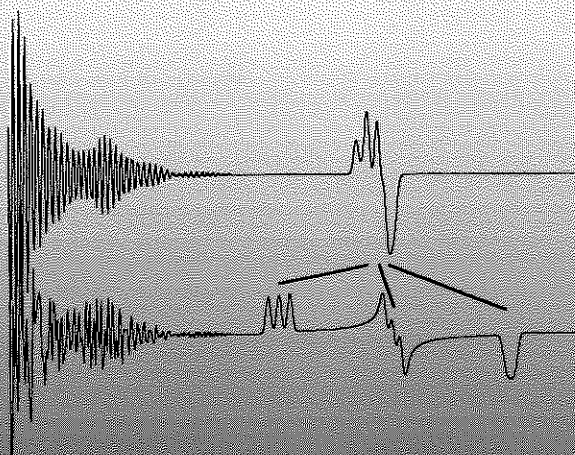




TU Delft

**Ninth Meeting of the
Benelux EPR Society**

**Programme
Book of Abstracts**



May 16, 2001

Location:

Aula Congrescentrum/Senaatszaal

TU Delft

Mekelweg 5

2628CC Delft

The organising committee:

W.R. Hagen and S. de Vries (TU Delft)

P. Gast (Secretary Benelux EPR Society)

E. Goovaerts (Chairman Benelux EPR Society)

Contents:

Programme page	2
Abstract of presentations	3
List of Posters	12
Poster abstracts	13

Programme

10.00-10.25

Registration and coffee

10.25-10.30

Opening by W.R. Hagen

10.30-11.00

A high-field EPR study of the anisotropic spin echo decay of $Q_A^{\bullet-}$ in bacterial photosynthetic reaction centers

S. V. Paschenko, P. Gast, A. J. Hoff, University of Leiden, Leiden, The Netherlands

11.00-11.30

Feasibility of low frequency EPR in radiation dosimetry

M. Zdravkova, B. Gallez, R. Debuyst, Catholic University of Louvain, Brussels, Belgium.

11.30-12.15 Keynote lecture

Quinone and tyrosine radicals in terminal oxidases as observed by multi-frequency cw- and pulsed-EPR spectroscopy

F. MacMillan, J.W. Goethe Universität, Frankfurt, Germany

12.15-12.45

Detection of charge compensating cation vacancies in ionic crystals: a Q-band ENDOR study of Rh^{2+} complexes in NaCl and AgCl

H. Vrielinck, F. Callens and P. Matthys Ghent University, Belgium

Lunch and Posters

14.00-14.30

Recent advances in EPR and multiple resonance instrumentation

P. Höfer, Bruker Analytik, Germany

14.30-15.00

A W-band EPR investigation of photoinduced charge transfer in conjugated polymer - fullerene composites

J. De Ceuster, E. Goovaerts, A. Bouwen, J.C. Hummelen, V. Dyakonov, University of Antwerpen, Antwerpen, Belgium

15.00-15.30

X-band and W-band EPR on the Active Centers of the Copper Protein Nitrite Reductase

M. Fittipaldi, H. J. Wijma, M. Ph. Verbeet, G. W. Canters, M. Huber and E. J.J. Groenen, Leiden University, Leiden, The Netherlands

15.30-16.00

The design of a CW and pulsed EPR spectrometer operating at 275.7 GHz.

H. Blok, J.A.J.M. Disselhorst, J. Schmidt, Leiden University, Leiden

16.00-16.15

General Meeting of the Benelux EPR Society

16.15-17.00 Tea and drinks

*A high-field EPR study of the anisotropic spin echo decay
of $Q_A^{-\bullet}$ in bacterial photosynthetic reaction centers*

S. V. Paschenko, P. Gast, A. J. Hoff

*Department of Biophysics, Huygens Laboratory, Leiden University,
P.O.Box 9504, 2300 RA Leiden, The Netherlands*

Electron spin echo experiments were performed on the reduced primary electron acceptor $Q_A^{-\bullet}$ in Zn-reconstituted photosynthetic reaction centers from *Rb. sphaeroides* R26 using a high-field, high-frequency D-band pulsed EPR spectrometer. The two-pulse electron spin echo decay was studied as a function of the time separation between the pulses, magnetic field strength and temperature. In the temperature range 80 – 120 K, the kinetics are exponential. The decay times in this range show a significant magnetic field anisotropy, being the longest at the magnetic field corresponding to the X principal direction of the $Q_A^{-\bullet}$ g-tensor, and shortest at Z-direction. At 40 K, the decays are essentially non-exponential. The possible mechanisms which could explain the experimental data are discussed. The mechanism of small amplitude librational motions of the $Q_A^{-\bullet}$ molecule, proposed earlier, is ruled out based on the results of the present work.

Feasibility of low frequency EPR in radiation dosimetry

Marieta Zdravkova, Bernard Gallez, Rene Debuyst
Laboratory of Biomedical Magnetic Resonance, Catholic University of Louvain,
Avenue Hippocrate, 10, B-1200 Brussels, Belgium.

Low frequency EPR provides possibilities of measuring the absorbed radiation dose in teeth in situ (without removing the teeth from the mouth). Some in vitro experiments with highly irradiated teeth were performed before starting in vivo dosimetry. They are necessary for checking the feasibility and the reliability of the method.

An L-band spectrometer ($\nu = 1.2$ GHz) with a loop antenna was used. A DPPH sample with small volume was used for studying the intensity of the EPR signal at different positions inside and outside the antenna. More bulky samples (licorice-flavored sweets, piled on each other) were also used to simulate teeth of different lengths.

The contribution of different components of the teeth (enamel, dentine and roots) to the EPR signal intensity was also studied. A reference sample of Li-phtalocyanine was used for these experiments, where roots and crown dentine were successively removed. The stability of the signal was also checked in these different components.

The lowest detectable radiation dose and dose increment with our spectrometer are discussed.

This work is supported by the European contract FIGD-CT-2000-00083

Quinone and tyrosine radicals in terminal oxidases as studied by multi-frequency cw- and pulsed-EPR spectroscopy

F. MacMillan

Institut für Physikalische und Theoretische
Chemie,

J. W. Goethe Universität Frankfurt,
D-60439 Frankfurt am Main,

Germany,

<http://www.prisner.de>; Email; fm@prisner.de

Cytochrome *c* oxidase from *Paracoccus denitrificans* (*aa₃*) catalyses the reduction of oxygen to water, a process which is accompanied by the pumping of four protons across the membrane. Elucidation of the structure of intermediates in these processes is crucial for understanding the mechanism of oxygen reduction. In the work presented here, the reaction of H₂O₂ with the fully oxidised protein has been investigated with multi-frequency electron paramagnetic resonance (EPR) spectroscopy. Here selectively-deuterated tyrosine has been incorporated into the protein and a drastic change in the lineshape of the EPR signal observed is detected. This would suggest that the observed EPR signal does indeed arise from a tyrosine radical species. It would seem also quite possible that this radical is an intermediate in the mechanism of oxygen reduction.

This radical is also observed in the related system ubiquinol oxidase (*bo₃*) from *E. coli* which in addition also contains a redox-active quinone, the so-called "high-affinity" Q_H quinone instead of Cu_A. The location of this cofactor and its binding site has yet to be accurately determined by x-ray crystallographic studies, however based on mutagenesis studies, a putative quinone binding site in the protein has been proposed.

Hyperfine Sub-level Correlation (HYSCORE) spectroscopy has been applied to investigate the Q_H binding site. Spectra revealed interactions of the unpaired electron of Q_H^{•-} with a single nitrogen ¹⁴N nucleus from the protein-surrounding matrix, which has been assigned to a protein-backbone nitrogen. This assignment is confirmed by Double Nuclear Coherence Transfer (DONUT)-HYSCORE spectroscopy. Performing HYSCORE spectroscopy on ¹⁵N/¹⁴N-exchanged quinol oxidases and using a simple point-dipole approximation, the distance between the coupled nitrogen and the closest carbonyl oxygen can be estimated.

In order to further characterise the binding site of the semiquinone, cw- and pulsed-high-field electron paramagnetic resonance (EPR) spectroscopy was applied to Q_H^{•-} using either native ubiquinone-8 or by exchanging for selectively ¹³C-labelled derivatives. Spectra provide evidence for potential one-sided hydrogen bonding of Q_H^{•-} within its protein-binding site. These results are compared with the corresponding information available on other protein binding sites and/or on model systems. They are discussed with regard to the location and potential function of Q_H in the enzyme.

**Detection of charge compensating cation vacancies in ionic crystals :
a Q-band ENDOR study of Rh^{2+} complexes in NaCl and AgCl**

H. Vrielinck, F. Callens and P. Matthys

Ghent University – Department of Solid State Sciences
Krijgslaan 281 – S1
B-9000 Gent, Belgium

EPR studies at X, Q and W-band and X-band ENDOR demonstrated that substitutional Rh^{2+} ions (with a low spin d^7 , 2A_1 ground state) in NaCl and AgCl can be associated with two, one or no charge compensating cation vacancies in next nearest neighbour positions. The presence and the stability of the $[RhCl_6]^{4-} \cdot nVac$ complexes ($n = 2, 1, 0$; Vac = next nearest neighbour cation vacancy) was found to be dependent on the type of crystal (NaCl or AgCl) and on the crystal growth conditions (solution-grown or melt-grown). The microscopic model for these centres was mainly deduced from their g tensor symmetry and their interconversion at elevated temperatures, attributed to the migration of the vacancies [1]. This all presented indirect evidence for the presence and the position of the vacancies.

The unpaired electron in $[RhCl_6]^{4-} \cdot nVac$ complexes is mainly localised in a d_{xy} orbital of the central ion and strongly interacts with the Cl ions along the g_z axis, perpendicular to the plane containing the vacancies. The purpose of the present study is to determine whether the presence and the position of the vacancies can be directly deduced from the ENDOR spectrum of these axial Cl ligands. It is shown that for the complexes with one vacancy and two vacancies the superhyperfine and quadrupole tensors are slightly tilted in the symmetry plane of the complex containing the axial ligands. This tilting can be directly observed through a splitting of the ENDOR transitions recorded in this plane. At conventional X-band frequencies, a correct analysis of the ENDOR spectra is hindered by the lack of g tensor resolution and by second order splitting of all ENDOR transitions. Therefore, the ENDOR analysis is carried out at Q-band. A physical interpretation of the tilting of the interaction tensors in terms of an off-centre displacement of the Rh^{2+} ion is proposed.

- [1] H. Vercammen, D. Schoemaker, H. Käß, E. Goovaerts, A. Bouwen, H. Vrielinck and F. Callens, J. Appl. Phys. 84 (1) 1998, p. 428

Recent Advances in EPR and Multiple Resonance Instrumentation

Peter Höfer, Bruker Analytik, 76287 Rheinstetten, Germany

The development efforts in EPR over the last years were mainly focused on improvements in signal-to-noise and resolution. The signal-to-noise issue in CW-EPR was tackled by the development of super high Q resonators and high purity solid state microwave oscillators. Improvements as large as a factor of 10 have been achieved by the combination of high purity sources and high-Q resonators. Another cornerstone of signal-to-noise gain was the development of a 94 GHz EPR spectrometer. At this frequency the reduction in resonator size (typically 3 mm) leads to a remarkable increase in absolute sensitivity.

In pulse EPR the long standing bottleneck of high speed averaging could be overcome by the development of a transient recorder tailored for the needs of EPR. The large spectrum dispersion and the short electron spin lattice relaxation times are taken into account by 250 MHz sampling rate and up to 1 million transients averaged per second.

The multiple resonance strategy was further utilized to increase spectral resolution. This was achieved by the development of pulsed ENDOR (Electron-Nuclear Double Resonance) resonators in X- and W-band. In addition, the second generation of the X-band FT/CW-EPR microwave bridge was introduced recently. With this new bridge we also introduced the first commercial pulsed ELDOR (Electron-Electron Double Resonance) system. Various novel pulsed electron-electron double resonance experiments like saturation-recovery ELDOR, DEER, ELDOR detected NMR and hyperfine selective ENDOR can now be performed. These key experiments provide new insight into relaxation mechanism, electron-electron dipolar coupling and electron-nuclear spin interaction.

A W-band EPR investigation of photoinduced charge transfer in conjugated polymer - fullerene composites

J. De Ceuster^a, E. Goovaerts^a, V. Dyakonov^b, A. Bouwen^a, and J. C. Hummelen^c

^a Department of Physics, University of Antwerp, Belgium

^b Energy and Semiconductor Research, University of Oldenburg, Germany

^c Stratingh Institute and MSC, University of Groningen, The Netherlands

The interest in organic donor-acceptor composites is steadily growing as a result of their possible use in active layers of the photovoltaic devices. The efficient generation of charge carriers and their fate after creation are important issues to be addressed. The nature of the charge carriers of these composites under illumination can be investigated by means of Electron Paramagnetic Resonance.

Light-induced Electron Spin Resonance (LESR) measurements are reported on composites of poly[2-methoxy-5(3', 7'-dimethyloctyloxy)-1, 4-phenylene vinylene] (MDMO-PPV) and [6,6]-phenyl-C₆₁-butyricacidmethylester (PCBM), a soluble derivative of C₆₀. Under illumination of the sample, two paramagnetic species are formed due to photoinduced charge transfer between conjugated polymer and fullerene. One is the positive polaron P⁺ on the polymer backbone and the other is the radical anion on the C₆₁-molecule. The results are an improvement to earlier LESR measurements¹, both in terms of resolution and of attribution of the signals. Using high-frequency (95 GHz) LESR it was for the first time possible to separate these two contributions on the basis of their g-factors, and moreover to resolve the g-factor anisotropy for both radicals. The positive polaron on the conjugated polymer chain possesses axial symmetry with principle values of the g-tensor $g_{||} = 2.0034(1)$ and $g_{\perp} = 2.0024(1)$. EPR on low doped polymer gave extra proof for the assignment to the positive polaron. The negatively charged C₆₁ molecule has a lower, rhombic symmetry with $g_x = 2.0003(1)$, $g_y = 2.0001(1)$, $g_z = 1.9982(1)$. Different spin lattice relaxation for both species was observed. It gives rise to a rapid passage effect for the positive polaron spectrum. Important for the photovoltaics is that the photogenerated radicals are remaining in the composites even after photoexcitation is off, implying stability of the trapping states at low temperatures.

¹ V. Dyakonov, G. Zorinants, M. Scharber, C.J. Brabec, R.A.J. Janssen, J.C. Hummelen and N.S. Sariciftci, Phys. Rev. B **59**, 8019 (1999)

X-Band and W-Band EPR on the Active Centers of the Copper Protein Nitrite Reductase

Maria Fittipaldi[§], Hein J. Wijma[#], Martin Ph. Verbeet[#], Gerard W. Canters[#], Martina Huber[§] and Edgar J. J. Groenen[§]

[§] Department of Molecular Physics, Huygens Laboratory, Leiden University, P.O. Box 9504, 2300 RA Leiden, The Netherlands

[#] Leiden Institute of Chemistry, Gorlaeus Labs, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands

Electron Paramagnetic Resonance, EPR, is a sensitive technique to determine the ligands and the electronic structure of paramagnetic metal centers in proteins. This can help to understand the function of the active site in metalloproteins. Here EPR is used to characterize the wild-type Nitrite Reductase (NIR) protein from *Alcaligenes faecalis* [2], which catalyzes the conversion of nitrite to NO. The NIR protein has a trimeric structure and in each monomer there are two copper centers. The two different centers (Type 1 and Type 2) have different functions in the protein determined by different environments (ligand structure) around the Cu-centers. The Type 1 center is a redox site, the other site is the catalytic one. Moreover the two different centers have peculiar spectroscopic features with distinct EPR parameters. Previously, from 95 GHz (W-band) EPR on NIR single crystals the full G-tensor of the Type 1 site had been obtained [3]. Here we report on continuous wave (cw) EPR at X-band and W-band frequencies on frozen solutions of wild-type and modified NIR. The W-band EPR spectra were particularly useful to discriminate between the signals of the two centers, and to obtain resolution of the signals in the g_{xx} , g_{yy} region of the spectra, which are superimposed in the X-band EPR spectra.

Mutants were investigated in which the Type 1 site was modified by replacing the copper ligand His145 with Ala (H145A) or Gly (H145G). The EPR spectra show that the signal of the Type 1 disappears. Upon adding imidazole to both mutants (H145A and H145G) a Type 1 EPR signal reappears showing that the Type1 site is reconstituted. Slight differences in the EPR parameters indicate that the (electronic) structure of the reconstituted site differs from the wild-type. Successful reconstitution is prerequisite to introduce external ligands.

We also investigated how binding of the substrate (nitrite) changes the electronic structure of the type 2 site, because this change in electronic structure may trigger the reduction of nitrite. The ultimate goal of these and future experiments planned, e.g. on single crystals, is to understand how the electronic structure determines the function of the active sites in metalloproteins.

2.M.E. Murphy, S. Turley, E. Adman, J.Biol. Chem. **45**, 28455 (1997)

3.M. van Gastel, M. J. Boulanger, G. W. Canters, M. Huber, M. E. P. Murphy, M. Ph. Verbeet, E. J. J. Groenen, J. Phys. Chem. **105**, 2236 (2001)

The design of a CW and pulsed EPR spectrometer operating at 275.7 GHz.

H. Blok, J.A.J.M. Disselhorst, J. Schmidt.

In this contribution we describe the design of a 275.7 GHz superheterodyne EPR spectrometer at Leiden University that will operate in CW as well as in pulsed mode. The source consists of a tripled 91.9 GHz Gunn oscillator which produces a power of about 5 mW at 275.7 GHz. A pseudo-optic system is used to transport the microwaves to and from the resonator. The signal from the resonator is pseudo-optically combined with microwaves from a second tripled Gunn oscillator to produce an IF signal at 6.6 GHz. This 6.6 GHz signal is subsequently demodulated to produce the final EPR signal. Some EPR spectra, realized with a single mode cavity at room temperature, will be shown.

List of Posters

Implementation of PL-detected ODMR measurements in a commercial W-band cylindrical EPR-probehead

G. Janssen, A. Bouwen, P. Casteels, E. Goovaerts

Single crystal HF-EPR of a Mn(III) compound: determination of higher order zfs parameters

M. Stefan, S. Mossin, E. Goovaerts, H. Weihe

An EPR investigation of the interaction between two spectral sensitizers in an AgX photographic emulsion

J. Van Nylen, E. Goovaerts, D. Schoemaker, P. Callant, K. Viane

α -L-Sorbose: A Preliminary Interpretation of its EPR Spectrum after X-irradiation at Room Temperature

G. C. A. M. Vanhaelewyn* and F. J. Callens

Coating of oxygen sensitive paramagnetic particles for EPR oximetry

J. He, N. Beghein and B. Gallez

In vitro effects of coal fly ashes: hydroxyl radical generation, iron release, and DNA damage and toxicity in rat lung epithelial cells

J. van Maanen

Magnetophotoselection Studies on the Triplet state in Photosynthetic bacterial reaction centers

I.V. Borovykh, I.I. Proskuryakov, I.B. Klenina, B. Joosten, P. Gast and A.J. Hoff

Implementation of luminescence-detected ODMR measurements in a commercial W-band cylindrical EPR-probehead

G. Janssen, A. Bouwen, P. Casteels, E. Goovaerts

Department of Physics, University of Antwerp, Universiteitsplein 1, B-2610 Antwerpen, Belgium

In an optically detected magnetic resonance (ODMR) experiment, the resonant absorption of microwaves is indirectly measured through the induced change of the optical absorption or emission spectrum of the examined material. Apart from the possibilities of the EPR technique, ODMR has additional advantages: ODMR is highly sensitive due to the detection in the optical region and it is able to examine species in their excited state, rather than in the ground state. Furthermore, magnetic resonance signals can be tagged to a specific species by selection of the corresponding optical band.

In order to perform an ODMR experiment detected via photoemission intensity, one needs optical access for both excitation of the sample and for collection of the emitted light. This is relatively easy to implement even in commercial equipment at conventional frequencies, but was performed at higher frequencies only in a small number of home-built spectrometers.

By means of a custom-made fiber bundle for optical excitation and collection we demonstrate the feasibility of W-band (95 GHz) ODMR measurements in a commercial high-frequency EPR spectrometer with standard cylindrical cavity probehead: the Bruker W-band E600 EPR system*.

Experiments prove the optical fiber bundle to be an efficient method for optical excitation and collection from the sample in the cylindrical cavity. Spectra will be presented of the self-trapped exciton in an AgCl crystal, of an octyl-substituted poly-phenylene vinylene (PPV) pentamer and of the excitons in InAs/GaAs quantum dots. Comparison will be made with our measurements in a dedicated Fabry-Perot cavity.

* D. Schmalbein, G. G. Maresch, A. Kamlowski, and P. Hofer, *Appl. Magn. Reson.* 16, 185 (1999).

SINGLE CRYSTAL HF-EPR OF A Mn(III) COMPOUND:
DETERMINATION
OF HIGHER ORDER ZERO-FIELD SPLITTING PARAMETERS

M. Stefan^{1,2}, S. Mossin³, P. ter Heerdt¹, A. Bouwen¹, E. Goovaerts¹ and H. Weihe³

¹Department of Physics, University of Antwerp (UIA), Universiteitsplein 1, 2610 Wilrijk, Belgium; ²National Institute for Materials Physics (IFA), POB MG-7, 76900 Bucharest, Romania; ³Institute of Chemistry, University of Copenhagen, Universitetsparken 5, 2100 Copenhagen, Denmark

High Field / High Frequency EPR spectroscopy has opened the possibility to study systems up to now difficult to reach with the conventional microwave frequencies for EPR. In particular, systems with a high-spin ground state, large zero field splitting and broad lines, or the usually EPR silent transition metal ions with integer spins could be investigated.

Single-crystal W-band EPR studies allowed us to obtain valuable information about the local structure and the magnetic properties of a Mn(III) compound, [Mn(cyclam)Br₂]Br, with the ground spin state $S = 2$. The complex crystallises as green prisms with tetragonal crystal symmetry ($P4_2/m$ space group) and two formula units in the unit cell [1]. The molecules have C_{2h} symmetry.

The measurements were performed with a continuous wave W-band EPR spectrometer (Bruker E600) with cylindrical cavity operating at 95 GHz, equipped with a superconducting magnet mounted on a rotating base.

Angular variations of the spectra were obtained in different planes, at 20K and at RT. The spectra showed the presence of two magnetically inequivalent centres, related by fourfold rotation, with the x and z axes in the (ab) plane and the y axis parallel to c . The spin – Hamiltonian parameters were determined up to the fourth order.

References

[1] P. A. Daugherty, J. Glerup, P. A. Goodson, D. J. Hodgson, K. Michelsen, Acta Chem. Scand. **45** (1991) 244

AN EPR INVESTIGATION OF THE INTERACTION BETWEEN TWO SPECTRAL sensitizers in an AgX photographic emulsion

J. Van Nylen*, E. Goovaerts*, D. Schoemaker*, P. Callant[†] and K. Viaene[†]

* Department of Physics, University of Antwerp (UIA), Universiteitsplein 1, B-2610 Antwerp, Belgium

[†] Agfa-Gevaert N.V., Septestraat 27, B-2640 Mortsel, Belgium

Silver halide technology forms the basis for photographic materials. Since silver halides (AgX) only absorb UV light, spectral sensitizers or dyes are adsorbed on the AgX microcrystals to expand their sensitivity into the visible region. Getting a good red sensitivity for these systems is a challenging task because the positioning of the energy levels of a red dye R is critical. Curiously, by adding an only UV-light absorbing supersensitizer (ss), the red (!) sensitivity can increase dramatically. Several explanations have been suggested, yet the molecular basis remains unclear. Since the light induced photoholes (resulting in dye radical cations) are playing a crucial role, EPR has proved a useful tool for studying these systems. [1][2] Three samples have been studied: Sample A (AgX/dye R and ss), sample B (AgX/dye R), and sample C (AgX/ss). In this way the system components can be studied separately and their interactions can be established. Excitation by Kr⁺ laser emission was performed at two wavelengths (647 or 350 nm). The first is a so-called spectral excitation in which only the R dye is excited. The second is a so-called intrinsic excitation by which the AgX and the ss are simultaneously excited. The EPR-intensity for sample A is much bigger than for sample B, which parallels the photographic behaviour mentioned above. Additional characterisation of the possible charge transfer processes was possible by studying the temperature dependence of the ESR signals. All samples were investigated at 298, 223 and 123 K. Upon cooling, the growth and decay kinetics are slowed down.

Based on these results, a structural mechanistic model will be proposed.

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[2] T. Ceulemans, D. Schoemaker, D. Vandebroucke, and R. De Keyser, *IS&T's 50th Annual Conference Proceedings*, 121 (1997)

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α -L-Sorbose: A Preliminary Interpretation of its EPR Spectrum after X-irradiation at Room Temperature

Gauthier C. A. M. Vanhaelewyn* and Freddy J. Callens

Ghent University, Department of Solid State Sciences
Krijgslaan 281-S1, B-9000 Gent, Belgium

Radiation-induced radicals in sugars have recently gained considerable interest with respect to both fundamental and applied research. E.g. sugar radicals seem to be plausible intermediates in the creation of defects in DNA molecules. Previous EPR, ENDOR and EI-EPR investigations of radiation-induced radicals in sucrose, D-glucose and D-fructose were very successful. Recently, Density Functional Theory (DFT) calculations on sucrose and D-fructose systems adequately explained the greater part of the involved hyperfine interactions and facilitated the identification of radical structures in the aforementioned sugar systems.

Up to now, ENDOR and EI-EPR experiments on sugar radicals were the most successful mainly when single crystals were available. Recently, L-sorbose single crystals have been grown from an aqueous solution. L-Sorbose has a molecular structure very similar to that of D-glucose and exhibits typical sugar-like EPR spectra after X-irradiation. However, no ENDOR detection of hyperfine interactions was possible up to now. Therefore, a preliminary interpretation has been attempted on the basis of X- and Q-band EPR data only, including results of heating experiments with a High Temperature Cavity. The presence of several radicals contributing to the global EPR spectrum could be demonstrated.

*Research Assistant of the Fund for Scientific Research - Flanders (Belgium) (F.W.O.)