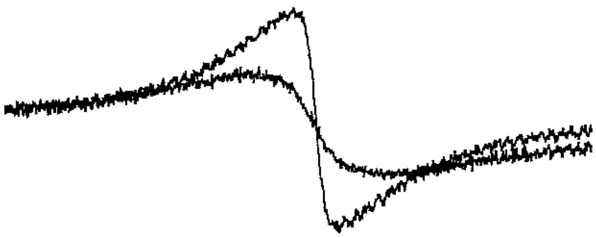
Tenth Meeting of the Benelux EPR Society

Program



May 15, 2002

Location:

Salle Couvreur
Van Helmont Tower (73, level 0)
Faculty of Medicine
Catholic University of Louvain
Avenue Mounier
B-1200 Brussels
Belgium

Organizing committee

R. Debuyst and B. Gallez

C. Baudelet, N. Beghein, B. Jordan, M. Zdravkova

With officers of the Benelux EPR society

E. Goovaerts (chair)

P. Gast (secretary)

Financial support : Bruker Belgium

Program

10-10.45	Registration – Coffee – Poster display
10.45	**Opening of the meeting** R. Debuyst (Brussels, B)
10.55	**In memoriam : Arnold Hoff** *P. Gast (Leiden, NL)*
	First session : Swing of spins Chair : F. Hagen (Delft, NL)
11.00	The electronic structure of donors, acceptors and vacancies in SiC as revealed by EPR and ENDOR spectroscopy at 95 GHz *J. Schmidt (Leiden, NL)*
11.30	An optically detected magnetic resonance study of triplet exciton formation in a series of conjugated polymers and oligomers with increased electron affinity *J. De Ceuster, E. Goovaerts, A. Bouwen, A. Charas, J. Morgado, L. Alacer, H. Detert (Antwerpen, B)*
12.00	Out-of-phase stimulated ESE appearing in the evolution of spin-correlated photosynthetic triplet-radical pair *I.V. Borovykh, L.V. Kulik, S.A. Dzuba, A.J. Hoff (Leiden, NL)*
12.30	**Lunch**
13.30	**Poster session**
	Second session : From low frequency to very high frequency Chair : B. Gallez (Brussels, B)
14.15	**Plenary lecture**
	In vivo EPR imaging of Free radicals in Biomedical Applications *J. Zweier (Baltimore, USA)*
15.00	Nitric oxide mediated pharmacological and physiological modulations of the tumor blood flow and oxygenation *B.F. Jordan, V. Grégoire, R. Demeure, P. Sonveaux, O. Feron, J. O'Hara, V. Vanhulle, N. Delzenne, and B. Gallez (Brussels, B)*
15.30	High frequency EPR in strongly correlated electron systems *P.J.M van Bentum and E. van der Horst (Nijmegen)*
16.00	**Coffee break and poster viewing**
	Third session : Applied EPR Chair : E. Goovaerts (Antwerpen, B)
16.30	Distance measurements by pulsed EPR *Martina Huber, Irene M.C. v. Amsterdam, Marcellus Ubbink, Gerard W. Canters (Leiden, NL)*
17.00	Review and recent advances in electron magnetic resonance on saccharides *G. Vanhaelewyn, E. Pauwels, M. Waroquier, and F. Callens (Ghent, B)*
17.30	**General assembly of the Benelux EPR society – Prospects for the next meeting** *E. Goovaerts (Antwerpen, B) and P. Gast (Leiden, NL)*
17.45	**Closing remarks** **Farewell drink**

Poster communications

G. Janssen, N. Zurauskiene, E. Goovaerts, A. Bouwen, D. Schoemaker, PM Koenraad, JH Wolter, M. Hopkinson (Antwerp, B)
Magnetic properties of InAs/GaAs quantum dots studied by optically detected magnetic resonance at 95 GHz

F. Stevens, H. Vrielinck, F. Callens, E. Pauwels and M. Waroquier (Gent, B)
DFT-EPR investigation of diatomic defects in ionic lattices

H. Vrielinck, K. Sabbe, M. Zdravkova, F. Callens and P. Matthys (Gent, B)
Single crystal EPR analysis of a trigonal Ir^{4+} centre in NaCl at X- and Q-band

S.V. Nistor, E. Goovaerts, S. Van Doorslaer, S. Dewilde and L. Moens (Antwerp, B)
EPR structure characterization of the ferric neuroglobin

I.V. Borovykh, L.V. Kulik, S.A. Dzuba, and A.J. Hoff (Leiden, NL)
Out-of-phase stimulated ESE appearing in the evolution of spin-correlated photosynthetic triplet-radical pair

B. Gallez and N. Beghein (Brussels, B)
Non invasive *in vivo* EPR monitoring of the methyl methacrylate polymerization during the bone cement formation

M. Lan, N. Beghein, and B. Gallez (Brussels, B)
Carbon blacks as EPR sensors for localized measurements of tissue oxygenation

A.Engalytcheff, R.Debuyst, H.Vrielinck, F.Callens and B.Tilquin (Brussels, B)
X- and Q-band EPR study of gamma-irradiated antihypertensive drugs : beta-blockers

M. Zdravkova, N. Crokart, E. Gaillard, F. Trompier, N. Beghein, B. Gallez and R. Debuyst (Brussels, B)
Accidentally irradiated fingers studied by L-band EPR spectroscopy

M. Zdravkova, JM Denis, B. Gallez, R. Debuyst (Brussels, B)
Sensitivity of whole human teeth to fast neutrons and gamma-rays estimated by L-Band EPR spectroscopy

P. Sonveaux, C. Dessy, A. Brouet, B.F. Jordan, V. Grégoire, B. Gallez, JL Balligand and O. Feron (Brussels, B)
Modulation of the tumor vasculature functionality by ionizing radiation accounts for tumor radio-sensitization and promotes gene delivery.

P. Mahy, B. Gallez, M. Debast, and V. Grégoire (Brussels, B)
In Vivo Calibration of the EF5 Immunofluorescence signal for tumor hypoxia with Electron paramagnetic resonance oximetry

A.Mouithys-Mickalad, S. Kohnen, M. Mathy-Hartert ,G. Du, F. Sluse, C.Deby, M. Lamy and G. Deby-Dupont (Liège, B)
Oxygen consumption and EPR studies of free radical production by alveolar cells exposed to anoxia: effects of the antibiotic ceftazidime.

In Memoriam : Arnold J. Hoff

Dear colleagues and friends of Arnold Hoff,

Arnold Jan Hoff passed away on 22 April 2002 at the age of 62. Until the very last days he choose to ignore the fatal cancer he was suffering from and continued to work with all available energy.

After his study in physics Arnold graduated with Johan Blok at the Free University in Amsterdam in 1969. In 1971 Arnold was introduced to the biophysics of photosynthetic reaction centers as a postdoc in George Feher's group at UCSD. Fully aware of the great potential of his specialization, magnetic resonance techniques, in the field of photosynthesis research, in 1974 Arnold joined Lou Duysens' biophysics laboratory at Leiden University, which at that time used mainly optical techniques. In 1985 he was appointed full professor in Biophysics.

The impact of Arnold's efforts for the development and application of magnetic resonance techniques for the study of primary reactions in photosynthesis is hard to overestimate. With his team of graduate students and post-docs he worked on electron spin polarization phenomena studied with cw and time-resolved EPR and ESE. Around 1982 he developed the technique of ADMR, an ODMR technique especially suited for photosynthetic samples. In recent years his attention turned to isotopically labeled reaction centers which were studied by NMR and EPR. Also, with his 'Russian connection' he worked on improving and re-evaluating magneto-photoselection and applying it to Radical Pair spin polarization. Future plans were the study of reaction centers with site directed spin labeling and developing the technique of CD-ADMR, but this was not to be. Arnold published more than 250 articles and 19 students graduated with him.

One of the things he loved most was to travel and to meet new people and to make new friends. He must have visited hundreds of symposia and congresses. Visitors to his office will remember the large collection of photographs on the wall of friends from all around the world. His joy for travel also led to a special relation with scientists in the field of EPR and photosynthesis from Russia. Since 1992 he was director of the Dutch-Russian Research Collaboration Network. In 1999 he was awarded the Voevodsky Gold Medal of the Russian Academy of Sciences. He was also the chairman of the highly successful ESF program 'Biophysics of Photosynthesis', that, in his own words, forged a network of cordial links between a great number of groups in practically all European countries. At Leiden University he was the initiator of BIOSPEC, a collaboration between physics, chemistry and biology, which led to the foundation of the research school BIOMAC.

This department and the biophysics community have lost a strong leader. We feel deep compassion for Zina and his children, who have lost much more.

Peter Gast
Hans van Gorkom
Thijs Aartsma
Thomas Schmidt

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ORAL

COMMUNICATIONS

The Electronic Structure of Donors, Acceptors and vacancies in SiC as revealed by EPR and ENDOR Spectroscopy at 95 GHz

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95 GHz pulsed EPR and ENDOR spectroscopy has been applied to study the electronic and geometric structure of donors, acceptors and vacancies in SiC. In particular the study of the boron acceptor has shown that precise information can be obtained owing to the high spectral resolution in the EPR as well as in the ENDOR measurements. The study shows that at liquid-helium temperatures there is a broken bond between the boron atom, which occupies a silicon position, and one of the carbon neighbors. The dangling bond of this carbon atom carries the main part of the spin density. The remainder of the spin density is distributed anisotropically in the crystal as revealed by an analysis of the ^{13}C ENDOR spectrum. A measurement of the spin-spin relaxation time as a function of temperature shows that the broken bond starts to hop around when increasing the temperature and that at temperatures above about 100 K the spin density distribution becomes axially symmetric. This distribution can be rationalized on the basis of effective mass theory.

An optically detected magnetic resonance study of triplet exciton formation in a series of conjugated polymers and oligomers with increased electron affinity.

De Ceuster, J., Antwerpen/B, Goovaerts, E., Antwerpen/B, Bouwen, A., Antwerpen/B, Charas, A., Lisboa/P, Morgado, J., Lisboa/P, Alcácer, L., Lisboa/P, Detert, H., Mainz/D

Jan De Ceuster, Department of Physics, University of Antwerp, Universiteitsplein 1, B-2610 Antwerpen

Since the discovery of electroluminescence in poly- (para phenylene vinylene) (PPV) [1], much effort has been dedicated to the development of new stable materials, which can act as the active layer in organic light emitting diodes (OLEDs). The formation of triplet excitons reduces the photo- and electroluminescence efficiency of the molecules. For optimal device performance it is furthermore necessary to reduce the energy barrier between LUMO and cathode in order to have good electron injection. In this work we investigate the efficiency of triplet exciton formation and its relation to PL quantum efficiency, bandgap and the position of the LUMO with respect to the vacuum level. Optically Detected Magnetic Resonance (ODMR) is a useful tool to study the non-luminescent states of conjugated molecules [2]. In the described measurements, performed in an X-band (9.4 GHz) spectrometer, the microwave-induced change in singlet luminescence is monitored as a function of magnetic field.

Two series of molecules have been studied in spincoated films:

(1) A series of three poly-fluorene based copolymers: poly- (9,9-bis (2-ethylhexyl) fluorene – *alt* - Ar); Ar = 1', 4'- phenylene / 2', 5'- thiophene / 2', 5'- thiophene - 1,1-dioxide, in order of increasing electron affinity. [3]

(2) A series of six PPV based oligomers, subdivided in three categories [4]:
   a. 2 oligomers (3 and 5 units), which are terminated by 1,3,4-oxadiazole
   b. 2 oligomers (3 and 5 units), which are terminated by –CN groups
   c. 2 oligomers (3 units), which are terminated by SO_2 groups.

The spectra consist of the superimposed signal of triplet excitons and triplet polaron pairs (around $g \approx 2$). The relative intensities of the signals of the different molecules are compared. It is concluded that in molecules with lower quantum efficiencies, the relative amount of triplet excitons, which are created is higher. In general, when the bandgap decreases, a higher triplet yield is observed. For the polymers, the triplet yield goes up when the LUMO lies lower but for the oligomers, the opposite is true. The question should be asked whether these changes are real changes in triplet yield or changes in ODMR efficiency. The ODMR measurements were performed as a function of incident light power. The relative changes in intensity and lineshape are discussed. The differences between ODMR and EPR transition probabilities show up in lineshape simulations.

[1] J. H. Burroughes, D. D. C. Bradley, A. R. Brown, R. N. Marks, K. Mackay, R. H. Friend, P. L. Burns, and A. B. Holmes, Nature **347** 539-541 1990

[2] V. Dyakonov, and E. Frankevich, Chem. Phys. **227** 203-217 1998

[3] A. Charas, N. Barbagallo, J. Morgado and L. Alcácer, Synth. Met. **122** 23-25 2001

[4] H. Detert and E. Sugiono, Synth. Met. **122** 15-17 and 19-21 2001

Out-of-phase stimulated ESE appearing in the evolution of spin-correlated photosynthetic triplet-radical pair

I.V. Borovykh[a], L.V. Kulik[b], S.A. Dzuba[b], and A.J. Hoff[a]

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Abstract

A strong out-of-phase stimulated electron spin echo is observed in the spin-correlated triplet-radical pair $^3PQ_A^-$ in photosynthetic bacterial reaction centers. The formation of this echo is shown to be induced by decay of the triplet state. The out-of-phase echo shows deep envelope modulation induced by electron-electron dipole interaction between the partners in the pair. The analysis of this modulation provides dipolar frequencies. The interspin distance in $^3PQ_A^-$ is shown to be the same as in the radical pair $P^+Q_A^-$. This new type of experiment appears to be widely applicable for the study of chemical reactions and intermolecular distances in solids.

Plenary Lecture

In vivo EPR imaging of free radicals in biomedical applications

Jay L. Zweier

The John Hopkins University School of Medicine
Baltimore, MD, USA

Nitric oxide mediated pharmacological and physiological modulations of the tumor blood flow and oxygenation

BF Jordan, V. Grégoire, R. Demeure, P. Sonveaux, O. Feron, J. O'Hara, V. Vanhulle, N. Delzenne, and B. Gallez

Université catholique de Louvain, Brussels, Belgium

Purpose/Introduction

Oxygen is a key environmental factor in the development of tumors and their response to treatment. The pO_2 plays important roles in the treatment of tumors. We tested the effects of pharmacological and physiological NO-mediated modulations on tumor bearing mice on the tumor blood flow, oxygenation and radiation sensitivity. These three treatments consisted in 1) i.p. injection of a NO donor (isosorbide dinitrate, 0.2 mg/kg); 2) i.v. insulin infusion (16 mU/kg/min, 25 min) and 3) electrical stimulation of the host tissue (5 Hz, 0.2 ms pulses, 15 min).

Subjects and Methods

The transplantable liver tumor model (TLT) and the syngeneic FSAII tumor model were implanted in the thigh of NMRI and C3H mice. Tumor oxygenation was monitored using EPR (Electron Paramagnetic Resonance) oximetry and OxyLite™. Tumor blood flow was evaluated by OxyFlo™ and contrast-enhanced MRI. Oxygen consumption was determined by EPR spectroscopy. The radiosensitizing properties of the treatments were studied in vivo via the determination of FSa II tumor regrowth delays.

Results

The three treatments induced a prolonged increase in tumor oxygenation on both tumor models. Isosorbide dinitrate administration induced an increase in tumor perfusion as a consequence of NO delivery that resulted in an increase in tumor pO_2. Contrarily, the increase in tumor pO_2 during and after insulin infusion was not due to an increase in flow (which was even decreased as shown using flash MRI), but to a decrease in tumor cells oxygen consumption. This mechanism turned out to be NO mediated (inhibition of complex I and IV of the mitochondrial respiratory chain) and was demonstrated by immunoblotting (eNOS phosphorylation), by the up-regulation of the tumor cGMP content and by inhibition of the effects using a NOS inhibitor. The electrical stimulation protocol induced NO production that resulted in a rapid increase in tumor oxygenation (flow effect) and that was relayed by a NO dependent decrease in tumor cells oxygen consumption after the end of the protocol. Isosorbide dinitrate injection[a] and insulin infusion[b] radiosensitized the FSa II tumor model with the same efficiency[a] or even with more efficacy[b] than carbogen breathing (reference treatment).

Discussion/Conclusion

These NO mediated modulators of the tumor oxygenation and blood flow have to be considered for their 1) radiosensitizing properties (for isosorbide dinitrate and insulin treatments), 2) improvement in tumor blood flow that could allow an increase in accessibility for chemosensitizing agents (for isosorbide dinitrate and electrical stimulation).

High frequency EPR in strongly correlated electron systems

P.J.M. van Bentum and E. van der Horst

High Field Magnet Laboratory,

University of Nijmegen

The Netherlands

We will give a brief presentation of the new high field magnet facility in Nijmegen, with the emphasis on prospects for very high field magnetic resonance studies.

This new European user facility will become operational in the beginning of 2003.
After this introduction we will describe some of our recent high frequency EPR results, mainly on strongly correlated electron systems. Examples will include linear AF Mn chains, the magnetic groundstate in solid molecular oxygen, low-high spin transitions in LaCo-oxides and frustrated spin lattices in 2D Cu-borates.

Distance measurements by pulsed EPR

Martina Huber, Irene M.C. v. Amsterdam[#], Marcellus Ubbink[#], Gerard W. Canters[#]

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For the interpretation of electron transfer rates and in relation to other biophysical problems, accurate distances are required. Electron paramagnetic resonance (EPR) combines specificity, due to exclusive sensitivity to paramagnetic centers, with being sensitive to a large range of distances. Modern EPR techniques now allow to target specifically longer distances. An overview over the existing techniques will be given.

Specifically, a pulsed ELDOR (electron electron double resonance) method, the four-pulse DEER[1] technique will be discussed. Previous applications of this technique were focussed on nitroxide spin labels. Here we show that it is also applicable to Cu(II) centers in proteins. This is of particular interest, as the large inhomogenous linewidths of Cu(II) EPR spectra make it impossible to resolve the dipolar splittings for longer distances in conventional continuous wave (cw) EPR spectra.

We have investigated covalently linked dimers of azurin. Azurin is a blue copper protein containing a single Cu(II) center. In one dimer the two proteins are directly linked via the cysteines of the N42C mutant[2,3] (**az-1**), in another dimer via a flexible linker (BMME)[2,3] (**az-2**). DEER modulation due to the dipolar coupling is observed for **az-1**, and the Fourier transform of the DEER response has a well defined peak at 2.7 MHz, which is in agreement with the distance of 25.9 Å obtained from X-ray data[2,3].

The larger g- and hyperfine anisotropy of Cu(II) compared to nitroxide spin labels makes it necessary to consider orientation selection due to the limited excitation bandwidth of the microwave pulses.

This dimer is part of a series to study self exchange ET via NMR[2,3]. The exponential distance dependence of ET rates and possible differences between the crystal structure and the (frozen) solution motivated the EPR study. The extension of DEER from previously used spin labels to Cu(II) allows to use copper as an intrinsic probe, avoids the problem of distributions of spin label orientations and is directly applicable to metallo-proteins, where metal-metal interactions often determine function.

References

1 Pannier et al. J. Magn. Reson. (142) 331-340 (2000)
2 van Amsterdam, et al. Chemistry, A European Journal 7 (11), 2398-2406 (2001)
3 van Amsterdam, et al. Nature Structural Biology 9 (1): 48-52

Review and Recent Advances in Electron Magnetic Resonance on Saccharides

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[1]Postdoctoral Fellow of the Fund for Scientific Research - Flanders (Belgium) (F.W.O.-Vlaanderen)

Electron Magnetic Resonance (EMR), such as Electron Paramagnetic Resonance (EPR), Electron Nuclear Double Resonance (ENDOR) and ENDOR-Induced EPR (EI-EPR) experiments on saccharides, have been carried out in the scope of either studying the radical formation in biological systems, applying sugar in accidental dosimetry or detecting irradiated sugar-containing food. The experimental acquisition of structural information on radiation induced radicals is somehow limited by the complexity of the saccharide systems themselves. In some cases, the EPR spectra of single crystals can be unravelled in terms of individual contributions each described by a set of spin Hamiltonian parameters. However, identification of the radicals is obstructed by the diversity of potential models. Therefore, regarding the growing success of quantum chemical calculations in molecular modelling, Density Functional Theory (DFT) calculations as a leading tool were applied to identify the experimentally detected radicals. Recently, the confrontation of DFT calculated magnetic resonance parameters with experimental values of radicals in saccharides appeared quite promising. In this work, a short review will be presented of the EMR studies obtained on irradiated saccharides by our group, and of recent combined EMR-DFT results.

POSTER

COMMUNICATIONS

Magnetic Properties of InAs/GaAs Quantum Dots Studied by Optically Detected Magnetic Resonance at 95 GHz

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The application of double resonance techniques with optical detection of microwave resonance makes it possible to carry out experiments with very high energy resolution and emission band selectivity. Optically detected cyclotron resonance (ODCR) has been applied to study the CR of carriers in low dimensional structures [1]. Optically detected magnetic resonance (ODMR) spectroscopy has already demonstrated its usefulness in studies of radiative and non-radiative recombination centers in semiconductors and of excitonic states in quantum wells, superlattices, and in *core-shell* quantum dots [2,3].
We report on ODMR measurements performed on MBE-grown InAs/GaAs quantum dot (QD) structures. Optical detection of magnetic resonance was carried out via the change of the QD photoluminescence intensity under W-band (95 GHz) microwave excitation. The InAs QDs are fabricated by the Stranski-Krastanov growth method. In one structure they are embedded in intrinsic GaAs layers, and emit at low temperature around 1.3 eV. In this structure with small-sized dots, cyclotron resonance of the electron in the 2D wetting layer is observed. In a second structure, the InAs QDs are embedded in the intrinsic region of a p-i-n doped structure. These larger dots emit at low temperature around 1.1 eV. For both structures, the ODMR spectra exhibit a strong anisotropy which is a consequence of carrier confinement.

[1] B.C. Cavenett, E.J. Pakulis, Phys. Rev. B **32**, 8449 (1985)
[2] N. G. Romanov, P. G. Baranov, I. V. Mashkov, P. Lavallard, and R. Planel, *Solid-State Electronics* **37** (4), 911 (1994).
[3] H. W. van Kesteren, E. C Cosman, W. A. J. A. van der Poel, and C. T. Foxon, *Phys. Rev. B* **41** (8), 5283 (1990).

DFT-EPR investigation of diatomic defects in ionic lattices

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Density functional methods are used to calculate the EPR parameters of X_2^- and XY^- (X and Y = S, Se) lattice defects in various alkali halide (MZ, M = Na, K, Rb and Z = Cl, Br, I) lattices.
In the literature, two models have been proposed for these diatomic chalogen defects. In the first model – mono-vacancy model - the X_2^- or XY^- molecular ion replaces a single halide ion. In the second model, the X_2^- or XY^- molecular ions replace two adjacent halide ions. It represents the di-vacancy model. In both models the bond axis of the diatomic molecular ion lies along a < 110 > direction.
Although a lot of EPR and ENDOR data are available, some questions remain unanswered. First, in the KCl:S_2^- case, two centres with different ground states have been encountered. Second, the occurrence of different ground state properties for different lattices containing the same lattice defect has not yet been fully explained.
The explanation of the former questions and the validation of the DFT methods for the description of paramagnetic defects in ionic lattices were the main stimulations for the present DFT study.
For all alkali halide lattices considered the calculated EPR parameters for the S_2^-, Se_2^- and SSe^- defects are in very good agreement with experiment when a mono-vacancy model is assumed. In KCl, the DFT results suggest that one of the S_2^- defects resides in a halide mono-vacancy, while the other occupies a di-vacancy site.

Single crystal EPR analysis of a trigonal Ir^{4+} centre in NaCl at X- and Q-band

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X-ray irradiation at 77 K and subsequent thermal anneal of Ir^{3+}-doped NaCl single crystals produces both Ir^{2+} ($5d^7$, S=1/2) and Ir^{4+} ($5d^5$, S=1/2) centres in these crystals [1]. One of the Ir^{4+} centres is stable up to room temperature. Its EPR spectrum is characterised by a large Ir-hyperfine splitting and smaller splittings attributed to neighbouring Cl^- ions. It exhibits a very small g anisotropy. As a result of the strong (ligand) hyperfine interactions, it is very difficult to resolve this anisotropy at X-band. The analysis of the angular dependence of the spectrum at Q-band in a {100} and a {110} plane demonstrates that the centre has trigonal symmetry and allows a very accurate determination of the principal g values. Possible models for the centre, in accordance with the EPR results, will be discussed.

[1] M. Zdravkova, K. Sabbe, F. Callens, E. Dobbeleir and P. Matthys, *Imag. Sci. J.* **47**, p. 63-70, 1999

EPR STRUCTURE CHARACTERIZATION OF THE FERRIC NEUROGLOBIN

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Abstract

We report the results of an X-band Electron Paramagnetic Resonance (EPR) spectroscopy study of the ferric form of mouse wild neuroglobin (Ngb), the newly discovered oxygen binding protein which is primarily expressed in the brain of mammals [1,2]. The low temperature study, performed on frozen solutions with pH varying from 5 to 10, is offering detailed structural information concerning the heme environment, essential for understanding its role in the nervous system of the vertebrate brain.

Two distinct, six-coordinated structural forms were found to be simultaneously present in a wide range of pH values: a rhombic low-spin specie with *g*-tensor principal values 3.15, 2.16 and 1.34, attributed to a His-Fe-His configuration, which is the dominant one and an axial aquomet high-spin form with $g_{\perp} = 5.95$ and $g_{||} \sim 2$, which exhibits an increased relative concentration at lower pH values.

[1] T. Burmester, B. Weich, S. Reinhardt, T. Hankeln, *Nature 407 (2000) 520.*

[2] C. Awenius, T. Hankeln, T. Burmester, *Biochem. Bioph. Res. Co. 287 (2001) 418.*

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Non invasive *in vivo* EPR monitoring

of the methyl methacrylate polymerization during the bone cement formation

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The curing of poly(methylmethacrylate) (PMMA) bone cement is done by a free radical polymerization. As the amount of free radicals present is a marker of the amount of unpolymerized chains present in the polymer, it is assumed that this could be related to the mechanical properties such as strength or density. In this study, the direct observation of the free radicals produced during the PMMA bone cement formation was obtained for the first time *in vivo* using low frequency EPR spectrometers (1.2 GHz). Low frequency permits measurements in live animals due to the increased microwave penetration. The amount of polymerization radicals was carried out non invasively over days on the same animals. The decay rates obtained *in vitro* and *in vivo* were compared: the decay rates were significantly lower when the curing process occurred *in vivo* compared to the situation *in vitro*. As the kinetics are rather different *in vitro* and *in vivo*, this emphasizes the value of the present method that permits the non invasive monitoring of the curing process directly *in vivo*.

Carbon blacks as EPR sensors for localized measurements of tissue oxygenation

Minbo Lan, Nelson Beghein, and Bernard Gallez

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Within the last few years several new paramagnetic particulate materials have been found to exhibit a pO_2-dependent EPR linewidth: lithium phthalocyanine crystals, lithium naphthalocyanine crystals, particles of natural coals such as fusinite or gloxy, analytical charcoals, and synthetic carbohydrate chars. In vivo EPR oximetry has already produced very useful results that have contributed significantly to solving important biological problems. It should be of major interest to transpose this technique into the clinic. For that purpose, there is a critical need for paramagnetic sensors that are fully biocompatible. Although there exist several strategies to improve the biocompatibility of the exisiting sensors, is should be more easy to use a material that is already being used in human subjects. That was first achieved in the pionnering works of the group of H.M. Swartz using India inks. They demonstrate the feasibility to measure the pO_2 in tissues using inks, and carried out the first human EPR oximetry study on a volunteer with an extensive tattoo on his upper arm. Although these studies open a great hope to the immediate application of India ink as oxygen sensor for the clinic, further development was hampered by the sub-optimal EPR characterisitics of these first probes, especially the low spin density and consequently the low signal-to-noise ratio obtained in vivo. Together with the group of H.M. Swartz, we have recently tried to select materials with more optimal EPR properties. Several dozens of drawing-ink and tattoo inks were screened for the presence of paramagnetic materials, oxygen sensitivity, and performances in vivo (B. Gallez et al, in preparation). Interesting results were obtained, and it is likely that some selected materials will be further investigated for possible human use in EPR oximetry. However, it also appeared that the EPR properties can vary from one batch to another within the same trademark of commercially available inks. Therefore, in order to get a long-term availability of optimal EPR sensors and distribute it in the EPR community, it should be very desirable to isolate the pure active component from ink that is sensitive to oxygen. The aim of the present study was to identify carbon blacks as possible EPR oxygen sensors. The carbon blacks are produced by the incomplete combustion or the thermal decomposition of aromatic oils or other hydrocarbons. Several classes of carbon blacks can be obtained from different production methods : gas blacks, furnace blacks, and lamp blacks. The three production processes result in a wide range of different carbon blacks with well characterized particle size, structure, surface area, rheology, and electrical conductivity. We screened the three classes of carbon blacks for the presence of oxygen sensitive paramagnetic centers. The steps used for the screening were : 1) to check the presence of paramagnetic centers in the material ; 2) to measure the sensitivity of the EPR linewidth to variations in oxygen environment, and calibrate the oxygen sensitive materials ; 3) to test the sensitivity and the stability of responsiveness in animals ; 4) to evaluate the usefulness of the probes to monitor variations of pO_2 in a murine tumor model. On 43 carbon blacks tested, hree compounds presented optimal properties as oxygen sensors : high sensitivity to small changes of pO2, high spin density, long stability of responsiveness in tissues, and large availability.

X- and Q-band EPR study of gamma-irradiated antihypertensive drugs : beta-blockers

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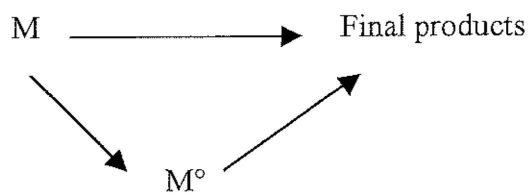
Beta-blockers have been irradiated by gamma rays from a ^{60}Co source.

It is well-known that degradation products are formed after irradiation of drugs though the mechanisms of production are not well established yet.

It is also well-known that radicals are trapped in solid state drugs after irradiation. Identification of these radicals could help us understanding the mechanisms of the irradiation of the solid state drugs. It would allow us to establish the radiolytic scheme of these drugs and to determine the contribution of the radicals to the formation of the final products.



We have therefore studied 4 beta-blockers by X-band EPR. As only little qualitative information could be obtained, we have extended the study to Q-band. The work presented here is the comparison between the X and the Q-band spectra of the drugs. The similarities are very important which makes us think that the spectra are dominated by hyperfine structures and that there might be only a slight anisotropy. The few changes could perhaps also be attributed to the increase of the linewidth that can occur when passing from X to Q-band.

Accidentally irradiated fingers studied by L-band EPR spectroscopy.

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Retrospective dosimetry is not limited to the use of teeth. Other irradiated tissues like bones also exhibit analogous EPR signals. Even if the radiosensitivity for bones is weaker than for teeth, significant signals could be detected in the case of accidental irradiation of limb extremities. Today, accident victims are generally amputated without a precise knowledge of the irradiated area which risks necrosis. In this respect, L-band EPR spectroscopy could be of great help : low frequency microwaves are less absorbed by water and penetrate more deeply into living material (~10 mm in tissues with L-band compared with ~1 mm with X-band). L-band EPR spectra of the irradiated extremities, for example a finger placed inside the antenna (surface-coil resonator) of the L-band spectrometer, could be used in order to estimate the absorbed dose. The substantial sensitivity loss (proportional to the third power of the microwave frequency) with L-band against X-band by approximately a factor of 500 could be partly offset by a gain in sample volume

Preliminary results shall be presented for this pilot project in collaboration with the Unit of Accident and Criticity Dosimetry of IRSN (France) which supplied us in a first step with highly irradiated (10-10^3 Gy) baboon and macaque fingers.

Sensitivity of whole human teeth to fast neutrons and gamma-rays estimated by L-band EPR spectroscopy.

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This work is the first attempt to use L-band spectroscopy for estimating the sensitivity of whole teeth to fast neutrons and gamma-rays. Three teeth were successively irradiated first with fast neutrons with a large energy spectrum (mean energy around 30 MeV) up to ~160 Gy and then with gamma-rays up to ~14 Gy. After each irradiation, L-band (~1 GHz) EPR spectra of each whole tooth surrounded by the surface-coil resonator were recorded, yielding a single composite line principally due to CO_2^- and native radicals. The sensitivities are estimated by the slopes of the linear dose response curves of the dosimetric CO_2^- radicals. The ratios of the gamma/neutron sensitivities were found to be in the range 8 to 9 (± 2) for the three teeth.

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Modulation of the tumor vasculature functionality by ionizing radiation accounts for tumor radio-sensitization and promotes gene delivery.

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In the presence of oxygen, irradiation-induced DNA radicals are converted into permanent damages that increase the probability of cell death. This is part of the rationale underlying for the effectiveness of fractionated radiotherapy which is thought to stimulate the cytotoxic effects of ionizing radiation by inducing tumor re-oxygenation. The tumor shrinking, which follows the removal of cells killed by irradiation, is, indeed, proposed to facilitate flow-dependent oxygen delivery and diffusion to cells that were formerly hypoxic, making them more radiosensitive for the next X-Ray exposure.

In the present study, however, we report that functional changes induced in the tumor vasculature by irradiation directly account for the reduction in hypoxia, as determined by using pre-implanted charcoal wood powder as EPR O_2-sensitive probe. First, the nitric oxide (NO)-mediated vasorelaxation that is defective in tumor small arteries was completely restored following irradiation. This was attributed to the expressional up-regulation of the endothelial NO synthase while the abundance of its physiological inhibitor, caveolin-1, was depressed. Second, this potentiation of the NO pathway was necessary and sufficient to induce a marked increase in tumor re-oxygenation that determined the higher sensitivity of the tumor to further irradiation and the resultant growth retardation. Third, experiments with isolated tumor cells allowed us to rule out NO-dependent changes in tumor cell respiration (i.e. O_2 consumption rate).

Interestingly, we also document that the irradiation-induced alteration in tumor vasculature reactivity led to an increased delivery of cationic lipid-DNA complex and consecutive (reporter) protein expression into the tumor in a NO-dependent fashion. Altogether, these data emphasize the critical role of the irradiated tumor vasculature in promoting both the effectiveness of fractionated radiotherapy and the selective gene and/or drug delivery to the tumor.

IN VIVO CALIBRATION OF THE EF5 IMMUNOFLUORESCENCE SIGNAL FOR TUMOR HYPOXIA WITH ELECTRON PARAMAGNETIC RESONANCE OXIMETRY.

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The use of the hypoxic cell binding probe EF5 has been shown to be a sensitive approach to detect tissue hypoxia by immunofluorescence (IF). However, IF only permits a relative quantitation of tissue hypoxia, limiting thus interpretation and multicenter comparison of the data. The objective of this study is to develop an experimental mouse tumor model for *in situ* calibration of the IF signal with Electron Paramagnetic Resonance (EPR) oximetry.

A syngeneic fibrosarcoma murine tumor line (NFSA) generated in the gastronecmius muscle in C3H mice was used. A paramagnetic charcoal (20 µl of a 100 mg/ml suspension of charcoal [EM Science charcoal wood powder, Gibbstown, USA]) was injected into 8.4 ± 0.6 mm diameter IM tumors. No difference was observed in the tumor growth curves of injected *vs* non injected mice. The EPR signal could not be detected in 4 of the 25 injected mice. Three to 4 days after charcoal injection, at a mean ± SD diameter of 10.6 ± 0.8 mm, EF5 (100 µM) was injected i.v, except for control animals ($EF5^-$). The intratumor oxygen partial pressure was then monitored every 20 min. with an EPR spectrometer (Magnettech, Berlin, Germany), under I.P. anaesthesia, performed with ketamine (140 mg/kg) and xylazine (1,3 mg/kg), with the animals breathing under 21 or 100% O_2 concentrations. Three hours after EF5 injection, tumors were harvested and freezed in liquid nitrogen. Frozen-sections were cut (n = 5-8 per tumor) in the region where the charcoal has been implanted, and processed for IF with (mean number of sections per tumor ± SD : 5.93 ± 1) or without (n = 1) the monoclonal antibody ELK3-51 conjugated to a Cy-3 fluorochrome. Slices were viewed with a fluorescence microscope (Zeiss, Berlin, Germany) at high magnification . Five tumors of the 21 harvested had to be discarded due to the fact that the charcoal was found to be partially or totaly in the tumor surrounding muscle. Areas (pictures of 190 x 140 µm, 55.8 ± 11.8 per tumor) surrounding the charcoal were digitized with a Sony DXC 950-P camera and saved as TIFF files. The red stacks of the red-green-blue TIFF files were analysed with the NIH-Image software. Quantification of hypoxia was based on fluorescence intensity 10µm around the area occupated by the charcoal in each picture (11.1 ± 10.6 % in the 896 pictures, determined by incandescent backlighting after each fluorescence acquisition).

EPR oximetry showed that tumors were more hypoxic under 21% than under 100% O_2 : mean (± SEM) pO_2 values reached 3.16 ± 2.1 (n = 5) and 9.77 ± 3.2 mmHg (n = 11), respectively. IF data were in agreement : corresponding mean (± SEM) fluorescence intensity reached values of 88.4 ± 5 (n = 5) and 76.4 ± 3 (n = 11), respectively. Moreover, excellent correlation was found between IF and EPR oximetry on the 16 individual tumors processed (r^2 0.47, Bravais -Pearson : $p < 0.01$) EF5 binding occured at low pO_2 values below 10 mm Hg, confirming published *in vitro* studies. In conclusions, we did demonstrate *in vivo* that the IF signal generated by EF5 adducts correlates statistically with pO_2, as measured by EPR oximetry.

Oxygen consumption and EPR studies of free radical production by alveolar cells exposed to anoxia: effects of the antibiotic ceftazidime.

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Pathological changes consequent upon reperfusion of an ischemic organ include oxidative stress due to the localized and short-lived generation of reactive oxygen species (ROS) (1). Several *in vitro* and *in vivo* models have been designed to simulate ischemia-reperfusion or anoxia-re-oxygenation (A/R), and to demonstrate the production of oxygen free radicals and reactive species, with subsequent lipid peroxidation, in organs (2) and cultured cells (3). However, the direct demonstration of free radical production by EPR spectroscopy at the cellular level remains difficult, and the kinetics of free radical release (during the ischemia/anoxic phase or at reperfusion/re-O_2) is still a matter of debate. It seems that in isolated human alveolar cells, the detection of free radical by EPR has never demonstrated, in parallel with cell respiration dysfunction. Consequently, this study was designed to investigate free radical production by cultured human alveolar epithelial cells subjected to anoxia/re-O_2 (A/R), and to evaluate the effects of ceftazidime, an antibiotic previously demonstrated to possess antioxidant properties (4). Two models of non-lethal A/R were performed on type II human pneumocytes (A549 cell line), either on cells attached to culture dishes (monolayer A/R model; 3,5h of anoxia, 30 min of re-O_2) or after cell detachment (suspension A/R model; 1h of anoxia, 10 min of re-O_2). Ceftazidime and selective inhibitors (SOD, Tiron, L-NMMA) were added before anoxia. Free radical production was assessed by the EPR spin trapping technique. Oxygen consumption was monitored, in parallel with EPR studies, in the suspension A/R model.

The production of free radical species was demonstrated by the generation of PBN-radical adducts: (a_N = 15.2 G) in the monolayer A/R model and a six-line EPR spectrum (a_N = 15.7 G and a_H = 2.7 G) in the suspension A/R model. A kinetic studies performed by oximetry, in parallel with EPR, demonstrated marked alterations of the cell respiratory function and that the free radical production started during anoxia and increasing during re-O_2. In the suspension A/R model, EPR spectra were decreased upon the addition of 200 U/ml SOD (37% inhibition), 0.1 mM Tiron (67% inhibition) and 1 mM L-NMMA (43% inhibition). Addition of 1mM ceftazidime decreased the amplitude of EPR spectra (37% inhibition) in both A/R models. Conclusively, these findings demonstrate that free radicals are produced by alveolar cells when subjected to anoxia-re-oxygenation sequence and that ceftazidime exerts an inhibiting effect as well as selective inhibitors (SOD, Tiron and L-NMMA).

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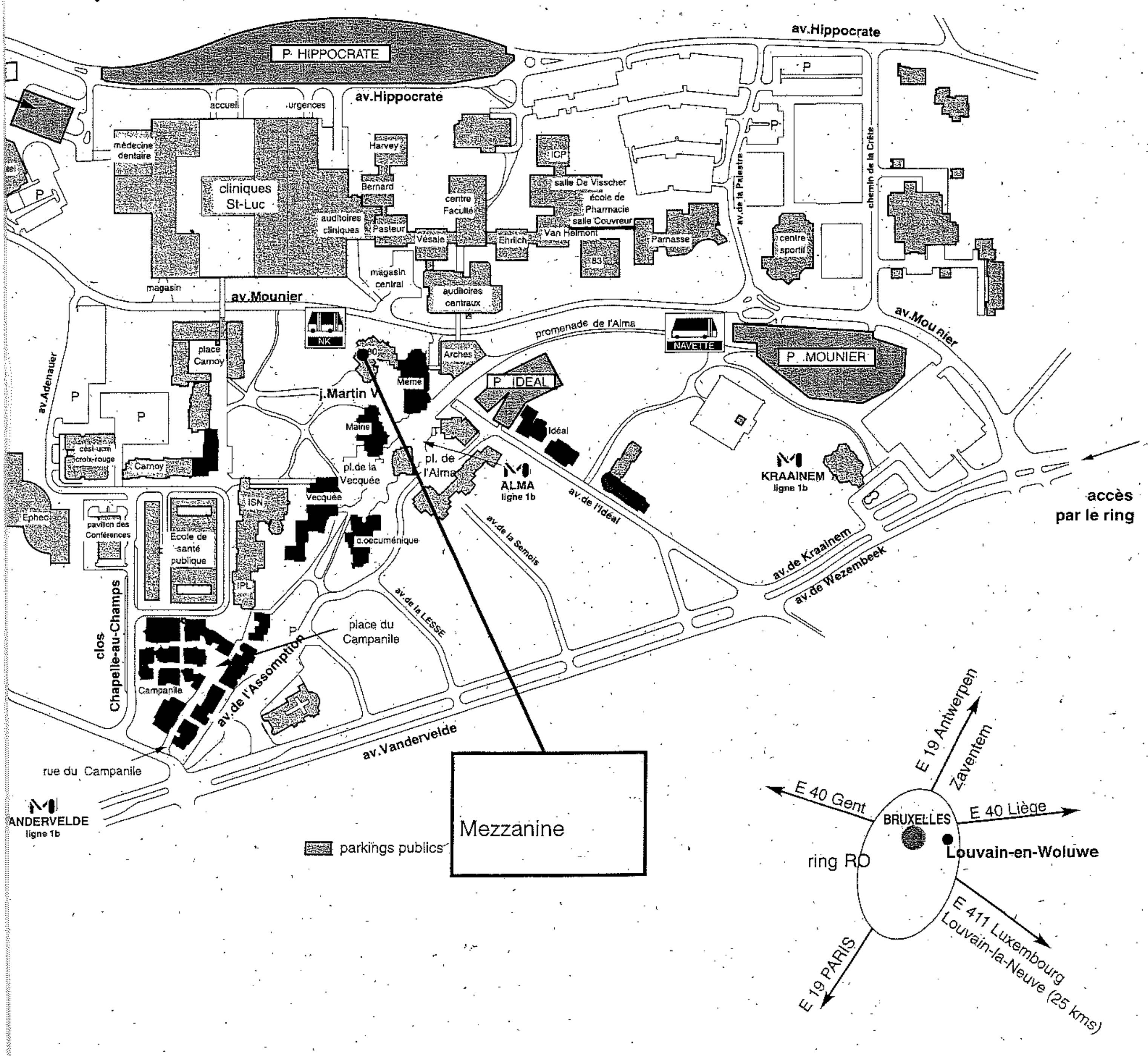
Plan :

A Louvain-en-Woluwé



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