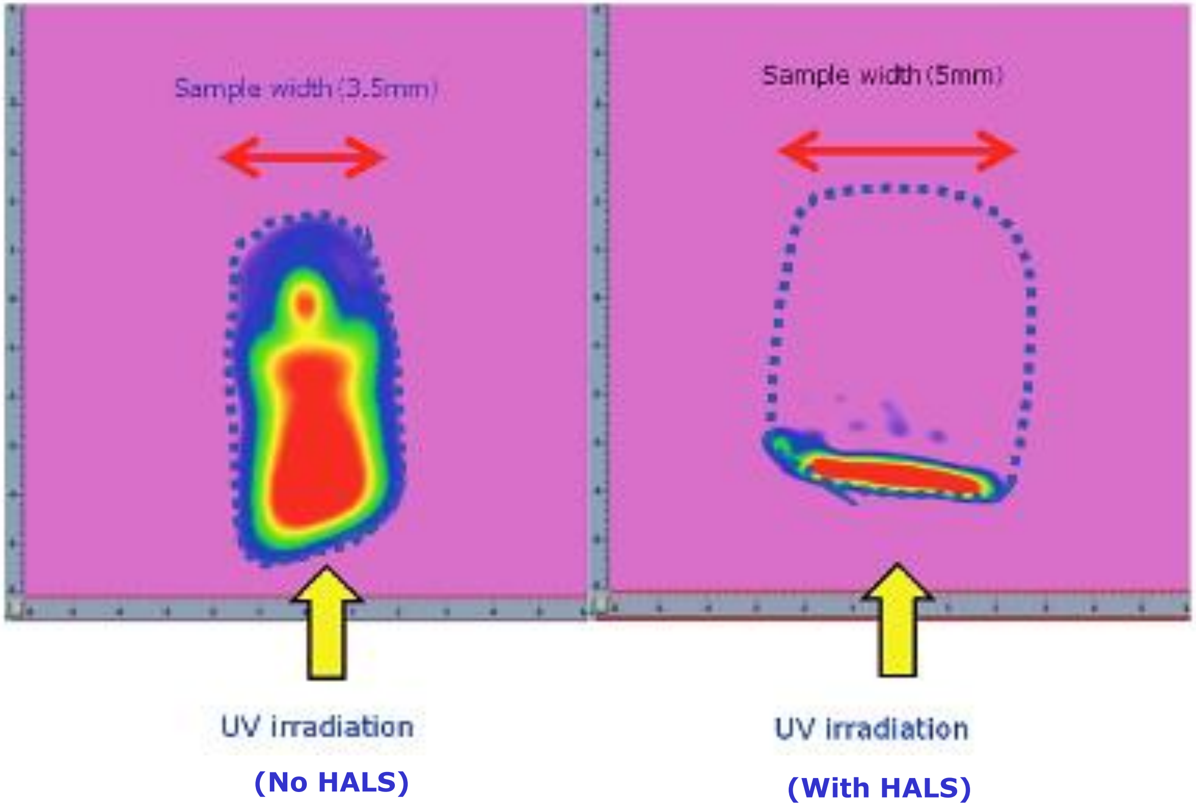


EPR solutions in polymer industry

Polymer photodegradation

- EPR imaging experiments provide details about the mechanism of polymer degradation
- The sample without HALS was irradiated from the bottom and the data show the radicals distribution
- Due to the sample transparency in the UV the irradiation penetrates the polymer completely meaning the degradation occurs throughout the whole sample
- After adding HALS EPR shows radicals only at the bottom of the sample indicating that polymer degradation was prevented by HALS by blocking the UV penetration

EPR imaging of polystyrene



EPR and paint

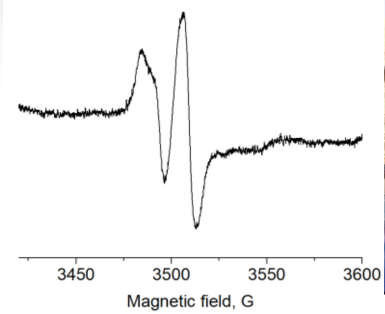
Photodegradation

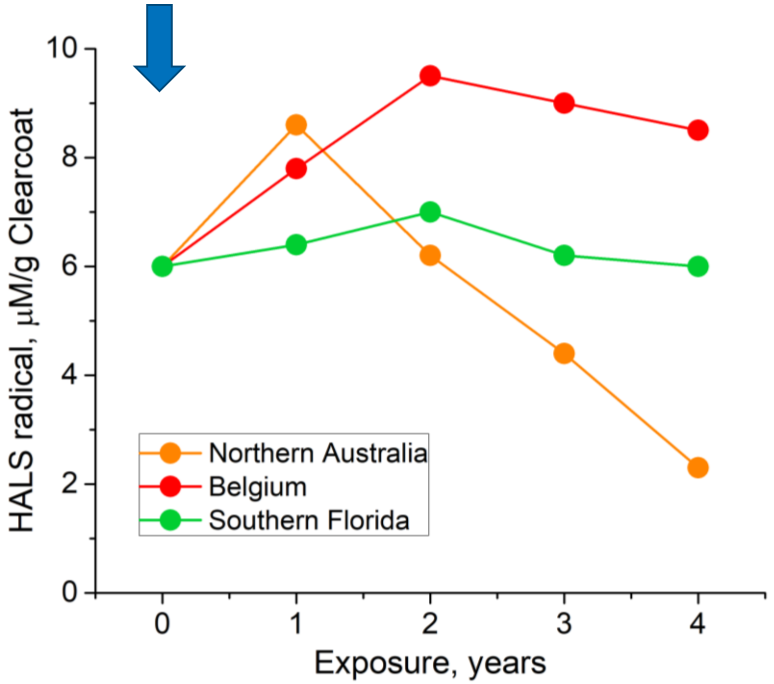
An example: Automotive paint deterioration

- The concentration of EPR-detected HALS radicals correlates to the amount of active HALS in automotive paint and quickly reaches a maximum during sun exposure at all locations

- The rate at which active HALS is lost is very dependent on exposure location. This behavior qualitatively matches the sun load associated with these exposure locations: Belgium, 9 MJ/m^2; Miami, Florida, 17 MJ/m^2 and, Townsville, Australia, 20 MJ/m^2

- Quantitative EPR data suggest that while the paint system may be over stabilized for service in Belgium, it is under stabilized for service in high sun load environments





Gerlock J.L. (Ford Motor Company) et al., Degrad. Stab. ***(2001)*** *73 201*

EPR and materials

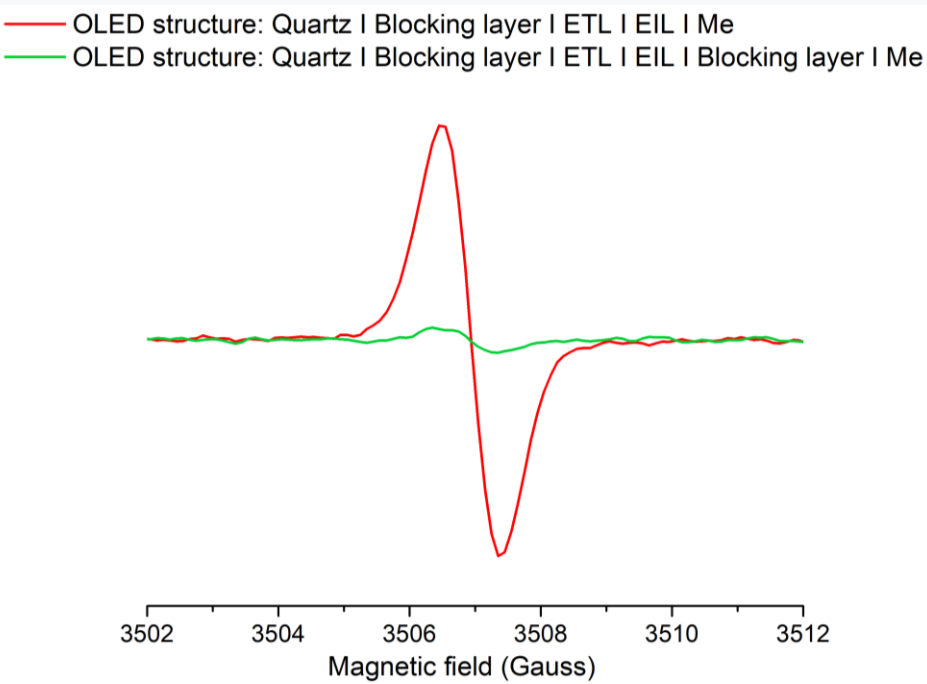
Photostability of OLEDs

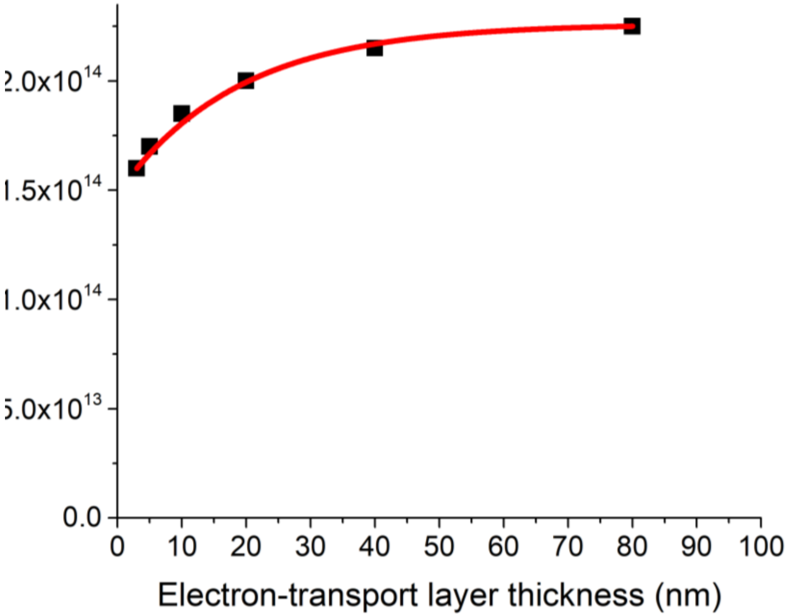
Charge carriers and charge-transfer reactions in OLED devices studied by electron paramagnetic resonance*

Thomas D. Pawlik
Denis Y. Kondakov
William J. Begley
Ralph H. Young

Abstract — Electron paramagnetic resonance (EPR) was used as a quantitative method to measure charge carriers that are present in OLED devices under various bias conditions. Charge-transfer reactions that occur at the cathode interface through the interaction of charge-injection layers and charge-transport layers were investigated.

Keywords — *OLED, charge carriers, charge-transfer reactions, electron paramagnetic resonance.*
DOI # 10.1889/JSID18.4.277



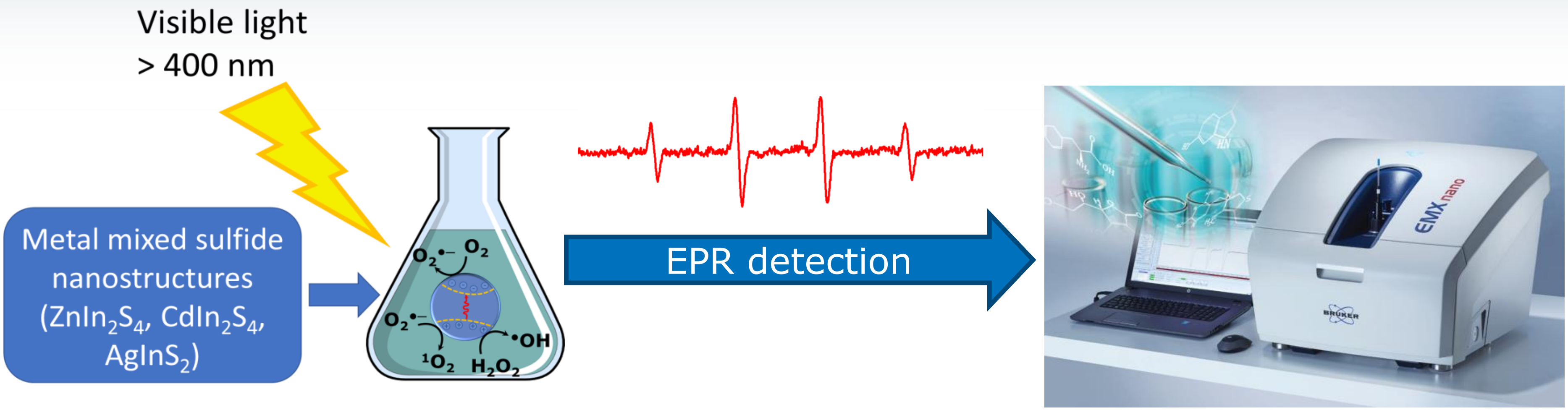


- Having a blocking layer between the electron-injection layer (EIL) and the cathode metal (Me) greatly reduces the number of organic radicals
- EPR results confirms that the second blocking layer reduces aging of OLEDs
- Quantitative EPR analysis provides information about the density of organic radicals as a function of the ETL layer

Pawlik T.D. et al., J. Soc. Inf. Disp. ***(2009)*** *18 277*

EPR and materials

Photoactivity of nanomaterials



- Photocatalytic nanomaterials ($ZnIn_2S_4$, $CdIn_2S_4$, $AgInS_2$) facilitate generation of reactive oxygen species (ROS) during irradiation with visible light
- EPR detects and quantifies the formation of three different ROS – superoxide ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), and singlet oxygen ($^1O_2$)
- The photocatalytic and photobiological activity of the nanomaterials correlates with the ROS concentration monitored by EPR
- The ability to promote ROS generation could be considered an intrinsic parameter of a given nanomaterial, similar to other physiochemical properties such as particle size, morphology, active surface area, etc.

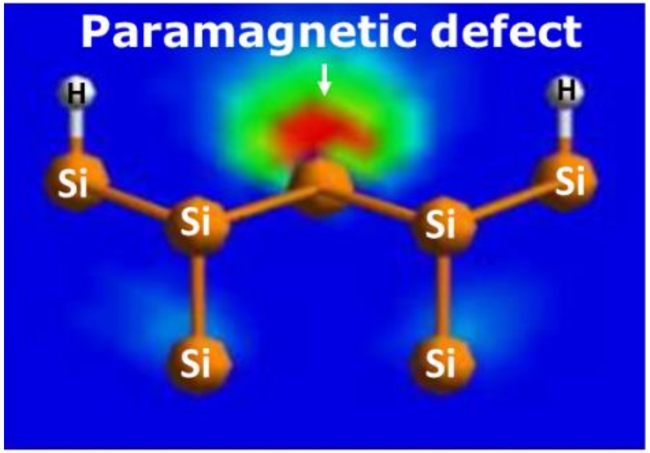
*He W. et al., ACS Appl. Mater. Interfaces (**2015**) 7 16440*

EPR and solar cells

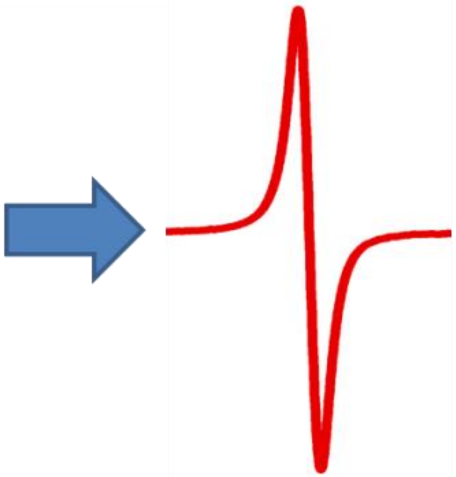
Photovoltaics photodegradation

Paramagnetic defects and impurities in photovoltaic materials include:

- E' centers in SiO_2
- Atomic H^0 in SiO_2 or c-Si
- D center defects in a-Si
- P_b centers at Si-SiO_2 interfaces
- Vacancies
- Transition metals
- Free radicals



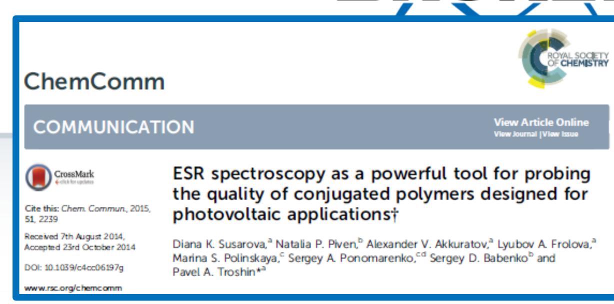
Structure of Si solar cell



EPR signal of paramagnetic defects or impurities

EPR and solar cells

Photovoltaics photodegradation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Received 7th August 2014, Accepted 23rd October 2014

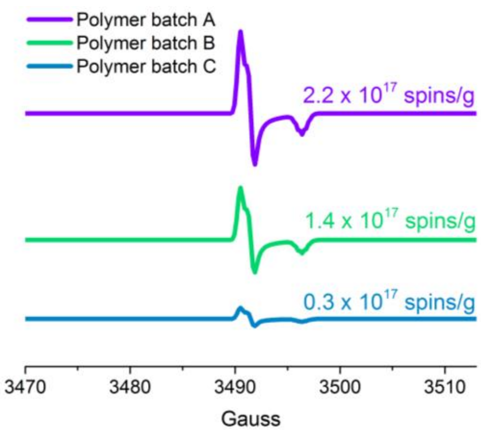
DOI: 10.1039/c4cc06197g

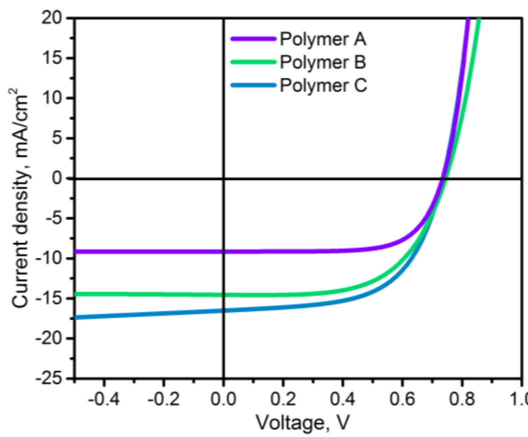
www.rsc.org/chemcomm

ESR spectroscopy as a powerful tool for probing the quality of conjugated polymers designed for photovoltaic applications†

Diana K. Susarova,[a] Natalia P. Piven,[b] Alexander V. Akkuratov,[a] Lyubov A. Frolova,[a] Marina S. Polinskaya,[c] Sergey A. Ponomarenko,[c,d] Sergey D. Babenko[b] and Pavel A. Troshin*[a]

An example: Organic solar cell degradation





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

- Conjugated polymers are used as electron donors in organic solar cells (organic photovoltaics)
- The amount of free radical defects in polymers varies between different batches, syntheses, or vendors
- EPR can detect, identify and quantify the free radical defects (impurities)
- Quantitative EPR analysis (spins/g) correlates with solar cell efficiency (PCE) and stability

Ding Z. et al., J. Mater. Chem. A ***(2016)*** *4 7274*

Photodegradation - a two-edged sword?

BRUKER

Photo-AOPs

- Photolysis (UV + H_2O_2)
- Photocatalysis (UV + catalyst)
- Photo-Fenton (UV + Fenton)

•OH

Hydroxyl radicals

Radical attack

Organic substances

Micropollutants

Microorganisms

Oxidized decomposition

Inactivated non-toxic final products

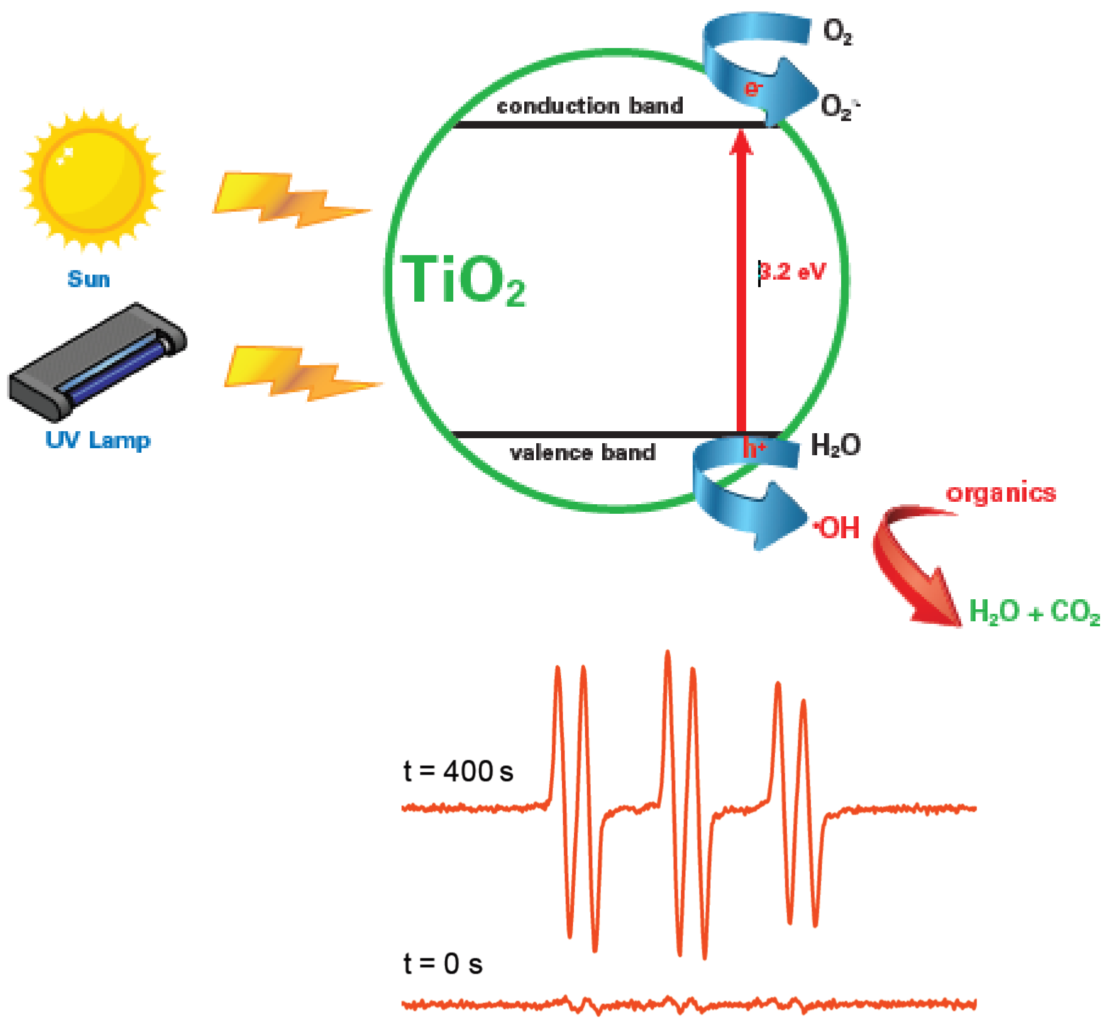
Detection

EPR spectrometer

EPR and photocatalysis

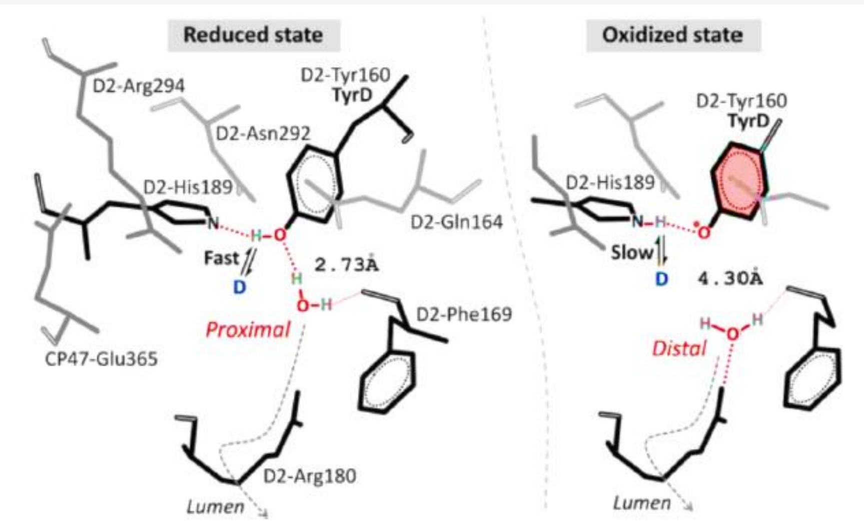
TiO_2

- Understanding of the photocatalytic mechanisms in TiO_2 photocatalysts is a prerequisite to improve their efficiency in a wide range of applications
- EPR detects and identifies key active species (hydroxyl radical, superoxide, singlet oxygen) produced in irradiated TiO_2 suspensions
- Monitoring the radical intermediates by EPR using various wavelengths and solvents provides complete characterization of TiO_2 photoactivity



EPR and photosynthesis

Photosystems

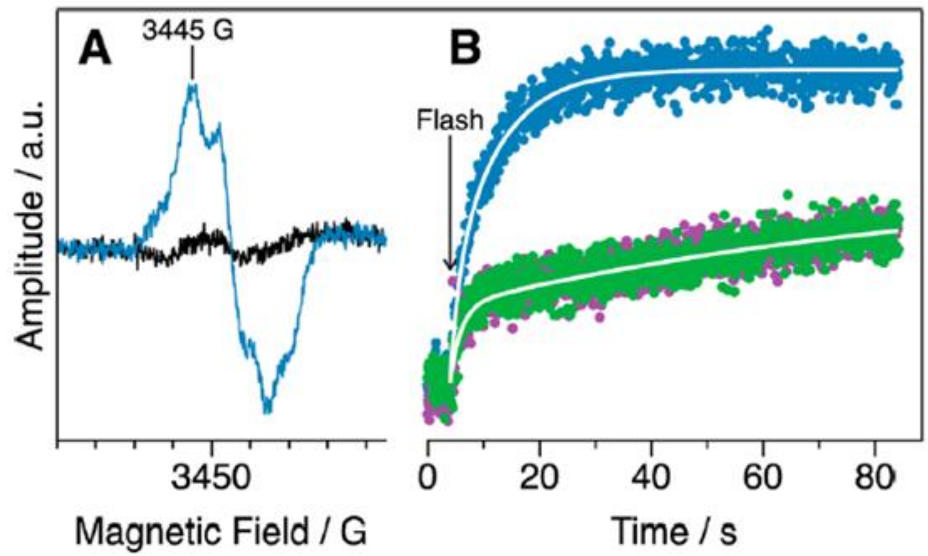


Environment around TyrD in its reduced and oxidized states

Spectroscopic Evidence for a Redox-Controlled Proton Gate at Tyrosine D in Photosystem II

Johannes Sjöholm,*,† Fikret Mamedov,* and Stenbjörn Styring

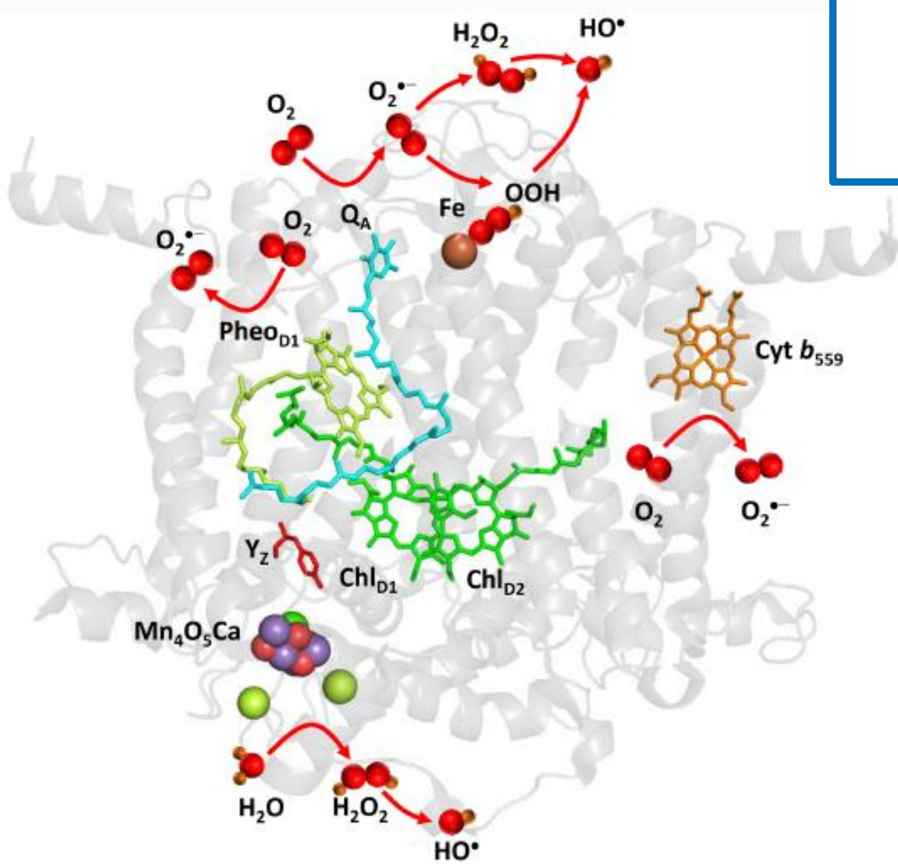
Molecular Biomimetics, Department of Chemistry-Ångström Laboratory, Uppsala University, P.O. Box 523, SE-751 20 Uppsala, Sweden

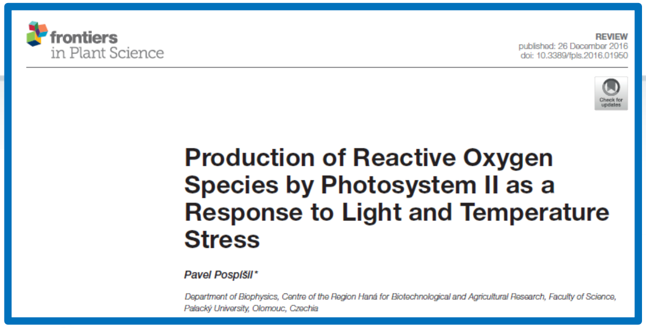


- EPR is used to detect protein radicals in PSII
- EPR shows almost no Tyr radical signal in dark meaning that TyrD is in its reduced state. After the laser flash the EPR signal represents ~80% of the maximal inducible radical
- The rate of the reaction allows the oxidation of TyrD to be followed by EPR time trace showing differences in the kinetics depending on the presence of deuterium

EPR and photosynthesis

Photosystems





frontiers in Plant Science

REVIEW
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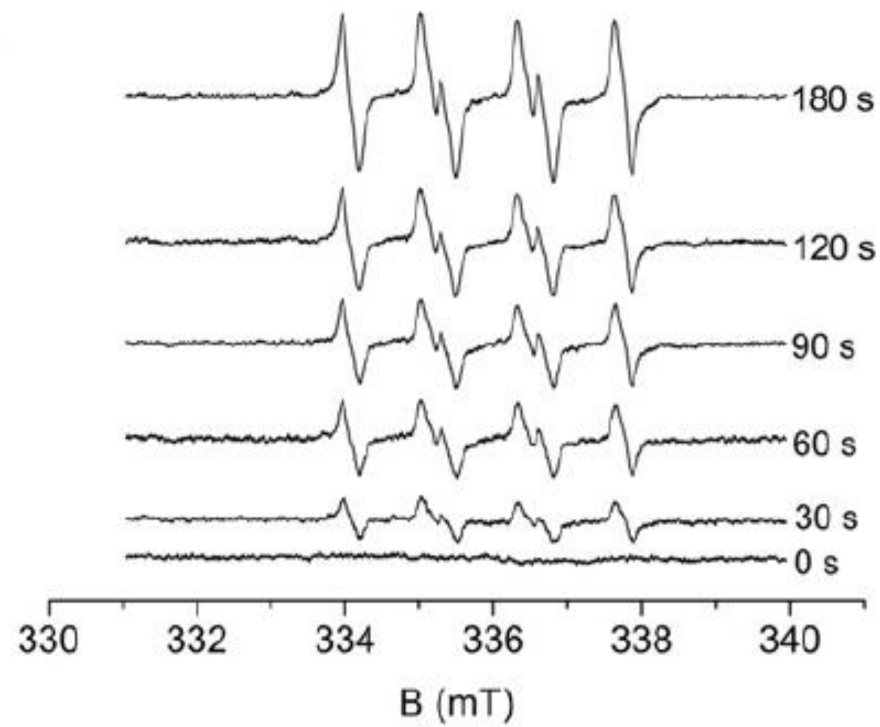
Production of Reactive Oxygen Species by Photosystem II as a Response to Light and Temperature Stress

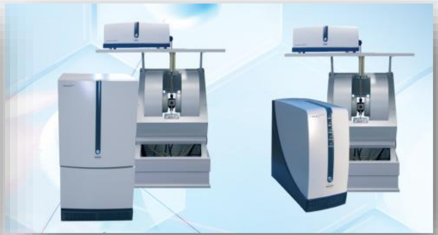
*Pavel Pospíšil**

Department of Biophysics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Olomouc, Czechia



Yadav D. et al. PLoS One ***9(12)****: e115466 (2014)*
Pospíšil P., Front. Plant Sci. ***7*** *1950 (2016)*

EPR Portfolio



microESR/EMXnano

Routine, Industrial and Education

- Single Frequency: X-Band
- Single probehead
- Variable temperature: 100 – 473K
- Sample irradiation
- No water cooling

EMXmicro/EMXplus

Routine Research

- Multiple frequency: L-, S-, X-, and Q-Band
- High sensitivity
- Specialized probeheads
- ENDOR
- Variable temperature: 4 – 1200K
- Sample irradiation
- Water cooling

ELEXSYS E500 / E580

ELEXSYS E680 & E780

Multi purpose & Specialized

- Multiple frequency: L-, S-, X-, Q- ,W-Band and 263 GHz
- Highest sensitivity in CW and pulse mode
- Dedicated probeheads for CW and pulse applications
- ENDOR, ELDOR, DEER
- Arbitrary Wave Form generator
- High power pulse microwave amplifiers
- Variable temperature: 4 – 1200K
- External triggering
- Water cooling

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Any questions?

Thank you!