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Université de Liège

24th Benelux EPR Society Meeting

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24th Benelux EPR Society Meeting-Program

10:00 Registration Coffee/Tea and Poster display

10:30 Opening of the meeting *M. Hoebeke and A. Mouithys (ULg, BEL)*

First Session: Co-Chairs: *B. Gallez (Louvain, BEL) & Jacco Briedé (Maastricht, NL)*

10:35 "EPR imaging: Success and challenge. An open field for new application."
Yves-Michel Frapart (Paris, FRA)

11:10 Endothelial nitric oxide uncoupling during lung ischemia-reperfusion injury.
Jan Gielis (Antwerp, BEL)

11:35 Contribution of EPR to magnetic cell tracking studies
Pierre Danhier (UCL-Louvain, BEL)

12:00 ESR spectroscopy for studying radical formation from different types of nanomaterials for assessment of toxicity.
Jacco Briedé (Maastricht, NL)

12:25 Lunch
13:45 Poster session

Second session: Co-Chairs: *S. Van Doorslaer (Antwerp, BEL) & H. Vrielinck (Ghent, BEL)*

14:00 "From Benchtop to Pulse, an overview of the latest Bruker EPR products"
Manuela Liberi (Bruker)

14:25 Towards Temperature cycle EPR
Gabriele Panarelli (Leiden, NL)

14:50 "Generalization of ENDOR-induced EPR for disordered systems: application to X irradiated sucrose"
Jevgenij Kusakovskij (Ghent, BEL)

15:15 Coffee Break and Poster session

15:45 The Strange Shapes of an Amyloid Protein Bound to Membranes
Pravin Kumar (Leiden, NL)

16:10 Light-induced EPR study of polarons in novel blends for organic solar cells
Melissa Van Landeghem (Antwerp, BEL)

16:35 General assembly of the Benelux EPR society – Prospects for the next meeting:
E. Groenen (Leiden, NL) and H. Vrielinck (Ghent, BEL)

17:00 Closing remarks and Drink

ORAL

COMMUNICATIONS

Bringing EPR to imaging :
Multidisciplinary approach for quantitative EPR spectroscopy and imaging

Dr. Yves-Michel FRAPART

Electron Paramagnetic Resonance (EPR) is a unique, non invasive methods to specifically detect paramagnetic species (transition ion, Reactive Oxygen Species, O_2 ....) in any sample (living organisms, materials ...).

XXIst century EPR as to face new challenge such as bringing EPR to imaging and to the clinical. Those challenges open new research field, and new results in the biomedical world, however some problems limit applications and have to be resolved for routine application :

- Anatomic location of the molecular EPR data obtained in the animal body, or the object.
- The relative instability of the EPR probes due to their possible metabolism and dispersion during acquisition.
- Getting quantitative data to obtain biomarkers for medicine and biological science with their reliability in spectroscopy *in vivo* and in imaging.
- Accurate transformation of EPR data to image.

A multidisciplinary approach combining mathematics, computer science, chemistry, electro-chemistry, X-ray micro-computed tomography and EPR methodology allow us to make break through progress for :

- High efficiency superoxide measurement on living cells[1].
- High resolution EPR images combined with anatomic micro-computed X-ray tomography images ([2], and dosimetry (unpublished results).
- Stabilizing tretrathiatriarylmethyl radical *in vivo* for accurate oximetry measurement (submitted publication).
- Combining EPR and electrochemistry for quantitative Oximetry [3].
- Obtention of EPR Measurement accuracy.
- New methodology to obtain images from the EPR data.

Our approach will be illustrated by recent example and application.

1. Abbas, K.; Hardy, M.; Poulhès, F.; Karoui, H.; Tordo, P.; Ouari, O.; Peyrot, F. Detection of superoxide production in stimulated and unstimulated living cells using new cyclic nitrone spin traps. *Free Radic. Biol. Med.* **2014**, *71*, 281–290.
2. Beziere, N.; Decroos, C.; Mkhitaryan, K.; Kish, E.; Richard, F.; Bigot-Marchand, S.; Durand, S.; Cloppet, F.; Chauvet, C.; Corvol, M.-T.; Rannou, F.; Xu-Li, Y.; Mansuy, D.; Peyrot, F.; Frapart, Y.-M. First combined in vivo X-ray tomography and high-resolution molecular electron paramagnetic resonance (EPR) imaging of the mouse knee joint taking into account the disappearance kinetics of the EPR probe. *Mol. Imaging* **2012**, *11*, 220–228.
3. Boutier-Pischon, A.; Auger, F.; Noël, J.-M.; Almario, A.; Frapart, Y.-M. EPR and electrochemical quantification of oxygen using newly synthesized para-silylated triarylmethyl radicals. *Free Radic. Res.* **2015**, 1–8.

JF GIELIS[1,2], D CAPPOEN[2], L QUIRYNEN[2], J BRIEDE[3], P COS[2], PEY VAN SCHIL[1]

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Endothelial nitric oxide synthase uncoupling during lung ischemia-reperfusion injury

Abstract:

Introduction

Lung ischemia-reperfusion injury (LIRI) is a necessary part of any organ transplantation and a key determinant of both acute and chronic graft failure. We used combined data gathered from electron spin resonance, western blotting and fluorescent microscopy to assess the contribution of endothelial nitric oxide synthase (eNOS) and eNOS uncoupling to oxidative and nitrosative stress during LIRI.

Materials and Methods

20 eNOS wild type mice (eNOS $^{+/+}$) and 20 eNOS knock-out mice (eNOS $^{-/-}$) were divided into four groups: a sham control group, and three groups receiving one hour of pulmonary ischemia followed by no, 1 hour or 24 hours of reperfusion. Following euthanasia, lung tissue samples were divided in three. One part was incubated with the NO spin trap Fe-DETC, one part was used for ascorbyl content as a marker of ROS content in tissue and a third was used to assess eNOS uncoupling and protein nitrosation with western blotting. In peripheral blood, arterial blood gases were taken and ROS content was determined using CMH spin probe.

Results

eNOS $^{+/+}$mice had lower ROS production in their peripheral circulation but worse blood gas values after 1 hour of reperfusion. Lung tissue of eNOS $^{-/-}$ mice showed lower ROS and NO production and lower protein nitrosation compared to wild-type. Structural eNOS uncoupling was present already after 1 hour of ischemia as shown by increased eNOS monomer-dimer ratios.

Conclusion

We have shown that eNOS uncoupling, both structurally and functionally, is present during pulmonary ischemia-reperfusion injury and contributes to an inflammatory reaction that ultimately leads to worse clinical outcome. Stabilizing eNOS may therefore be a new approach to extend pulmonary graft survival.

Contribution of EPR to magnetic cell tracking studies

Pierre Danhier[1], Bernard Gallez[1]

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Magnetic resonance imaging (MRI) cell tracking is a promising technique for monitoring the fate of cells in cell-based therapies and in pathophysiological situations such as cancer metastasis. For this purpose, cells are first labeled *ex vivo* with MRI contrast agents (superparamagnetic iron oxides, SPIO). After injection into the small animal, SPIO-labeled cells induce signal voids on T_2-, T_2*-weighted MR images and can be tracked *in vivo* using MRI.

Here we aim to describe the applications of EPR to validate magnetic cell tracking approaches. First EPR was used to optimize SPIO labeling protocols. An efficient labeling of cancer cells with iron oxides is a prerequisite step for valid MRI cell tracking studies. We successfully used and validated EPR for quantifying the intracellular iron oxide content in SPIO-labeled cells. We showed that the specificity of EPR for measuring superparamagnetic forms of iron is a major advantage of the technique. EPR was found to be sensitive and reliable compared to standard iron quantification methods such as inductively coupled plasma mass spectroscopy (ICP-MS).

We next used EPR to quantify *ex vivo* the iron oxide content in tissues to validate MRI cell tracking experiments. In experimental liver metastasis assays, it was found that labeled cancer cells entrapped in the liver could not be visualized on T_2-,T_2*-weighted MR images, whereas *ex vivo* EPR measurements confirmed the presence of SPIO-labeled cancer cells in this organ. EPR was also instrumental to characterize the loss of SPIO after injection in tissues owing to iron oxide metabolism. We also aimed to monitor the homing of SPIO-labeled breast cancer cells in the mouse brain following intracardiac injection. MRI was found to be sensitive to detect iron oxide-labeled cells in the brain parenchyma. Moreover, the complementary role of *ex vivo* EPR to quantify the number of iron oxide-labeled cells in MRI cell tracking studies was highlighted.

In MRI cell tracking experiments, the dilution of the intracellular iron oxide content with cell division as well as the degradation of iron oxides by macrophages were shown to limit the follow-up period. Using EPR to quantify superparamagnetic forms of iron and ICP-MS to quantify the total iron pools in cells, we observed that macrophages could quickly take up and degrade iron oxides. In conclusion, EPR is a complementary technique in cell tracking studies to (*i*) validate cell labeling protocols, (*ii*) estimate the number of SPIO-labeled cells in tissues and (*iii*) to characterize the metabolism of SPIO.

ESR spectroscopy for studying radical formation from different types of nanomaterials for assessment of toxicity

Jacco J. Briedé, Héloïse Proquin, Penny Nymark, Theo M. de Kok, Henk van Loveren, Jos C.S. Kleinjans

Department of Toxicogenomics, GROW institute of Oncology and Developmental Biology, Maastricht University, the Netherlands

Nanotechnology has led to the development of a wide array of new materials that can be found in many products. The nanotechnology industry is promising significant scientific, economic and societal benefits, but concerns are raised about safety and toxicity of nanomaterials. Current safety tests are not appropriate for nanomaterials due to their specific properties as well as their differences in physical appearance like shape and surface, giving these materials unique characteristics. We have assessed the cellular as well as acellular radical formation of different types of nanomaterials, like TiO2, silver nanoparticles and multi-walled carbon nanotubes (MWCNT) by electron spin resonance (ESR/EPR) spectroscopy in combination with the spin trapping technique in order to study their toxic and carcinogenic properties. The results of radical and reactive oxygen species (ROS) formation of nanoparticles measured by ESR spectroscopy provides specific information about surface reactivity in terms of oxygen radical reactivity and this was related to cytotoxicity observed in different types of cells. But also other types of radicals detected by ESR indicated toxicological behavior of nanoparticles. Studying the physicochemical properties of nanomaterials is required to reinforce the conventional toxicological evaluations. In addition to current safety assays, ESR spectroscopy seems to be a valuable tool to gather information about expected nanomaterial-induced cellular toxicity and carcinogenicity.

1) Park MV, Lynch I, Ramirez-Garcia S, Dawson KA, de la Fonteyne L, Gremmer E, Briede JJ, Slob, W, Elsaesser A, Howard CV et al. In vitro evaluation of cytotoxic and inflammation properties of silica nanoparticles of diferent seizes in murine RAW264.7 macrophages. J Nanopart Res 13 (2011) 6775-87.
2) Park MV, Neigh AM, Vermeulen JP, de la Fonteyne LJ, Verharen HW, Briedé JJ, van Loveren H, de Jong WH. The effect of particle size on the cytotoxicity, inflammation, developmental toxicity and genotoxicity of silver nanoparticles. Biomaterials. 32 (2011) 9810-7
3) Nymark P, Jensen KA, Suhonen S, Kembouche Y, Vippola M, Kleinjans J, Catalán J, Norppa H, van Delft J, Briedé JJ. Free radical scavenging and formation by multi-walled carbon nanotubes in cell free conditions and in human bronchial epithelial cells. Part Fibre Toxicol. 11 (2014) 9810-7.
4) Nymark P, Wijshoff P, Cavill R, van Herwijnen M, Coonen ML, Claessen S, Catalán J, Norppa H, Kleinjans JC, Briedé JJ. Extensive temporal transcriptome and microRNA analyses identify molecular mechanisms underlying mitochondrial dysfunction induced by multi-walled carbon nanotubes in human lung cells. Nanotoxicology 9 (2015) 624-35.

"From Benchtop to Pulse, an overview of the latest Bruker EPR products"

Dr. Manuela LIBERI

Bruker

Our presentation will focus on our expandable instrumentation. We make an overview that goes from the benchtop system to some accessories for CW-EPR, and from the AWG for the pulse EPR to the cryogen-free variable temperature systems: solutions with the aim to accommodate the changing needs of researchers and laboratories.

Towards Temperature-Cycle EPR

Gabriele Panarelli[1], Mykhailo Azarkh[2], Peter Gast[1], and Edgar Groenen[1]

Most (bio)chemical reactions do not occur as a one-step process yielding products directly from reagents. On the contrary, most of the times reactions involve at least an intermediate stage where short-lived, possibly paramagnetic species are generated, which eventually lead to the final products. Knowing the physicochemical details of such reaction intermediates is paramount to understand and characterize the reactivity and kinetics of virtually any chemical system, from enzymatic catalysis to polymer degradation.

In principle, high-frequency Electron Paramagnetic Resonance can be a powerful tool to study the evolution of paramagnetic intermediate species of chemical reactions, because of its high *g*-resolution and of the small sample volume. In order to study chemical kinetics in a controlled way by EPR, it is most convenient to freeze a reaction to a suitable temperature and then heat it directly in the spectrometer's cavity, thus letting the reaction unfold for a limited period of time. The setup we use is an infrared laser coupled to our 275-GHz EPR spectrometer, which allows controlled and reproducible *in-situ* heating of the sample.

Work is in progress to apply our technique, which we call *Temperature-cycle EPR*, to investigate chemical kinetics. We will show its potential advantages as compared to the well-known Rapid-Freeze-Quench EPR.

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2. Department of Chemistry, University of Konstanz, 78464 Konstanz, Germany.

Title "The Strange Shapes of an Amyloid Protein Bound to Membranes"

Pravin Kumar[1], Ine M. J. Segers-Nolten[2], Nathalie Schilderink[2], Vinod Subramaniam[2,3], Martina Huber[1]*

1 Department of Physics, Huygens-Kammerlingh-Onnes Laboratory, Leiden University, Leiden, The Netherlands
2 Nanobiophysics, MESA+ Institute for Nanotechnology, University of Twente, Enschede, The Netherlands
3 FOM Institute AMOLF, Amsterdam, The Netherlands

Abstract:

As an intrinsically disordered protein, α-Synuclein is extremely flexible. Human α-Synuclein is a natural protein in the brain, where it associates with neuronal junctions (synapses), however with so far unexplained function. The protein became notorious because it seems involved in Parkinson's disease. The binding of α-Synuclein to membranes, and more specifically to natural membranes is thought to be crucial in relation to its pathological and physiological function. Spin-label EPR is one of the few approaches to study this question. By continuous-wave, room temperature EPR on proteins with single spin labels we determine the local binding of the protein and by double electron-electron resonance (DEER) on the mutant spin labeled at positions 27 and 56 the conformation.

Reference: Parkinson's Protein α-Synuclein Binds Efficiently and with a Novel Conformation to Two Natural Membrane Mimics Pravin Kumar, Ine M.J.Segers-Nolten, Nathalie Schilderink , Vinod Subramaniam, Martina Huber
PLOS One (2015) Published: November 20, 2015 DOI: 10.1371/journal.pone.0142795

Generalization of ENDOR-induced EPR for disordered systems: application to Xirradiated sucrose

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The EPR spectra of radiation-induced radicals in organic solids are composed of multiple components that largely overlap because of similar weak *g* anisotropy and a large number of HF interactions. Such properties make these systems extremely difficult to study using standard cw EPR spectroscopy alone. ENDOR spectroscopy, which offers species discrimination, orientation selectivity and high HF resolution, is a very popular complementary technique. In single crystals, decomposition of complex spectra is possible by means of ENDOR-induced EPR (EIE) experiments. For $S = ½$, $I = ½$ systems, recording of the intensity of particular ENDOR lines as a function of the magnetic field, yields absorption-like EPR spectra of corresponding spectral components. In this contribution, we propose a generalization of EIE that is applicable to disordered systems and considerably broadens its application possibilities.

The proposed method is validated for the case of stable radicals in X-ray irradiated sucrose, where the radical composition was established by single crystal EPR and ENDOR studies. It must be noted that, sucrose is interesting in its own right as a model system for studying radiation damage to sugarcontaining biomolecules and as an affordable and a versatile dosimetric material. It has recently been shown that four distinct radical species explain its stable spectrum and that one of these radicals exhibits a distinctively large HF interaction [1,2]. Isolation of the ENDOR spectrum of this interaction from other spectral components made it possible to extract the radical's EPR absorption spectrum from a multicomponent powder pattern (Fig. 1).

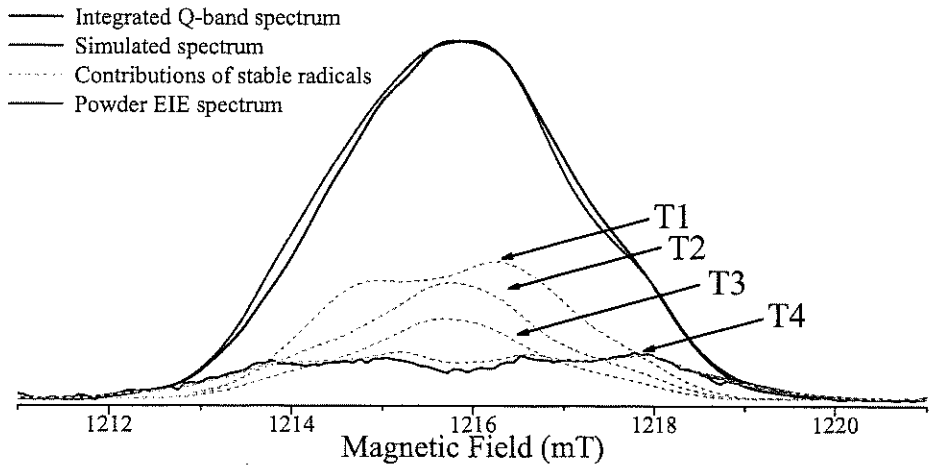

Figure 1: Decomposition of the integrated Q-band EPR spectrum of X-ray irradiated sucrose powder at 110 K (black line). The sum spectrum of stable radicals (blue line) consists of four components (light grey dashed lines), which are scaled here by appropriate weights.

1. H. De Cooman, J. Keysabyl, J. Kusakovskij, A. Van Yperen-De Deyne, M. Waroquier, F. Callens, and H. Vrielinck, J. Phys. Chem. B 2013, 117, p. 7169.
2. J. Kusakovskij, I. Caretti, S. Van Doorslaer, F. Callens, and H. Vrielinck. PCCP, 2016, DOI: 10.1039/C6CP01118G.

Light-induced EPR study of polarons in novel blends for organic solar cells

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[2] *BIMEF Laboratory, Department of Physics, University of Antwerp.*
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Over the last decade, the power conversion efficiencies of bulk heterojunction organic solar cells have increased steadily demonstrating their great potential for future photovoltaic applications. Today, a major part of the ongoing research efforts in organic photovoltaics involves the development of new materials to further improve solar cell efficiencies and to address stability problems. In particular, the development of alternative acceptor materials attracts great scientific interest motivated by the high cost, poor absorption properties and limited chemical versatility of the widely used fullerene acceptors.

In this context we investigated a recently synthesized 2,5-dithienylthiazolo[5,4-d] thiazole (DTTzTz) molecule as an alternative acceptor in combination with the standard donor polymer MDMO-PPV. Successful charge transfer in similar systems was already established by photoluminescence quenching and photo-induced absorption [1]. We are now further characterizing charge transfer processes in this system by EPR techniques. To that end light-induced EPR is particularly suited to study photogenerated radicals (polarons) in the blends.

Despite the demonstrated efficiency of charge transfer in donor:acceptor blends with diCN-DTTzTz, solar cells based on this material have shown poor device performance. Therefore we chose to perform parallel measurements on FBR, a novel nonfullerene small molecule acceptor synthesised by Iain McCulloch et al. [2]. Solar cell devices based on FBR and P3HT as donor polymer yielded promising power conversion efficiencies of 4.1%. Hence FBR:P3HT serves as a good reference system for our results.

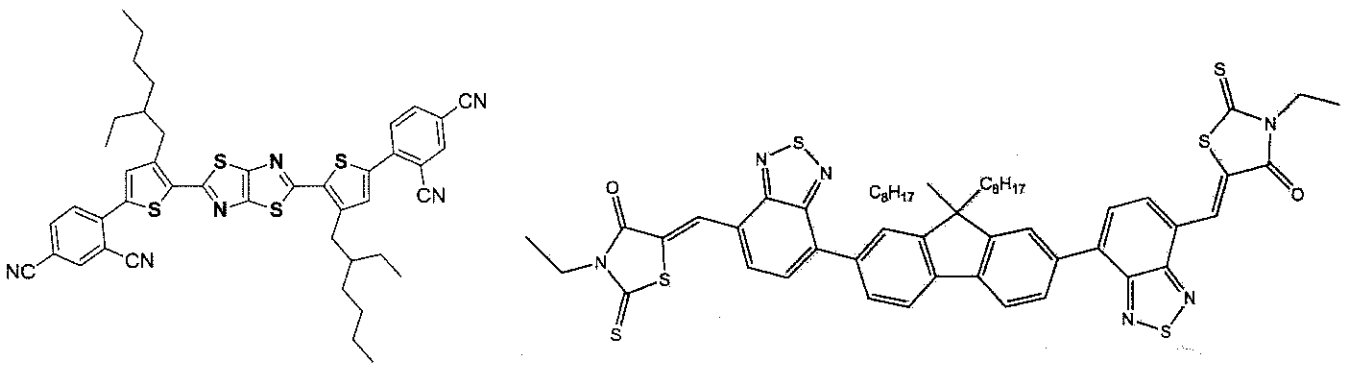

Figure 1: Left: Molecular structure of a DTTzTz small molecule acceptor with benzodinitrile side groups (diCN-DTTzTz). Right: Molecular structure of FBR.

[1] N. Nevil, Y. Ling, S. Van Mierloo, J. Kesters, F. Piersimoni, P. Adriaensens, L. Lutsen, D. Vanderzande, J. Manca, W. Maes, S. Van Doorslaer, E. Goovaerts. *Phys. Chem. Chem. Phys.* 2012, **14**, 15774.

[2] S. Holliday, R. S. Ashraf, C. B. Nielsen, M. Kirkus, J. A. Röhr, C. Tan, E. Collado-Fregoso, A. Knall, J. R. Durrant, J. Nelson, I. McCulloch. *J. Am. Chem. Soc.* 2014, **137**, 898–904.

POSTER

COMMUNICATIONS

Breathing effect in the V-doped Metal Organic Framework MIL-53(Al) monitored *in situ* by Electron Paramagnetic Resonance and X-ray diffraction

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Metal-Organic Frameworks (MOFs) are crystalline porous materials constructed of metal ions connected by organic linkers. These materials possess many interesting features, like well-defined pore size, pore shape and ultra-high porosity. A characteristic example of MOFs with one dimensional pores is MIL-53(Al) ([Al(OH)(BDC), BDC = terephthalate or 1,4-benzenedicarboxylate]. One interesting property of MIL-53(Al) is structural flexibility called breathing phenomenon, the structure can reversibly change from a large open pore (LP) to a narrow pore form (NP) by changing the temperature and/or pressure conditions.

The breathing effect triggered by temperature was investigated in MIL-53(Al) doped with vanadium ions. Since the V^{IV} dopant ions have one unpaired electron ($3d^1$ configuration, S=1/2) they exhibit an Electron Paramagnetic Resonance (EPR) spectrum.

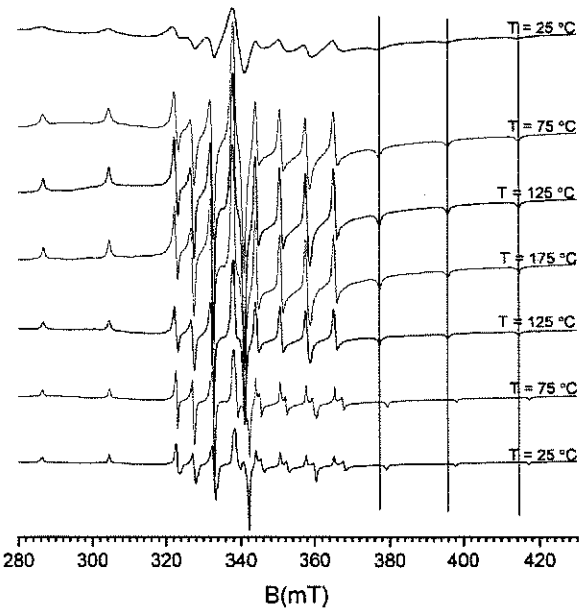

Figure 1 – Breathing effect in V-doped MIL-53(Al) in air monitored with *in situ* EPR. The first spectrum recorded in the cycle is shown as the bottom trace and is associated with the NP form. The vertical lines are present to guide the eye and indicate small shifts in specific EPR features.

The temperature-induced breathing effect in MIL-53(Al) was monitored with *in situ* EPR and powder X-ray Diffraction (XRD), recording spectra as a function of temperature in air and in vacuum. In Figure 1 EPR spectra of V^{IV} during the temperature cycle in air is presented. The first spectrum recorded in the cycle is associated with the NP form (bottom trace). When heating the sample above 100 °C water is released from the structure and the LP form is obtained which is visible in the EPR spectrum at 125 °C. Cooling the sample down to room temperature water from the air contracts the structure again to NP form. Additional feature is observed, below 100°C EPR spectrum broadens due to exchange interaction between EPR probes and paramagnetic oxygen (top trace). Heating cycle in vacuum showed similar results to one in air with difference while cooling. Since water cannot occupy pores, framework stays in LP form even at RT. We show that V^{IV} centers can be used as local probe to detect phase transitions from NP to LP form and back in the MIL-53(Al) framework.

Oxygenation (measured by EPR) is a predictive marker of wound healing in diabetic mice

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(2) Advanced Drug Delivery and Biomaterials Research group, Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium

Introduction : Impaired wound healing is a frequent complication of diabetes. Indeed, about 15 % of diabetic patients develop foot ulcers and amputation must be required in 25 % of cases as a result of healing impairments. To date, it still lacks efficient treatment to improve ulcers healing. Oxygen is known to play a key role during wound healing and hypoxia is described as a cause of wound healing impairment. EPR oximetry is a technique that allows repeated measurements of the absolute tissue pO_2 in pedicled skin flaps during the healing process. The pO_2 is determined from the linewidth of the EPR signal recorded with a biocompatible oxygen sensor such as crystals of lithium phthalocyanine (LiPc) previously implanted in the tissue.

Aims : To evaluate if LL37 (an antimicrobial peptide promoting angiogenesis) will favor wound healing in diabetic mice and to test EPR oximetry as a predictive marker of response to a treatment.

Materials and methods : LiPc crystals were implanted in the skin of male 7 and 12-week-old BKS(D)-Lepr^{db}/JOrlRj mice before the surgery. A 30 x 8 mm pedicled flap (containing the LiPc crystals) and a 4 mm diameter excisional skin wound were realized on the back of mice. The electroporation of a plasmid expressing the LL37 peptide was realized on excisional wounds and flaps. A plasmid expressing GFP was used as control. EPR signal was recorded in the flap repeatedly during the healing process. The excisional wound kinetics of closure was determined by quantification of wound area on digital photographs taken repeatedly during wound healing.

Results : After a same decrease induced by the surgery, the pO_2 slightly increased the first days after the surgery in the 7-week-old mice. Interestingly, from day 7 to day 11, pO_2 was higher in the LL37-treated group compared to the control group where pO_2 returned to very low values. Also, excisional wound kinetics of closure was faster in the treated group. In the 12-week-old mice, LL37-treated and control flaps remained hypoxic during all the monitoring. The treatment does not favor significantly the kinetics of closure of the excisional wound.

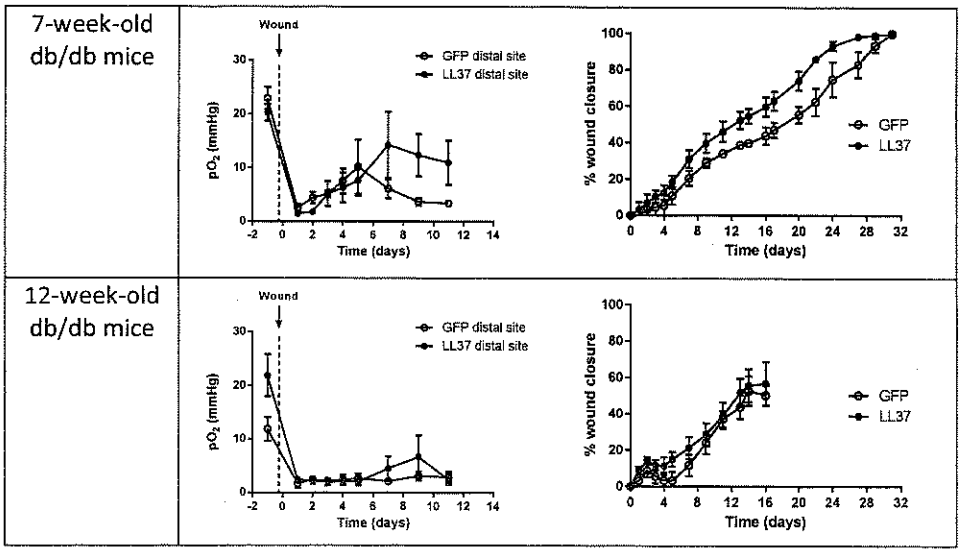

Fig 1 : pO_2 variations in the distal part of the pedicled flap (left) and excisional wound closure kinetics (right) in LL37-treated or GFP-treated (control) 7- and 12-week-old BKS(D)-Lepr^{db}/JOrlRj mice.

Conclusions : LL37 favors wound healing in 7-week-old-mice but not in 12-week-old diabetic mice. Results obtained by EPR oximetry were consistent with the kinetics of closure of excisional wounds. EPR oximetry is a suitable tool to predict the response to a treatment.

EPR detection of superoxide: comparison of different approaches

Scheinok Samantha[1], Philippe Levêque[1], Pierre Sonveaux[2], Bernard Gallez[1].

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Introduction :

Superoxide radical anion is implicated in many pathological conditions such as hypertension[1], cancer[2], neurodegenerative diseases[3], ... Porporato et al. showed that superoxide production could promote cell migration, invasion and metastasis of cancer in mice. MitoTempol, a superoxide scavenger, blocked this aggressiveness of cancer cells[4]. Because it was reported that MitoTempol is not specific of the superoxide[5], the overall aim of this project is to confirm non unambiguously the implication of the superoxide in this protective effects. Nowadays there are two main techniques to detect superoxide anion: the use fluorescent probes and the EPR-based techniques. The latter include spin trapping (monitoring of the formation of a spin adduct after reaction with superoxide) and the decay of stable free radicals in the presence of superoxide.

Aim: We compared the different techniques in terms of sensitivity and specificity towards the superoxide radical. We used the classical spin traps (DMPO, EMPO, DEPMPO, DIPPMPO). We also monitored the decay of the EPR signal of nitroxides (Tempol, mitoTempo) and trityl radical (CT-03) in the presence of superoxide anion.

Materials and methods: The experiments have been performed in vitro at 310K. The superoxide radical was produced using a hypoxanthine (1mM)/xanthine oxidase system. Three concentrations of xanthine oxidase (0,05; 0,02; 0,01 U/ml) were used to compare the sensitivity of the different probes. Hydroxyl radical was also produced with a Fenton reaction (H_2O_2:2mM) to test the specificity of the probes towards the superoxide radical.

Results: All the spin traps detected superoxide even when the enzyme concentration is low. The relative sensitivity for the detection of superoxide is DIPPMPO>EMPO>DEPMPO. CT-03 was sensitive to the presence of superoxide radical, but the specificity was low. MitoTempo and Tempol did not show any loss of EPR signal in the presence of superoxide alone. However, their EPR signal decreased in the presence of NADH (1mM). With hydroxyl radical the signal decreased even without NADH.

Conclusions: DIPPMPO seems to be the most promising probe for the detection of the superoxide radical because the stability of his adduct is greater than the EMPO. The specificity of CT-03 needs to be confirm in the future. MitoTEMPO and TEMPOL are not specific of superoxide radical but there reaction in presence in NADH needs to be clarified.

References

1 Anna E. Dikalova, Alfiya T. Bikineyeva, Klaudia Budzyn, Rafal R. Nazarewicz, Louise McCann, William Lewis, David G. Harrison, and Sergey I. Dikalov. "Therapeutic Targeting of Mitochondrial Superoxide in Hypertension." *Circ Res.; 107(1): 106–116*, 2010.

2 Rafal R. Nazarewicz, Anna Dikalova, Alfiya Bikineyeva, Sergey Ivanov, Igor A. Kirilyuk, and Igor A. Grigor'ev, and Sergey I. Dikalov. "Does Scavenging of Mitochondrial Superoxide Attenuate Cancer Prosurvival Signaling Pathways?" *Antioxidants & Redox Signaling, Vol 19, No 4: 344-349*, 2013.

3 Zhivko Zhelev, Rumiana Bakalova, Ichio Aoki, Dessislava Lazarova, and Tsuneo Saga. "Imaging of Superoxide Generation in the Dopaminergic Area of the Brain in Parkinson's Disease, Using Mito-TEMPO." *ACS Chem. Neurosci., 4: 1439–1445*, 2013.

4 Paolo E. Porporato, Valéry L. Payen, Jhudit Pérez-Escuredo, Christophe J. De Saedeleer, Pierre Danhier, and Tamara Copetti, Suveera Dhup, Morgane Tardy, Thibaut Vazeille, Caroline Bouzin, Olivier Feron, Carine Michiels, Bernard Gallez, and Pierre Sonveaux. "A Mitochondrial Switch Promotes Tumor Metastasis." *Cell Reports 8: 754–766*, 2014.

5 Amram Samuni, Sara Goldstein, Angelo Russo, James B. Mitchell, Murali C. Krishna, and Pedatsur Neta. "Kinetics and Mechanism of Hydroxyl Radical and OH-Adduct Radical Reactions with Nitroxides and with Their Hydroxylamines." *J. Am. Chem. Soc., Vol. 124, No. 29: 8719-8724*, 2002.

Clinical Application of Electron Paramagnetic Resonance Spectroscopy: Impact of Oxidative Stress on blood Heme-Nitrosylated Hemoglobin and Endothelial Function in Young Women Consuming Oral Contraceptives.

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During the last decade, clinical studies using a post-hoc statistical approach identified an increased risk of venous thromboembolism in females under oral contraceptive pills (OCPs), possibly through disturbed vascular homeostasis [1]. However, the impact of OCPs on endothelial function and redox status of the vasculature was not properly analyzed.

We assessed the bioavailability of nitric oxide, a main mediator of vascular homeostasis by measuring the level of hemoglobin nitrosylated at heme-Fe(II) in venous erythrocytes (5-coordinate-α-HbNO, HbNO) using a subtraction EPR method developed previously [2]. The vascular oxidative status was assessed through measurement of plasma peroxides. Whole plasma peroxides were determined by a spectrophotometric assay based on 3,3',5,5'-tetramethylbenzidine oxidation by horseradish peroxidase in presence of peroxides.

Young female subjects (N=104) were selected from a group of reportedly healthy volunteers and separated into sub-groups defined as user or not of OCPs containing ethinyl estradiol and different types of progestogens: levonorgestrel, desogestrel, gestodene, drospirenone and ciproterone.

We first observed that the HbNO level was significantly lower in venous erythrocytes of female subjects consuming OCPs versus control female subjects (162.1+/-9.0; N=61, and 215+/-13 nmol/L; N=43 respectively; P = 0.0003). The plasma level of biological peroxides was determined in 46 subjects and was found to be significantly increased in the subjects consuming contraceptives (from 1.051+/-0.132 mmol/L, N=22 to 1.918+/-0.155 mmol/L N=24; P = 0.001). Interestingly, the level of paramagnetic form of oxidized ceruloplasmin-Cu(II) was also significantly higher in the group under OCP administration, but was reduced to the level of controls after ex vivo addition of ascorbic acid (10mmol/L, 15min.). We observed that the index of endothelial function measured by digital reactive hyperemia pulse tonometry (EndoPat) showed a trend towards impairement in all subjects consuming OCPs, but was significantly lower specifically in the subjects consuming pills containing drospirenone (FRHI in control 0.67+/-0.07; N=43 versus 0.41+/-0.1; N=16 in OCPs subjects). Notably, this group of subjects demonstrated the lowest HbNO level (120.1+/-8.0 nmol/L).

Conclusion: This cohort study demonstrates that a decrease of 5-coordinate-α-HbNO measured by quantitative EPR in human venous erythrocytes is correlated with the development of the endothelial dysfunction under OCPs consumption, in parallel with increased plasma oxidative stress.

[1] Lidegaard O., Milsom I., Skovlund C., Skjeldestad F., Lokkegaard E BMJ 2011; 343: d6423.
[2] Lobysheva II, Biller P, Gallez B, Beauloye C, Balligand JL. PLoS One. 2013 8(10):e76457.

Temperature dependence of X-irradiated sucrose powder EPR spectrum

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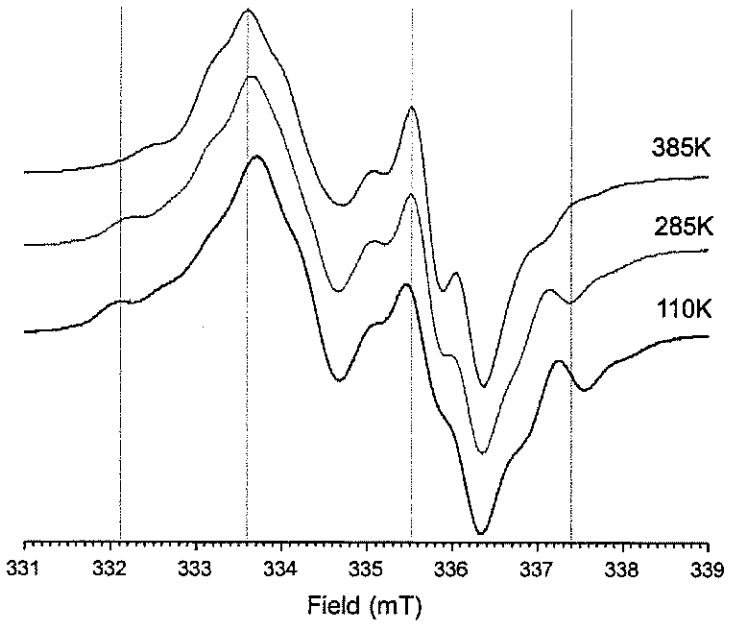
Sucrose is the main component of table sugar and it is present in many sugar-containing foodstuffs. High-energy radiation induces stable radicals in sucrose, which are detectable with EPR at room temperature. Their concentration is proportional to the absorbed dose in the 0.2 Gy – 10 kGy range[1]. This makes sucrose a perfect candidate for emergency dosimetry or characterization of radiation-sterilized foodstuffs using EPR spectroscopy. From a fundamental point of view, it can also serve as a model system for studying the radiation chemistry of other sugar-containing molecules like DNA and RNA.

Multiple radical species contribute to the stable EPR spectrum of irradiated sucrose. Our group identified three radical species contributing to the centre of the radiation-induced EPR spectrum and recently the fourth species, contributing to the "wings" has been thoroughly characterized and tentatively identified. These results followed from single crystal EPR and ENDOR studies at 110 K[2, 3], combined with density functional theory calculations. Dosimetric measurements on sucrose, however, are performed at room temperature on powder samples: decomposing and reliably simulating such spectra still presents a challenge. In this work we attempt to expand our knowledge about the temperature dependence of the sucrose powder spectrum in the range 110K - 385K. Preliminary results immediately revealed interesting effects: the central part of the spectrum shows only small changes, while features in the wings gradually move towards the centre, causing a noticeable narrowing of the total spectrum. Using simulations with single crystal data, the effects of temperature on the spectra of the known stable radicals are studied in more detail.



1. Y. Karakirova, N. D. Yordanov, H. De Cooman, H. Vrielinck and F. Callens, Radiation Physics and Chemistry **79** (5), 654-659 (2010).
2. H. De Cooman, J. Keysabyl, J. Kusakovskij, A. Van Yperen-De Deyne, M. Waroquier, F. Callens and H. Vrielinck, J Phys Chem B **117** (24), 7169-7178 (2013).
3. J. Kusakovskij, I. Caretti, S. Van Doorslaer, F. Callens and H. Vrielinck, Phys Chem Chem Phys **18** (16), 10983-10991 (2016).

Length-dependence of cardiac fibers EPR spectra: a new experimental setup

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Length-dependent activation is a fundamental property of the cardiac muscle that underlies the Frank-Starling law of the heart. However, the molecular mechanisms responsible for this length-dependent activation are still not fully understood. EPR is a powerful technique to analyse the dynamics of a molecular process and has already been used to study the contraction of skeletal and cardiac spin-labeled fibers [1]–[3]. A new experimental setup is proposed to study the length-dependence of EPR spectra from spin-labeled cardiac fibers.

Cardiac muscle fibers were prepared from papillary muscle of pig hearts and cut out along the principal axis of contraction. They were skinned and labeled following the spin labeling procedure described in [2].The experimental setup for the fiber length variations is built as follows: both ends of the fiber were attached to nylon threads and the end of the lower thread is attached to a micrometer screw (Thorlabs) allowing for small extensions of the fiber length (one complete rotation of the screw = 0.5 mm). The end of the upper thread was attached to a fixed shelf.The fiber was driven down the EPR cavity with a lab jack that carries the whole experimental setup. EPR spectra were acquired for cardiac fibers in relaxing solution [2] with progressive stretching of the fiber. A two-component spectrum is obtained, one component being more ordered than the other one [3], and the relative contribution of the two components varies with the stretching of the fiber (Fig. 1). Future work with this experimental setup includes the study of contracting instead of relaxing fibers and a comparison between the length effects (with stretching) and the osmotic compression at constant length (with dextran).

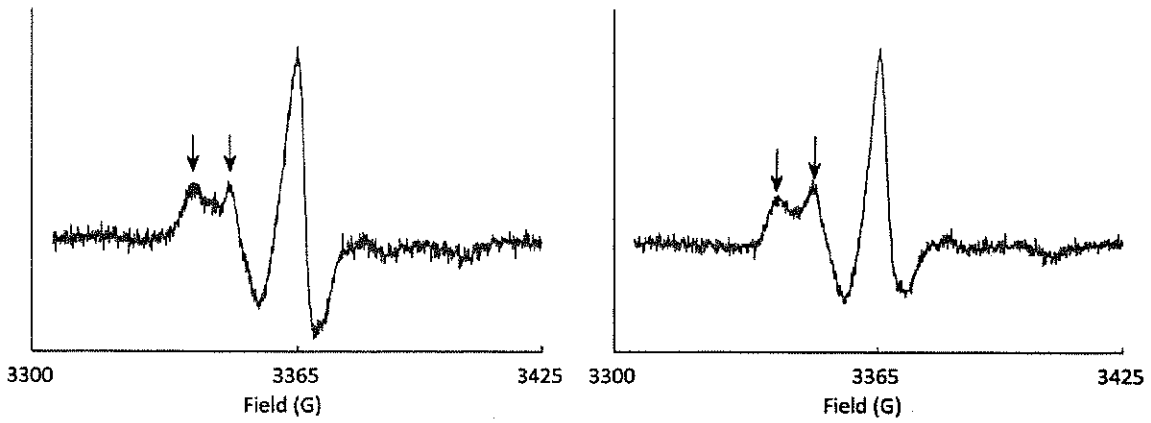

Fig. 1 Left: EPR spectrum of spin-labeled cardiac fibers in relaxing solution. Right: EPR spectrum of the same fibers with a 2 mm stretch. Arrows indicate the two spectral components.

[1] J. Belágyi and E. Röth, "Orientation dependence and motional properties of spin labels in cardiac and skeletal muscle fibres," *Gen. Physiol. Biophys.*, vol. 6, no. 6, pp. 571–581, 1987.

[2] D. Lorinczy and J. Belágyi, "Internal flexibility of cardiac myosins," *J. Therm. Anal.*, vol. 47, no. 2, pp. 503–514, 1996.

[3] N. Naber, T. J. Purcell, E. Pate, and R. Cooke, "Dynamics of the Nucleotide Pocket of Myosin Measured by Spin-Labeled Nucleotides," *Biophys J*, vol. 92, no. 1, pp. 172–184, 2007.

Study of the antioxidant action of morphine on the peroxidase cycle of MPO and HRP

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Inflammation is a complex physiological phenomenon involving chemical and enzymatic mechanisms. During this event, Polymorphonuclear Neutrophil Leukocytes (PMNs) play an important role by producing reactive oxygen species (ROS) and releasing myeloperoxidase (MPO), an oxidant enzyme. The latter one has two main activities : chlorination and peroxidase, which participate in the host defence against micro-organisms like bacteria and virus. However, an excessive amount of ROS and MPO released in the extracellular medium can cause damages on the surrounding tissues. A possible pathway to control this excessive inflammation is to regulate the neutrophil functions including MPO activity [4] [6] .

Besides its analgesic action, morphine presents antioxidant properties and has been shown to inhibit the ROS production and the PMN degranulation [1] [2] [3] [5]. However, there are few data about the potential effect of morphine on MPO activity

The aim of the study was to investigate the potential antioxidant activity of morphine on MPO activity in comparison to another peroxidase : Horseradish Peroxidase (HRP). Herein, we investigated the action of morphine on the different intermediates of the peroxidase cycle of MPO, using two spectroscopic techniques : EPR and UV-Visible absorption. As HRP belongs to the peroxidases and is characterized by a quite similar peroxidation cycle to MPO, the comparison with results obtained are also presented and can provide additional information on the mechanisms of action of morphine. The results show that morphine acts as a reducing agent in the peroxidase cycle of the two enzymes, like ascorbic acid. Morphine protects both enzymes from the adverse effect of their natural substrate, H_2O_2, which can act as a suicide inactivator at high concentration.

In conclusion, our findings show that morphine inhibits the formation of compound III for both enzymes and accelerates their peroxidase cycle, providing a competitive effet at high concentration for other substrates, like ABTS, having a high oxidant potential when oxidized by the enzymes.

[1] L. Elyasi, S. H. Eftekhar-Vaghefi, and S. Esmaeili-Mahani. *Rejuv. research*, 17 :255, 2014.

[2] I. Gülçin, S. Beydemir, H. A. Alici, M. Elmastas, and M. E. Büyükokurôglu. *Pharmacological Research*, 49 :59, 2004.

[3] T. Kanesaki, M. Saeki, Y. Ooi, M. Suematsu, K. Matsumoto, M. Sakuda, K. Saito, and S. Maeda. 372 :319, 1999.

[4] C. C. King, M. M. Jefferson, and E. L. Thomas. *Journal of Leukocyte Biology*, 61 :293, 1997.

[5] G. Minguet, T. Franck, A. Mouithys-Mickalad, J. Joris, C. Sandersen, and D. Serteyn. The 9th International Human Peroxidase Meeting, Cologne Germany, 2015.

[6] J. A. Smith. *Journal of Leukocyte Biology*, 56 :672, 1994.

Antioxidant properties of tree herbal teas investigated by EPR spectroscopy and chemiluminescence technique.

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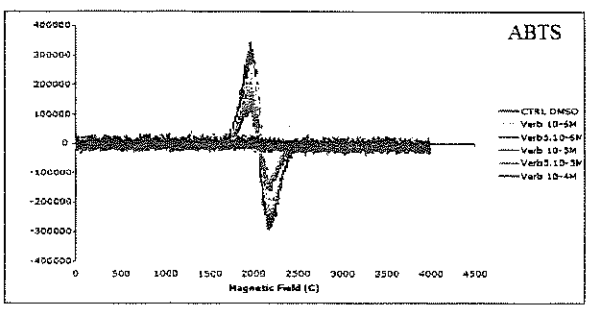
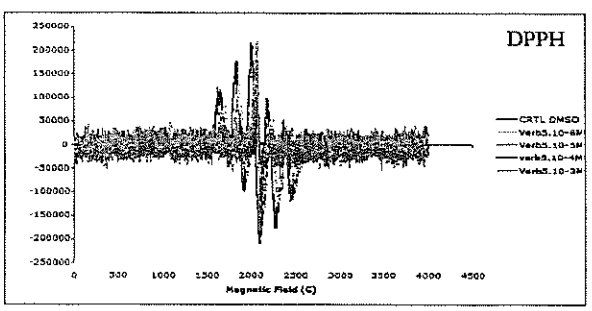
Herbal teas have been reported to be useful sources of new biologically active compounds. Three herbal teas: *Lantana montevidensis, Lippia multiflora and Ocimum gratissimum*, widely consumed at Kahemba city in Democratic Republic of Congo (DRC), were collected and investigated for their potential antiradical and antioxidant properties. The Kahemba's region is also known for a disease named Konzo, which is related to oxidative stress. Finding alternative and complementary ways to reduce the redox processes might have beneficial interest in context of developed countries. Herein, we were particularly interested to establish the correlation between radical-scavenging and cellular antioxidant activities of organic extracts of *L. montevidensis, L. multiflora and O. gratissimum* leaves using electron paramagnetic resonance (EPR) for ABTS and DPPH assays and lucigenin-enhanced chemiluminescence technique to follow the production of superoxide anion ($O_2^{\cdot-}$) by phorbol myristate acetate (PMA)-stimulated neutrophils.

The organic extracts were phytochemically screened by Thin Layer and High Perfomance Liquid Chromatography techniques, revealing the presence in the three plants of verbascoside as major phenolic compound.

All extracts displayed a good radical-scavenging activity at the concentration range 1 – 20µg/mL and a cellular antioxidant activity at 0.05 – 10 µg/mL, in the following order: *L. multiflora > O. gratissimum >L. montevidensis.* Verbascoside, at 10^{-5} M, strongly inhibited EPR signals in both ABTS and DPPH models. Interestingly, when verbascoside was used at the highest concentration of 5.10^{-4} M in DPPH system, a phenoxy radical signal was observed and attributed to the radical verbascosil. *L. multiflora* which contains more verbascoside, inhibited completely the radical signal at 10 µg/mL and showed the highest cellular antioxidant activity with IC_{50} of 0.40 ± 0.09 µg/mL. The antioxidant activity of the herbal teas extracts was correlated to the content of verbascoside and could be partly attributed to their radical scavenging capacities.





These herbal teas may be used as nutraceuticals for their potent radical scavenging and antioxidant activities that can prevent oxidative damage under different disease conditions include konzo.

Electron paramagnetic resonance and fluorescence studies on potential anticancer properties of two new Ru(II) complexes

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Fight against cancer is a priority of today's research. According to WHO[1], more or less 10 million people died every year because of this disease. Since the discovery of the anticancer properties of cisplatin (CisPt) in 1965 by Rosenberg [1], the treatment of cancer by chemotherapy has known great improvements. Unfortunately, CisPt has several side effects and is not effective against all kinds of cancer. Nevertheless its use highlights the great potential of organometallic compounds in the treatment of cancer [2]. Here we investigated the potential anticancer properties of two new organometallic compounds based on ruthenium II : $[RuCl(p\text{-}cymene)(S_2C.IDip)]^+(PF_6)^-$ and $[RuCl(p\text{-}cymene)(S_2C.Icy)]^+(PF_6)^-$, named as LDO436 and LDO437 respectively.

The cytotoxicity of the Ru-complexes and CisPt on keratinocyte (HaCat) cell line was determined by MTT assay. LDO437 shows higher cytotoxicity than LDO436 and both were found much higher than CisPt. CisPt is a well-known DNA-interacting molecule and the current thinking is that the inhibition of DNA replication is an essential first step of the cytotoxicity of the organometallic compound [3]. In order to understand the mechanism of action leading to cell death, fluorescence spectroscopy was used to measure the interaction between Ru-complexes and DNA. To gain more information, the pharmacodynamics of Ru-complexes was investigated by interaction with albumin.

The antioxidant properties of Ru-complexes were determined by electron paramagnetic resonance spectroscopy : DPPH and ABTS assays show significant decrease in the EPR spectra when incubated with Ru-complexes.

References:

1. Nafees M., Zijian G., Current Opinion in Chemical Biology (2014), 144-153.
2. Dasari S., Tchounwou P., European Journal of Pharmacology 740 (2014), 364-378.
3. Gonzalez V., Fuertes M., Alonso C., Perez J., Mol Pharmacol 59 (2001), 657-663.

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