



XXV RNE - IX CIE ESPECTROSCOPIA

**XXV NATIONAL SPECTROSCOPY
MEETING (XXV RNE)**

**IX IBERIAN SPECTROSCOPY
CONFERENCE (IX CIE)**

Alicante, 20th-22nd July 2016

Book of abstracts

Organizers



Sponsoring Institutions



**GENERALITAT
VALENCIANA**

CONSELLERIA D'EDUCACIÓ, INVESTIGACIÓ,
CULTURA I ESPORT



Sponsors



XXV NATIONAL SPECTROSCOPY MEETING

IX IBERIAN SPECTROSCOPY CONFERENCE

The XXV National Spectroscopy Meeting and IX Iberian Spectroscopy Conference (XXV RNE - IX CIE) has been organized by:

Sociedad de Espectroscopia Aplicada ([SEA](#))
Comité de Espectroscopia de la Sociedad Española de Óptica
([SEDOPTICA](#))
Sociedad Española de Química Analítica ([SEQA](#))
Sociedade Portuguesa de Bioquímica ([SPB](#))
Sociedade Portuguesa de Química ([SPQ](#))
Universidad de Alicante ([UA](#))

Scientific Committee:

PRESIDENT: Luis Fermín Capitán Vallvey, SEQA.

SECRETARY: Manuel Hernández Córdoba, SEQA.

VOCALS:

Demetrios Anglos, Universidad de Creta.

Carlos Bendicho Hernández, SEQA.

José Manuel Costa Fernández, SEA.

João Carlos Lima, SPQ.

María Rosa López Ramírez, SEDOptica

Paula Matos Marques, SPB.

Luca Prodi, Universidad de Bolonia.

María Teresa Tena Vázquez, UNIRIOJA.

Luís Batista de Carvalho, SPB.

Pilar Bermejo Barrera, SEQA.

Laura María Ilharco, SPQ.

Ángel López Molinero, SEQA.

Belén Maté Naya, SEDOPTICA.

María Cruz Moreno Bondi, SEA.

María Paz Sevilla Sierra, SEDOPTICA.

José Miguel Vadillo Pérez, SEA.

Organizing Committee:

PRESIDENT: Antonio Canals Hernández, UA.

VOCALS:

Miguel Ángel Aguirre Pastor, UA.

Luciana Costa Dos Reis Cruz, UA.

Elena Fernández Martínez, UA.

João P. C. A. Prates Ramalho, SQP.

Lorena Vidal Martínez, UA.

Paola Baile Pomares, UA.

Elena Domínguez Cañas, SEQA.

Montserrat Hidalgo Núñez, UA.

Laura Ripoll Seguer, UA.

Index

SCIENTIFIC PROGRAM	9
PLENARY AND INVITED LECTURES.....	17
ORAL PRESENTATIONS	27
POSTER PRESENTATIONS.....	65
Nanoparticles.....	65
Food Science	75
Sensors.....	99
Advances in instrumental and methodological analysis.....	113
Environment.....	133
Historic and artistic heritage.....	141
Sample preparation.....	147
Process control	153
Chemometrics.....	157
Imaging techniques	161
Forensic analysis	165
Speciation	171
Others.....	175
LIST OF AUTHORS	189

SCIENTIFIC PROGRAM

Tuesday, 19th July

19:00-21:00	Registration
20:30-21:00	Welcome cocktail

Wednesday, 20th July

09:00-09:30	Opening Plenary lecture Chair: Luis Fermín Capitán Vallvey
9:30-10:15	PL-1: D. Diamond <i>Rethinking biochemical sensing – biomimetic fluidics based on stimuli-responsive materials</i> Oral communications (I): Advances in instrumental and methodological analysis (I) Chairs: Manuel Hernández Córdoba and Sergio Armenta
10:15-10:30	O-01: <u>D. Gallart</u> , L. Elbal, S. Armenta, M. de la Guardia <i>Passive exposure to nicotine from e-cigarettes</i>
10:30-10:45	O-02: <u>F. Laborda</u> , I. Abad-Álvaro, E. Bolea, E. Peña-Vázquez, P. Bermejo-Barrera, J.R. Castillo <i>Analysis of nanomaterials by fast scanning single particle ICP-MS: A user's manual</i>
10:45-11:00	O-03: <u>C. Álvarez-Llamas</u> , J. Pisonero, N. Bordel <i>Quantification of fluorine traces in solid samples using CaF molecular emission bands in atmospheric air LIBS</i>
11:00-11:15	O-04: <u>M. García</u> , M. A. Aguirre, A. Canals <i>As, Hg and Se determination in gasoline samples by on-line chemical vapor generation using a Flow Blurring® multiple nebulizer in ICP-OES</i>
11:15-11:45	Coffee break Invited lecture Chair: Ángel López Molinero
11:45-12:15	IN-1: R. Martínez-Máñez <i>Chromo-fluorogenic optical probes</i>
12:15- 13:45	Poster session
13:45-15:30	Lunch break Plenary lecture Chair: Teodomiro López Navarrete
15:30-16:15	PL-2: V. G. Baonza <i>Extreme Spectroscopy</i> Oral communications (II): Advances in instrumental and methodological analysis (II) Chairs: María Teresa Tena Vázquez and María Paz Sevilla Sierra
16:15-16:30	O-05: <u>L. Ripoll</u> , M. Hidalgo, A. Canals <i>Electrospray pyrolysis deposition followed by laser-induced breakdown spectroscopy: a new method for trace elemental analysis in liquid samples</i>

Scientific Program

16:30-16:45	O-06: <u>P. Purohit</u> , F. J. Fortes, J. J. Laserna <i>OC-OT-LIBS: A novel approach to the chemical characterization of single particles</i>
16:45-17:00	O-07: <u>J. M. Vadillo</u> , I. M. Carrasco-García, M. López-Claros, J. J. Laserna <i>Femtosecond time-resolved phase-change microscopy and ablation threshold calculations to understand ultrafast laser ablation</i>
17:00-17:15	O-08: <u>S. Medina</u> , J. M. Vadillo, J. J. Laserna <i>Femtosecond laser ionization time-of-flight mass spectrometry of size-sorted aerosolized matter</i>
17:15-17:45	Coffee break Oral communications (III): Environmental analysis, sensors and imaging techniques Chairs: Concepción Pérez Conde and Carlos Bendicho Hernández
17:45-18:00	O-09: J. Anzano, <u>D. Paules</u> , R. Mur, R. J. Lasheras, M. Escudero, J. del Valle, O. Tapia, S. Hamida, E. Gaspar, J. Cajal, C. Bello, J. Casas, S. Sangüesa, F. Bayo, P. González-Blasco, A. Calvo, J. Cáceres, S. Moncayo, J. Anwar, A. Dar <i>Analytical Potential of Laser Induced Breakdown Spectroscopy in Environmental Studies</i>
18:00-18:15	O-10: <u>M. M. Erenas</u> , A. Martínez-Olmos, N. López-Ruiz, I. de Orbe-Payá, A. J. Palma, L. F. Capitán-Vallvey <i>HF RFID label for simultaneous oxygen, ammonia, carbon dioxide and humidity determination</i>
18:15-18:30	O-11: <u>E. Benito-Peña</u> , F. Salis, A. B. Descalzo, M. C. Moreno-Bondi, G. Orellana <i>Immunoassay for immunosuppressant drugs using conjugated strongly near-infrared fluorescent magnetic nanoparticles</i>
18:30-18:45	O-12: <u>A. López-Molinero</u> , P. Berlin <i>The lighting Influence in Digital Image Based methods</i>
18:45-19:30	Meeting the Experts
20:00	Alicante city guided tour

Thursday, 21st July

	Plenary lecture Chair: José Manuel Costa Fernández
09:00-09:45	PL-3: L. B. Oddershede <i>Optical manipulation and applications of HOT nanoparticles</i>
	Invited lecture Chair: José Miguel Vadillo
09:45-10:15	IN-2: <u>M. T. Fernández-Argüelles</u> , N. Vasimalai, M. Prado, B. Espiña, L. Diéguez, V. Vilas, K. Böhme, K. Zagorovsky, W. Chan <i>Bioanalytical strategies based on nanoparticles</i>
	Oral communications (IV): Nanoparticles (I) Chairs: María Rosa López Ramírez and Pilar Bermejo Barrera
10:15-10:30	O-13: <u>A. I. López-Lorente</u> , P. Wang, B. Mizaikoff <i>In-situ formation of bare gold nanoparticles for surface enhanced infrared spectroscopic studies on bovine serum albumin</i>
10:30-10:45	O-14: <u>D. R. Abujetas</u> , R. Paniagua-Domínguez, A. D. Van Dam, B. J. M. Brenny, A. Polman, J. Gómez Rivas, J. A. Sánchez-Gil <i>Optical Spectroscopy of Semiconductor Nanowires: Tailoring Light Harvesting and Emission at the Nanoescale</i>
10:45-11:00	O-15: <u>M. Berenguel</u> , A. Salinas, B. Fernández, C. S. Martínez, J. Alonso, L. F. Capitán-Vallvey, M. Puyol <i>Microfluidic Hydrothermal Synthesis of Highly Luminescent Carbon Dots</i>
11:00-11:15	O-16: <u>J. M. Costa-Fernández</u> , M. Menéndez-Miranda, D. Bouzas-Ramos, A. Sanz-Medel <i>Field-flow fractionation coupled to different detectors as a tool for the characterization of engineered nanoparticles</i>
11:15-11:45	Coffee break
	Invited lecture Chair: Luís Batista de Carvalho
11:45-12:15	IN-3: <u>P. D. Vaz</u> , C. D. Nunes, C. I. Fernandes, A. Bento, S. Rudic <i>Looking inside catalysts through neutrons</i>
12:15-13:45	Poster session
13:45-15:30	Lunch break
	Invited lecture Chair: Rafael Escribano
15:30-16:00	IN-4: <u>E. Moreno</u> , R. Martín-Doménech, G. M. Muñoz-Caro <i>UV irradiation of H₂O:CO:NH₃ ice analog mixtures</i>
	Oral communications (V): Nanoparticles (II) Chairs: Belén Maté Naya and María Cruz Moreno Bondi
16:00-16:15	O-17: <u>M. M. Gómez-Gómez</u> , K. Ramos, L. Ramos <i>Characterization and quantification of silver nanoparticles in chicken meat subjected to in-vitro human gastrointestinal digestion using single particle ICP-MS</i>

Scientific Program

16:15-16:30	O-18: <u>D. Bouzas-Ramos</u>, M. Menéndez-Miranda, Z. Gajdosechova, E. Krupp, J. Feldmann, J. R. Encinar, J. M. Costa-Fernández, A. Sanz-Medel <i>Use of a pool of spectroscopic tools for the determination of the nanoparticle concentration and ligand density of engineered water-soluble HgSe nanoparticles</i>
16:30-16:45	O-19: <u>M. Menéndez-Miranda</u>, J. M. Costa-Fernández, J. Ruiz, A. Sanz-Medel, W. J. Parak, C. Carrillo-Carrion <i>Determination of the ratio of fluorophore/nanoparticle for fluorescence-labelled nanoparticles</i>
16:45-17:00	O-20: <u>S. López</u>, N. Rodríguez, R. Serrano, R. C. Rodríguez, A. Ríos <i>Gold nanoparticles and dissolved gold species determination in culture medium and cells by coupled hydrodynamic separation techniques to ICP-MS</i>
17:00-17:30	Coffee break Vendor session and other oral communications (VI): Chairs: María Teresa Fernández Argüelles and Elena Moreno Atahonero
17:30-17:45	O-21: <u>M. L. Fernández-Sánchez</u>, R. González de Vega, J. Pisonero, N. Eiró, F. J. Vizoso, A. Sanz-Medel <i>Mapping of Zn and MMP-11 in breast tissues by LA-MS</i>
17:45-18:00	O-22: <u>A. Lobato</u>, M. Cáceres, L. Bonales, O. R. Montoro, M. Taravillo, V. G. Baonza <i>Anomalies in the structure of liquid water under pressure and temperature as revealed by Raman Spectroscopy</i>
18:00-18:15	O-23: <u>R. Santos</u>, S. Wünscher, H. Gleisner, R. Chemnitzer <i>Analysis of REE in basalt, cement, shale, rock and stream sediment CRMS by ICP-QMS</i>
18:15-18:30	O-24: M. Yamanaka, T. Itagaki, S. Wilbur, S. Nunez, H. Goenaga-Infante, <u>N. Kovachev</u> <i>New frontiers in very small size nanoparticle analysis: Triple quadrupole ICP-MS (ICP-MS QQQ) for single nanoparticle detection of SiO₂ and Au materials</i>
18:30-19:30	Societies meeting
20:30	Conference dinner

Friday, 22nd July

	Plenary lecture Chair: João P. C. A. Prates Ramalho
09:00-09:45	PL-4: D. M. Bassani <i>Spectroscopy of supramolecular species: How organisation affects electronic transitions in self-assembled molecular systems</i>
	Oral communications (VII): Speciation, food science and historic and artistic heritage Chairs: Antonio Moreda Piñeiro and Isela Lavilla
09:45-10:00	O-25: <u>F. A. Esteve-Turrillas</u> , S. Armenta, M. de la Guardia <i>Low cost extraction in Analytical Chemistry. What else?</i>
10:00-10:15	O-26: <u>A. I. Corps-Ricardo</u> , F. J. Guzmán-Bernardo, M. Zougagh, R. C. Rodríguez-Martín-Doimeadios, A. Ríos-Castro <i>Monitoring of polycyclic aromatic hydrocarbons in water by magnetic solid phase extraction based on magnetic nanoparticles coated with multi-walled carbon nanotubes</i>
10:15-10:30	O-27: <u>M. Jiménez-Moreno</u> , M. Jiménez-Moreno, G. P. F. Arrifano, M. E. Crespo-López, R. C. Rodríguez-Martín-Doimeadios <i>Fast and simultaneous mercury speciation analysis in hair samples by gas chromatography coupled to atomic fluorescence spectroscopy</i>
10:30-10:45	O-28: <u>J. M. Hernández-Hierro</u> , J. Nogales-Bueno, B. Baca-Bocanegra, F. J. Rodríguez-Pulido, F. J. Heredia <i>Use of near infrared hyperspectral tools for the screening of extractable polyphenols in red grape skins</i>
10:45-11:00	O-29: <u>J. A. Tuñón</u> , A. Sánchez, D. J. Parras, M. Montejo, P. Vandenabeele, M. O. Rodríguez, C. Rísquez, A. Martínez, M. P. G. Rodríguez <i>Analysis and evolution of black decoration in ceramics of the Iberians by MRS and EDXRF</i>
11:00-11:15	O-30: <u>M. López-Claros</u> , F. J. Fortes, S. Guirado, J. J. Laserna <i>LIBS in cultural heritage: recognition and identification of objects in an underwater archaeological shipwreck</i>
11:15-11:45	Coffee break
	Oral communications (VIII): Molecular synthesis and characterization and other topics Chairs: María Vega Cañamares and Maria Paula Marques
11:45- 12:00	O-31: <u>S. F. Prazeres</u> , F. Ortega, I. A. Pavel, A. Pasc, N. Canilho, C. García-Ruiz, G. Montalvo <i>Vibrational spectroscopy applied to the study of β-galactosidase-silica support interactions</i>
12:00-12:15	O-32: <u>G. Molpeceres</u> , M. Jiménez-Redondo, V. Timón, M. A. Moreno, V. J. Herrero, R. Escribano, B. Maté, I. Tanarro <i>Modelling and irradiation experiments of cosmic dust analogues</i>
12:15-12:30	O-33: <u>M. Martín-Garrido</u> , S. Martínez-Ramírez <i>Calcium Silicate Hydrate Synthesis</i>
12:30-12:45	O-34: <u>M. Moreno-Oliva</u> , J. T. López-Navarrete <i>Ultrafast transient absorption spectroscopy: principles and applications</i>

Scientific Program

12:45-13:00	O-35: <u>M. Abidi</u> , S. López-Bernabeu, F. Huerta, F. Montilla, S. B. Hentati, E. Morallón <i>In situ spectroelectrochemical techniques to the characterization of polyaniline derivatives</i>
13:00-14:00	Closing ceremony and conference awards Best poster prize, awarded by SEQA Best oral presentation prize, awarded by SEQA Best oral presentation prize, awarded by Inycom Best poster prize, awarded by Agilent Technologies "Francisco Álvarez 2016" prize, awarded by SEA

PLENARY AND INVITED LECTURES

Rethinking Biochemical Sensing - Biomimetic Fluidics based on Stimuli-Responsive Materials

L. Florea, W. Francis, A. Dunne, A. BenAzouz, S. Coleman, D. Bruen, **D. Diamond***.
Insight Centre for Data Analytics, National Centre for Sensor Research, School of Chemical Sciences, Dublin City University, Dublin 9, Ireland.
*dermot.diamond@dcu.ie

Since the initial breakthroughs in the 1960's and 70's that led to the development of the glucose biosensor, the oxygen electrode, ion-selective electrodes, and electrochemical/optochemical diagnostic devices, the vision of very reliable, affordable chemical sensors and bio-sensors capable of functioning autonomously for long periods of time (years) remains unrealized. This is despite massive investment in research and the publication of many thousands of papers in the literature. It is over 40 years since the first papers proposing the concept of the artificial pancreas, by combining glucose monitoring with an insulin pump¹. Yet even now, there is no chemical sensor/biosensor that can function reliably inside the body for more than a few days, and such is the gap in what can be delivered (days), and what is required (years) for implantable devices, it is not surprising that in health diagnostics, the overwhelmingly dominant paradigm for reliable measurements is still single use disposable sensors. Realising disruptive improvements in chem/bio-sensing platforms capable of long-term independent operation requires a step-back and rethinking of strategies, and considering solutions suggested by nature and materials science, rather than incremental improvements in existing approaches².

Through recent developments in 3D fabrication technologies in recent years, we can now build and characterize much more sophisticated 3D platforms than was previously possible. We can create regions of differing polarity and hydrophobicity, mix passive and binding behaviours, and regions of differing flexibility/rigidity, hardness/softness. In addition, we can integrate materials that can switch between these characteristics, enabling the creation of biomimetic microfluidic building blocks that exhibit photoswitchable characteristics such as programmed microvehicle movement (chemotaxis), switchable binding and release, switchable actuation (e.g. valving), and photodetection. These building blocks can be in turn integrated into microfluidic systems with hitherto unsurpassed functionalities that can contribute to bridging the gap between what is required for many applications, and what we can currently deliver³. The transition from the current paradigm from engineering inspired 2D fluidics to bioinspired 3D fluids is a major milestone in the evolution of microfluidics. Another lesson we can learn from biofluidics is that the entire system is active. Currently, the only role of the substrate in a microfluidic chip is to define the channels. In biology, the channel walls have a very active role, as has the surrounding tissue. Walls can sense, and respond e.g. open pores and release active agents such as functionalised micro/nanoparticles, vesicles, droplets). Implementation of these disruptive concepts may open the way to biochemical sensing systems with performance characteristics far beyond that of current devices. A key development will be the integration of biomimetic functions like self-diagnosis of condition and self-repair capabilities to extend their useful lifetime⁴.

References

- [1] Albisser, A. M.; Leibel, B. S.; Ewart, T. G.; Davidovac, Z.; Botz, C. K.; Zingg, W.; Schipper, H.; Gander, R. Clinical Control of Diabetes by the Artificial Pancreas. *Diabetes* **1974**, 23 (5), 397–404.
- [2] Diamond, D.; Byrne, R.; Lopez, F. B.; Cleary, J.; Maher, D.; Healy, J.; Fay, C.; Kim, J.; Lau, K.-T. Biomimetics and Materials with Multiple Personalities - The Foundation of Next Generation Molecular Sensing Devices. In *2010 IEEE SENSORS*; IEEE Sensors; IEEE, 2010; pp 1079–1082.
- [3] Benito-Lopez, F.; Byrne, R.; Răduță, A. M.; Vrana, N. E.; McGuinness, G.; Diamond, D. Ionogel-Based Light-Actuated Valves for Controlling Liquid Flow in Micro-Fluidic Manifolds. *Lab Chip* **2010**, 10 (2), 195–201.
- [4] Florea, L.; Wagner, K.; Wagner, P.; Wallace, G. G.; Benito-Lopez, F.; Officer, D. L.; Diamond, D. Photo-Chemopropulsion - Light-Stimulated Movement of Microdroplets. *Adv. Mater.* **2014**, 26 (43), 7339–7345.

Extreme Spectroscopy

V. G. Baonza.

*Universidad Complutense de Madrid e Instituto de GeoCiencias IGEO (UCM-CSIC), Ciudad Universitaria S/N, 28040-Madrid, Spain.
vgbaonza@ucm.es*

Spectroscopy plays a prevalent role for studying a wide range of phenomena promoted by the application of extreme pressures and/or temperatures. Novel phase transitions, structural transformations and chemical reactivity [1] are among the most exciting phenomena to be probed using both vibrational and absorption/emission spectroscopies.

In this talk, we focus our analysis on water-related [2] and carbon-based [3] related materials/systems, due to their implications in physics and chemistry, geological and biological processes, and materials science. We shall put emphasis on the particular suitability of Raman spectroscopy coupled to diamond anvil cell devices [1] to interpret the physico-chemical changes induced by pressure and temperature on these prototypical systems.

Examples covering the last two decades [1-3] will be used to illustrate the current opportunities offered by the spectroscopic methods under extreme conditions in many scientific fields.

Acknowledgements

The results presented here have been funded by MICINN/MINECO under several past and active (CSD2007-00045/MAT2015-71070-REDC, CTQ2015-67755-C02-01-R) projects, and several PFU and FPU grants.

References

- [1] V. G. Baonza *et al.*, *J. Chem. Phys.* **124**, 044508 (2005); *J. Chem. Phys.* **121**, 11156 (2004); *J. Raman Spectrosc.* **34**, 264 (2003); V. G. Baonza, *High Press. Res.* **18**, 379 (2000).
[2] A. Arencibia *et al.*, *J. Chem. Phys.* **123**, 214502 (2005); *Phys. Rev. Lett.* **89**, 195504 (2002); V. G. Baonza, F. Rull, J. Dubessy, *Raman Spectroscopy of Gases, Water and other Geological Fluids*, EMU Notes on Mineralogy, Ch. 8., European Mineralogical Union (2012).
[3] M. Peña-Alvarez *et al.*, *Faraday Discuss.* **173**, 157 (2014); *Angew. Chem. Intl. Ed.* **53**, 7033 (2014); *J. Phys. Chem. C*, **118**, 25132 (2014); Del Corro *et al.*, *Phys. Rev. B* **85**, 033407 (2012); *Carbon* **50** (2012); *Nano Lett.* **8**, 2215 (2008).

Optical manipulation and applications of HOT nanoparticles

L. B. Oddershede.

Niels Bohr Institute, University of Copenhagen, Blegdamsvej 17, 2100 Copenhagen, Denmark.
oddershede@nbi.dk

A tightly focused laser beam can trap and manipulate individual metallic nanoparticles both in liquid [1] and in air [2]. Not only the position, but also the orientation of a single nanoparticle can be controlled and such precise optical control of metallic nanoparticles has huge potential, e.g., for aerotaxy, for nano-architectural purposes or for amplifying spectroscopic signals. Due to their plasmonic properties, metallic nanoparticles will absorb part of the incident light and the energy will be released as heat into their local surroundings. The heating associated with resonant irradiation of metallic nanoparticles can be extreme. Moreover, this heating of an irradiated nanoparticle cannot be theoretically predicted, as the precise focal intensity distribution on the nanoscale is unknown and typically highly aberrated [3]. We developed a novel membrane-based assay to directly quantify the absorbance and temperature profile of an individual irradiated metallic nanoparticle and show how the temperature depends on laser power and particle size, shape, orientation and composition [4,5]. Laser induced heating of metallic nanoparticles can be advantageously used in a controlled manner, for instance to fuse membranes and cargos of two selected giant unilamellar vesicles [6]. Another promising application of these hot metallic nanoparticles is within cancer therapy, where novel results show that laser irradiated metallic nanoparticles can be used to mediate targeted drug delivery and for photothermal tumor treatment.

References

- [1] P.M. Hansen, V.K. Bhatia, and L.B. Oddershede, *Nano Letters*, **5**, 1937-1942 (2005).
- [2] L. Jauffred, S.M-R. Taheri, R. Schmitt, H. Linke, and L.B. Oddershede, *Nano Letters*, **15**, 4713-4719 (2015).
- [3] A. Kyrsting, P.M. Bendix, and L.B. Oddershede, *Nano Letters*, **13**, 31-35 (2013).
- [4] P.M. Bendix, S.N.S. Reihani, and L.B. Oddershede, *ACS Nano*, **4**, 2256-2262 (2010).
- [5] H. Ma, P. Tian, J. Pello, P.M. Bendix, and L.B. Oddershede, *Nano Letters*, **14**, 612-619 (2014).
- [6] A. Rørvig-Lund, A. Bahadori, S. Semsey, P.M. Bendix, and L.B. Oddershede, *Nano Letters*, **15**, 4183-4188 (2015).

Spectroscopy of supramolecular species: How organisation affects electronic transitions in self-assembled molecular systems

D. M. Bassani.

*Inst. of Molecular Sciences, Univ. of Bordeaux.
dario.bassani@u-bordeaux.fr*

The rational design of supramolecular structures endowed with tunable electronic properties is a cornerstone towards bridging the bottom-up and top-down approach to molecule-based electronics.^[1,2] With this in mind, we investigate the use of molecular recognition motifs to direct the formation of selected architectures possessing unique ground- and excited-state electronic properties. Several examples of this approach, including the spontaneous generation of highly emissive organic nano-spheres (Fig. 1) and the formation of fullerene double cables (Fig. 2) will be presented.^[3,4] A unique property of this material, that we have dubbed *photopolism*, is the capability of undergoing a photoinduced switch in the polarization emission. The process is diffraction-limited, and can be used to write information on single crystals that can be read-out from the polarization component of the emitted light (Fig. 3).

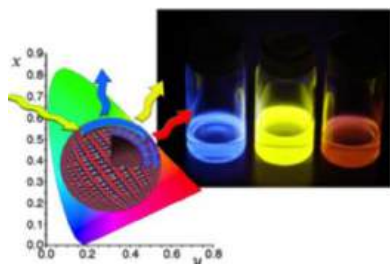


Fig. 1. Self-assembly of luminescent nano-spheres.

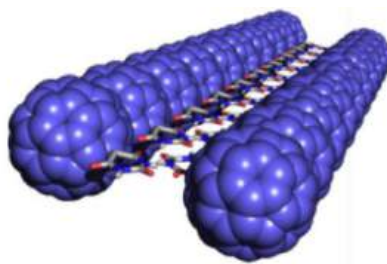


Fig. 2. X-ray structure of a fullerene double-cable.



Fig. 3. Photopolitic lithography (80 X 80 μm)

References

- [1] *Chem. Soc. Rev.* 2014, 43, 4179.
- [2] *NPG Asia Mat.* 2014, 6, e116.
- [3] *ACS Nano*, 2016, 10, 998; *Macromolecules* 2013, 46, 1591.
- [4] *Angew. Chem. Int. Ed.* 2011, 50, 9584; *Phys. Chem. Chem. Phys.* 2012, 14, 8859.

Chromo-fluorogenic optical probes

R. Martínez-Máñez.

Instituto Interuniversitario de Investigación de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Unidad Mixta Universidad Politécnica de Valencia-Universidad de Valencia, Spain.

Departamento de Química, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022, Valencia, Spain.

CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN).

rmaez@qim.upv.es

The development of new molecular systems for the detection of anions, cations, or neutral molecules has gained importance in the last years due to the importance of detecting target species in environmental and biological samples. In this field designed receptors are able to transform, upon coordination, host-guest interactions into a measurable signal which allows analyte recognition and sensing. In particular optical outputs are attractive because it is possible to use low-cost and widely extended instrumentation [1].

Moreover, apart from the traditional design and synthesis of sometimes complex hosts, enhanced selectivity can be achieved by using selected probes coupled to inorganic supports.[2,3] This procedure has proved useful for the detection of analytes for which no functional receptor group is available or selectivity is hard to achieve with conventional synthetic methods.

Selected examples toward the development of classical chromo-fluorogenic probes and sensory materials that use a combination of nanotechnology and supramolecular recognition principles will be shown.[4] In this later case the design strategies in most cases involve synergisms of bio-concepts and nanoscale inorganic supports which create functional sensing systems showing enhanced functions and properties.

Acknowledgements

Financial support from the Spanish Government (Project MAT2015-64139-C4-1), from the Generalitat Valencia (Project PROMETEOII/2014/047) and from CIBER-BBN is gratefully acknowledged.

References

- [1] R. Martínez-Máñez, F. Sancenón, *Chem. Rev.*, **2003**, *103*, 4419.
- [2] A. B. Descalzo, R. Martínez-Máñez, F. Sancenón, K. Hoffmann, K. Rurack, *Angew. Chem., Int. Ed.*, **2006**, *45*, 5924.
- [3] K. Rurack, R. Martínez-Máñez (Eds), *The supramolecular chemistry of organic-inorganic hybrid materials*. John Wiley & Sons, 2010.
- [4] See for instance: (a) E. Aznar, M. Oroval, L. Pascual, J. R. Murguía, R. Martínez-Máñez, F. Sancenón, *Chem. Rev.* **2016**, *116*, 561–718; (b) F. Sancenón, L. Pascual, M. Oroval, E. Aznar, R. Martínez-Máñez, *ChemistryOpen*, **2015**, *4*, 418-437.

Bioanalytical strategies based on nanoparticles

M. T. Fernández-Argüelles^{1,*}, N. Vasimalai¹, M. Prado¹, B. Espiña¹, L. Diéguez¹, V. Vilas¹, K. Böhme², K. Zagorovsky³, W. Chan³.

¹*Life Sciences Department, International Iberian Nanotechnology Laboratory (INL), Braga, Portugal.*

²*Centro Tecnológico Alimentario (CETAL), Lugo, Spain.*

³*Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Canada.*

**maria.fernandez-arguelles@inl.int*

Recent developments in nanotechnology have led to a new generation of high-value optical probes that are being exploited in order to overcome the limitations of traditional dyes and fluorophores. Their great potential has allowed the development of new analytical assays with unprecedented analytical performance characteristics, related to sensitivity, multiplexing capability, sample throughput, cost-effectiveness and ease of use.

The high specific surface area of nanoparticles, and the possible functionalization with different biochemical moieties as peptides, DNA sequences, antibodies or aptamers, make them suitable as platform for the detection of a range of relevant analytes. Hence, nanoparticles with excellent optical properties are being used in a wide variety of research areas including food and environmental analysis, medicine, bioimaging, proteomic and genomic studies, etc.

This presentation aims to show recent applications of different types of nanoparticles based on changes on their optical properties. Among the various types of nanoparticles that are being explored in the nanobiotechnology area, gold nanoparticles are highly appealing, because besides their physicochemical properties derived from the localized surface plasmon resonance, they present advantages such as well-known synthesis and functionalization with a different biomolecules. Thus, applications on early infectious disease diagnosis, food adulteration detection and environmental analysis will be presented. Moreover, fluorescent carbon dots are emerging as promising nanoparticles due to excellent photoluminescent properties, poor photobleaching, low toxicity and good biocompatibility. Application of food-based carbon dots for the study of in-vitro cancer growth inhibition and bioimaging will be also presented.

Acknowledgements

This work has been supported by the NSERC Steacie Fellowship Grant, the P.O Norte CCDR-N / ON.2 programme, and the Marie Curie COFUND Programme.

Looking inside catalysts through neutrons

P. D. Vaz^{1,2*}, C. D. Nunes¹, C. I. Fernandes¹, A. Bento¹, S. Rudić².

¹CQB, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal.

²ISIS Neutron & Muon Source, Rutherford Appleton Laboratory, Chilton, Didcot, Oxfordshire OX11 0QX, United Kingdom.

*pmvaz@fc.ul.pt

Vibrational spectroscopy is a very powerful and useful tool to address several issues across several fields of research. In the specific case of heterogeneous catalysis this becomes even more important given the complexity of the systems under study, which not rare times are defying. In this context, Diffuse Reflectance Infrared Fourier Transform (DRIFT) and Raman spectroscopy are widely available and well-understood techniques for studies of heterogeneous catalysts [1]. Despite this, vibrational spectroscopy can also be performed very efficiently using neutron techniques. Inelastic Neutron Scattering (INS) Spectroscopy can be seen as a complementary technique to Infrared and Raman techniques. In a similar way to Raman, INS relies on scattered neutrons by the atomic nuclei. The scattering intensity depends on the incoherent inelastic scattering cross section and the amplitude of vibration. While both of these quantities are large for protium, they are relatively small for all remaining nuclei; as such the scattered intensity is dominated by hydrogen motion.

In this talk some examples of the use of INS applied to heterogeneous catalysis will be presented, stressing out its capabilities and limitations as well.

The present focus of research is on styrene oxidation mediated by Mo-based heterogeneous catalysts (Fig 1) that can yield selectively styrene oxide or benzaldehyde as products [2-4]. INS (combined with DRIFT when appropriate) allows gaining insights on possible mechanistic proposals by assessing not only the adsorbed species at the catalyst's surface (Fig. 2) [5], but also by providing information about how a given reagent interacts with the catalyst's surface in oxidation catalysis.

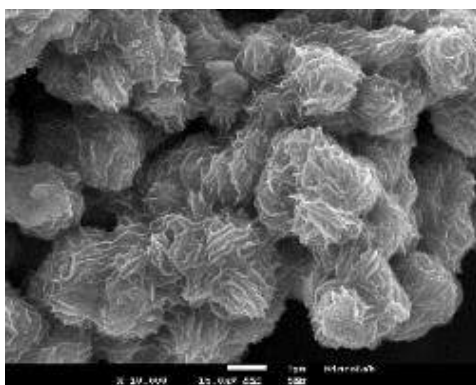


Fig. 1 – SEM image of a Mo heterogeneous catalyst

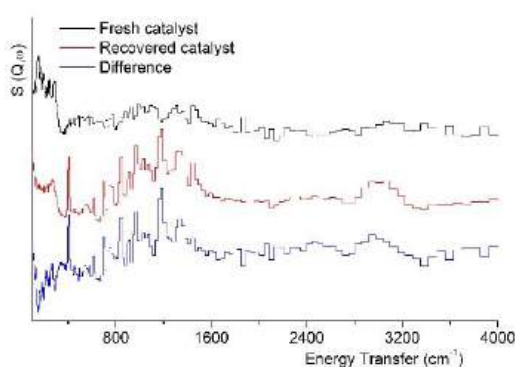


Fig. 2 – INS spectra of Mo heterogeneous catalyst used in styrene oxidation.

Acknowledgements

Authors thank FCT, POCI and FEDER (project UID/MULTI/00612/2013) for financial support. CIF also thanks FCT for a research grant (SFRH/BD/81029/2011) and RSC for a visiting scientist fellowship at ISIS. The authors thank the STFC Rutherford Appleton Laboratory for access to ISIS Pulsed Neutron and Muon Source facility. Authors also thank support by the European Commission under the 7th Framework Programme through the 'Research Infrastructures' action of the 'Capacities' Programme, NMI3-II Grant number 283883. Contract No. 283883-NMI3-II (for experiments run from March 2012 onwards).

References

- [1] P. D. Vaz, et al., *Chem. Eur. J.*, **13**, 7874-7882 (2007).
- [2] C. I. Fernandes et al. *Applied Catal A: Gen*, **384**, 84–93 (2010).
- [3] N. U. Silva et al. *Applied Catal A: Gen*, **408**, 105-116 (2011).
- [4] A. Bento et al., *Applied Catal A: Gen*, **504**, 399–407 (2015).
- [5] C. I. Fernandes, et al., *Phys. Chem. Chem. Phys.*, submitted for publication.

UV irradiation of H₂O:CO:NH₃ ice analog mixtures

E. Moreno^{1,2,*}, R. Martín-Doménech², G. M. Muñoz-Caro².

¹Instituto of Materials Science of Madrid, (CSIC), Cantoblanco, 28049 Madrid, Spain.

²Center of Astrobiology (INTA-CSIC), Ctra. de Ajalvir km 5, Torrejón de Ardoz, Madrid, Spain.

*elena.moreno@cab.inta-csic.es

Dense molecular clouds in the interstellar medium (ISM) have densities of 10^4 - 10^6 particles cm⁻³ and temperatures as low as 10 K in the interior owing to shielding from UV radiation. Dust grains in dense clouds, less than 1 micron in size, accrete ice mantles, with estimated thicknesses of hundredths of a micron, composed mainly of H₂O, and other species like CO, CH₃OH, CO₂, and NH₃ [1,2]. Cosmic rays are able to penetrate into the cloud and produce UV photons by excitation of molecular hydrogen (known as the "secondary UV field", about 10^3 - 10^4 photons cm⁻² s⁻¹). Some studies indicate that the energy deposited in ice mantles by secondary UV photons is about 10 times larger than the energy deposited directly by cosmic rays [3]. The accretion and desorption processes of gas molecules on cold grains play an important role in the evolution of dense clouds and circumstellar regions around YSOs, see e.g. Bisschop et al. (2006) [4].

Not only there are species such as H₂O, CO, CO₂, NH₃ in the ISM, but also organic molecules of considerable complexity were observed in dark interstellar clouds and in localized regions of hot cores, protoplanetary nebulae and disks, and circumstellar envelopes [5]. In particular, there exist significant abundances of large partly hydrogen-saturated molecules, many of them being of prebiotic interest [6]. These species challenge the completeness of the standard ion-neutral scheme in interstellar chemistry, suggesting that reactions on dust grains are involved in their formation.

Various combinations of ice mixtures (containing generally H₂O, CO, CH₃OH, CO₂, CH₄ and NH₃) were previously irradiated in laboratory simulations. We report our results on UV irradiation of H₂O:CO:NH₃ ice analog mixtures using the ISAC set-up to explore the reaction pathways leading to formation of complex organic molecules, among them are amino acids, carboxylic acids and N-heterocycles.

The ultra-high vacuum conditions of ISAC with a base pressure around 4×10^{-11} mbar, the equivalent of a particle density that falls within the dense cloud values, drastically reduce background water contamination, allowing the study of processes on a clean ice surface like photodesorption and a more efficient monitoring of low-abundance photoproducts in the experiments [7].

References

- [1] E. L. Gibb, D. C. B. Whittet, and J. E. Chiar., *ApJ*, **558**, 702-716 (2001).
- [2] E. Dartois, *Space Science Rev.*, **119**, 293-310. (2005).
- [3] C. J. Shen, J. M. Greenberg, W. A. Schutte, and E. F. van Dishoeck., *A&A*, **415**, 203-215 (2004).
- [4] S. E. Bisschop, H. J. Fraser, K. I. Öberg, E. F. van Dishoeck, and S. Schlemmer, *A&A*, **449**, 1297-1309 (2006).
- [5] A. Dalgarno, *Faraday Discuss*, **133**, 9-25 (2006).
- [6] L. E. Snyder, *Proc. Natl. Acad. Sci.*, **103**, 12243-12248 (2006).
- [7] G. M. Muñoz Caro, A. Jiménez-Escobar, J. A. Martín-Gago, C. Rogero, C. Atienza, S. Puertas, J. M. Sobrado, and J. Torres-Redondo, *A&A*, **522**, A108 (2010).

ORAL PRESENTATIONS

Passive exposure to nicotine from e-cigarettes

D. Gallart-Mateu, L. Elbal, S. Armenta*, M. de la Guardia.

Department of Analytical Chemistry, University of Valencia, 50th Dr. Moliner St., 46100 Burjassot, Valencia, Spain.

*Sergio.Armenta@uv.es

A procedure, based on ion mobility spectrometry (IMS) after liquid-liquid microextraction (LLME), has been successfully used for the determination of passive exposure to nicotine from cigarette and e-cigarette smoking. Nicotine has been determined in exhaled breath and oral fluids of both, active and passive smokers and vapers (**Figure 1**). The exposure studies, made in closed environments, evidenced that the exhaled breath after smoking conventional blend cigarette provides nicotine levels of the order of 220 ng per puff, in the case of experienced smokers, being exhaled only 32 ng in the case of e-cigarettes. Regarding passive exposure to nicotine, a set of 16 non-smokers oral fluids were sampled before and after smoking/vaping exposition at 30 minutes intervals. Exposition to blend class A cigarettes, rolling tobacco and two different e-cigarette devices were evaluated and compared. The nicotine content in oral fluids of non-smokers exposed to conventional yellow blend tobacco presented higher concentrations, from 24 to 111 $\mu\text{g L}^{-1}$, than those obtained after exposition to e-cigarettes, varied from 7 to 16 $\mu\text{g L}^{-1}$. Nicotine levels of oral fluids of non-smokers exposed to rolling tobacco, from 18 to 56 $\mu\text{g L}^{-1}$, provide an intermediate situation between classical cigarettes and e-cigarettes exposition. On the other hand, the oral fluid nicotine content in active traditional tobacco smokers and e-cigarette vapers was monitored at 30 min intervals during a smoking session. The nicotine concentration immediately increased after smoking and, after that, decreased due to the absorption, dilution and metabolism processes.

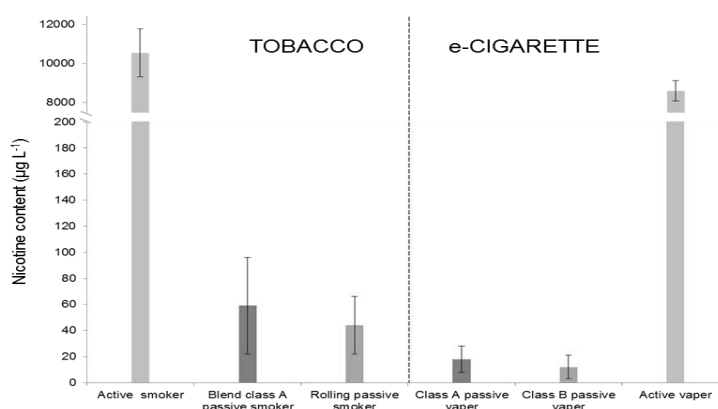


Figure 1. Averaged nicotine content, expressed as $\mu\text{g L}^{-1}$, in oral fluids of active and passive smokers/vapers

The developed procedure provided a comparable accuracy to that of a reference methodology based on gas chromatography- mass spectrometry (GC-MS) [1] for the analysis for spiked oral fluid samples. The precision of the procedure, expressed as relative standard deviation (RSD), ranged from 2 to 4 % for spiked oral fluid samples at 40 and 75 $\mu\text{g L}^{-1}$ nicotine.

Acknowledgements

Authors acknowledge the financial support of Generalitat Valenciana (Project PROMETEO II 2014-077) and Ministerio de Economía y Competitividad-Feder (Project CTQ 2014-52841-P and Project CTQ 2012-38635).

References

[1] C.N. Man, S. Ismail, G.L. Harn, R. Lajis, R. Awang, *J. Chromatogr. B*, **877**, 339-342 (2009).

Analysis of nanomaterials by fast scanning single particle ICP-MS: A user's manual

F. Laborda^{1*}, I. Abad-Álvarez¹, E. Bolea¹, E. Peña-Vázquez², P. Bermejo-Barrera², J. R. Castillo¹.

¹Group of Analytical Spectroscopy and Sensors (GEAS). Institute of Environmental Sciences (IUCA). University of Zaragoza, Pedro Cerbuna 12, 50009, Zaragoza (Spain).

²Group of Trace Elements, Spectroscopy and Speciation (GETEE). Department of Analytical Chemistry, Nutrition and Bromatology. University of Santiago de Compostela, Avda. Das Ciencias, s/n, 15782, Santiago de Compostela (Spain).

*flaborda@unizar.es

Inductively coupled plasma mass spectrometry (ICP-MS) is an atomic spectrometric technique used routinely for the determination of total element contents at ultra-trace levels. Alternatively, the technique can be used in the mode called single particle detection (SP-ICP-MS). By using this ICP-MS mode, inorganic nanoparticles and dissolved forms can be detected and quantified without any previous separation with number concentration detection limits of 1000 particles mL⁻¹ (part-per-trillion levels and below); additionally, nanoparticles can also be size characterized down to 10-20 nm size [1].

In single particle detection mode, when one nanoparticle is introduced into the plasma, a pack of gaseous ions is generated, being measured as a single pulse by the detector. The number of pulses is proportional to the number concentration of nanoparticles, whereas the intensity of each pulse is proportional to the number of atoms in the nanoparticle, hence to the mass of element and the size of the nanoparticle if its composition, shape and density are known. Detection of individual nanoparticle involves working at nanoparticle number concentrations low enough (ca. 10⁸ nanoparticles L⁻¹ or lower) and data acquisition frequency high enough (ca. 100 Hz or higher) in order to get a linear relationship between number of pulses and nanoparticle number concentration.

The packet of ions generated by a single nanoparticle has a length of about 500 µs. Until very recently, available quadrupole ICP-MS instruments could just work with reading times in the range of milliseconds (recommended reading times: 3-10 ms), what means that ion packs are recorded as single pulses. However, the last generation of ICP-MS instruments are featured with fast scanning quadrupoles (data acquisition frequencies up to 100 kHz), with reading times in the range of microseconds (10-100 µs). Under these conditions, ion packets are recorded as single transient signals. The use of fast scanning quadrupoles involves a number of features when working in SP-ICP-MS, namely more than one isotope can be measured in a nanoparticle, detection of dissolved species is limited and the precision of the results can be improved.

A comparative study by using conventional and fast scanning SP-ICP-MS will be presented, evaluating the performance of this novel SP-ICP-MS mode. The study carried out shows that users must select number concentrations properly, depending on the scanning mode, to get results of the highest quality.

Acknowledgements

This work was supported by the Spanish Ministry of Economy and Competitiveness, projects CTQ2015-68094-C2-1-R and CTQ2015-68094-C2-2-R.

References

[1] F. Laborda, E. Bolea and J. Jiménez-Lamana, *Anal. Chem.*, **86**, 2270–2278 (2014).

Quantification of fluorine traces in solid samples using CaF molecular emission bands in atmospheric air LIBS

C. Álvarez-Llamas¹, J. Pisonero¹, N. Bordel^{1,*}.

¹Department of Physics, University of Oviedo, c/ Gonzalo Gutiérrez Quirós, Campus de Mieres, 33600 Mieres, Spain.

*bordel@uniovi.es

There is a great interest in the development and improvement of different analytical methods for fast, accurate and precise quantitative detection of fluorine and fluoride compounds, both in industrial or scientific applications. Fluorine and fluoride compounds are employed in fire extinguishers, pharmaceutical and agrochemical products, dental care products, polymers, etc.[1] Spectroscopic detection of fluorine in solid samples, and in particular using Laser-Induced Breakdown Spectroscopy (LIBS), is a challenging task due to the low excitation efficiency of this element. Helium atmosphere LIBS might be used to increase the signal-to-background ratio of fluorine atomic lines in the infrared spectral region (680-710 nm) [2] [3]. Alternatively, the measurement of molecular compounds formed in the LIBS plasma might be used to indirectly detect fluorine [4], [5].

In this work, the detection of CaF molecular emission bands is investigated to improve the analytical capabilities of atmospheric air LIBS for the determination of fluorine traces in solid samples. In a first step, it was investigated the quantification capabilities of LIBS in powdered samples with calcium content. In particular, Cu matrix samples containing different fluorine concentration (between 50 and 600 µg/g), and variable amounts of Ca, are used to demonstrate linear relationships between CaF emission signal and F concentration. Limits of detection for fluorine are improved by more than 1 order of magnitude using CaF emission bands versus F atomic lines, in atmospheric-air LIBS. Furthermore, a toothpaste powder sample is used to validate this analytical method. For the analysis of fluorine in calcium-free samples, it was also investigated the possibility of using an in-line external source of Ca to form the CaF molecular band.

Acknowledgements

The authors would like to acknowledge the financial support from the Government of the Principality of Asturias through the project FC-15-GRUPIN14-040.

References

- [1] W. R. Dolbier, J. Fluor. Chem., vol. 126, no. 2, pp. 157–163, 2005.
- [2] J. E. Sansonetti, W. C. Martin, and S. L. Young, 2005. [Online]. Available: <http://physics.nist.gov/Handbook>.
- [3] C. D. Quarles, J. J. Gonzalez, L. J. East, J. H. Yoo, M. Morey, and R. E. Russo, J. Anal. At. Spectrom., vol. 29, no. 7, p. 1238, 2014.
- [4] M. Gaft, L. Nagli, N. Eliezer, Y. Groisman, and O. Forni, Spectrochim. Acta Part B At. Spectrosc., vol. 98, pp. 39–47, Jun. 2014.
- [5] C. Álvarez Llamas, J. Pisonero, and N. Bordel, Spectrochim. Acta Part B At. Spectrosc., vol. 100, pp. 123–128, Oct. 2014.

As, Hg and Se determination in gasoline samples by on-line chemical vapor generation using a *Flow Blurring*[®] multiple nebulizer in ICP-OES

M. García-Martínez, M. A. Aguirre*, A. Canals.

Departamento de Química Analítica, Nutrición y Bromatología e Instituto Universitario de Materiales, Universidad de Alicante, P.O. BOX: 99, Alicante E-03080, Spain.

**aguirre.pastor@ua.es*

Conventional liquid sample introduction systems in ICP-based techniques, as pneumatic nebulization, present many limitations due to their low transport efficiency. In order to overcome these limitations, analytes are derivatized into the proper volatile specie and then are introduced into the plasma. This procedure requires a suitable batch or flow system for promoting chemical reactions to generate volatile species. In the present work, a simple methodology for on-line chemical vapor generation directly into the spray chamber is described. By this way, the analyte transport efficiency is improved and consequently, the sensitivity. The aim is to evaluate this new methodology for the analysis of arsenic, mercury and selenium as model analytes in organic samples (*i.e.*, gasoline samples). For this purpose, a multiple nebulizer based on *Flow Blurring*[®] technology is used [1]. The nebulizer includes three independent nebulization units: one of them is used for sample or standards introduction and the remaining two are used for introducing the reagents needed for volatile species generation (*i.e.*, acid and reducing agent) [2]. Using the proposed sample introduction system, the use of complex FIA systems, typically used for this purpose, is avoided.

The chemical vapor generation system was optimized in order to obtain the highest sensitivity using a multivariate approach. The optimization has been done in two steps: (i) a preliminary evaluation using a Plackett-Burman factorial design of all variables, in order to know the significant ones, and (ii) a circumscribed central composite design to assess the optimal experimental conditions for chemical vapor generation. Under optimum conditions, the applicability of the analytical method in ICP-OES was studied by determining arsenic, mercury and selenium in spiked commercial gasoline samples.

Acknowledgements

The authors kindly acknowledge the financial support from the Government of Spain (CTQ2011-23968), OneNeb (division of Ingeniatics Tecnologías S.L.) for the multinebulizer prototypes provided and Agilent Technologies for the donation of the spectrometer.

References

- [1] M. A. Aguirre, N. Kovachev, M. Hidalgo and A. Canals, *J. Anal. At. Spectrom.*, **27**, 2102-2110 (2012).
- [2] C. D. Pereira, M. A. Aguirre, J.A. Nóbrega, M. Hidalgo and A. Canals, *Microchem. J.*, **112**, 82-86 (2014).

Electrospray pyrolysis deposition followed by laser-induced breakdown spectroscopy: a new method for trace elemental analysis in liquid samples

L. Ripoll, M. Hidalgo*, A. Canals.

Department of Analytical Chemistry and Food Sciences and University Materials Institute, University of Alicante, P.O. Box 99, E-03080 Alicante, Spain.

**montserrat.hidalgo@ua.es*

Since its introduction, LIBS has found a large number of useful applications in the field of elemental analysis, mainly due to the special features characterizing the technique. LIBS analysis only needs optical access to the sample by means of a tightly focused laser beam, which allows just a very small quantity of sample to be directly analyzed without the need of sample preparation. This fact, together with the small size of the instrumentation, makes LIBS ideal for *in situ* and on line applications on practically any kind of sample. However, in spite of its huge potential, the low sensitivity of LIBS when compared with other conventional techniques constitutes its most important limitation for (ultra)trace elemental analysis, especially when liquid samples are the target.

The limitation in sensitivity and reproducibility of LIBS analysis of liquid matrices has been frequently surmounted with the aid of a sample preparation procedure that, in many cases, consist on the conversion of the liquid sample to a solid matrix by a simple drying process. This approach avoids the experimental difficulties usually associated with LIBS analysis of liquids and lead to an increase in the analyte concentration in the generated solid residue, therefore improving both sensitivity and reproducibility. Still, for these sample preparation procedures to be considered fully useful, they should be fast and easy to automate and integrate with the LIBS detection system, in order to maintain the on-line and *in situ* capabilities characterizing this technique.

In this work, a simple sample preparation procedure based on electrospray pyrolysis deposition is presented. Electrospray is produced by applying a strong electric field to a liquid sample passing through a capillary tube. The charged liquid in the capillary nozzle becomes unstable as it is forced to hold more and more charge, breaking in a mist of highly charged droplets, which prevents their coagulation and promotes self-dispersion. The generated droplets are attracted to a grounded heated substrate, where they settle and dry, generating a homogeneous solid layer that can be easily analyzed by LIBS.

In order to evaluate the viability of the proposed procedure, different experimental parameters affecting the morphology of the generated solid residue were studied and optimized. Under optimum conditions, analytical figures of merit for the analysis of several metals in aqueous samples were obtained. Results show that electrospray pyrolysis deposition could be a useful method for overcoming LIBS limitations in liquid analysis, providing a fast and automated sample preparation procedure to improve sensitivity and reproducibility of LIBS analysis of liquid samples.

Acknowledgements

The authors would like to thank the Regional Government of Valencia (Spain) (PROMETEO/2013/038) for the financial support.

OC-OT-LIBS: A novel approach to the chemical characterization of single particles

P. Purohit*, F. J. Fortes, J. J. Laserna.

¹ Departamento de Química Analítica, Universidad de Málaga, Campus de Teatinos s/n, 29071 Málaga, Spain.

purohit@uma.es

Spectral identification of individual micro- and nano-sized particles by the sequential intervention of optical catapulting, optical trapping and laser-induced breakdown spectroscopy is presented¹. The three techniques are used for different purposes. Optical catapulting (OC) serves to put the particulate material under inspection in aerosol form²⁻⁴. Optical trapping (OT) permits the isolation and manipulation of individual particles from the aerosol, which are subsequently analyzed by laser-induced breakdown spectroscopy (LIBS). Once catapulted, the dynamics of particle trapping depends on the laser beam characteristics (power and intensity gradient) and on the particle properties (size, mass and shape). Particles are stably trapped in air at atmospheric pressure and can be conveniently manipulated for a precise positioning for LIBS analysis. The spectra acquired from the individually trapped particles permit a straightforward identification of the inspected material.

The current work focuses on the development of a procedure for simultaneously acquiring dual information about the particle under study via LIBS and time-resolved plasma images by taking advantage of the aforementioned features of the OC-OT-LIBS instrument to align the multiple lines in a simple yet highly accurate way. The plasma imaging does not only further reinforce the spectral data, but also allows a better comprehension of the chemical and physical processes involved during laser-particle interaction. Also, a thorough determination of the optimal excitation conditions generating the most information out of each laser event was run along the determination of parameters such as the width of the optical trap, its stability as a function of the laser power and the laser wavelength. The extreme sensibility of the presented OC-OT-LIBS technology allows a detection power of attograms for single/individual particle analysis.

Acknowledgements

The authors gratefully acknowledge the funding support from the Ministerio de Economía y Competitividad (Project CTQ-204-56058) and the concession of a FPI grant to one of the authors (PP).

References

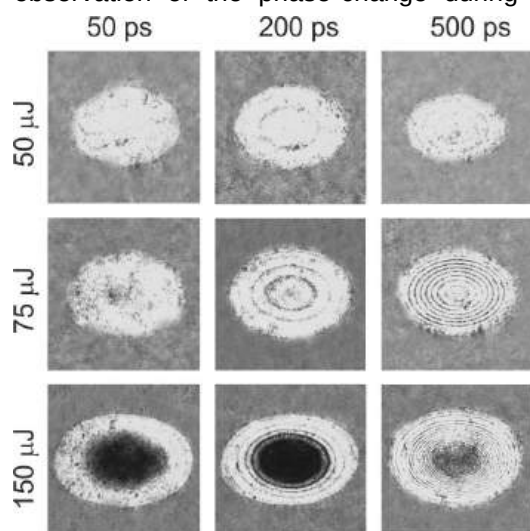
- [1] F.J. Fortes, A. Fernández-Bravo and J.J. Laserna, *Spectrochim. Acta B*, **100**, (2014) 78–85.
- [2] F.J. Fortes, L.M. Cabalín and J.J. Laserna, *Spectrochim. Acta B*, **64**, (2009) 642–648.
- [3] F.J. Fortes and J.J. Laserna, *Appl. Surf. Sci.*, **256**, (2010) 5924–5928.
- [4] M. Abdelhamid, F.J. Fortes, M.A. Harith and J.J. Laserna, *J. Anal. Atom. Spectrom.*, **26**, (2011) 1445–1450.

Femtosecond time-resolved phase-change microscopy and ablation threshold calculations to understand ultrafast laser ablation

I. M. Carrasco-García, M. López-Claros, **J. M. Vadillo**, J. J. Laserna.
UMALASERLAB, Universidad de Málaga, C/ Jimenez Fraud 4, 29010 Málaga.
samara@uma.es

Ultrashort laser pulses have proven to be a powerful tool for the solids spectroscopy. The particular coupling of energy and high peak power enabled by ultrashort laser pulses facilitates energy coupling inside materials by multi-photon or tunneling ionization mechanisms. From the analytical point of view, ultrashort laser ablation opens new possibilities due to the significantly different properties when compared with their nanosecond counterpart, where the concurrence of photochemical and photothermal processes taking place during the photon absorption govern the process. During nanosecond ablation the incoming laser beam and the expanding plasma interact, impairing the visualization of the different phenomena occurring until mass transfer occurs. Such hidden events are on the basis of the physics governing the ablation process and are in close contact with analytical techniques as LIBS, LIMS, MALDI or LA-ICP.

The present communication details the design, construction and evaluation of a microscope with time-resolved imaging capabilities. With such instrument, femtosecond-resolved micrographies of the surface of samples exposed to ultrashort laser pulses are obtained, allowing the dynamic observation of the phase-change during subthreshold laser-matter interaction. The results



presented demonstrates the appearance of dynamic Newton rings at the surface of the sample that corresponds to the formation of a thin laser-induced surface layer resulting in constructive and destructive interference of the light reflected from the surface with the light reflected from the layer interface (see figure inserted). Additionally, accurate calculation of the ablation thresholds required in different materials and pure elements will allow to demonstrate the existence or not of matrix effects that condition the selective appearance of specific elements in the expanding plume.

Acknowledgements

This work has been funded by project CTQ2011-24433.

Femtosecond Laser Ionization Time-of-Flight Mass Spectrometry of Size-Sorted Aerosolized Matter

S. Medina*, J. M. Vadillo, J. J. Laserna.

UMALASERLAB, Universidad de Málaga, C/ Jimenez Fraud 4, 29010 Málaga.

*samara@uma.es

There are few doubts about the increasing interest in aerosol characterization. Particles with aerodynamic diameters in the low micrometer range can be transported by suspension for long distances and represent an important risk for health due to their easy breathability. In addition, the riskiness of this material not only depends on its size, but also on its chemical composition. For this reason, the availability of analytical tools to correlate particles sizes and its chemical components is fundamental in order to know and prevent human and ecological impact of such pollutants.

Laser Ionization Time-of-Flight Mass Spectrometry (LI-TOF-MS) exhibits important advantages for such task due to the limited sample pretreatment, and the easy data interpretation. On top of that, the use of lasers as ionization probes brings the opportunity of localized analysis, and makes possible obtaining different mass spectra at selected laser energies, allowing the operation under gentle or hard ionization conditions.

Acknowledgements

This work is funded by project CTQ2014-56058P.

Potential of Laser Induced Breakdown Spectroscopy in Environmental Studies

J. Anzano^{1,*}, D. Paules¹, R. Mur¹, R. J. Lasheras¹, M. Escudero², J. del Valle², O. Tapia², S. Hamida³, E. Gaspar¹, J. Cajal¹, C. Bello¹, J. Casas¹, S. Sangüesa¹, F. Bayo⁴, P. González-Blasco³, A. Calvo⁵, J. Cáceres⁶, S. Moncayo⁶, J. Anwar⁷, A. Dar⁷.

¹Laser Laboratory, Department of Analytical Chemistry, Faculty of Sciences. University of Zaragoza. Pedro Cerbuna 12, 50009 Zaragoza, Spain.

²Centro Universitario de la Defensa (CUD) de Zaragoza, E-50090 Zaragoza, Spain.

³University of Bouchaib Belhadj Ain Temouchent, Algeria.

⁴Laboratorio de Sanidad Animal, Gobierno de Aragón, Zaragoza, Spain.

⁵Confederación Hidrográfica del Ebro, Zaragoza, Spain.

⁶Department of Analytical Chemistry, Complutense University, Madrid, Spain.

⁷Department of Chemistry, University of Punjab, Lahore, Pakistan.

*janzano@unizar.es

There are only few techniques which match with Laser Induced Breakdown Spectroscopy, (LIBS), in its advantages like speed, accuracy, precision, possibility of on-site analysis and no or little effort for sample preparation. That is why it has been successfully employed for the qualitative as well as quantitative characterization of various types of samples. By using Nd: YAG pulsed laser we analyzed a number of geological, environmental and food samples chiefly for metal analysis. During this work it has been noticed that matrix has a pronounced effect on determination of analyte. Calibration curves for metal ions obtained in different matrixes showed different slopes. We are working extensively to investigate this issue in detail.

One environmental problem, which Southern Europe facing now a days is presence of particulate matter in air. This particulate matter is mainly composed the fine dust particles from African Sahara which are transported to Europe through different routes. This particulate matter, consisting of mineral particles of very small size cause a variety of adverse effects on visibility, climate, ecosystems and human health. In this context, the development of in-situ measurement technologies for speciation and complete analysis of atmospheric particulate is a demand of time. LIBS can provide a satisfactory solution in this regard. It is non-destructive and fast analytical technique which can provide precise and accurate results in minimum possible time. In this regard we employed Laser Induced Breakdown Spectroscopy for the qualitative and quantitative analysis of natural and irradiated diatomite. This natural rock is used as a filtration aid, mild abrasive in products such as metal polishes, adsorbent for liquids, and porous support for chemical catalysts. The morphology studies and microstructure of this material has also been investigated by Field Emission Scanning Electron Microscopy (FESEM).

Acknowledgements

This work was supported by the Department of Innovation, Research and University of the Aragon Regional Government and the European Social Found (group E75), the University of Zaragoza (proposal #UZ2015-CIE-01) and General Services Research Support -SAI of University of Zaragoza.

HF RFID label for simultaneous oxygen, ammonia, carbon dioxide and humidity determination

M. M. Erenas¹, A. Martínez-Olmos², N. López-Ruiz², I. de Orbe-Payá¹, A. J. Palma², L. F. Capitán-Vallvey^{1,*}.

¹ECSENS, Department of Analytical Chemistry, Faculty of Science, University of Granada, 18071, Granada

²ECSENS, CITIC-UGR, Department of Electronics and Computer Technology, University of Granada, 18071 Granada.

*lcapitan@ugr.es

Intelligent packaging as an extension of the communication function of traditional packaging is based on its ability to sense, detect, or record external or internal changes in the product's environment. One of the types of intelligent packaging is based on measuring the headspace of the packaging as an indicative marker of the quality and/or safety of the packed food. The parameters used to detect the status of the packed food are the content in different gases depending on the packed product. The most usual gases are oxygen (0.5-2%), carbon dioxide (5-20%), ammonia (< 15 mg/L in fish) and finally humidity (around 85% for cut fruits and vegetables).

In this work we present a flexible HF RFID label [1] for the optical determination of the four gases above mentioned. The HF RFID tag is designed and screen printed on a flexible substrate containing on one side all the electronic components needed: one white LED and four digital colour detectors to perform the optical measurement; a microcontroller, and a RFID chip, as it is presented in Figure 1. On the other side of the flexible substrate, and located opposite to the colour detectors, four membranes sensitive to the presence of oxygen, carbon dioxide, ammonia and humidity are placed. This RFID tag can operate in passive mode, with no external battery, powering from the RF interrogation signal of the reader.

The chemistry selected for the determination of the gases can be classified in two different groups: quenching of luminescence of immobilized Pd(II) meso-tetra (pentafluorophenyl) porphine complex for oxygen and change in colour for the rest of gases. Namely membranes containing pH indicators for carbon dioxide and ammonia and methylene blue membrane for humidity. The luminescence quenching by oxygen is quantified using the red coordinate of the RGB colour space whereas for the other three membranes for the determination of ammonia, carbon dioxide and ammonia the change in colour measured by the H parameter of the HSV colour space is used.

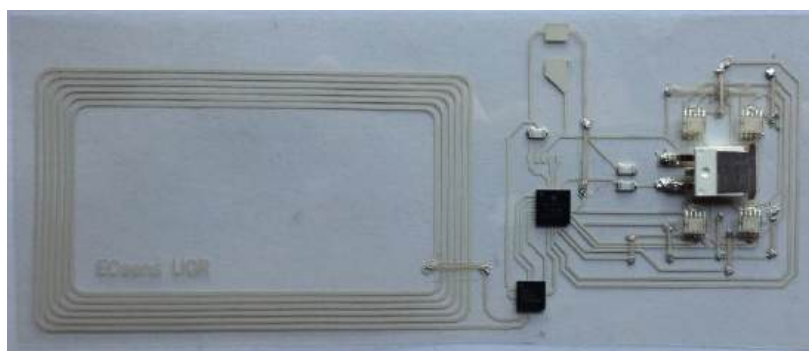


Figure 1. HF RFID tag

Acknowledgements

This work was funded by Spanish Ministerio de Economía y Competitividad under Project CTQ2013-44545-R project that was partially supported by European Regional Development Funds (ERDF).

References

[1] A. Martínez-Olmos, J. Fernández-Salmerón, N. López-Ruiz, A. Rivadeneyra Torres, L. F. Capitán-Vallvey, and A. J. Palma, *Anal. Chem.*, **85**, 11098–11105 (2013).

Immunoassay for immunosuppressant drugs using conjugated strongly near-infrared fluorescent magnetic nanoparticles

E. Benito-Peña², F. Salis¹, A. B. Descalzo², M. C. Moreno-Bondi^{*,2}, G. Orellana^{*,1}.

¹Dpmt. of Organic Chemistry, Complutense University of Madrid, 28040 Madrid, Spain.

²Dpmt. of Analytical Chemistry, Complutense University of Madrid, 28040 Madrid, Spain.

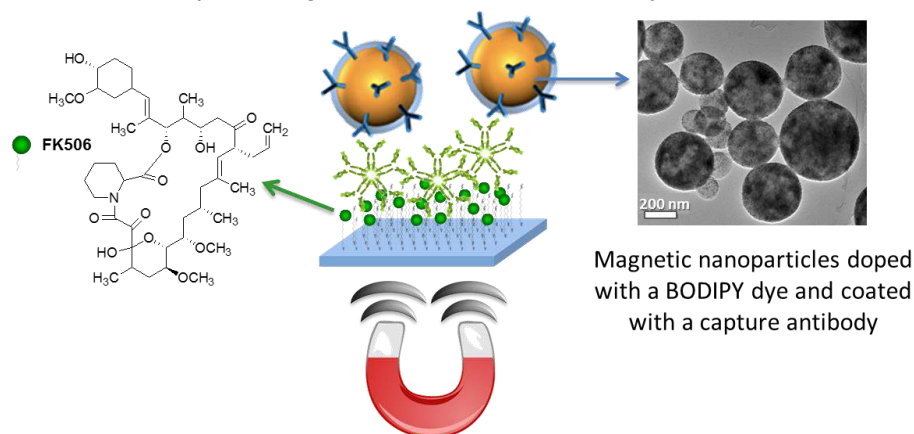
*orellana@ucm.es and *mcmbondi@ucm.es

Tacrolimus (FK506) is a hydrophobic macrolide lactone produced by *Streptomyces tsukubaensis* and is widely used as immunosuppressant after organ transplantation. The free fraction of this drug in serum (1–2%) correlates with its accumulation in blood cells. Taking into account its narrow therapeutic window, semicontinuous monitoring of FK506 in transplanted patients will be most helpful to boost its therapeutic efficiency and avoid potential adverse effects. In this context, optical immunosensors could achieve the required quantification of FK506 and its metabolites in plasma thanks to its intrinsic high sensitivity and selectivity.

The use of luminescent magnetic nanoparticles (NPs) as labels could allow a significant improvement of the bioassay sensitivity and facilitate sample handling [1].

The nanoparticles we have used are ca. 300 nm polystyrene beads decorated with magnetic ferrite nanograins (8–10 nm). For conferring fluorescent properties, these magnetic beads have been doped with novel BODIPY dyes prepared in our laboratory. Boron-dipyrromethene (BODIPY) dyes are fluorophores well known for the tuneability of their very bright emission, photostability and their versatile chemistry [2,3]. On the negative side, they display a small Stokes shift. For working with serum samples and minimize autofluorescence, we prepared a BODIPY dye with a π -extended structure that shows an absorption maximum at 641 nm and emission at >650 nm, with a fluorescence quantum yield of 0.64 (in ethanol). Bioconjugation of the analytes or the antibodies can be performed through the –COOH groups available on the surface of the magnetic nanoparticles.

The power of our approach has been demonstrated by the development of a heterogeneous fluorescence magnetic immunoassay to detect FK506. The latter provides a very low limit of detection (1.8 ng/mL) and IC₅₀ (13 ng/mL), together with a wide dynamic range (3.6 – 37 ng/mL) that fits the therapeutic requirements. Moreover, this strategy is superior to other conventional methods thanks to the improved signal-to-noise ratio obtained by the use of the novel NPs.



Acknowledgements

The authors are grateful for the financial support from the European Union under the program FP7-ICT-2011-8 (contract no. 318372 "NANODEM"), as well as the Spanish Ministry of Economy and Competitiveness (grant no. CTQ2015-69278-C2-1-R and CTQ2015-69278-C2-2-R).

References

- [1] D. Issadore, R.M. Westervelt in *Point-of-Care Diagnostics on a Chip*, Springer: Heidelberg, 2013.
- [2] A. Loudet and K. Burgess, *Chem. Rev.* **107**, 4891-4932 (2007).
- [3] U. Gilles, R. Ziessel and A. Harriman, *Angew. Chem. Int. Ed.* **47**, 1184-1201 (2008).

The lighting Influence in Digital Image Based methods

A. López-Molinero^{1,*}, P. Berlin-Larque.

¹University of Zaragoza, Department of Analytical Chemistry, 50009 Zaragoza.

*anlopez@unizar.es

The Calcium content, in pharmaceutical formulations for osteoporosis disease treatment, has been determined by a new method of digital images captured with a portable camera.

The method was based in the formation of a red-orange complex with the reagent Glioxal bis(2-hydroxyAnil) [1]. This chromatic study was addressed to evaluate the influence of the lighting color.

Chemometric methods of ANOVA and Multivariate Analysis were applied to characterize the chromaticity. They allowed to evaluate and model the influence of lighting color but also other experimental factors, such as the camera, and other practical conditions.

Thus it was demonstrated that, even in very casual conditions and white lighting, the analytical potential of this method, and in the RGB color space, was characterized by: the analyte as the most influent factor which deals with the 84% of chromatic variance. And at the same time, it demonstrated low influence of external factors: camera and the method conditions

The change to a green illumination increased the total chromatic variance of the chromogenic system at level of 250%. And also, and more important, in these conditions the analyte might justify the 95% of chromatic variance. In these conditions, the camera and the method effects lowered their influences from 16% to 5% of the total chromatic variance.

The analytical performance, after the lighting change, White-> green, showed a sensitivity increase in the order of 70%, given as the slope of the linear calibration. The LD and LQ were diminished 6%. The linear calibration increased its performance and presented a lower value in the standard deviation of residuals.

Multivariate analysis allowed to characterize the color behavior. The reduction and projection of basic RGB color to PC1 and PC2 principal components showed only significant colors. Thus, it was demonstrated that with White lighting: G and B were confounded and consequently the color was dominated by only two components. However, with green illuminant all colors were significant and may be differentiated.

The best performance with green lighting and detection of the G reflected color allowed to obtain the following data of merit: limit of detection was in the order of 0.4 mg/l; the linear range was obeyed up to 15 mg/l Ca(II); the reproducibility was lower than 4%, as % rsd, for 6 mg/l Ca (II).

In the previous best conditions, the method may be applied for a ready determination of Ca in pharmaceutical pills. The results were statistically compared with FAAS and the declared content. At 95% of confidence differences were not significant. Consequently the method could be said as free of systematic errors.

Acknowledgements

These works were funded by MINECO in the project CTQ20012- 34774 and by DGA in the DGA-FEDER project.

References

[1] A.Lopez-Molinero, V. T. Cubero, R. D.Irigoyen, D. S. Piazuolo, *Talanta*, **103**, 236-244 (2013).

***In-situ* formation of bare gold nanoparticles for surface enhanced infrared spectroscopic studies on bovine serum albumin**

A. I. López-Lorente¹, P. Wang¹, B. Mizaikoff^{1,*}.

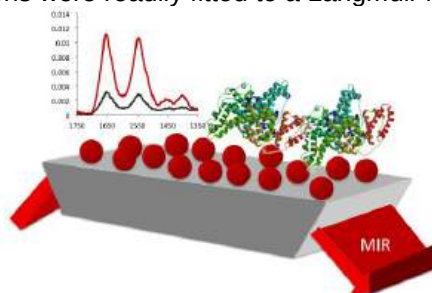
¹Institute of Analytical and Bioanalytical Chemistry, Ulm University, 89081 Ulm, Germany.

*boris.mizaikoff@uni-ulm.de

Mid-infrared (MIR) spectroscopy investigates the interaction of MIR photons with molecules via the excitation of vibrational and rotational modes with inherent molecular selectivity, thus providing an ideal platform for optical sensing applications including the analysis of proteins. The characteristic mid-infrared signature of proteins includes bands that predominantly arise from vibrations within the peptide backbone such as the amide I and amide II features, among others. The amide I band (1600-1700 cm⁻¹) is related to C=O stretching vibrations, whereas the amide II band (1500-1600 cm⁻¹) is associated with CN stretching and NH in-plane bending [1].

Surface enhanced infrared absorption spectroscopy (SEIRAS) using an attenuated total reflection (ATR) configuration has been used to analyze the protein bovine serum albumin (BSA) adsorbed onto bare gold nanoparticles (AuNPs). The bare gold nanoparticles forming the SEIRA-active substrate were synthesized *in-situ* inside the ATR compartment taking advantage of the stainless steel walls of the liquid cell acting as reducing agent, as previously demonstrated by our research team [2]. Thereby, a thin layer of AuNPs is deposited at the waveguide surface within the evanescent field. The *in-situ* formation of AuNPs at the substrate surface facilitates the preparation of a new SEIRA surface prior to each measurement, and the formation of AuNPs can be directly monitored via correspondingly enhanced water absorption bands [3].

BSA has a high affinity towards gold nanoparticles due to external thiol functions available for conjugation to the AuNP surface [4]. Consequently, SEIRA studies take advantage of the affinity of the protein to bare AuNPs present within the evanescent field at the ATR crystal. The absorbance of BSA on top of AuNPs deposited at the Si waveguide is distinctly enhanced vs. bare Si, thus improving the sensitivity of the detection. Furthermore, it was shown that the presence of an AuNPs layer does not affect the secondary structure of the protein. Measurements were carried out both in water and D₂O, which conveniently shifts the strongly absorbing background of H₂O from the spectral regime of interest for protein studies. Measurements at different protein concentrations were readily fitted to a Langmuir isotherm for quantification.



Acknowledgements

A.I. López-Lorente wishes to thank the Alexander von Humboldt Foundation for the award of a Postdoctoral Fellowship. This work was in part supported by the Boehringer Ingelheim Ulm University BioCenter (BIU).

References

- [1] P. Wang, W. Bohr, M. Otto, K.M. Danzer, B. Mizaikoff, *Anal. Bioanal. Chem.*, **407**, 4015-4021 (2015).
- [2] A.I. López-Lorente, B.M. Simonet, M. Valcárcel, S. Eppler, R. Schindl, C. Kranz, B. Mizaikoff, *Talanta*, **118**, 321-327 (2014).
- [3] A.I. López-Lorente, M. Sieger, M. Valcárcel, B. Mizaikoff, *Anal. Chem.*, **86**, 783-789 (2014).
- [4] D.H. Tsai, M. Davila-Morris, F.W. DelRio, S. Guha, M.R. Zachariah, V.A. Hackley, *Langmuir*, **27**, 9302-9313 (2011).

Optical Spectroscopy of Semiconductor Nanowires: Tailoring Light Harvesting and Emission at the Nanoscale

D. R. Abujetas^{1,*}, R. Paniagua-Domínguez², A. D. Van-Dam³, B. J. M. Brenny⁴, A. Polman⁴, J. Gómez-Rivas^{3,5}, J. A. Sánchez-Gil¹.

¹*Instituto de Estructura de la Materia, Consejo Superior de Investigaciones Científicas (IEM-CSIC), Serrano 121, 28006 Madrid (Spain).*

²*Data Storage Institute, Agency for Science, Technology and Research, 117608, Singapore.*

³*COBRA Research Institute, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven (The Netherlands).*

⁴*Center for Nanophotonics, FOM-Institute AMOLF, Science Park 104, 1098XG Amsterdam (The Netherlands).*

⁵*FOM Institute DIFFER, P.O. Box 6336, 5600 HH Eindhoven (The Netherlands).*

*diego.romero@iem.cfmac.csic.es

Dielectric and semiconductor nanostructures are becoming increasingly important as components in nanoscale integrated circuits and optoelectronic devices. Such resonant structures can exhibit complex interactions with light, controlling the wavelength, momentum and polarization of emission. Concretely, the directional/spectral optical response of nanowires (NWs) is a fundamental property that depends on the material, geometry and dimensions of the nanostructures and that needs to be considered in the design of NW optical devices [1, 2]. In this contribution, we investigate emission and absorption from finite semiconductor NWs, theoretically and experimentally (Fourier microscopy and cathodoluminescence polarimetry) demonstrating their behavior as optical absorbers [3, 4] and nanoantennas [5,6].

The description of light absorption at finite semiconductor NWs based on Mie (theory) resonances prevails over a wide angular range, however failing at grazing incidences where leaky/guided modes begin to play a relevant role [3]. Conversely, we have also demonstrated that directional emission of polarized light is governed by the Fabry-Perot-like guided/leaky mode resonances that mediate the emission process [5,6]. Our results show that semiconductor NWs can efficiently emit and absorb electromagnetic radiation in defined direction/polarization/frequency, providing a solid theoretical framework to tailor the NW response to electromagnetic radiation, relevant to the design of: broad band and broad angle nanowire-based solar cells and photodetectors; and lighting devices such as LEDs, nanolasers, or single-photon source.

Acknowledgements

The authors acknowledge the Spanish “Ministerio de Economía y Competitividad” for financial support through the grants NANOPLAS+ (FIS2012-31070) and LENSBEAM (FIS2015-69295-C3-2-P), the “Stichting voor Fundamenteel Onderzoek der Materie (FOM)” as well as the Dutch technology foundation STW, which are financially supported by the “Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO)” and the Dutch Ministry of Economic Affairs. It is also part of NanoNextNL, a nanotechnology program funded by the Dutch Ministry of Economic Affairs, part of an industrial partnership program between Philips and FOM, and is supported by the European Research Council (ERC).

References

- [1] L. Cao, J. S. White, J. S. Park, J. A. Schuller, B. M. Clemens, and M. L. Brongersma, *Nat. Mater.*, **8**, 643 (2009).
- [2] J. Claudon, N. Gregersen, P. Lalanne, and J-M. Gérard, *Chem. Phys. Chem.*, **14**, 2393 (2013).
- [3] G. Grzela, R. Paniagua-Domínguez, T. Barten, D. Van Dam, J. A. Sánchez-Gil, and J. Gómez Rivas, *Nano Lett.*, **14**, 3227 (2014).
- [4] D. R. Abujetas, R. Paniagua-Domínguez, and J. A. Sánchez-Gil, *ACS Photonics*, **2**, 921 (2015).
- [5] D. Van Dam, D. R. Abujetas, R. Paniagua-Domínguez, J. A. Sánchez-Gil, Erik P. A. M. Bakkers, Jos E. M. Haverkort, and J. Gómez Rivas, *Nano Lett.*, **15**, 4557 (2015).
- [6] B. J. M. Brenny, D. R. Abujetas, D. Van Dam, J. A. Sánchez-Gil, J. Gómez Rivas and A. Polman, submitted.

Microfluidic Hydrothermal Synthesis of Highly Luminescent Carbon Dots

M. Berenguel-Alonso¹, A. Salinas-Castillo^{2,*}, B. Fernández³, C. S. Martínez-Cisneros¹, J. Alonso-Chamarro¹, L. F. Capitán-Vallvey², M. Puyol¹.

¹Group of Sensors and Biosensors, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain.

²ECsens, Dept. of Analytical Chemistry, University of Granada, 18071 Granada, Spain.

³Department of Inorganic Chemistry, Faculty of Sciences, University of Granada.

*alfonsos@ugr.es

Carbon dots (Cdots) have recently drawn great attention from the scientific community due to their excellent luminescent properties, high biocompatibility and low cost, compared to other luminescent nanomaterials [1]. These properties make Cdots suitable for a wide range of applications, such as (bio)sensing, bioimaging or nanomedicine [2].

The suitability of performing the direct carbonization of ascorbic acid in a heated microfluidic system was previously demonstrated, although low quantum yield Cdots were obtained [3]. In this sense, herein we present a ceramic microfluidic reactor for the synthesis of highly luminescent Cdots. The microreactor is fabricated using Low Temperature Co-fired Ceramics (LTCC), which enables the monolithic integration of both the fluidics and the heating systems. Moreover, we were able to monitor the reaction progress along the micromixer by using a thin ceramic layer that allowed the light to go through –although the bulk material is completely opaque– as well as seal the microreactor. The synthesis is based on a hydrothermal method and therefore a backpressure regulator was used in order to control the pressure inside the microsystem. Different reaction parameters have been studied, e.g. flow rates, temperature and pressure. The Cdots obtained exhibit a strong fluorescence emission band at around 450 nm.

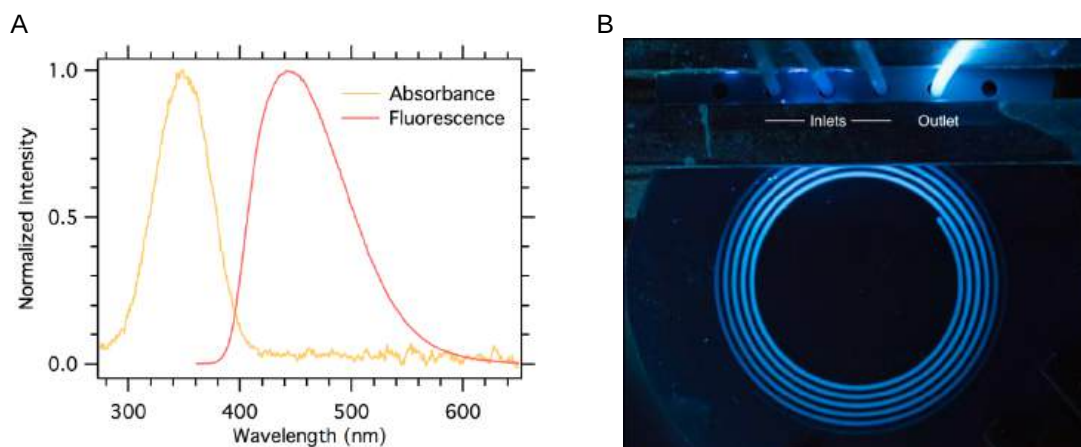


Figure 1. A) Normalized absorption and fluorescence spectra of the Cdots obtained. B) Microfluidic reactor during the synthesis. The increasing fluorescence along the mixer indicates the progress of the reaction.

Acknowledgements

This study was supported by projects from the Spanish MINECO and FEDER (CTQ2013-44545-R and projects CTQ2012-36165), Junta de Andalucía (P10-FQM-5974), the Government of Catalonia (SGR 2014-837), the "Fortalecimiento de Grupos", and "Reincorporación de Doctores" programs.

References

- [1] J. Esteves da Silva, H. Gonçalves, *TRAC Trend. Anal.Chem*, **30**, 1327 – 1336 (2011).
- [2] S.Lim, W. Shen and Z. Gao, *Chem. Soc. Rev.*, **44**, 362 – 381 (2015).
- [3] S. Gómez-de Pedro, A. Salinas-Castillo, M. Ariza-Avidad, A. Lapresta-Fernández, C. Sánchez-González, C.S. Martínez-Cisneros, M. Puyol, L.F. Capitan-Vallvey and J. Alonso-Chamarro, *Nanoscale*, **6**, 6018 – 6024 (2014).

Field-Flow Fractionation coupled to different detectors as a tool for the characterization of engineered nanoparticles

J. M. Costa-Fernández*, M. Menéndez-Miranda, D. Bouzas-Ramos, A. Sanz-Medel.
Department of Physical and Analytical Chemistry. University of Oviedo. Avda. Julián Clavería, 8. 33006 Oviedo, Spain.
*jcostafe@uniovi.es

The development of robust and sensitive bioanalytical applications of engineered nanoparticles (NPs) demands for analytical tools able to: characterize the nanoparticle core, guide the solubilization process and control the bioconjugation reaction of the nanoparticles to recognition units, such as antibodies and aptamers, which are specific for the target biomolecules of interest [1]. Traditional NP characterization techniques, such as UV-Vis, Fluorescence, Dynamic light Scattering (DLS) and Transmission Electron Microscopy (TEM) are typically used to get information about size, size distribution, NP concentration and hydrodynamic radius. However, sensitivity and information about NP composition in complex samples is very limited.

Asymmetrical flow field-flow fractionation (AF4) is one of the most promising separation techniques able to achieve size-dependent separation of nanoscale particles ranging from <1 nm up to several μm [2]. Hybridation of the AF4 with different detectors could provide nanoparticle sizing and compositional analysis: information of great value to achieve a proper characterization of a nanoparticle colloidal solution.

Here, on-line coupling of AF4 to fluorescence and ICP-MS detectors is proposed as a powerful diagnostic tool for control the quality of the product of the synthesis of quantum dots (QDs) nanoparticles. Besides, a strategy is presented for both, qualitative and quantitative assessment of the bioconjugation of CdSe/ZnS QDs to proteins based on the use of an optimized AF4 separation. In particular, conjugation effectiveness between a "model" monoclonal IgG antibody (Ab) and CdSe/ZnS core-shell Quantum Dots (QDs), surface-coated with an amphiphilic polymer, has been monitored here by such hybrid technique. The hyphenated technique used provided nanoparticle size-based separation, elemental detection, and composition analysis capabilities that turned out to be instrumental in order to investigate in depth the Ab-QDs bioconjugation process [3].

Moreover, the analytical strategy here proposed allowed us not only to clearly identify the bioconjugation reaction products but also to quantify nanoparticle: antibodies bioconjugation efficiency. This is a key issue in future development of analytical and bioanalytical photoluminescent QDs applications.

References

- [1] Jose-Maria Montenegro, V. Grazu, A. Sukhanova, S. Agarwal, J. M. de la Fuente, I. Nabiev, A. Greiner, W. J. Parak, *Adv. Drug Delivery Reviews*, **65**, 677 (2013).
- [2] A.J. Bednar, A.R. Poda, D.M. Mitrano, A.J. Kennedy, E.P. Gray, J.F. Ranville, C.A. Hayes, F.H. Crocker, A.J. Steevens, *Talanta*, **104**, 140 (2013).
- [3] M. Menéndez-Miranda; J. Ruiz Encinar; J. M. Costa Fernandez, A. Sanz-Medel, *Journal of Chromatography A*, **1422**, 247 (2015).

Characterization and quantification of silver nanoparticles in chicken meat during the *in-vitro* human gastrointestinal digestion by single particle ICP-MS

K. Ramos¹, L. Ramos², **M. M. Gómez-Gómez^{1,*}**.

¹Department of Analytical Chemistry, Faculty of Chemistry, Universidad Complutense de Madrid, Avenida Complutense s/n, 28040 Madrid, Spain.

²Department of Instrumental Analysis and Environmental Chemistry, IQOG-CISC, Juan de la Cierva 3, 28006 Madrid, Spain.

*mmgomez@ucm.es

Many food sectors are taking advance of the antibacterial properties of silver nanoparticles (AgNPs) to develop food supplements, animal feed products and packaging materials with improved properties. However, these new uses can result in their presence of AgNPs in food. Once AgNPs reach humans, their fate and possible transformation remains unclear. In this study, a chicken meat containing AgNPs (CRMs Nanolyse 14) [1] was used as a model matrix to study the fate of AgNPs upon oral ingestion following an *in-vitro* gastrointestinal digestion model. The behaviour of AgNPs standard solutions of 60 nm was evaluated in parallel. Sample preparation conditions were optimized to prevent AgNPs oxidation and/or aggregation. At each stage of the digestion process (salivary, gastric and intestinal) the total silver released from the tested sample and standard was determined by ICPMS. The presence of dissolved silver forms was evaluated by ultrafiltration and analysis of the filtrate. The presence of both AgNPs and dissolved silver was confirmed in the three extracts of both the 60 nm AgNPs standards and the chicken meat extracts by evaluation of the raw transient signal data obtained by single particle (SP)-ICPMS analysis [2]. AgNPs was characterized and quantified in terms of size distribution and particle number concentration. The studies show a silver released of 35% in the saliva extract, which increases to 54% and 57% in the case of the gastric and intestine stages, respectively. AgNPs in salivary extracts have a maximum size of 28-30 nm, but in the stomach and intestine extracts these value increased to 36-38 nm. The proposed methodology provided accurate information regarding silver speciation and AgNPs size distribution and number concentration in complex extracts, with minimum sample manipulation and involving short analysis time, which minimized the risk of AgNPs agglomeration and/or oxidation.

Acknowledgements

The authors thank MINECO (CTQ2014-55711-R and CTQ2012-32957), the Community of Madrid (S2013/ABI-3028) and the European FEDER program for founding.

References

- [1] R.J.B. Peters, et al. *Ana. Bioanal Chem*, **406**, 3875-3885 (2014).
- [2] K. Ramos, M.M. Gomez-Gómez, C. Cámara, L. Ramos. *Talanta*, **151**, 83-90 (2016).

Use of a pool of spectroscopic tools for the determination of the nanoparticle concentration and ligand density of engineered water-soluble HgSe nanoparticles

D. Bouzas-Ramos^{1,*}, M. Menéndez-Miranda¹, Z. Gajdosechova², E. Krupp², J. Feldmann², J. R. Encinar¹, J. M. Costa-Fernández¹, A. Sanz-Medel¹.

¹Department of Physical and Analytical Chemistry, University of Oviedo, Julián Clavería 8, 33006, Oviedo, Spain.

²University of Aberdeen, Meston Walk, Aberdeen, AB24 3UE, United Kingdom.

*dbouzasr@gmail.com

The toxicological antagonism between selenium (Se) and mercury (Hg) in animals has long been under comprehensive study up to the present day [1]. Nowadays it is well-known that the accumulation of Hg often accompanies Se accumulation at an equimolar ratio, often resulting in the formation of HgSe nanoparticles (NPs) in the tissues of biological organism. These NPs have been recognized as the ultimate product of mercury detoxification in biological systems [2]. However, the exact mechanism of their formation and distribution in animals is not understood and thus well-characterized engineered NPs would be invaluable to investigate such metabolic products.

In this work, HgSe NPs were sonochemically synthesized and transferred for the first time to aqueous media using dihydrolipoic acid (DHLA) as nanoparticle capping ligand. A thorough characterization of the engineered HgSe NPs was carried out. Different complementary techniques, including high resolution-transmission electron microscopy (HR-TEM) and X-ray powder diffraction (XRD), provided information about the morphology and crystal structure of the NPs. X-ray photoelectron spectroscopy (XPS) and energy dispersive X-ray spectrometer (EDX) analysis were used in order to assess the chemical composition and purity of the same HgSe NPs. Moreover, fourier transform infrared spectroscopy (FT-IR), nuclear magnetic resonance (NMR), dynamic light scattering (DLS), zeta potential determinations and asymmetric flow field-flow fractionation (AF4) coupled on-line to fluorescence and inductively coupled plasma-mass spectrometry (ICP-MS) confirmed that the DHLA ligands were bound onto the surface of the NPs making them stable in aqueous solutions. Fluorescence measurements evidenced that the engineered NPs exhibit a photoluminescence emission centred at 575 nm.

Finally, the Se/Hg molar ratio of the NPs was accurately determined by ICP-MS. Such valuable information was used to precisely determine the nanoparticle concentration in aqueous solution (in terms of number of nanoparticles per unit volume) and the ligand density (in terms of number of DHLA molecules per nanoparticle).

Acknowledgements

This work was supported by the Spanish Ministry of Education (MINECO-13-CTQ2013-49032-C2-1-R) and Principado de Asturias (FC-15-GRUPIN14-092). Agilent Technologies Foundation is also acknowledged. D. Bouzas-Ramos and M. Menéndez-Miranda acknowledge the Ph.D. grants (BP14-137 and BP12-046, respectively) from Principado de Asturias (Spain).

References

- [1] D. Y. Yang, Y. W. Chen, J. M. Gunn and N. Belzile, *Environ. Rev.*, **16**, 71 – 92 (2008).
- [2] M. A. K. Khan and F. Wang, *Chem. Res. Toxicol.*, **23**, 1202 – 1206 (2010).

Determination of the ratio of fluorophore/nanoparticle for fluorescence-labelled nanoparticles

M. Menéndez-Miranda^{1,2,*}, J. M. Costa-Fernández², J. R. Encinar², A. Sanz-Medel, W. J. Parak¹, C. Carrillo-Carrion¹.

¹*Fachbereich Physik, Philipps Universität Marburg, 35037 Marburg, Germany.*

²*Department of Physical and Analytical Chemistry, University of Oviedo, 33006.*

**mariomemi@gmail.com*

Optical analysis based on fluorescence labelling has been extensively used for the selective tagging of a wide range of biomedical important targets or for sensing purposes. Fluorescent nanoparticles (NPs) offer interesting properties as labels, as they can be also used as active labels that change their properties upon changes in the environment, such as pH- or distance-dependent fluorescence. In case NPs are not intrinsically fluorescent, they can be made fluorescent by attaching fluorophores to their volume and/or surface. Dye-labelled NPs can produce a highly amplified optical signal compared to a single dye molecule, as there are many dye molecule attached to each NP, providing a great improvement in analytical sensitivity. However, an appropriate control to quantify the fluorophore/NP ratio is required to succeed in the preparation of quantitative platforms matching the required application. Here a methodology to determine such parameter, the fluorophore/NP ratio, is presented. The methodology combines data obtained from UV/Vis absorption spectroscopy for determination of the dye concentration and inductively coupled plasma mass spectrometry (ICP-MS) analysis for determination of the NP concentration. To validate the approach, it has been applied to the analysis of different sets of fluorophore-NP conjugates prepared using diverse fluorescent dyes (i.e. fluorophores with different structures and emissions) and several types of NPs (i.e. PbS QDs, Au NPs and FePt NPs). The fluorophore-NP conjugates hereby were designed to incorporate the dye directly into an amphiphilic polymer coating. The developed methodology allows for quantification of fluorophore-NP coupling, and therefore, opens up the possibility of selecting controlled conjugates.

Acknowledgements

This work was supported by the European Commission (project FutureNanoNeeds to WJP). MMM acknowledges the Ph.D. grant (BP12-046) from Principado de Asturias (Spain). CCC acknowledges the Alexander von Humboldt Foundation for a PostDoc fellowship. We thank to Dr. Pablo del Pino and Mrs. Marta Gallego for assisting with the TEM imaging.

Gold nanoparticles and dissolved gold species determination in culture medium and cells by coupled hydrodynamic separation techniques to ICP-MS

S. López-Sanz^{1,*}, N. Rodríguez-Fariñas¹, R. Serrano-Vargas²,
R. C. Rodríguez-Martín-Doimeadios¹, A. Ríos-Castro³.

¹Department of Analytical Chemistry and Food Technology, Environmental Sciences Institute (ICAM), University of Castilla-La Mancha, Avda. Carlos III s/n, 45071 Toledo, Spain.

²Department of Inorganic Chemistry, Organic and Biochemistry, Faculty of Environmental Sciences and Biochemistry, University of Castilla-La Mancha, Avda. Carlos III s/n, 45071 Toledo, Spain.

³Department of Analytical Chemistry and Food Technology, Faculty of Chemical Sciences and Technologies, University of Castilla-La Mancha, Avda. Camilo José Cela s/n, 13071 Ciudad Real, Spain.
*sara.lopez@clm.es

The use of gold nanoparticles (AuNPs) in different fields has rapidly grown in the last decades, but at the same time, there is also a growing concern about their potential toxicity on biological systems. Nanotoxicity test in vitro with cells are applied to evaluate this risk. Dynamic formation of protein coating around the NPs and liberation of ions may impact the biological response and the characterization of NPs in this media represents a challenging issue. For this purpose, the use of separation techniques (such as HPLC) coupled to specific detectors (such as UV-Vis and ICP-MS) is a promising option. Therefore, in this study a methodology has been developed for the characterization and identification of AuNPs and gold dissolved species (Au^{3+}) in culture medium (DMEM) and HeLa cells used in nanotoxicity tests. The presence of the culture medium produces a shift towards larger hydrodynamic diameters, which suggests the formation of a protein corona. These results were confirmed in the analysis of cells and supernatants obtained from the nanotoxicity test with AuNPs and Au^{3+} . Moreover, the AuNPs could suffer an oxidation process in the supernatants. Only a small amount of AuNPs and Au^{3+} standards was internalized by cells. To evaluate the biological impact of AuNPs, a classical viability assay onto HeLa cells was performed using cellular media DMEM in the presence of increasing dosage of AuNPs. The results showed that AuNPs exhibit a slight toxic effect.

Acknowledgements

Project MINECO CTQ-2013-48411-P and predoctoral contract MINECO BES-2014-069095, and project PEIC-2014-001-P from Junta de Comunidades de Castilla – La Mancha.

Mapping of Zn and MMP-11 in breast tissues by LA-MS

M. L. Fernández-Sánchez^{1*}, R. González de Vega¹, J. Pisonero², N. Eiró³, F. J. Vizoso³, A. Sanz-Medel¹.

¹Department of Physical and Analytical Chemistry, University of Oviedo, Spain.

²Department of Physics, University of Oviedo, Spain.

³Research Unit, Hospital Jove Foundation, Gijón, Spain.

*marisafs@uniovi.es

The matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases. The main functions of this metalloproteinases are the degradation of the stromal connective tissue and basement membrane components]. In healthy tissues the activity of the metalloproteinases is regulated by specific endogenous tissue inhibitors (TIMPs). Recent data from model systems suggest that MMPs are involved in breast cancer (BC) initiation, invasion and metastasis[1].

Particularly, MMP-11 (Stromelysin-3) is expressed in stromal fibroblasts adjacent to epithelial tumor cells, and high levels of this metalloproteinase were associated with tumor progression and poor prognosis of BC [2]. Consequently, MMP-11 involved in these processes can be a candidate to be identified as new prognostic biomarker in BC.

In other hand, several studies have shown that concentrations of essential trace elements (Fe, Cu, Zn and Ca), naturally present in breast tissues may be significantly increased in BC tissues. Bioimaging analytical techniques, based in laser ablation and mass spectrometry LA-MS, have been rapidly growing in biology and medicine for studies of biological systems to provide information of biomolecules and metals with lateral resolution at the micrometer scale.

Elemental mass spectrometry based analytical methods (e.g. laser ablation inductively coupled plasma mass spectrometry, LA-ICP-MS) have been developed for bioimaging of essential trace metals (Ca, Fe, Cu and Zn) and no metals (S, P and Se) in biological tissues. Moreover, combinations of immunohistochemistry (IHC) assays with LA-ICP-MS technique and/or matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) have been widely applied for imaging and mapping of expression of protein biomarkers (MMPs, HER2, MUC-1) implicated in breast cancer with high sensitivity and high local resolution.

The aim of this work is the development of new strategies based on LA-ICP-MS and/or MALDI-MS to investigate the distribution of Zn and MMP-11 in normal and cancer breast tissues. The results obtained showed that the levels of Zn and MMP-11 in cancer breast tissues were significantly higher than those found in normal ones. The elemental and molecular spatial distributions were correlated with histological aspects in breast tissues. The results usefulness for diagnostic, prognostic and treatment purposes of breast cancer will be discussed.

References

- [1] M.J. Duffy, T. Maguire, A. Hill, E. McDermott and N. O'Higgins. *Breast cancer research*, **2**, 252-257, (2000).
- [2] L.O. González, I. Pidal, S. Junquera, M.D. Corte, J. Vázquez, J.C. Rodríguez, M.L. Lamelas, A.M. Merino, J.L. García-Muñiz and F.J. Vizoso. *British Journal of Cancer*, **97**, 957-963, (2007).

Anomalies in the structure of liquid water under pressure and temperature as revealed by Raman Spectroscopy

A. Lobato^{1*}, M. Cáceres¹, L. Bonales², O. R. Montoro¹, M. Taravillo¹, V. G. Baonza¹.

¹MALTA-Consolider Team, Departamento de Química-Física I, Facultad de Ciencias Químicas, Universidad Complutense, Madrid, 28040, Spain.

²Unidad de Residuos de Alta Actividad, Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT), Madrid, 28040, Spain.

*a.lobato@ucm.es

Raman spectroscopy have been proved to be a suitable technique to study inter/intramolecular interactions and structural information [1]. Frequency changes of a given stretching vibration are directly related to the strengthening / weakening of those bonds involved with the molecular environment. On the other hand, hydrogen-bonding is responsible of the anomalous properties of water. Its rich phase diagram, the maxima and minima found in its thermodynamic response functions or the possible existence of two interconvertible species generally known as High Density Water and Low Density Water are only some examples of the outstanding properties of water, originated by this interaction [2]. Thus, the debate about the structure of water is still an open question.

Here, we present a study of the pressure (up to 1 GPa) and temperature dependence (260-500 K) of the Raman OH-stretching band of water. Assuming that this band can be split in five contributions, which depend on the different donor-acceptor character of the hydrogen bond [3], we are able to explain the changes on water structure with varying pressure and temperature. Our results reveal that pressure produces the collapse of the molecules of the second shell into the first shell of the water, whereas the temperature produces a unique sequential rupture of the hydrogen bonds. This analysis is in excellent agreement with the pressure analysis of the radial distribution function given by Soper et al. [4], whereas the temperature analysis reproduces the maximum density and it is consistent with the X-ray spectroscopy data, thus providing a global picture about the structure and anomalous behavior of liquid water.

Acknowledgements

Financial support from Ministerio de Economía y Competitividad of Spain (MAT2015-71070-REDC) and CT2015-67755-C2-1-R is acknowledged. A.L. is grateful to the Spanish Ministerio de Educación, Cultura y Deporte for an FPU grant.

References

- [1] W. B. Bosma, L. E. Fried and S. Mukamel, *J. Chem. Phys.*, **98**, 4413-4421 (1993).
- [2] J. R. Errington and P. G. Debenedetti, *Nature*, **409**, 318-321 (2001).
- [3] Q. Sun, *Vib. Spectrosc.*, **51**, 213-217 (2009).
- [4] A. K. Soper and M. A. Ricci, *Phys. Rev. Lett.*, **84**, 2881-2884 (2000).

Analysis of REE in basalt, cement, shale, rock and stream sediment CRMS by ICP-QMS

R. M. Santos*, S. Wünscher, H. Gleisner, R. Chemnitzer.

Analytik Jena AG, Analytical Instrumentation, Konrad-Zuse-Strasse 1, 07745 Jena / Germany.

**rui.santos@analytik-jena.com*

Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) is at the present time the most used instrumental method for rare earth elements (REE) quantification. Nevertheless, its applicability in the analysis of geological samples exhibits some constraints, since the accuracy of the data is largely dependent, both on complete sample dissolution and adequate correction of polyatomic species MO⁺ and MOH⁺. Within this method development, five geological reference materials, three from Geopt proficiency-testing program: HPT-1, OPC-1, SBC-1, and two from MC Chinese Reference Materials: GBW 7103 and GBW 7359, were employed to validate the options made for sample digestion and polyatomic corrections. The obtained analytical results for samples of different matrices demonstrate the applicability of this method and instrument in various geological studies such as petrology and mineralogy. The PlasmaQuant® MS Elite allows for a significant reduction of oxide / hydroxide levels in collision gas mode while achieving high sensitivity. This results in limits of detection of 0.1 to 2 ppt in the presence of Na₂O₂ matrix and allows for running the analysis of REE without pre-defined correction equations. It is therefore a perfect tool for the analysis of REE in different geological applications.

Acknowledgements

Analytik Jena AG wants to thank the Geological Survey of Portugal (LNEG) for performing the digestion procedure of the investigated geological reference materials.

New frontiers in very small size nanoparticle analysis: Triple quadrupole ICP-MS (ICP-MS QQQ) for single nanoparticle detection of SiO₂ and Au materials

M. Yamanaka¹, T. Itagaki¹, S. Wilbur¹, S. Nunez², H. Goenaga-Infante², **N. Kovachev¹**.

¹Agilent Technologies.

²LGC Limited.

*nikolay.kovachev@agilent.com

Nanomaterials are increasingly used in industrial processes, manufactured goods, medicines, and consumer products such as cosmetics, sunscreen, and food. The measurement of nanoparticles (NPs) is the focus of attention because the fate of NPs in the environment and the potential for toxic effects once absorbed into the body are not yet fully understood.

ICP-MS is a well-established technique for measuring the elemental content of materials; the relatively recent development of Single Particle acquisition mode (spICP-MS) now provides a powerful method to characterize the NP content of a sample.

However, the detection of very small nanoparticles (<20 nm) remains challenging for spICP-MS, due to the low signal generated from such particles. Particle mass decreases as the cube of the diameter, so a factor of 2 reduction in particle diameter (for example from 60 nm to 30 nm) results in an 8x reduction in mass and therefore signal.

In addition, some nanoparticles are difficult to detect because of the inherent low sensitivity of the elements they are composed by, or due to the presence of strong polyatomic interferences. The Agilent 8900 Triple Quadrupole ICP-MS (ICP-QQQ) addresses these drawbacks due its background, high sensitivity and effective control of interferences with MS/MS, which ensure that even very small NPs can be easily distinguished from background signal.

Au and SiO₂ NPs were measured in spICP-MS mode using MS/MS on the Agilent 8900 ICP-QQQ, equipped with Single Nanoparticle Application Module software. The capabilities of the instrument for the measurement of these materials will be shown and discussed.

Fast and simultaneous mercury speciation analysis in hair samples by gas chromatography coupled to atomic fluorescence spectroscopy

M. Jiménez-Moreno^{1,*}, G. P. F. Arrifano², M. E. Crespo-López²,
R. C. Rodríguez-Martín-Doimeadios¹.

¹Department of Analytical Chemistry and Food Technology, Faculty of Environmental Sciences and Biochemistry, University of Castilla-La Mancha, Avenida Carlos III, s/n – 45071 Toledo, Spain

²Laboratório de Farmacologia Molecular, Instituto de Ciências Biológicas, Universidade Federal do Pará, Av. Augusto Correa, 01. Campus do Guamá, 66075110 Belém, Brazil.

*maria.jimenez@uclm.es

Mercury (Hg) is a naturally occurring pollutant that affects human health, especially when it is in form of organic compounds. The main route of human exposure to Hg is the consumption of fish and seafood (where Hg is accumulated as the very important neurotoxin monomethylmercury, MeHg) but the inhalation of Hg vapour (mainly Hg⁰) is also important in mining areas. To evaluate the exposure of population to these Hg sources there are different approaches but hair is one of the best options for different reasons. It is known that hair samples are representative of cumulative exposure to Hg, the sampling is not invasive and the transport and conservation of the sample is easy. Therefore, analytical methods enabling the determination of different Hg species (mainly MeHg and inorganic Hg) in hair are necessary in order to assess potential Hg health risks. Traditional sample preparation procedures for Hg speciation in hair usually involve very tedious and time-consuming extraction procedures leading to potential errors such as artifact formation of Hg forms. Thus, this work was aimed to develop a fast and simple method for the simultaneous and quantitative determination of Hg species in hair samples. The extraction of Hg species by closed-vessel microwave extraction was carefully optimized evaluating different parameters, such as extracting agent, irradiation temperature, heating time or sample weight. The finally proposed methodology consists in an acidic (6 N nitric acid) microwave extraction and a subsequent analysis by gas chromatography coupled to atomic fluorescence detection after derivatization by ethylation. A preconcentration step using a nitrogen stream was also included when Hg species concentrations were not detectable. The procedural detection limits, after preconcentration, were 2.4 and 2.0 ng/g for MeHg and inorganic Hg, respectively. The optimized method was validated using the hair certified reference material ERM-DB001 obtaining MeHg (254±23 ng/g) and inorganic Hg (119±28 ng/g) concentrations in good agreement with the certified concentration for total Hg (365±28 ng/g) and the proposed value for MeHg (240 ng/g). An additional validation was also conducted by analysing real hair samples using the proposed method and an independent method which is based on the pyrolysis of the sample and provides information only about total Hg. The sum of Hg species obtained by the developed method were in excellent agreement with the total Hg concentration provided by the independent method. With the new proposed procedure a considerable reduction of the time of analysis was achieved compared to other methods giving information about individual Hg species.

Acknowledgements

The authors acknowledge to the *Spanish Ministry of Economy and Competitiveness* (CTQ2013-48411-9) and the *Brazilian Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) (467143/2014-5 and 447568/2014-0) for financial support.

Low cost extraction in Analytical Chemistry. What else?

S. Armenta, M. de la Guardia, **F. A. Esteve-Turrillas***

Department of Analytical Chemistry, University of Valencia, 50th Dr. Moliner St., 46100 Burjassot, Spain.

**Francesc.A.Esteve@uv.es*

The use of a Nespresso coffee machine for the pressurized extraction of polycyclic aromatic hydrocarbons (PAHs) in soils has been employed as a model for the evaluation of the exciting possibilities of this popular system. Nespresso devices works for coffee extraction at 72°C and 19 bars and, thus based on that we have evaluated the use of different acetonitrile:water mixtures and experimental conditions to do the complete extraction of PAHs from polluted soils.

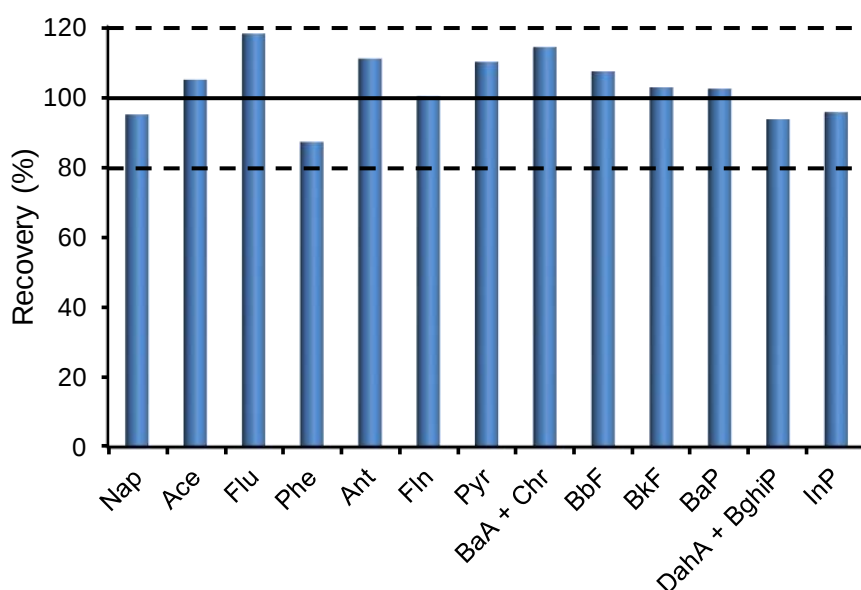
Preliminary studies on aged spiked samples evidenced that 75 mL of 40:60 acetonitrile:water mixtures are enough to do the complete extraction of 16 priority PAHs in a single step from 5 g of soil mixed with Spe-ed matrix hygroscopic dispersing agent and using a borosilicate filter to avoid sample losses. After extraction, PAHs were determined by liquid chromatography (LC) using molecular fluorescence detection (FD). The whole extraction process were made in 20 seconds and the chromatography time involved 25 minutes for each run.

The method has been applied to the evaluation of the presence of PAHs in contaminated soils, sediments and road dusts and the accuracy was checked using two different certified reference materials.

The limit of detection (LOD) of the developed procedure ranged between 2 and 128 $\mu\text{g kg}^{-1}$ with relative standard deviation (RSD) values of the order of 5% at $\mu\text{g kg}^{-1}$ concentration level.

In short, the use of this system is a cheap and fast green alternative to the reference procedure which involves the use of 100 mL pure acetonitrile for 30 minutes in an ultrasound water bath.

This study opens new possibilities for the laboratory use of the Nespresso coffee machines for the extraction of samples and additional works are in due course in our laboratory for food, industrial and environmental samples to do the determination of mineral elements and their chemical species and organic compounds like pesticides, pharmaceuticals and detergents.



Acknowledgements

Authors gratefully acknowledge the financial support of the Ministerio de Economía y Competitividad (CTQ-2012-38635 and CTQ-2014-52841) and Generalitat Valenciana (PROMETEO-II 2014-077).

Monitoring of polycyclic aromatic hydrocarbons in water by magnetic solid phase extraction based on magnetic nanoparticles coated with multi-walled carbon nanotubes

A. I. Corps-Ricardo¹, F. J. Guzmán-Bernardo¹, M. Zougagh²,
R. C. Rodríguez-Martín-Doimeadios^{1,*}, A. Ríos-Castro².

¹University of Castilla-La Mancha, Faculty of Environmental Sciences and Biochemistry. Carlos III s/n Ave. 45071, Toledo, Spain.

²University of Castilla-La Mancha, Faculty of Chemical Sciences and Technologies. 9, Camilo José Cela Ave. 13071 Ciudad Real, Spain.

*rosacarmen.rodriguez@uclm.es

Magnetic solid phase extraction (MSPE) is one of the emerging techniques for sample preparation. It avoids time-consuming and tedious on-column SPE procedures and it provides a rapid and simple analyte separation without the need for centrifugation or filtration steps [1]. This approach is especially interesting for the monitoring of organic pollutants in environmental samples.

Polycyclic aromatic hydrocarbons (PAHs) are most worrying organic pollutants. These are hazardous chemical compounds due to their high toxicity, mutagenicity, carcinogenicity, persistency and bioaccumulation properties [2]. High performance liquid chromatography–ultraviolet detection coupled to fluorescence detection (HPLC-FLD) is among the most used techniques for the PAHs determination in different environmental matrices. Concerning to sample preparation, because of their planar structure, PAHs can be adsorbed onto carbon nanotubes by means of a strong π - π interaction.

In this work, we have used Fe₃O₄ magnetic nanoparticles coated with multi walled carbon nanotubes (MNP-MWCNT) as MSPE sorbent material for the determination of seven PAH congeners in water. The congeners monitored were naphthalene (NAPH), acenaphthene (ACE), fluorene (FLU), phenanthrene (PHEN), anthracene (ANTH), pyrene (PYR) and flouranthene (FLT).

HPLC-FLD was used for the identification and quantification of the analytes. The separation was carried out in a Zorbax Eclipse PAH column (2.1 x 50 mm x 1.8 μ m) and water:acetonitrile was used as mobile phase at 0.42 mL/min. The gradient was as follows: 40% acetonitrile (t=0 to 0.45 min) and then increased to 100% (t = 6 min) for 3 min. After that, the mobile phase was returned to initial conditions in 0.5 min and kept for 3.5 min. The total run time was 13 min. Oven temperature was set at 25°C. The excitation wavelength was 260 nm and the emission wavelengths were 350 nm (NAPH, ACE, FLU and PHEN), 420 nm (ANTH, PYR) and 500 nm (FLT). The instrumental limits of detection and quantification were 0.01 – 0.73 μ g/L and 0.04 – 2.4 μ g/L, respectively.

The parameters affecting the sorption and desorption steps were optimized. After checking that the adsorption of the analytes was complete, the supernatant was separated from the MNP-MWCNTs by means of an external magnet. The recovery studies showed values from 73 to 107 %. The methodology was applied to several samples of synthetic water spiked with PAHs at different concentrations and containing different amounts of humic acids and ionic strength, with satisfactory results.

ü

Acknowledgements

The authors thank the Ministerio de Economía y Competitividad (Project CTQ2013-48411-P) and the Junta de Comunidades de Castilla – La Mancha (Project PEIC-2014-001-P) for supporting this work.

References

- [1] C. Herrero, J. Barciela, S. García, R.M. Peña and J. Otárola, *Anal Chim Acta*, **892**, 10 – 26 (2015).
- [2] H. Costa, S. M. Resende, D. Freire, A. Dias, B. Fernades, A. P. Carvalho, R. Monteiro, P. Serp, Z. L. Cardeal, *Anal Chim Acta*, **873**, 51 – 56 (2015).

Use of near infrared hyperspectral tools for the screening of extractable polyphenols in red grape skins

J. Nogales-Bueno¹, B. Baca-Bocanegra¹, F. J. Rodríguez-Pulido¹, F. J. Heredia¹,
J. M. Hernández-Hierro^{1,*}.

¹ Food Colour & Quality Laboratory, Department of Nutrition & Food Science, Universidad de Sevilla.

*jmhhierro@us.es

Red grapes (*Vitis vinifera* L.) contain about four grams of phenolic material per kilo. There is a substantial variation in the levels of phenolic compounds which depends on a number of factors including the variety of grape, high or low skin:volume ratio, growing region, climate, and growth conditions [1]. It is also really important to know the amount of these phenols that may be extracted from grapes to wine. Hyperspectral imaging is an emerging and rapid technique for non-destructive food analysis usually carried out in either the visible-short near infrared or near infrared spectral regions [2].

Hyperspectral images of intact grapes were recorded at harvest time using a near infrared hyperspectral imaging system (900 - 1700 nm). Spectral data have been correlated with red grape skin extractable polyphenols (total phenolic, anthocyanins and flavanols) by modified partial least squares regression (MPLS) using a number of spectral pretreatments. The obtained results (coefficient of determination (RSQ) and standard error of prediction (SEP) respectively) for the developed models were: 0.82 and 0.92 mg g⁻¹ of grape skin for extractable total phenolic content, 0.79 and 0.63 mg g⁻¹ of grape skin for extractable anthocyanin content, 0.82 and 0.45 mg g⁻¹ of grape skin for extractable flavanol content [3]. The procedure reported here using near infrared hyperspectral imaging presents a good potential for a fast and reasonably inexpensive screening of the extractable polyphenolic compounds in intact grapes. In addition, the heterogeneity of extractable polyphenols within the same ripeness stage has been observed. This methodology allows sorting the berries according to their polyphenol extractable contents and then the same samples could be used in further studies for other destructive analyses or purposes. Furthermore, differences between Syrah and Tempranillo extractable polyphenols have been also found. Nonetheless, a comprehensive study should be made in order to evaluate factors, such as different production areas and grape varieties, in the complete development of these models.

Acknowledgements

The Spanish MINECO is thanked for J. Nogales-Bueno FPI grant (BES-2012-060192) and project AGL2014-58486-C2. Universidad de Sevilla is thanked for J.M. Hernández-Hierro extension of Juan de la Cierva postdoctoral contract and also for B. Baca-Bocanegra predoctoral grant (VPPI-Universidad de Sevilla). The authors also thank the technical staff of Biology Service [Servicios Generales de Investigación (SGI), Universidad de Sevilla].

References

- [1] A. Crozier, M. N. Clifford, and H. Ashihara. Plant Secondary Metabolites. Occurrence, Structure and Role in the Human Diet, Blackwell Publishing: Oxford, 2006.
- [2] A. A. Gowen, C. P. O'Donnell, P. J. Cullen, G. Downey and J. M. Frias, *Trends Food Sci Tech*, **18**, 590 – 598 (2007).
- [3] J. Nogales-Bueno, B. Baca-Bocanegra, F. J. Rodríguez-Pulido, F. J. Heredia and J. M. Hernández-Hierro, *Food Chem*, **172**, 559 – 564 (2015).

Analysis and evolution of black decoration in ceramics of the Iberians by MRS and EDXRF

J. Tuñón¹, A. Sánchez^{1,*}, D. J. Parras¹, M. Montejo², P. Vandenabeele³, M. O. Rodríguez¹, C. Rísquez¹, A. Martínez¹, M. P. G. Rodríguez².

¹University Research Institute for Iberian Archaeology. University of Jaén. University Campus, Building C6, 23071, Jaén, Spain.

²Department of Physical and Analytical Chemistry. University of Jaén. University Campus, Building C6, 23071, Jaén, Spain.

³Department of Archaeology. University of Ghent. Blandijnberg 2, B-9000, Ghent, Belgium.

*vizcaino@ujaen.es

The knowledge and dissemination of the culture of Iberians (Iron Age, south, east and north of the Iberian Peninsula, 6th-1st century B.C.) have experienced great progress within the last two decades, becoming one of the most important fields in the study of the Protohistory of the Iberian Peninsula. Aspects such as the processes of manufacturing and decoration of different kind of artifacts, and the choice of raw materials, including pigments, have focused our attention and have been the subject of detailed studies [1, 2, 3]. The analyzes performed have focused on the study of the mineral and elemental composition of the materials selected using instrumental techniques such as micro Raman spectroscopy (MRS) and energy dispersive X-ray fluorescence (EDXRF). This work consists of the analysis of black decorated Iberian potteries from 10 archaeological sites in the Spanish provinces of Jaen, Granada, Málaga and Ciudad Real. This selection of samples covers a long temporary range (6th-3rd ct. BC.), and several contexts (funerary, ritual, military and domestic).

The origin and elaboration of black decoration in Iberian ceramics is here identified for the first time using MRS and EDXRF. A distinction must be made between black in oxidation firing pottery vs. black in reduction firing pottery. Once amorphous carbon was abandoned in 6th ct. BC., black decoration in oxidation firing, usually combined with red bands, was obtained from some kind of manganese oxide (jacobite, hausmannite, bixbyite).

A second stage in the use of black decoration opens when use of manganese oxide is abandoned from the 4th ct. BC. From that period onwards, red and black combinations result from use of hematite and a double (oxidation and reduction) firing. This is why the Raman spectrum of black colour is magnetite. This type of decoration has a clear territorial meaning: it is characteristic of the eastern province of Jaen and the south of Ciudad Real in the third century B.C.

All information obtained will be shared by means of on-line catalogue and database currently under development that will provide information on archaeological contexts, chemical composition, and details of the analytical methodology, also incorporating 3D models of the materials studied using 3D viewer on the Sketchfab website.

Acknowledgements

This work has been developed under the Arquiberlab Project (Project of Excellence Junta de Andalucía. HUM-7459).

References

- [1] Parras D, Montejo M, Ramos N, Sánchez A. Analysis of pigments and coverings by X-Ray Diffraction (XRD) and Micro Raman Spectroscopy (MRS) in the cemetery of Tútugi (Galera, Granada, Spain) and the settlement Convento 2 (Montemayor, Córdoba, Spain). *Spect. Acta Part A*, 64, (2006): 1133.
- [2] D, Parras. P, Vandenabeele. A, Sánchez. M, Montejo, L, Moens. N, Ramos. Micro-Raman spectroscopy of decorated pottery from the Iberian archaeological site of Puente Tablas (Jaén, Spain, 7th-4th century B.C.). *Journal of Raman Spectroscopy* 41, (2010): 68.
- [3] A. Sánchez, J. Tuñón, M. Montejo, D. Parras, Micro Raman spectroscopy (MRS) and energy dispersive x-ray microfluorescence (μ EDXRF) analysis of pigments in the Iberian cemetery of *Tutugi* (from the fourth to the third century B.C., Galera, Granada, Spain). *Journal of Raman Spectroscopy*; 43 (11), (2012): 1788.

LIBS in cultural heritage: recognition and identification of objects in an underwater archaeological shipwreck

M. López-Claros, F. J. Fortes, S. Guirado, J. J. Laserna*

Department of Analytical Chemistry, Faculty of Sciences, University of Malaga, Campus de Teatinos s/n, 29071, Malaga. <http://www.laser.uma.es>

*laserna@uma.es

Nowadays, one of the most important areas of interest in archeology is the characterization of the submersed cultural heritage. Mediterranean Sea is rich in archaeological findings due to storms, accidents and naval battles since prehistoric times. Chemical analysis of submerged materials is an extremely valuable source of information on the origin and precedence of the wrecks, and also the raw materials employed during the manufacturing of the objects found in these sites. Nevertheless, sometimes it is not possible to extract the archaeological material from the marine environment due to size of the sample, the legislation or preservation purposes. In these cases, the in-situ analysis turns into the only alternative for obtaining information. In spite of this demand, no analytical techniques are available for the in-situ chemical characterization of underwater materials.

The versatility of laser-induced breakdown spectroscopy (LIBS) has been successfully tested in oceanography ¹. Advantages such as rapid and in situ analysis with no sample preparation make LIBS a suitable alternative for field measurements. To further exploit the inherent advantages of the technology, a mobile fiber-based LIBS platform capable of performing remote measurements up to 50 meters range has been designed for the recognition and identification of artworks in underwater archaeological shipwrecks. The LIBS prototype featured both single-pulse (SP-LIBS) and multi-pulse excitation (MP-LIBS) ². The use of multi-pulse excitation allowed an increased laser beam energy (up to 95 mJ) transmitted through the optical fiber. This excitation mode results in an improved performance of the equipment in terms of extended range of analysis (to a depth of 50 m) and a broader variety of samples to be analyzed (i.e., rocks, marble, ceramics and concrete). In the present work, the design and construction considerations of the instrument are reported and its performance is discussed on the basis of the spectral response, the remote irradiance achieved upon the range of analysis and its influence on plasma properties, as well as the effect of the laser pulse duration and purge gas to the LIBS signal. Also, to check the reliability and reproducibility of the instrument for field analysis several robustness tests were performed outside the lab. Finally, the capability of this instrument was successfully demonstrated in an underwater archaeological shipwreck (San Pedro de Alcántara, Malaga).

Acknowledgements

The authors acknowledge the funds from projects P11-FQM-7193. One of the authors (MLC) thanks the Junta de Andalucía for providing a fellowship. Furthermore, they thank the collaboration with the Subaquatic Archaeological Centre (CAS).

References:

- [1] S. Guirado, F.J. Fortes, V. Lazic and J.J. Laserna, *Spectrochim. Acta Part B*, **137**, 74-75 (2012).
- [2] S. Guirado, F.J. Fortes and J.J. Laserna, *Talanta*, **137**, 182–188 (2015).

Vibrational spectroscopy applied to the study of β -galactosidase-silica support interactions

S. F. Prazeres¹, F. Ortega², I. A. Pavel³, A. Pasc³, N. Canilho³, C. García-Ruiz^{1,2}, G. Montalvo^{1,2,*}.

¹Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering, University of Alcalá, Ctra. Madrid-Barcelona Km. 33.600, Alcalá de Henares 28871, Spain.

²University Institute of Research in Police Sciences, University of Alcalá, Ctra. Madrid-Barcelona Km. 33.600, Alcalá de Henares 28871, Spain.

³SRS MC UMR 7565, Université de Lorraine, F-54506 Vandoeuvre-lès-Nancy cedex, France.

*gemma.montalvo@uah.es

The enzyme immobilization is attracting general scientific and industrial interest because of the improvements in the thermal and pH stability of the modern enzymes. Furthermore, the immobilization process facilitates their reuse. Different techniques, such as, small angle x-ray scattering (SAXS) and nitrogen adsorption can help to identify the presence of the enzyme adsorbed on the support material. However, it is still a challenge to study the interactions between the functional groups of the enzyme and the support. In this context, vibrational spectroscopy can play an important role, since reported studies showed that the interaction enzyme-support can be confirmed with the displacement and/or increment of some spectral bands' intensity, or the appearance of new ones.[1]

The aim of this work was to use vibrational spectroscopy, specifically Infrared and Raman, to study the interaction between β -galactosidase (EC 3.2.1.23), an interesting enzyme in the production of low-lactose products, and a meso-macroporous silica support. The ATR-FTIR spectra showed the presence of the amide I at 1651 cm^{-1} ($\nu\text{C=O}$), and amide II at 1535 cm^{-1} ($\delta\text{N-H}$), which are representative of the enzyme. Some displacement of the $\nu\text{Si-O-Si}$ and $\nu\text{Si-OH}$ bands, characteristic of silica support, also reinforced the interaction between β -galactosidase and the silica support. Regarding to the Raman spectra, they exhibited the amine band at 3060 cm^{-1} , and a broad band between 1710 and 1510 cm^{-1} . In this range, it is possible to identify the amide band at 1670 and 1586 cm^{-1} . It is extremely interesting the difference in this band conformation for different concentrations of immobilized enzyme. Afterwards, chemometric analysis (extended canonical variate analysis, ECVA) were carried out to inspect the variability of each sample with different enzyme concentration. The ECVA algorithm improves the canonical variate analysis (CVA) as a technique designed to emphasise the differences between data classes according to a statistical criterion. Interestingly, this method clearly identified the spectral difference among the raw silica particles and both low and high concentrations of immobilized enzyme.

Acknowledgements

The research leading to these results has received funding from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme FP7/2007-2013/ under REA grant agreement n° 606713 – BIBAFOODS.

References

[1] S. F. Prazeres, C. G. Ruiz and G. M. García, *Appl Spectrosc Rev*, **50**, 797 – 821 (2015).

Modelling and irradiation experiments of cosmic dust analogues

G. Molpeceres*, M. Jiménez-Redondo, V. Timón, M. A. Moreno, V. J. Herrero, R. Escribano, B. Maté, I. Tanarro.

Instituto de Estructura de la Materia, IEM-CSIC, Serrano 123, 28006 Madrid, Spain.

**german.molpeceres@csic.es*

Carbonaceous compounds are found in diverse astronomical media [1], many of them found in small dust grains. Mostly formed during the last stage of evolution of carbon rich stars, their skeleton is thought to be mainly composed of carbon and hydrogen [2]. They are thus characterized by several IR features which act as fingerprints of their presence, namely aliphatic, aromatic and olefinic functional groups [3].

The present study is devoted, from the theoretical and experimental point of view, to the study of structural and spectroscopic features of Hydrogenated Amorphous Carbon (HAC) whose absorptions have a very good degree of agreement with the astronomical observations.

Ab initio calculations of a series of randomly packed Polycyclic Aromatic Hydrocarbons (PAH) derivatives (Fig 1) are performed in order to understand, in an atomistic context, the spectral profile of these species. Among other factors: amorphization, inhibition of bending modes and number of functional groups are considered in these calculations. Simulation cells are constructed using a Metropolis Monte Carlo procedure. The level of theory chosen is Density Functional Theory (DFT) in its Generalized Gradient Approximation (GGA) formulation of the exchange and correlation energy using a plane wave expansion of the total electronic wave function.

Irradiation experiments with high energy electrons (5 keV) are conducted in an attempt to obtain quantitative data on the rate of destruction of these materials under extraterrestrial conditions. Upon collision with the sample, high energy electrons produce a cascade of processes whose destruction effect is similar to the one measured for cosmic rays analogues (MeV protons). The samples are generated by Plasma Enhanced Chemical Vapour Deposition (PECVD) and the irradiation is monitored by IR spectroscopy, taking into special consideration the 3.4 μ m absorption band (Fig 2).

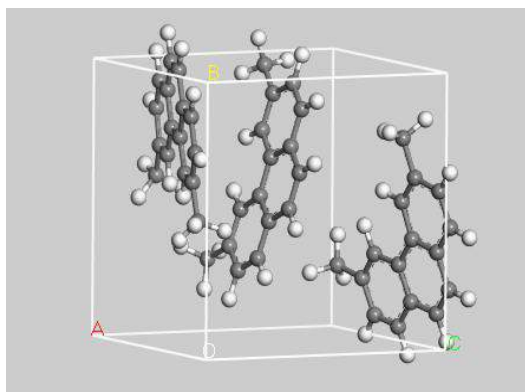


Figure 1: Amorphous cell containing three methylated derivatives of phenanthrene.

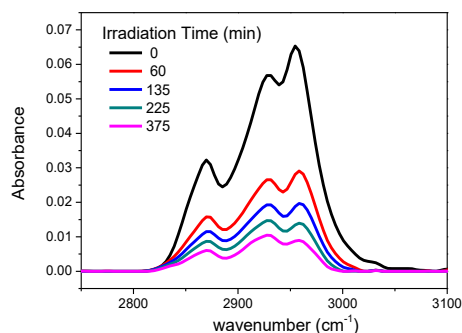


Figure 2: Decay of the CH aliphatic stretching region upon electron irradiation.

Acknowledgements

This research has been supported by the Spanish MINECO, Project FIS2013-48087-C2-1-P. G.M. acknowledges MINECO PhD grant BES-2014-069355. Calculations have been performed at Trueno-CSIC and CESGA.

References

- [1] A.G.G.M. Tielens, *Rev. Mod. Phys.*, **85** (2013) 1021.
- [2] J. E. Chiar, A. G. G. M. Tielens, A. J. Adamson and A. Ricca, *Astrophys. J.*, 2013,**770**,78.
- [3] S. Kwok, in *The Molecular Universe*, J. Cernicharo and R. Bachiller ed, IAU Symposium 280, Cambridge University Press, 2011, p 203.

Calcium Silicate Hydrate Synthesis

M. Martín-Garrido*, S. Martínez-Ramírez.

Instituto de Estructura de la Materia (IEM-CSIC), C/Serrano 121, 28006 Madrid.

**moises.martin@iem.cfmac.csic.es*

The main component of the binders present in the Cultural Heritage mortars is an amorphous hydrated calcium silicate (called C-S-H[†] gel) whose structure resembles that of the tobermorite and jennite minerals. However, while the C-S-H gel has no well-defined stoichiometry, the other two minerals are crystalline with a well-established chemical formula, $\text{Ca}_5\text{Si}_6\text{O}_{16}(\text{OH})_2 \cdot 4\text{H}_2\text{O}$ for tobermorite and $\text{Ca}_9\text{Si}_6\text{O}_{18}(\text{OH})_6 \cdot 8\text{H}_2\text{O}$ for jennite. Both minerals have a Ca/Si ratio of 0.83 and 1.5 respectively. Instead, the gel C-S-H has a variable stoichiometry, presenting relations Ca/Si ranging from 0.5 to 2.1 [1].

The synthesis of the C-S-H gel can be done in different ways, which can influence the Ca/Si ratio and therefore their properties. In this work the C-S-H gel was synthesis by three methods: the double decomposition [2], hydrothermal [3] and mechanochemical [4]. The formation of the amorphous compound was monitored by Raman and Infrared Spectroscopy. Through thermal-thermogravimetric analysis the percentage of the phases formed, gel C-S-H, calcite and amorphous carbonates in each synthesis was determined. The method of double decomposition leads to the formation of a larger amount of gel C-S-H at the same reaction time.

Acknowledgements

The authors wish to thank the Thermal Analysis Service of the ICMM-CSIC, for the ATD/TG analysis in the samples.

This research was supported by the Comunidad de Madrid and the European Social Fun (Program Geomateriales 2-S2013/MIT-2914).

References

- [1] B. Lothenbach and A. Nonat, *Cem. Concr. Res.*, **78**, 57 – 70 (2015).
- [2] J.J.F. Chen, J.J. Thomas, H.F.W. Taylor and H. M. Jennings. *Cem. Concr. Res.* **34**, 1499-1519 (2004).
- [3] S. Grangeon, F. Claret, C. Lerouge, F. Warmont, T. Sato, S. Anraku, C. Numako, Y. Linard and B. Lanson. *Cem. Concr. Res.* **52**, 31-37 (2013).
- [4] F. Saito, G. Mi, M. Hanada. *Solid State Ionics* **101-103** 37-43 (1997).

[†]Cement Chemistry Notation, C = CaO, S = SiO₂, H = H₂O.

Ultrafast transient absorption spectroscopy: principles and applications

M. Moreno-Oliva^{1,*}, J. T. López-Navarrete¹.

¹University of Málaga, Department of Physical Chemistry, Campus de Teatinos s/n, Málaga, 29071, Spain.

*mmorenoo@uma.es

The study of photophysical and photochemical processes crosses the interest of many fields of research in physics, chemistry and biology. In particular, the photophysical and photochemical reactions, after light absorption by a photosynthetic pigment-protein complex, are among the fastest events in biology, taking place on timescales ranging from tens of femtoseconds to a few nanoseconds. Among the experimental approaches developed for this purpose, the advent of ultrafast transient absorption spectroscopy has become a powerful and widely used technique.[1,2]

Focusing on the process of photosynthesis, it relies upon the efficient absorption and conversion of the radiant energy from the Sun. Chlorophylls and carotenoids are the main players in the process. Photosynthetic pigments are typically arranged in a highly organized fashion to constitute antennas and reaction centers, supramolecular devices where light harvesting and charge separation take place. The very early steps in the photosynthetic process take place after the absorption of a photon by an antenna system, which harvests light and eventually delivers it to the reaction center. In order to compete with internal conversion, intersystem crossing, and fluorescence, which inevitably lead to energy loss, the energy and electron transfer processes that fix the excited-state energy in photosynthesis must be extremely fast. In order to investigate these events, ultrafast techniques down to a sub-100 fs resolution must be used. In this way, energy migration within the system as well as the formation of new chemical species such as charge-separated states can be tracked in real time. This can be achieved by making use of ultrafast transient absorption spectroscopy.

The basic principles of this notable technique, instrumentation, and some recent applications to photosynthetic systems[3] will be described.

Acknowledgements

M. Moreno Oliva thanks the MINECO for a “Juan de la Cierva-Incorporación” research contract.

References

- [1] U. Megerle, I. Pugliesi, C. Schrieffer, C.F. Sailer and E. Riedle, *Appl. Phys. B*, **96**, 215 – 231 (2009).
- [2] R. Berera, R. van Grondelle and J.T.M. Kennis, *Photosynth. Res.*, **101**, 105 – 118 (2009).
- [3] T. Nikkonen, M. Moreno Oliva, A. Kahnt, M. Muuronen, J. Helaja and D.M. Guldi, *Chem. Eur. J.*, **21**, 590 – 600 (2015).

***in situ* Spectroelectrochemical Techniques to the Characterization of Polyaniline Derivatives**

M. Abidi^{1,2,*}, S. López-Bernabeu¹, F. Huerta³, F. Montilla¹, S. B. Hentati², E. Morallón¹.

¹Dept. Química Física e Instituto Universitario de Materiales. Universidad de Alicante. Spain.

²Laboratoire de chimie des matériaux, Faculté des Sciences de Bizerte. 7021, Zarzouna Université de Carthage, Tunisie. laboratoire de Chimie des Matériaux. Université de Carthage. Tunisie.

³Dept. Ingeniería Textil y Papelera. Universidad Politécnica de Valencia. Spain.

*abidi.maali@gmail.com

Several studies have been concerned with the mechanistic aspects of the electropolymerization of o-aminophenol. The synthesized poly(o-aminophenol), poly(OAP) shows a ladder structure with phenoxazine-type units [1, 2].

2-amino-4-tert-butylphenol monomer was polymerized from aqueous solution using either electrochemical or chemical oxidation procedures. *In situ* spectroscopic characterization techniques were employed to gain information on the chemical structure and redox behavior of the electroactive material obtained. A possible chemical structure of such material has been depicted in Fig. 1.

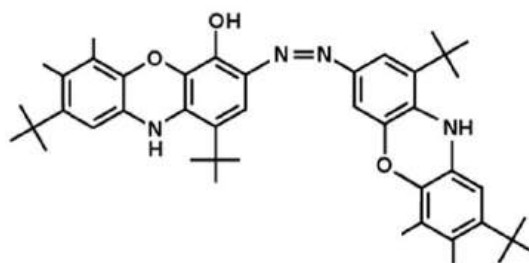


Fig. 1. Representation of the reduced form of a poly(2-amino-4-tert-butylphenol) fragment, as derived from *in situ* spectroscopic results.

The combination of electrochemical methods with *in situ* FTIR and *in situ* UV-vis spectroscopies strongly suggest that the chemical structure of poly(2-amino-4-tert-butylphenol) contains both phenoxazine rings and azo moieties. The former, which are more prevalent, arise from the usual *para*-coupling of aniline-derivative monomers [3], while the latter are probably stimulated by the presence of a voluminous alkyl group adjacent to the more reactive *para*- position. No significant structural differences were found between the chemically or electrochemically synthesized materials.

Acknowledgements

Financial support from the Spanish Ministerio de Economía y Competitividad and FEDER funds (MAT2013-42007-P) and from the Generalitat Valenciana (PROMETEO2013/038) is gratefully acknowledged. M. Abidi thanks the Ministry of Higher Education and Scientific Research of Tunisia for funding her stay at the University of Alicante.

References

- [1] H.J. Salavagione, J. Arias, P. Garces, E. Morallon, C. Barbero, J.L. Vazquez, *J. Electroanal. Chem.*, **565**, 375–383 (2004).
- [2] H.J. Salavagione, J. Arias-Pardilla, J.M. Perez, J.L. Vazquez, E. Morallon, M.C. Miras, C. Barbero, *J. Electroanal. Chem.*, **576**, 139–145 (2005).
- [3] Y. Wei, R. Hariharan, S.A. Patel, Chemical and electrochemical copolymerization of aniline with alkyl ring-substituted anilines. *Macromolecules*, **23**, 758–764 (1990).

POSTER PRESENTATIONS Nanoparticles

Detection of Hg by total reflection X-ray fluorescence following photochemical generation of Hg vapor and trapping onto Pd nanoparticles

C. Bendicho^{1,*}, I. De La Calle¹, V. Romero¹, M. Gryglicka², I. Lavilla¹.

¹Departamento de Química Analítica y Alimentaria, Área de Química Analítica, Facultad de Química, Universidad de Vigo, Campus As Lagoas-Marcosende s/n, 36310 Vigo, Spain.

²Department of Analytical Chemistry, Faculty of Chemistry, Maria Skłodowska-Curie University, Wydział Chemii UMCS w Lublinie pl. M. Curie-Skłodowskiej 2, 20-031 Lublin, Poland.

*bendicho@uvigo.es

The high toxicity of mercury (Hg) even at extremely low concentration makes it necessary the development of very sensitive methodologies for the preconcentration of this element. The maximum content of Hg established in drinking water by the European Commission and USEPA is 1 and 2 µg/L Hg, resp. [1, 2].

In recent years, nanomaterials have been introduced in sample preparation procedures due their attractive properties. One of the potential applications of noble metal nanoparticles is their use as sorbents of metal species due to their high specific surface area and sorption capacity. Hg can be efficiently retained onto noble metal nanoparticles as a result of amalgamation. Thus, we have developed a technique based on previous publications related with photochemical vapour generation and adapted for total reflection X-ray fluorescence (TXRF) analysis. Special attention has been paid to the challenges that pose the analysis of Hg by TXRF [3]. The main objective of this work lies in the improvement of detection capability for Hg by TXRF, overcoming the problem of Hg losses occurring typically due to volatilization. The method consists on the photogeneration of Hg vapor in the presence of a precursor and trapping onto quartz substrates previously coated with Pd nanoparticles. Size and morphology of the Pd NPs were studied by transmission electron microscopy (TEM). Pd nanoparticles were spherical-shaped with an average particle size of 3 nm. Operation parameters in the continuous flow-photochemical vapor generation (CF-photo CVG) system were optimized. Acetic acid was chosen as precursor for reducing species.

A trapping time of 30 min and an acetic acid concentration of 2 M were found as optimal for detection of Hg by TXRF. Under these conditions, the enrichment factor and the limit of detection achieved were 127 and 54 ng/L, respectively. The repeatability of the method (expressed as the relative standard deviation) for a Hg standard of 1 µg/L was 3 %. Recovery studies were performed in water samples spiked with 10 µg/L of Hg. Recoveries in the range of 96 - 101 % were obtained. The reported approach could open the door to on-site detection of Hg at sub-ppb level using a portable TXRF instrument.

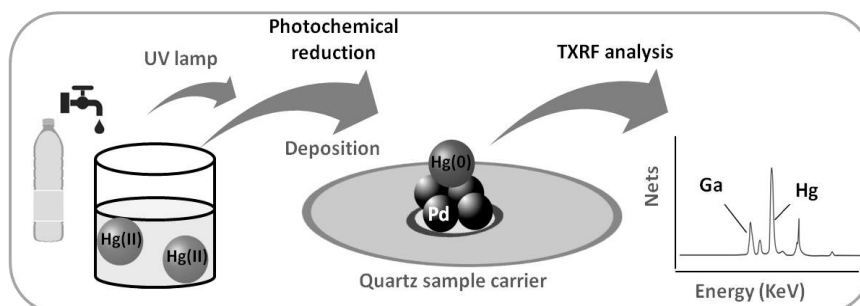


Figure 1. Scheme of the CF-photo CVG approach coupled to TXRF

References

- [1] National Primary DrinkingWater Regulations, EPA 816-F-09-004, 2009. <http://water.epa.gov/drink/contaminants/upload/mcl-2.pdf> (updated 2015).
- [2] EU (European Communities) Commission Directive 2003/40/EC on natural mineral waters, 2003.
- [3] C. Bendicho, F. Pena, M. Costas, S. Gil, I. Lavilla. Photochemistry-based sample treatments as greener approaches for trace-element analysis and speciation. Trends in Analytical Chemistry 29 (2010) 681.

Luminescence and Photoconductance of a Novel 3D Potassium-MOF based on Perylene-3,4,9,10-tetracarboxylate

A. Salinas¹, B. Fernández², S. Castellanos³, B. Biel⁴, L.F. Capitán-Vallvey¹, A. Rodríguez-Diéguez^{2,*}.

¹Ecsens, Department of Analytical Chemistry, Faculty of Sciences, University of Granada.

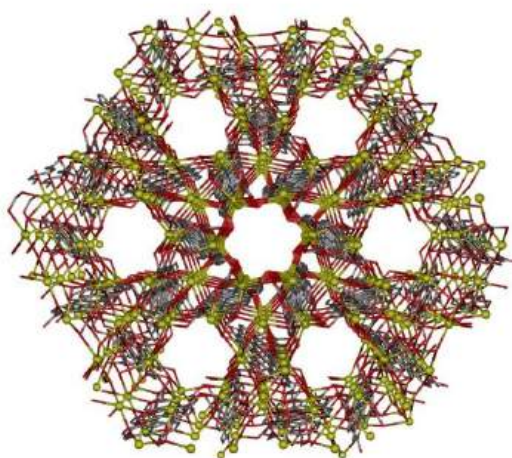
²Department of Inorganic Chemistry, Faculty of Sciences, University of Granada.

³Catalysis Engineering, Chemical Engineering Department, Delft University of Technology, 2628 BL Delft, The Netherlands.

⁴Department of Electronics and Computer Science, Faculty of Sciences, University of Granada.

*antonio5@ugr.es

Metal-organic frameworks (MOFs) are a relatively new class of materials that have received great interest due to their structural and topological diversity, as well as to the properties that arise from their structural features because the combination of metal centers and organic ligands provides fantastic possibilities for the construction of materials with various structure and functionality [1]. In particular, the study of s-block-based MOFs hasn't been explored in the field of MOFs, however, these metal centers are cheap, non-toxic, and essential in many biological processes, and their complexes have shown a wealth of interesting properties, ranging from catalysts to ferroelectrics. For these reasons, we decided to synthesize new MOFs by using potassium as metal center and to study some of the physical properties exhibited by these materials. In this work, we have synthesized a novel MOFs based on the perylene-3,4,9,10-tetracarboxylate linker and potassium as metallic center. We report the formation of this K-based MOF using conventional routes with water as solvent. This material displays intense photoluminescence properties in liquid and solid state at room temperature. What is more interesting, we have realized photoconductance measurements. To the best of our knowledge, this is the first example of 3D-MOF with this interesting octadentate ligand and one of the few examples of three-dimensional potassium MOF materials.



Acknowledgements

This study was supported by projects from the Spanish MINECO (CTQ2013-44545-R), Junta de Andalucía (P10-FQM-5974 and FQM-1484), and the "Fortalecimiento de Grupos" and "Re-incorporación de Doctores" Programs.

References

[1] R.J. Kuppler, D.J. Timmons, Q.R. Fang, J.R. Li, T.A. Makal, M.D. Young, D. Yuan, D. Zhao, W. Zhuang, H.C. Zhou. *Coordination Chemistry Reviews*, **253**, 3042-3066, (2009).

The role of the electrolyte on the SERS spectra of pyridine

I. López-Tocón*, J. Soto, J. C. Otero.

Department of Physical Chemistry, Faculty of Science, University of Málaga. Campus de Excelencia Internacional Andalucía Tech. Campus de Teatinos s/n E-29071 Málaga, Spain.

*tocon@uma.es

The electrolyte plays an important role in electrochemical SERS experiments given that the overall intensity of the spectrum and the vibrational wavenumber of the enhanced bands can be affected. This is because the behavior of an adsorbate in the presence of different electrolytes is related to the relative strength of adsorption processes. The competitiveness established between the anions of the electrolyte and the molecules to diffuse from the bulk solution to the electrode surface has proved to be decisive in obtaining intense SERS spectra. It has been shown that SERS of aromatic acids such as benzoic acid and even aromatic alpha-amino acids as phenylacetic have stronger intensity when the most commonly used electrolyte in SERS, potassium chloride, is changed by sodium sulphate [1,2]. In addition, the type of electrolyte can also affect to the electrode potential to which the maximum relative intensity of the enhanced bands is reached, being able to determine the relative adsorption strength of each anion [3]. Moreover, the anions of the electrolyte seem to be also involved in the formation of ion pair with protonated and positively charged chemical species [4] favoring their adsorption on the metallic surface and thus giving SERS spectra.

SERS spectra of pyridine has been recorded on a silver electrode in a potential range from 0.0 to -1.20 V with a saline solution, pyridine / KCl (0.1M / 0.1M), by using the 514.5 nm exciting line by us [5]. Under these experimental conditions, the maximum intensity of the enhanced 8a and 9a bands is reached at -0.75 V and -1.20 V, respectively, being the 9a band what dominates the spectrum at negative electrode potential. Although this behavior has been explained under a resonant charge transfer mechanism, the nature of the electronic resonance processes involved in the enhancement of each band is different. The 8a band is enhanced due to an electronic excitation between the ground and excited charge transfer electronic state of the metal-adsorbate surface complex, while the activity of the 9a band is due to a plasmon-like excitation taking into account an overall electronic structure of small metal clusters [6] which is able to selectively modify the relative intensities of specific SERS band.

We intend now to record pyridine SERS spectra under the same experimental conditions but varying only the type of electrolyte in order to check how it affects the relative intensities and vibrational wavenumbers of the bands as well as the electrode potential to which the enhanced bands reach the maximum intensity. Different electrolytes like KCl, NaCl, KBr, NaBr and Na₂SO₄, have been selected in such way that allows us to compare SERS spectra in which changes only the cation or the anion of the electrolyte. From the analysis of all these SERS spectra, it can be concluded that no significant wavenumbers shifts have been detected, while the relative intensities of the bands and the electrode potential to which the maximum intensity is reached are slightly modified. NaBr electrolyte requires more negative electrode potential in order to enhance the 8a and 9a bands and to resolve the 12 and 1 pair.

Acknowledgements

We are grateful to the Spanish MINECO (CTQ2012-31846) and Junta de Andalucía (FQM-5156 and 6778), for financial support.

References

- [1] Y.J. Kwon, S.S. Park, K.Kim and M.S. Kim, *J. Raman Spectrosc.* **25**, 655-661 (1994).
- [2] M.R. Lopez-Ramirez, C. Ruano, J.L. Castro, J.F. Arenas, J. Soto and J.C. Otero *J. Phys. Chem. C* **114**, 7666–7672 (2010).
- [3] D.S. Kellogg and J.E. Pemberton, *J. Phys. Chem.* **91**, 1120-1126 (1987).
- [4] M.S. Kim, M.K. Kim, C.J. Lee, Y.M. Jung and M.S. Lee, *Bull. Korean Chem. Soc.* **30**, 2930-2934 (2009).
- [5] J.F. Arenas, I. López Tocón, J.C. Otero, and J.I. Marcos, *J. Phys. Chem.* **100**, 9254-9261 (1996).
- [6] J. Román-Pérez, I. López-Tocón, J.L. Castro, J. F. Arenas, J. Soto and J.C. Otero, *Phys. Chem. Chem. Phys.* **17**, 2326-2329 (2015).

Spectroscopic characterization of piroxicam (non-steroidal and anti-inflammatory drug) on solution, adsorbed on silver nanoparticles and complexed with cucurbiturils

P. Sevilla^{1,2*}, M. Hernández², E. Corda², J. V. García-Ramos².

¹Departamento de Química Física II, Facultad de Farmacia, UCM. 28040, Madrid. Spain.

²Instituto de Estructura de la Materia, IEM-CSIC. C/Serrano 121. 28006, Madrid. Spain.

*paz@ucm.es

Silver nanoparticles exhibit Localized Surface Plasmon Resonances (LSPR) that are responsible of the enhanced spectroscopies (SERS and SEF) under several conditions. They present also anti-inflammatory effects and as additional advantage, they can act as drug nanocarriers adsorbing the pharmaceutical molecules on their surface. Consequently, a combination of drugs and silver nanoparticles will constitute an ideal system to include in multi-step drug delivery systems acting as "magic bullets" to achieve the disease cells.

Piroxicam (PX), (4-hydroxy-2-methyl-N-(pyridin-2-yl)-2H-1,2-benzothiazine-3-carboxamide1,1-dioxide), is a non-steroidal anti-inflammatory drug (NSAID) that has numerous applications as antipyretic or painkiller. It works by inhibiting the synthesis of prostaglandins which preserve the integrity of the stomach lining and maintain normal renal function in a compromised kidney, producing gastrointestinal side effects and serious skin reactions. This is the reason why the European Medicines Agency has restricted their uses from 2007. Thus, development of novel delivery systems which will permit targeted and controlled release of drugs will avoid several of those undesired complications. Before launching such a project it is necessary to characterize the physicochemical behavior of these systems in solution.

From the point of view of chemistry PX is a complex molecule that presents an acid-base equilibrium with, at least, five species in equilibrium with different biomedical implications. In this work, we present the spectroscopic properties of three different systems:

- i) Raman and fluorescence of PX either in water at different pH values or in solvents with different polarities, and also its SERS and SEF characterization in gold and silver colloids, trying to imitate several physiological conditions. Our results [1] show that SERS spectra of PX in gold and silver colloids exhibit similar trends indicating same conformer and orientation of drug to both noble metal NPs. At acidic pH=1 bands of open anionic and zwitterionic conformers are identified. At pH's in the range 2-4, bands dominating the spectra correspond to zwitterionic form with a relative parallel orientation of the aromatic moieties to the metal NPs surface. At pH=7 few and weak bands appear in the SERS spectrum, its analysis indicating the presence of the anionic form of PX differently oriented to the metal surface.
- ii) UV-visible, fluorescence and NMR of PX-cucurbituril systems. Our results indicate formation of complexes with CB8, at pH=4.0 and pH=2.0 where the drug is in its neutral and cationic form respectively. NMR data show only one group of signals, almost coincident for both pHs, what indicates similarity between both complexes formed. Absence of signals for protons from benzothiazine in the spectra obtained at pH=2 could be caused by the presence of a second host molecule in this environment of the guest as Job's plots indicates.
- iii) SERS of PX-cucurbituril complexes adsorbed on silver nanoparticles. Predominant signal is related to the CB8, but can be also seen some peaks attributable to PX probably located near the rims as UV, fluorescence and NMR have previously indicate.

Acknowledgements

This work was supported by the *Spanish Ministerio de Economía y Competitividad* (grant FIS2014-52212-R). Recording of the NMR spectra at the "Laboratorio de RMN de alto campo" (Instituto de Química Física Rocasolano, CSIC) by David Pantoja-Uceda is gratefully acknowledged.

References

- [1] M. Hernandez, E. Corda, J.V. Garcia-Ramos, C. Domingo and P. Sevilla, *JRS*, doi: 10.1002/jrs.4839 (2015).

Catalytic gold deposition on quantum dot tags for ultrasensitive detection of cancer biomarkers

M. García-Cortés*, J. R. Encinar, J. M. Costa-Fernández, A. Sanz-Medel.

Department of Physical and Analytical Chemistry (University of Oviedo), Julián Clavería 8, 33006, Oviedo, Spain.

**garciacormarta@uniovi.es*

Omics researchers have reported many new potential biomarkers for early alarm diagnosis, for evaluating new therapies efficiency and to assess the prognosis of different cancers and diseases. Therefore, their absolute quantification at very low concentration levels in biological samples is a must for clinical decision-making allowing not only a prompt diagnosis but also monitoring the disease progression and evaluating therapeutic interventions. Ultrasensitive bioanalytical methods are thus urgently required to assess such biomarkers at ultralow levels in very complex samples such as fluids or tissues.

In order to reach the required very low detection limits, deposition of gold and silver onto gold nanoparticles and clusters surface has been proposed as amplification strategy of the analytical signal [1]. Although gold nanoparticles are very attractive for bioanalytical applications, they are not fluorescent and luminescent signal is especially suited for optical imaging studies without the need of perform a biopsy for analytical detection. Metal nanoclusters have luminescent properties but fluorescent emission is affected by autofluorescence of biological media that produces a lack of sensitivity and selectivity. In this sense, phosphorescent QDs stands out for having very advantageous properties such as large separation between absorption and emission wavelength and long emission lifetime that allows time-gated detection in order to easily avoid autofluorescence of biological media.

In this context, a Mn-doped ZnS quantum dots-based immunoassay platform is presented for highly sensitive detection of cancer biomarkers. Ultrahigh sensitivity is achieved through gold deposition on the surface of the nanoparticle tags acting as catalytic seeds, thus effectively amplifying the size of the metallic nanoparticles after the immunoassay and before the tag detection. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) has been used as detection technique of the amplified labels due to its extremely sensitivity for metals, minimum matrix effects and multielemental and isotopic detection [2].

Acknowledgements

This work has been supported by MINECO-13-CTQ2013-49032-C2-1-R project from the Spanish Ministry of Economy and Competitiveness, FC-15-GRUPIN14-092 from Gobierno del Principado Asturias and Agilent Technologies Foundation. M.G.C. acknowledges the Ph.D. grant (BP13-110) from Gobierno del Principado de Asturias (Spain).

References

- [1] C. S. Thaxton, R. Elghanian, A. D. Thomas, S. I. Stoeva, J.-S. Lee, N. D. Smith, A. J. Schaeffer, H. Klocker, W. Horninger, G. Bartsch, and C. A. Mirkin, *Proc. Natl. Acad. Sci. U.S.A.*, **106**, 18437–42 (2009).
- [2] A. Sanz-Medel, M. Montes-Bayón, J. Bettmer, M. L. Fernández-Sánchez, and J. Ruiz Encinar, *TrAC Trends Anal. Chem.*, **40**, 52–63 (2012).

UV-Vis Spectrophotometry as an alternate technique for fast determination of CuNPs *via* complexation with DDTC

M. Aboal-Somoza^{1,*}, B. Besada-Romero¹, J. Sanmartín-Matalobos²,
P. Bermejo-Barrera¹.

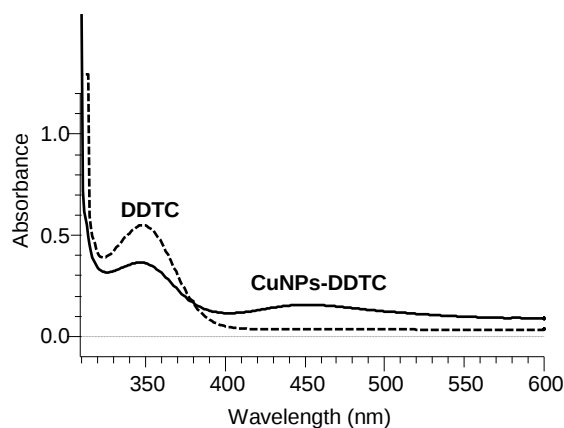
¹GETEE Group. Department of Analytical Chemistry, Nutrition and Bromatology; Faculty of Chemistry; Universidade de Santiago de Compostela, Santiago de Compostela, Spain.

²Department of Inorganic Chemistry; Faculty of Chemistry; Universidade de Santiago de Compostela, Santiago de Compostela, Spain.

*m.aboal@usc.es

Nowadays, nanomaterials are used and applied in very different fields. This increases the interest on the determination of these materials in diverse matrices and samples. Among the reasons supporting this interest, the possible toxicity of such nanomaterials can be highlighted. Thus, as an alternative to expensive and specialized techniques and methods, the work presented here explores the application of a simple technique, such as UV-Vis spectrophotometry, to achieve the determination of copper nanoparticles (CuNPs).

The method is based on the well-known ability of diethyldithiocarbamate (DDTC) to complex a variety of metallic ions, such as Cu(II). Moreover, the possible formation of a compound (some kind of “complex”) between CuNPs and DDTC is explored. That CuNPs-DDTC complex is formed just by adding DDTC solution to the sample, and exhibits an absorption band with a maximum at about 454 nm. The calibration line extends up to –at least- 60 µg/ml and the results of the validation studies performed suggest that the reliable determination of CuNPs is a real possibility. Finally, the application of the method to real samples is carried out.



Acknowledgements

The financial support by both the Ministerio de Economía y Competitividad, Ref: CTQ2015-68094-C2-2 and the Xunta de Galicia (Grupo de Referencia Competitiva 6RC2014/2016) is gratefully acknowledged.

Cloud Point Extraction of copper oxide nanoparticles and subsequent determination by GFAAS with Zeeman background correction

M. Aboal-Somoza^{1,*}, I. Blanco-Liaño¹, P. Bermejo-Barrera¹.

¹GETEE Group, Department of Analytical Chemistry, Nutrition and Bromatology; Faculty of Chemistry; Universidade de Santiago de Compostela, Santiago de Compostela, Spain.

*m.aboal@usc.es

Due to different, well-known, reasons, the interest on the determination of nanomaterials in quite different matrices and samples is increasing day by day. In this context, the development of analytical methods aimed at such determinations is always important. In addition, in view of the low concentrations at which those nanomaterials are present, the concentration power of the diverse approaches, as it is the case of cloud point extraction (CPE) procedures, is especially welcome.

This work deals with the CPE of copper oxide nanoparticles (CuONPs) as a previous step to copper determination by GFAAS, using Zeeman effect background correction.

The development of the CPE process, given the variety of factors likely to be considered, required a preliminary experimental design, from which the pH and the amount of surfactant (Triton X-114) were concluded to need a more careful investigation. Other factors, such as the possible addition of a salt to improve phase separation (usually recommended in the literature) or the incubation time and temperature revealed to be not significant. Therefore, the study of the best pH interval and amount of Triton X-114, as well as the adjustment of the GFAAS conditions enabled the proposal of a method that was finally applied to the determination of CuONPs in real water samples.

Acknowledgements

The financial support of both the Ministerio de Economía y Competitividad, Ref: CTQ2015-68094-C2-2 and the Xunta de Galicia (Grupo de Referencia Competitiva 6RC2014/2016) is gratefully acknowledged.

Spectroscopic and theoretical studies on self-assembling processes of π -conjugated discotic molecules

A. Garzón^{1,*}, I. Bravo¹, P. J. Pacheco¹, J. Fernández¹, E. Niza¹, J. Albaladejo¹, J. Tolosa², J. C. García-Martínez², J. Rodríguez-López², A. Navarro³, M. P. Fernández-Liencres³, T. Peña-Ruiz³, J. M. Granadino-Roldán³, J. Canales-Vázquez⁴, M. Moral⁴.

¹Dpto. Química Física, Facultad Farmacia (Albacete) y Facultad de Ciencias y Tecnologías Químicas (Ciudad Real), Universidad de Castilla-La Mancha (UCLM), Spain.

²Área de Química Orgánica, Facultad Farmacia (Albacete) y Facultad de Ciencias y Tecnologías Químicas (Ciudad Real), UCLM, Spain.

³Dpto. Química Física y Analítica, Facultad de Ciencias Experimentales, Universidad de Jaén, Spain.

⁴Instituto de Energías Renovables, UCLM, Albacete, Spain.

*andres.garzon@uclm.es

Optical properties and applications of π -conjugated molecules depend on their supramolecular ordering [1-4]. For instance, light emitting properties of π -conjugated molecules employed in OLEDs also strongly depend on the molecular packing. In solution, self-assembly of π -conjugated molecules used as fluorescent sensors can change their performance in biological systems as live cells [5]. Therefore, the understanding of the supramolecular interactions between individual π -conjugated molecules and the control of the molecular packing is crucial for the development of these applications [1,2].

We have chosen the 1,3,5-tristyrylbenzene molecule with C_3 symmetry as a model to shed light on the self-assembly of π -conjugated discotic molecules. A combined experimental and theoretical study on self-assembling processes of 1,3,5-tristyrylbenzene was carried out. UV-Vis absorption spectroscopy, stationary-state fluorescence spectroscopy, time-resolved fluorescence spectroscopy and dynamic light scattering spectroscopy were some of the techniques used in this study. Calculations at the TD-DFT level were employed to analyse the spectra of 1,3,5-tristyrylbenzene and its aggregated states in solution. The supramolecular structure of 1,3,5-tristyrylbenzene in solid phase was studied by means of transmission and scanning electron microscopies.

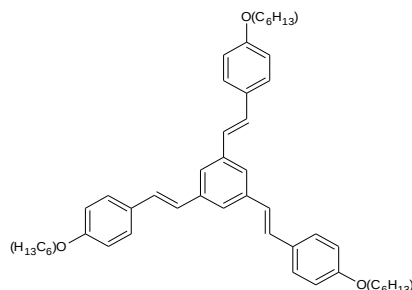


Figure 1. Molecular formula of 1,3,5-tristyrylbenzene

Acknowledgements

The authors would like to thank the Consejería de Educación y Ciencia de la Junta de Comunidades de Castilla-La Mancha (FEDER projects, PEII11-0279-8538 and PEII-2014-043-P (FOTOCINE)), Ministerio Español de Economía y Competitividad (CTQ2013-48411-P), Universidad de Castilla-La Mancha (project GI20152964), Universidad de Jaén (Programa operativo FEDER Andalucía UJA2014/02_FT/04) and the computational resources supplied by the Centro de Servicios de Informática y Redes de Comunicaciones CSIRC-Universidad de Granada for supporting this research.

References

- [1] E. Busseron, Y. Ruff, E. Moulin and N. Giuseppone. *Nanoscale*, **5**, 7098-7140 (2013).
- [2] F. J. M. Hoebe, P. Jonkheijm, E. W. Meijer and Albertus P. H. J. Schenning. *Chem. Rev.*, **105**, 1491-1546 (2005).
- [3] Y. Yamamoto. *Sci. Technol. Adv. Mater.*, **13**, 033001 (2012).
- [4] L. Feng, C. Zhu, H. Yuan, L. Liu, F. Lva and S. Wang. *Chem. Soc. Rev.*, **42**, 6620-6633 (2013).
- [5] D. Aigner, R. I. Dmitriev, S. M. Borisov, D. B. Papkovsky and I. Klimant. *J. Mater. Chem. B*, **2**, 6792-6801 (2014).

POSTER PRESENTATIONS Food Science

Use of oleic-acid functionalized nanoparticles for the magnetic solid-phase microextraction of alkylphenols in fruit juices using liquid chromatography-tandem mass spectrometry

M. Hernández-Córdoba*, M. Pastor, A. Torres P. Viñas, N. Campillo.

Department of Analytical Chemistry, Faculty of Chemistry, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, E-30100 Murcia, Spain.

*hcordoba@um.es

Alkylphenols (APs) are considered as environmentally persistent contaminants, and are classified as endocrine disrupting chemicals (EDCs) [1], being subjected to EU controls [2]. The use of industrial and waste waters for irrigation purposes may lead to the presence of these compounds as food contaminants, especially in fruits and vegetables. The exposure route for these chemicals in humans is mainly through water, although there is also some exposure through soil and foods [3].

Magnetic nanoparticles of cobalt ferrite with oleic acid as the surfactant (CoFe₂O₄/oleic acid) were used as sorbent material for the determination of the APs, 4-*tert*-butylphenol (TBP), 4-pentylphenol (PP), 4-hexylphenol (HP), 4-*tert*-octylphenol (TOP), octylphenol (OP), 4-nonylphenol (NP), and the alkylphenol ethoxylates (APEOs), 4-*tert*-octylphenol monoethoxylate (TOP₁EO), 4-*tert*-octylphenol diethoxylate (TOP₂EO), 4-nonylphenol monoethoxylate (NP₁EO) and 4-nonylphenol diethoxylate (NP₂EO) in fruit juices.

The fruit juice was homogenized and centrifuged for 5 min at 3000 rpm. Then, 10 mL of fruit juice supernatant diluted to 25 mL with water and spiked at 80 ng mL⁻¹ with a solution of benzo(k)fluoranthene (Bkf, internal standard) was added to 50 mg of CoFe₂O₄/oleic acid nanoparticles and shaken for 10 min at room temperature for the adsorption of APs and APEOs. Desorption was carried out with 3 mL of methanol by orbital shaking for 5 min. Finally, the extract was filtered and evaporated to dryness under an argon flow. The residue was reconstituted with 100 µL of acetonitrile, and 30 µL was injected into the LC/MS in positive mode and 30 µL in negative mode. An Eclipse XDB-C18 (5 cm x 0.46 cm x 5 µm) analytical column was used. The mobile phase was a mixture of acetonitrile and 0.1 % (v/v) formic acid, operating under gradient elution with the optimized program being: an isocratic step with 55:45 acetonitrile:formic acid (v/v) for 3.5 min; a linear gradient to 70:30 (v/v) in 0.5 min, which was held for 1.5 min; a linear gradient to 80:20 (v/v) in 1.5 min, which was held for 13 min. The analytes eluted with retention times between 4.2 and 14.1 min corresponding to TBP and NP₂EO, respectively, under a flow-rate of 0.4 mL min⁻¹.

Electrospray-ion trap-tandem mass spectrometry (ESI-IT-MS/MS) was selected for ionization. TBP, PP and HP were not ionized using the ESI system and were detected by diode-array (DAD). OP, TOP and NP showed better sensitivity in negative mode, meanwhile maximum sensitivity for the ethoxylated APs was obtained operating in ion positive mode. Good sensitivity and selectivity were achieved when operating in the SRM mode, with two transitions for each compound.

Detection limits were in the range 1.1 to 38 ng mL⁻¹. The recoveries obtained were in the 91-119% range, with RSDs lower than 14%. Different fruit juice samples were analyzed to determine the AP content by the proposed method. The most frequently found compounds were TOP, NP and TOP₁EO, which were present at very low concentrations.

References.

- [1] B. Kudlak, J. Namieśnik, Environmental fate of endocrine disrupting compounds-analytical problems and challenges, *Crit. Rev. Anal. Chem.* **38**, 242–258 (2008).
- [2] Directive 2003/53/EC of the European Parliament and of the Council of 18 June 2003, amending for the 26th time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (nonylphenol, nonylphenol ethoxylate and cement), 2003.
- [3] K. Guenther, V. Heinke, B. Thiele, E. Kleist, H. Prast, T. Raecker, Endocrine disrupting nonylphenols are ubiquitous in food, *Environ. Sci. Technol.* **36**, 1676–1680 (2002).

CO₂ sensor for food application

I. M. Pérez de Vargas-Sansalvador¹, D. Diamond², L. F. Capitan-Vallvey^{1,*}.

¹ECsens group, Department of Analytical Chemistry, University of Granada, Spain.

²Adaptive Sensors Group, Insight, National Centre for Sensor Research, Dublin City University, Ireland.

*lcapitan@ugr.es

The carbon dioxide levels inside meat packages can be used as indicator of freshness. If the CO₂ concentration changes during storage it is a clear indicator that bacteria are growing inside the container and / or the package is not well sealed and the modified atmosphere has been compromised. However, a non-destructive method for determining the CO₂ concentration within the package has not, as yet, been reported.

To this end, the objective of the SmartPack project is to exploit the development and integration of a CO₂ sensor in meat packages using the imaging and communications capabilities of Smartphones for freshness detection.

Optical CO₂ sensors based on the acidity of this molecule, are normally solvent-based sensors, the drawback of this approach in the food packaging industry is due to the long-term instability of the sensors, arising from the quaternary ammonium hydroxides decomposition. However, in this project we avoid the use of these compounds. Water based sensors are prepared using meta cresol purple sodium salt as indicator, glycerol as plasticizer and sodium bicarbonate as buffer in a matrix of hydroxyethyl cellulose. In this way, the lifetime is increased and also this composition creates an easily printable ink. Moreover, ionic liquids have been included in the matrix making the sensor more selective to CO₂ than other gases due to its higher solubility.

This new water-based sensor has been characterised in terms of carbon dioxide sensitivity, dynamic response, and stability under different conditions.

The sensor responds up to 100% of carbon dioxide. In Figure 1 can be observed the change in colour from 0 to 100% of CO₂. Moreover, it has been demonstrated that the stability is much higher than the solvent-based sensors making them suitable for smart packaging application.

The sensitive ink has been optimised and characterized using bench-top instrumentation. Moreover, the RGB and HSV readout of standard digital photographic cameras have been used as a simple imaging technique

The next steps include testing of the printed sensors integrated in meat packages using a mobile phone application as a detector to progress the study towards the intended application as a smart packaging tool.

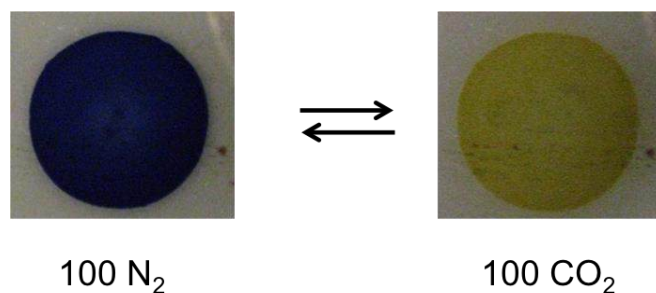


Figure 1. Water based CO₂ sensor colour variation at 100% Nitrogen and 100% CO₂ atmospheres.

Acknowledgements

This research is supported by Talentia Postdoc Fellowship (Junta de Andalucía - EU funded FP7 Marie Curie People, Grant agreement 267226).

Discrimination of DOP Andalusian Vinegars by combining Multidimensional Fluorescence and Chemometric approaches

R. Ríos-Reina¹, R. M. Callejón^{1*}, J. A. Ocaña², M. L. Morales¹, D. L. García-González³, J. M. Amigo⁴.

¹Área de Nutrición y Bromatología, Fac. Farmacia, Univ. Sevilla, C/P. García Gonzalez no. 2, E-41012 Sevilla, Spain.

²Dept. Química Analítica. Fac. Química. Univ. Sevilla. C/ P. García Gonzalez s/n, E-41012 Sevilla, Spain.

³Instituto de la Grasa (CSIC), Campus University Pablo de Olavide - Building 46, Ctra. de Utrera, km. 1 E-41013, Sevilla, Spain.

⁴Department of Food Sciences, Spectroscopy and Chemometrics, Faculty of Sciences, Univ. Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark.

*rcallejon@us.es

Nowadays, there is a growing demand for high quality food products in the market. This is the case of wine vinegar, which is a product that is becoming more appreciated by the consumer for its organoleptic characteristics and versatility in modern cooking. In fact, some wine vinegars, traditionally linked to a certain geographical area, have been protected by the European Union with a legislative system known as "Protected Designation of Origin" (PDO), obtaining a protection of their specifications related to their chemical and sensory features, their production system as well as a certification [1]. In Andalucía, there are three types of wine vinegars protected by a PDO: "Vinagre de Jerez", "Vinagre Condado de Huelva" and "Vinagre Montilla-Moriles". All of them are produced by the so-called "criaderas y solera" ageing system which provides a remarkable complexity and organoleptic characteristics [2]. Within each PDO, there are different categories according to their ageing time in wood barrels. Thus, these vinegars have different prices in the market according to their quality, being the longer ageing time and hence, the higher cost of their production. In consequence, these products are more subjected to frauds [2]. In addition, the growing consumer demand as well as the increasing diversity of wine vinegars, has generated a need to characterize them and to ensure an adequate quality to demonstrate and defend their identity. For this purpose, there is an increasing need to develop rapid, inexpensive and effective analytical methods, as well as requiring low sample manipulation for the characterization of DOP wine vinegars. Fluorescence spectroscopy is emerging as a competitive technique for this purpose, since provides in a few seconds an excitation-emission landscape that may be used as a fingerprint of the vinegar. Multi-way analysis, specifically Parallel Factor Analysis (PARAFAC), is a powerful tool for simultaneous determination of fluorescent components, because they extract the most relevant information from the data and allow building robust models. Moreover, the information obtained by PARAFAC can be used to build robust and reliable classification and discrimination models (e.g. by using Support Vector Machines and Partial Least Squares-Discriminant Analysis models). In this context, the aim of this work was to study the possibilities of multi-way fluorescence linked to PARAFAC and to classify the different Andalusian DOP wine vinegars. The results demonstrated that the use of the proposed analytical and chemometric tools are a perfect combination to extract relevant chemical information about the vinegars as well as to classify and discriminate them considering their corresponding DOP and the different ageing.

Acknowledgements

This work was supported by an FPU scholarship of the "Ministerio de Educación, Cultura y Deporte" (MECD, Spanish Government) and by the "Consejería De Economía, Innovación Y Ciencia, Junta de Andalucía" under the project P12-AGR-1601.

References

- [1] F. Chinnici, E. D. Guerrero, F. Sonni, N. Natali, R. N. Marín and C. Riponi. *J. Agric Food Chem.*, **57**(11), 4784–4792 (2009).
- [2] R. M. Callejón, J. M. Amigo, E. Pairo, S. Garmón, J. A. Ocaña and M. L. Morales. *Talanta*, **88**, 456–462 (2012).

ATR-FTIR Spectroscopy as a useful tool for characterizing wine vinegars with Protected Designation Of Origin

R. Ríos-Reina¹, C. Oliver-Pozo², J. M. Amigo³, R. M. Callejón¹, D. L. García-González^{2,*}.

¹Área de Nutrición y Bromatología, Fac. Farmacia, Univ. Sevilla, C/P. García Gonzalez no. 2, E-41012 Sevilla, Spain.

²Instituto de la Grasa (CSIC), Campus University Pablo de Olavide - Building 46, Ctra. de Utrera, km. 1 E- 41013, Sevilla, Spain.

³Department of Food Sciences, Spectroscopy and Chemometrics, Faculty of Sciences, Univ. Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark.

*dluisg@cica.es

Some wine vinegars are traditionally linked to a certain geographical area, and the European Union protects them with a legal framework that provides the category of "Protected Designation of Origin" (PDO). These vinegars have high prices in the market due to their high quality, the long aging time and the high cost of their production. That explains that these products are vulnerable to frequent fraud [1]. In addition to that, the growing consumer demand as well as the increasing diversity of wine vinegars has raised a need to characterize them and to provide an adequate quality control to defend their identity. For this reason, new tools are required to fight against falsification or mislabeling.

Fourier Transform mid infrared spectroscopy (FTIR), equipped with Attenuated Total Reflectance (ATR) has proven to be an appropriate method for the authentication of several liquid foods [2,3]. However, in regards to vinegar authentication, scarce research has been done to develop a method with a rapid technique, such as ATR-FTIR, to characterize vinegars belonging to different PDOs and different categories of aging time.

For this purpose, new spectroscopy techniques such as ATR-FTIR has been used to investigate the potential of this technique as a rapid, cost-effective and non-destructive tool for vinegar characterization considering the three major PDO from Andalusia ("Jerez", "Condado de Huelva" and "Montilla-Moriles"). Spectra from 84 wine vinegars belonging to the three PDO including different categories have been analyzed and compared in the infrared region of 4000-600 cm⁻¹. The best wavelength regions for predicting the authenticity criteria were selected and a proper chemical explanation of the assignment was provided to help in the interpretation of results. Changes associated to aging and categories were observed in the bands located in the "fingerprint" region of the spectrum (1800-900 cm⁻¹). These bands were assigned to certain specific compounds that increase during aging (e.g. alcohols, esters, ethers) or due to special compounds of Pedro Ximenez categories (e.g. sugars, furfural). The ability of these bands for assessing the aging and categories of PDOs was checked by Principal Component Analysis. This analysis revealed the importance of the region associated to acetic acid (1800-1680 and 1475-1230 cm⁻¹) and the bands at 1085, 1045 and 1015 cm⁻¹, characteristics of ethanol, alcoholic and esters compounds. The advantages of this technology (e.g. fast, no-destructive methodology and no-sample preparation) would allow implementing this measurement as an additional control for PDO councils and producers to assess the authenticity of PDO, categories, and to monitor the aging process.

Acknowledgements

This work was supported by an FPU scholarship of the "Ministerio de Educación, Cultura y Deporte" (MECD, Spanish Government) and by the "Consejería de Economía, Innovación y Ciencia, Junta de Andalucía" under the project P12-AGR-1601.

References

- [1] R. M. Callejón, J. M. Amigo, E. Pairo, S. Garmón, J. A. Ocaña, and M. L. Morales. *Talanta*, **88**, 456–462 (2012).
- [2] D. Dong, W. Zheng, L. Jiao, Y. Lang, and X. Zhao. *Food Chem.*, **194**, 95–100 (2016).
- [3] I. Gouveias, J. M. M. de Almeida, T. Carvalho, N. Machado, and A. I. R. N. a. Barros. *Food Chem.*, **174**, 226–232 (2015).

Front-face fluorescence spectroscopy for the monitoring of intact extra virgin olive oils

I. Durán-Merás*, J. Dominguez-Manzano .

Department of Analytical Chemistry and IACYS, University of Extremadura, 06006, Badajoz, Spain.

*iduran@unex.es

The application of fluorescence spectroscopy in food analysis has significantly increased because of its many advantages compared to other spectroscopic techniques: 100–1000 times better sensitivity [1], easy adaptation to on-line monitoring, little sample preparation, and rapid and relatively low-cost instrumentation and use. Food storage can deeply modify the intrinsic quality parameters of raw fresh products by destroying the natural bioactive and nutritional compounds or by generating neoformed compounds some of them being potentially health damaging. Rapid and non-destructive quality assessment methods are needed to objectivize the naturalness and authenticity of organic food.

Fluorescence typical for phenolic compounds of olive oils was reported [2]. On the other hand the effect of the dilution on the total fluorescence spectrum of extra virgin olive oils, has been also reported [3]. Fluorescence spectroscopy of oil samples diluted in *n*-hexane was proposed for the monitoring of extra virgin olive oil during storage [4].

Front-face fluorescence technique give us the opportunity of use a non-destructive technique and the analysis of intact foods avoiding the changes interactions that may occur when samples are diluted.

The intrinsic fluorescence of olive oils takes the advantage that it is possible obtaining spectroscopy images and use its evolution for evaluate the conditions during the storage. The purpose of this work is to use this signal as fingerprint of the olive oils and to facilitate monitoring of the oil quality only through images, without the need to assess or quantify other parameters.

With the object to avoid that the signal from the fluorophores of interest lies close to, or on top of the Rayleigh scatter, two different spectroscopy images of intact extra virgin olive oils were obtained, one of them corresponding to tocopherols and phenols and the other corresponding to chlorophylls and pheophytins. The changes produced under different storage conditions were evaluated and in this sense variables such as the influence of UV-irradiation, exposure to sun-light, and the effect of the temperature storage have been studied.

The fluorescent profile of the tocopherols and phenols is principally affected by the UV-irradiation and by the temperature.

The effect of sun-light is higher in the fluorescence image corresponding to pigments and it is negligible in the spectral range of tocopherols and phenols.

Acknowledgements

MEyC of Spain (CTQ2014-52309P) and Gobierno de Extremadura (GR15090-FQM003), co-financed by European FEDER funds.

References

- [1] G.M. Strasburg, R.D. Ludescher, *Trends Food Sci. Tech.*, **6**, 69-75 (1995).
- [2] N. Dupuy, Y. Le Dréau, D. Ollivier, J. Artaud, C. Pinatel, J. Kister., *J. Agric. Food Chem.*, **53**, 9361-9368 (2005).
- [3] E. Sikorska, I.V. Khmelinskii, M. Sikorski, Analysis of olive oils by fluorescence spectroscopy: Methods and applications, in *Olive oil: Constituents, health properties and bioconversions*, Ed. D. Boskou, 63-88 (2012).
- [4] E. Sikorska, I.V. Khmelinskii, M. Sikorski, F. Caponio, M.T. Bilancia, A. Pasqualone, T. Gomes, *Intern. J. Food Sci. and Technol.*, **43**, 52–61 (2008).

Smartphone as an economic and reliable tool for monitoring sparkling wine browning process

R. M. Callejón^{1*}, M. Villar-Navarro², C. Ubeda¹, **M. L. Morales¹**, J. L. Pérez-Bernal².

¹Área de Nutrición y Bromatología, Fac. Farmacia, Univ. Sevilla, C/P. García Gonzalez no. 2, E-41012 Sevilla, Spain.

²Dept. Química Analítica. Fac. Química. Univ. Sevilla. C/ P. García Gonzalez s/n, E-41012 Sevilla, Spain.

*rcallejon@us.es

Nowadays, sparkling wine is produced almost everywhere in the world and is becoming more appreciated and demanded by the consumer. The most famous sparkling wines include champagne from France or cava from Spain among others [1]. Thus, cava is a highly-quality sparkling wine (designation of origin) produced by the traditional method, which undergoes a biological ageing for at least 9 months in contact with lees under anaerobic conditions in bottle. In addition, there are different categories according the biological aging: Reserva, with at least 15 months in contact with the lees, and Gran Reserva, with at least 30 months in contact with the lees.

As wine is a dynamic product in terms of its physicochemical and sensory properties, sparkling wine undergoes many complex chemical changes during the biological aging and storage, which are accompanied by alterations of the organoleptic characteristics, specially aroma or color [2]. Browning is an oxidative process involving sugars, lipids, amino acids and phenols, which decreases the sensorial quality of wines (loss of colour, flavour and aroma, and increment of astringency) [3]. Thus, since quality is of prime relevance for sparkling wines, browning has to be controlled during processing and storage. Therefore, there is a need to find fast and accurate methods for monitoring the extent of browning reaction as well as alternative quality markers for sparkling wines.

In this context, a fast simple and reliable method to study the wine browning based on colorimetric measurements is proposed. Using a smartphone camera and a suitable lightbox as measurement device, is possible to obtain digital images of different wine stages and, by means of digital image analysis data, they are gathered and analysed in the RGB color system. Preliminary results show that browning process affects mainly to the Blue channel with a time dependent decay, while Red and Green channel remained nearly constant. That corresponds to a shift to darker (higher absorbances) and "brownier" color. Correspondingly, a very close correlation with results obtained by measuring absorbance at 420 nm has been found in all the cases, showing the possible application of the proposed methodology to the study of browning process. Among others, the advantages of the proposed methodology are the lack of sample treatment, the affordable instrumentation and it is easy to use.

Acknowledgements

The authors want to thank CONICYT for the financial support provided through project FONDECYT 11140275 and the Universidad Autónoma de Chile.

References

- [1] B. Kemp, H. Alexandre, B. Robillard and R. Marchal. *J. Agric. Food Chem.*, **63**, 19–38 (2015).
- [2] S. Kallithraka, M.I. Salacha and I. Tzourou. *Food Chem.*, **113**, 500–505 (2009).
- [3] V. Ferreira, A. Escudero, P. Fernández and J. F. Cacho. *Eur. Food Res. Technol.*, **205**, 392–396 (1997).

Assessment of Arsenic Species in Rice Samples

A. Jiménez Rodríguez, M.C. Barciela-Alonso, M. R. Domínguez-González,
P. Bermejo-Barrera*

GETEE Group, Department of Analytical Chemistry, Nutrition and Bromatology. Faculty of Chemistry,
Universidade de Santiago de Compostela. Avenida das Ciencias s/n, 15782 – Santiago de Compostela,
Spain.

*pilar.bermejo@usc.es

Rice is one of the most consumed cereals in the world and the principal food in many countries for billion of people. Rice plant has the ability to bioaccumulate trace elements such as arsenic and may contain several species with different toxicity. The inorganic forms of arsenic are more toxic than organic forms and the toxicity increases with decreasing oxidation states [1]. Children under three years of age are the consumers of rice in Europe most exposed to inorganic arsenic thorough the diet (rice-based food). Recently, the European Commission established the maximum levels of inorganic arsenic in foodstuffs. This regulation establishes a maximum level of inorganic arsenic of 0.10 mg/Kg in rice destined for the production of food for infants and young children [2].

The aim of this work was the development of a method for the determination of arsenic species in rice samples. The arsenic species were extracted from rice samples using microwave-energy and Milli-Q® water as extraction solution. Arsenic species were then determined in the extracts by high performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (HPLC-ICP-MS). A Hamilton PRP-X100 (150 x 4.1 mm, 5 µm particle size) column was used for the separation of arsenic species, using 25 mM NH₄H₂PO₄ (pH 6) as mobile phase operating at a flow rate of 1 mL min⁻¹. Working in these conditions, the arsenic species were separated in less than 10 minutes. The method developed was sensitive (LODs of 25, 15, 13 and 31 ng g⁻¹ for As(III), DMA, MMA and As(V), respectively) and precise (RSD<10%). The accuracy of the method was evaluated using the certified reference material ERM-BC211 Rice (Institute for Reference Materials and Measurements, IRMM). The method developed was applied for arsenic speciation in several rice samples.

Acknowledgements

The authors wish to thank the *Xunta de Galicia (Grupo de Referencia Competitiva 6RC2014/2016)* for financial support.

References

- [1] A. Leonard. Arsenic metals and their compounds in the environment. Occurrence, analysis and biological relevance. Weinheim: Wiley, VCH, (1991).
- [2] Commission Regulation (EU) 2015/1006 of 25 June 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels of inorganic arsenic in foodstuffs.

Assessment of antioxidants bio-availability in edible nuts and seeds

P. Herbello-Hermelo¹, M. R. Domínguez-González¹, J. P. Lamas², M. Lores², P. Bermejo-Barrera¹, **A. Moreda-Piñeiro^{1,*}**.

¹GETEE - Department of Analytical Chemistry, Nutrition and Bromatology. Faculty of Chemistry. Universidade de Santiago de Compostela. Avenida das Ciencias, s/n. 15782, Santiago de Compostela. Spain.

²LIDSA - Department of Analytical Chemistry, Nutrition and bromatology. Faculty of Chemistry. Universidade de Santiago de Compostela. Avenida das Ciencias, s/n. 15782, Santiago de Compostela. Spain.

*antonio.moreda@usc.es

A bio-availability study based on an *in vitro* dialyzability approach has been applied to assess the bio-available fraction of antioxidants in edible nuts and seeds. The *in vitro* digestion procedure consisted of two sequential stages, which simulate gastric and intestinal digestion. The first step requires 0.15g of a freshly prepared gastric solution [6.0%(m/v) pepsin in 6.0 M hydrochloric acid], and incubation at 37°C with an orbital – horizontal shaking at 150rpm for 120min. The second step uses 5.0 mL of intestinal solution (4.0 %(m/v) pancreatine and 2.5%(m/v) bile salts dissolved in 0.1 M sodium hydrogen carbonate), and also incubation at 37°C with an orbital – horizontal shaking at 150rpm for 120min]. During this second stage, dialysis membranes (10 kDa cut-off) filled with 20mL of 0.15N PIPES (pH 7.5) were used for simulating bio-absorption. Total antioxidants in nuts/seeds and dialysates were extracted by pressurized liquid extraction (PLE) using sand as dispersant and 63% aqueous methanol as extraction solvent in two cycles at 105 °C. Antioxidants in the dialysates were determined in the form of total polyphenols content by the Folin-Ciocalteu spectrophotometric method ($\lambda= 760$ nm), and the results were expressed as mg of gallic acid equivalents per g of dry weight (GAE/g·dw). Bio-availability ratios (dialyzability) were assessed by calculating the percentages of total antioxidant concentrations in the PIPES solution inside the dialysis membranes (bio-available fraction), and those found in the foodstuff after PLE. In general, high dialyzability ratios were assessed in most of nuts/seeds (dialyzability percentages within the 25 – 91 % range). The highest dialyzability ratios were found in fried *Marcona* almonds (91%); whereas, percentages of 78% were assessed in raw pine nuts, 76% in toasted peanuts (a legume), and 75% in raw *Largueta* almonds. Moderate antioxidant dialyzability was found in raw hazelnuts (56%), toasted pistachio (48%), Macadamia nuts (47%), toasted *Largueta* almonds (42%), toasted sunflower seeds (46%), fried cashews (35%), and raw pecans (34%). However, low dialyzability was found in Goji berries (30%), nuts (25%), raw pumpkin seeds (20%), Brazil nuts (17%), and chestnuts (15%). Finally, the effect of major nutrient constituents (protein, fat, carbohydrate, and dietary fiber) of nuts/seeds on the dialyzability of antioxidants was statistically evaluated.

Acknowledgements

The authors are grateful for the financial support provided by the Dirección Xeral de I+D – Xunta de Galicia (Project number 6RC2014/2016 *Grupos de Referencia Competitiva*, and project number R2014/013 Consolidated Research Units Program), and European Regional Development Fund (FEDER), projects number 6RC2014/2016 and GPC2014/035.

Use of trace and ultratrace metals for geographical origin differentiation of Chilean “natural wines” from Itata Valley

M. Latorre¹, C. Peña-Farfal¹, Y. Neira¹, P. Herbello-Hermelo², P. Bermejo-Barrera²,
A. Moreda-Piñeiro^{2,*}.

¹Department of Instrumental Analysis. Faculty of Pharmacy. University of Concepción. PO Box 237,4070043, Concepción. Chile.

²GETEE - Department of Analytical Chemistry, Nutrition and Bromatology. Faculty of Chemistry. Universidade de Santiago de Compostela. Avenida das Ciencias, s/n. 15782, Santiago de Compostela. Spain.

*antonio.moreda@usc.es

The term “natural wine” relates a wine made without chemical and minimum technological intervention in growing grapes, and also in winery practices when preparing the product. Itata Valley is one of the most important winemaking areas in Chile, located in the South Valley, a region comprising a vast area bordered by the Andes and a lower coastal mountain. Some wine producers from this area have recently started to change growing grapes and winery practices for marketing natural wines. Chemical characterization of these wines is scarce, and also attempts for geographical classification based on chemical data have not yet been addressed.

In this communication thirty-eight trace and ultratrace metals have been determined in seventy natural red and white wine samples from the same vintage (2014), and found concentrations have been used as discriminating features for assessing geographical classification. Metals have been assessed by inductively coupled plasma – mass spectrometry (ICP-MS), and sample pre-treatment consisted of sample dilution (1:10) with 1%(m/v) nitric acid. Determinations have been performed using the standard addition technique, and accuracy of method was mainly tested by analytical recovery assays. Principal components analysis (PCA), and Linear Discriminant Analysis (LDA) were used for exploratory and classification purposes.

An accumulated variance accounting 70% was obtained when using the first two principal components (PCs), and five groupings were observed when examining the two-dimensional plot of the samples defined by the first two PCs. The groupings correspond with the several vineyards located at areas close to the coast and to Andes. LDA results showed a successful classification of samples (100% of correctly classified cases in the training and prediction set). A cross validation test was used to validate the results of the classification. The metals that offered the highest discriminating power along the first two linear discriminant functions were Li, B, Mg, V, Zn, Cu, As, Mo, Cd, and Sn.

Acknowledgements

The authors are grateful for the financial support provided by the Dirección Xeral de I+D – Xunta de Galicia (Project number 6RC2014/2016 *Grupos de Referencia Competitiva*), and to *Comisión Nacional de Investigación Científica y Tecnológica*, CONICYT – Chile.

Comparison between NIR and MIR efficiency for assessing the impact of freezing conditions on phytochemicals in legumes

N. Machado^{1,*}, R. Dominguez-Perles¹, A. Ramos¹, A. Barros¹, E. Rosa¹.

¹Centre for the Research and Technology of Agro-Environmental and biological Sciences, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal.

*nmachado@utad.pt

The value of legumes as a source of bioactive compounds (nutrients and non-nutrients) depends on a plethora of factors, among which storage conditions present a preponderant relevance, modulating the value of edible seeds and vegetative material as a dietary source of nutrients and phytochemicals. Thus, additional approaches are required, in order to allow rapid evaluations of the effect of storage at freezing temperatures compatible with the close timing linked to industrial activities. In this concern, this work deals with the implementation of new analytical tools (NIR and MIR spectroscopic analyses, resorting to the DRIFT and ATR accessories, respectively), for the evaluation of dried pods of cowpea (*Vigna unguiculata* L.), regarding their phytochemical composition and radical scavenging power, aiming to the development of new approaches to assess the effect of freezing for distinct periods (6, 9 or 12 months) at different temperatures (-18 or -40 °C).

To achieve these objectives, PLS-R multivariate analyses were applied in order to produce discriminative models for plant materials undergoing distinct freezing periods and temperatures [1]. In addition, the joint application of spectroscopic analysis and multivariate tools allowed the development of prediction models, which were validated resorting to the CV (Cross-Validation) leave-one-out procedure. Furthermore, distinct spectral treatments such as the 1st derivative or the Savitzky-Golay (SGS) simultaneous derivative/smoothing were also assessed.

Concerning the discrimination between the distinct samples assessed, the DRIFT module (NIR) evidenced a better performance than the ATR (MIR). Hence, the former allowed the correct classification of all samples resorting to the first 4 Factors extracted, when the 1st derivative is used, whilst 5 Factors were necessary when the SGS treatment was applied to the spectra. In the case of the MIR spectra, the best performance was also observed for the 1st derivative, corresponding to a misclassified samples rate of 3.3%, when the maximum number of Factors (5, corresponding to the minimum number of replicates) was used.

Concerning the parameters evaluated on cowpea pods (total phenols, flavonoids, and the antiradical activity measured by two distinct assays, DPPH and ABTS) both spectroscopic techniques (ATR and DRIFT) showed a good performance, generally leading to regressions between predicted and experimental values of around $R^2 = 0.99$. Moreover, the NIR spectra consecutively led to lower errors in the RMSECV procedure, while the best performances were observed using either the spectra, or the 1st derivative, depending on the parameter to be assessed. In the case of ATR, the usage of the spectra corresponded always to lower errors, while concerning total phenols, this technique failed to provide a good calibration model, with the best correlation observed corresponding to $R^2 = 0.78$.

Finally, it can be elated that the spectra reflect specific variations of pods' composition, since it was possible to develop content evaluation models, besides being also shown the feasibility of this approach for the assessment of these plant materials, concerning freezing time and temperature, and the advantage of DRIFT (NIR) for this purpose.

Acknowledgements

N. F. L. Machado kindly acknowledges a post-doctoral research grant from the Centre for the Research and Technology of Agro-Environmental and Biological Sciences, with the reference BI/CITAB/UTAD/QUI/2015. This work is supported by: European Investment Funds by FEDER/COMPETE/POCI– Operacional Competitiveness and Internacionalization Programme, under Project POCI-01-0145-FEDER-006958 and National Funds by FCT - Portuguese Foundation for Science and Technology, under the project UID/AGR/04033/2013, and the European Project EUROLEGUME (Seventh Research Framework Programme of the European Union – FP7 research project nº 61378)

References

[1] S. Wold, M. Sjostrom, L. Eriksson, *Chem. Intell. Lab. Systems*, **58**, 109 – 130 (2001).

Cloud point extraction and atomic absorption spectroscopy for speciation analysis of manganese in bioaccessible fraction: nutritional relevance

F. Cámara-Martos*, J. Sevillano-Morales, M. A. Amaro-López, R. Moreno-Rojas.

Departamento de Bromatología y Tecnología de Alimentos. Universidad de Córdoba. Campus de Rabanales s/n. Edificio C1 – Darwin.

**bt2camaf@uco.es*

Trace element bioaccessible fraction is defined as a fraction of the trace element concentration initially present in the food which is solubilized in the intestinal lumen. Later, it will be efficiently absorbed, metabolized by physiological routes and used by the body for typical physiological functions or deposited in storage compounds [1]. Its determination is highly important to assess the true nutritional value of a meal. This trace element bioaccessibility is conditioned by several factors such as chemical form of element.

Mn activates many enzymes involved in several metabolic processes. It is needed for protein and fat metabolism, healthy nervous and immune systems, and for blood sugar regulation. Mn is involved in the utilization of vitamins B1 and E and it is required for normal bone growth [2]. It can be found in foods as Mn(II), Mn (VII) or Mn bounded to other molecules (peptides, polyphenols, fiber fractions). This later chemical form is poorly absorbed.

The procedure described by Cámara et al. [3] with slight modifications was used to estimate Mn bioaccessibility (solubility). The simplified process is based on mimicking the physiological conditions of the gastrointestinal tract. Thus, 3 g of lyophilized sample of food were submitted to a simulated digestion with pepsin and pancreatin – biliary salts during 4 hours. Finally the pH was adjusted to 7.2 with 0.5M NaOH. Aliquots of the digested sample were transferred to polypropylene centrifuge tubes and were centrifuged for 1 h at 3000 rpm and 4° C. Finally, the supernatant (soluble fraction) was collected, and submitted directly to the manganese speciation analysis. This procedure avoids possible transformations of manganese species due to sample manipulation.

Cloud Point Extraction (CPE) is based on the use of a surfactant such as Triton X-100. In much diluted solutions of non-ionic surfactant, the monomers are dispersed in the solvent. However, heating and choosing a compatible pH buffer, these monomers associate spontaneously, forming aggregates of colloidal dimensions, due to the reduced solubility of the surfactant in water. Choosing an appropriate chelating agent, able to form a hydrophobic complex entrapped by micellar structures with the Mn ion, extraction of Mn in the surfactant rich phase can be achieved. The phase separation can be accelerated by centrifuging and cooling. Aqueous phase is discarded and surfactant – rich phase is picked with HNO₃ 0.1 M. Finally, Mn could be analyzed by Atomic Absorption Spectroscopy.

In the present study chelating agents such as 1 – phenyl – 3 – methyl – 4 – benzoyl – 5 – pyrazolone (PMBP) and 8 – quinolinol (HOx) were assayed to different pH buffers (hydrochloric acid/ glycine; acetic/acetate; ammonium/chloride ammonia). It was also controlled several parameters such as [Triton X – 100], [PMBP], [HOX], pH, Temperature and time. Optimization method allows to identify Mn species in soluble fraction and relate them to their bioaccessibility

References

- [1] F. Cámara – Martos, JR. Marval – León and R. Moreno-Rojas, in: Selenium: dietary sources, properties and role in human health, W. Morrison (Ed.), Nova Publishers: New York, 1 – 28, 2015.
- [2] L. Ruzik, *Talanta*, **93**, 18 – 31 (2012).
- [3] F. Cámara, MA. Amaro, R. Barberá, G. Clemente, *Food Chem*, **92**, 481 – 489 (2005).

Influence of bioactive compounds on Fe, Zn and Mn bioaccessibility in quinoa (*Chenopodium quinoa*)

Z. L. Valencia-Reyes¹, R. Moreno-Rojas², F. Quispe³, F. Cámara-Martos^{2*}.

¹Universidad Cesar Vallejo, Av. Alfredo Mendiola 6232 Lima, Perú.

²Departamento de Bromatología y Tecnología de Alimentos. Universidad de Córdoba. Campus de Rabanales s/n. Edificio C1 – Darwin.

³Instituto Nacional de Innovación Agraria – INIA, Av. La Molina 1981 Lima, Perú.

*bt2camaf@uco.es

Introduction

Quinoa (*Chenopodium quinoa*) is a cereal cultivated in the Andean region for thousand years. Currently, it is being expanded because it represents a great potential to improve the living conditions of the Andes population. It is a good source of bioactive compounds such as polyphenols, betalains and flavonoids. Moreover, it could also provide significant amounts of trace elements such as Fe, Zn and Mn. However, bioaccessibility of these trace elements present in Quinoa species remains unknown.

Methodology

Five Quinoa varieties were studied (Blanca Junin, Amarillo Maracani, Negra Collana, Pasankalla and Altiplano). This later variety cultivated in different localizations, Lima and Puno.

Samples were cooked by boiling in water during 20 minutes and Fe, Zn and Mn concentrations were analyzed by Atomic Absorption Spectroscopy prior destruction of organic matter.

Likewise, polyphenols (765 nm), betalains (476, 538 and 600 nm) and flavonoids (510 nm) concentrations in samples were determined by UV – Visible Spectroscopy using the wavelengths above indicated [2-3].

Bioaccessibility assays: The procedure described by Cámara et al. [1] with slight modifications was used to estimate trace element bioaccessibility. The simplified process is based on mimicking the physiological conditions of the gastrointestinal tract. Thus, 3 - 5 g of lyophilized sample of food was submitted to a simulated digestion with pepsin and pancreatin – biliar salts during 4 hours. Moreover, these bioaccessibility assays allow us to distinguish between soluble fraction (that remaining soluble in the lumen, thus able to be absorbed) and dialyzable fraction (which besides solubilized, it is capable of crossing a membrane with a certain pore size) [4].

Soluble and dialyzable fractions were collected, its organic matter was destroyed, and the trace element content was measured by Atomic Absorption Spectroscopy.

Results

Trace element concentrations in Quinoa varieties studied ranged between 33.9 – 7.9 µg/g for Fe; 24.2 – 8.7 µg/g for Zn and 22.2 – 6.6 µg/g for Mn. However the bioaccessibility (solubility and dializability) of these trace elements were extremely low, with percentages around 1%. The high concentrations of bioactive compounds present in Quinoa species may impair Fe, Zn and Mn bioaccessibility. Different strategies should be developed to enhance this bioaccessibility in order to improve its nutritional value

References

[1] F. Cámara, MA. Amaro, R. Barberá, G. Clemente, *Food Chem*, **(92)**, 481 – 489 (2005).

[2] I. Dini, G. Tenore, A. Dini, *LWT Food Sci Tech* **(43)**, 447 – 451 (2010).

[3] F. Quispe, K. Arroyo, G. Gorriti, *Rev Soc Quim Perú* **(77)**, 205 – 207 (2011).

[4] F. Cámara – Martos, JR. Marval – León and R. Moreno-Rojas, in: Selenium: dietary sources, properties and role in human health, W. Morrison (Ed.), Nova Publishers: New York, 1 – 28, 2015.

Effect of oak wood chips in the anthocyanin extraction from grape skins previously classified using near infrared hyperspectral image

B. Baca-Bocanegra¹, J. Nogales-Bueno¹, F. J. Rodríguez-Pulido¹, F. J. Heredia¹, J. M. Hernández-Hierro¹.

¹Food Colour & Quality Laboratory, Department of Nutrition & Food Science, Universidad de Sevilla, Spain.
*bbaca1@us.es

Anthocyanins extracted from grape skin are the principal compounds involved in the color of red wines and their interactions with other phenolic compounds (called copigments), normally colorless, allow improving the color stabilization of aged wines by copigmentation reactions [1]. Therefore, it is important to know the amount of anthocyanins that may be extracted from grapes to wine. Several factors can influence this extractability [2]. The extractability of anthocyanins could also be affected due to the addition of sources of copigments.

The main goal of this study was to study the effect of the addition of oak wood chips on the extractability of anthocyanins from heterogeneous grapes. Those grapes were previously classified by hyperspectral image analysis according to the amount of anthocyanins transferred to the extraction media [3].

Ten *Vitis vinifera* L. cv. Syrah grape skins from grapes that had been previously classified as low extractable anthocyanin content were underwent to simulated maceration in wine-like solution, with or without French oak (*Quercus petraea* L.) wood light toasted chips. Oak wood chips were added to the wine-like solution in a 4 g L⁻¹ ratio. Grape skins were added to extraction media in a 1:20 ratio. For each sample, anthocyanin composition of the extracts was measured at the third day of maceration. Anthocyanins chromatographic analysis was carried out and results were expressed as mg of malvidin-3-O-glucoside equivalents per gram of grape skin [4-5]. All analyses were performed in duplicate.

Univariate analysis of variance (ANOVA) was applied to discriminate among the means of chemical data (i.e. individual, acylated, non acylated and total anthocyanins) taking into account pairs of control wines and wines with addition of oak wood chips. There were not significant differences among wines and for each variable. Furthermore, principal component analysis was used as an unsupervised pattern recognition method in order to obtain a general overview. The presence of oak wood chips in the extraction media did not cause significant changes in the anthocyanin extractability for all samples. Taking into account, the addition of oak wood chips may be a good technique to obtain red wines that will present high color quality and stability due to the copigmentation procedure.

Acknowledgements

The Spanish MINECO is thanked for J. Nogales-Bueno FPI grant (BES-2012-060192) and project AGL2014-58486-C2. Universidad de Sevilla is thanked for J.M. Hernández-Hierro extension of Juan de la Cierva postdoctoral contract and also for B. Baca-Bocanegra predoctoral grant (VPPI-Universidad de Sevilla). The authors also thank the technical staff of Biology Service [Servicios Generales de Investigación (SGI), Universidad de Sevilla].

References

- [1] B. Gordillo, M.J. Cejudo-Bastante, F.J. Rodríguez-Pulido, M.L. Gonzalez-Miret, F.J. Heredia, *Food Chem*, **141**, 2184-2190. (2013).
- [2] P. Ribereau-Gayon, Y. Glories, A. Maujean, D. Dubourdieu, *Handbook of Enology*, vol. 2, the Chemistry of Wine: Stabilization and Treatments. John Wiley & Sons Ltd., Baffins Lane, Chichester, 2000.
- [3] J. Nogales-Bueno, B. Baca-Bocanegra, F. J. Rodríguez-Pulido, F. J. Heredia and J. M. Hernández-Hierro, *Food Chem*, **172**, 559 – 564. (2015).
- [4] M. García-Marino, J. M. Hernández-Hierro, J. C. Rivas-Gonzalo and M. T. Escribano-Bailón, *Anal. Chim. Acta*, **660**, 134 – 142. (2010).
- [5] J. M. Hernández-Hierro, J. Nogales-Bueno, F. J. Rodríguez-Pulido and F. J. Heredia, *J. Agr. Food. Chem*, **61**, 9804–9809. (2013).

Use of near infrared hyperspectral tools for the screening of relevant parameters in coffee beans

B. Baca-Bocanegra*, J. Nogales-Bueno, L. Romero-Molina, M. L. Escudero-Gilete, F. J. Heredia, J. M. Hernández-Hierro.

Food Colour & Quality Laboratory, Department of Nutrition & Food Science, Universidad de Sevilla.

**bbaca1@us.es*

Coffee (*Coffea* sp.) is one of the most-consumed beverages in the world being aroma and taste their main sensory characteristics. The chemical composition of coffee beans, affected by a huge number of factors (bean production, coffee type, roasting process), and the quantity of compounds that would be extracted into the beverage may determine its quality and sensory profiles [1-3]. The established analytical methods usually employed in the coffee industry for determination of these descriptors are time-consuming, requiring sample preparation and even chemical manipulations. Therefore, simple and rapid methods, capable of being easily implemented in routine analyses, are needed.

In the present work, the potential of near infrared hyperspectral imaging to determine extractable caffeine and total extractable phenolic levels in coffee has been evaluated. Hyperspectral images of coffee beans were recorded using a near infrared hyperspectral imaging device covering the spectral range between 900 and 1700 nm. Spectral data have been correlated with reference values (extractable caffeine content and extractable total phenolic content) by modified partial least squares regression (MPLS) using a number of spectral pre-treatments. The obtained results (coefficient of determination (RSQ) and standard error of prediction (SEP) respectively) for the developed models were: 0.97 and 1.80 mg g⁻¹ of coffee for extractable caffeine content, 0.58 and 6.28 mg g⁻¹ of coffee for extractable total phenolic content.

Thus, near infrared hyperspectral imaging has been shown to provide a very suitable strategy for modelling and predicting two coffee attributes (extractable caffeine content and extractable total phenolic content) of great relevance from a quality assurance point of view in coffee samples. The promising results obtained will allow a similar methodology to be considered in future applications related to further coffee quality parameters.

Acknowledgements

The Spanish MINECO is thanked for J. Nogales-Bueno FPI grant (BES-2012-060192) and project AGL2014-58486-C2. Universidad de Sevilla is thanked for J.M. Hernández-Hierro extension of Juan de la Cierva postdoctoral contract and also for B. Baca-Bocanegra predoctoral grant (VPPI-Universidad de Sevilla). The authors also thank the technical staff of Biology Service [Servicios Generales de Investigación (SGI), Universidad de Sevilla].

References

- [1] A. S. Franca, J. C. F. Mendonça, S. D. Oliveira, *LWT Food Sci. Technol.*, **38**, 709-715 (2005).
- [2] I. Esteban-Díez, J. M. González-Saiz, C. Pizarro, *J. Near Infrared Spectrosc.*, **12**, 287-298 (2004).
- [3] R. J. Clarke, R. Macrae, *Coffee*, Vol. 1, Chemistry. Elsevier Applied Science Publishers, London, U.K., 1985.

Effect of white grape seed in the anthocyanin extraction from red grape skins previously classified by near infrared hyperspectral tools

J. Nogales-Bueno¹, B. Baca-Bocanegra¹, M. J. Jara-Palacios¹, F. J. Rodríguez-Pulido¹, F. J. Heredia¹, J. M. Hernández-Hierro¹.

¹Food Colour & Quality Laboratory, Department of Nutrition & Food Science, Universidad de Sevilla.

*julionogales@us.es

Anthocyanins are important polyphenols present in red grapes and play a relevant role in the sensory characteristics of red wines. They are red-coloured phenols that provide to red wine its characteristic colour. This colour is based on the fully conjugated flavylum chromophore [1]. The colour of red wine is an important quality parameter, it is usually the first characteristic perceived by consumers, who usually tend to prefer wines with a deep colour and hue. Thus, wine colour plays a key role in the decision-making process of consumers [2].

Anthocyanins are transferred to wine from red grape skin during the fermentation process. It is really important to know the amount of these phenols that may be extracted from grapes to wine, i.e., the extractable anthocyanin content. This extraction is influenced by a range of factors such as grape ripeness, variety, characteristics of the maceration stage, total anthocyanin content, etc. The addition of copigments improves wine colour, however, copigment sources, such as white grape seeds, might affect anthocyanin extraction [3].

In this work, a feasibility study on the effect of natural copigments from white grape seeds on anthocyanin extraction of grape skin has been developed. Firstly, a stock model wine solution was made up of 4 g L⁻¹ tartaric acid and 12.5% ethanol, adjusted at pH 3.6 with NaOH 0.5 M. Then, white grape seeds that had been previously characterised [4] were macerated in the model wine solution for three days. The concentration of total phenols in this stock solution was 2.6 g L⁻¹ expressed as gallic acid equivalents. From this solution, two model wine solutions were made up with different concentration of total phenols (0.1 and 0.2 g L⁻¹ expressed as gallic acid equivalents). Additionally, another model wine solution without copigments (0 g L⁻¹) was also used in the study as control.

Secondly, Tempranillo and Syrah (*Vitis vinifera* L.) whole grape were collected and a hyperspectral characterisation was performed [3]. On the basis of this hyperspectral analysis, a number of grapes with different levels of predicted extractable anthocyanin content were selected. Then, each grape skin was divided into three parts and each one was macerated in a different model wine solution with different copigment concentrations. Macerations were carried out for a three-day period in a constant ratio of volume solution to weight of skin (20:1 mL g⁻¹).

After the three-day period of skin maceration, skins were removed, the supernatant was analysed and extractable anthocyanin contents were obtained. As results, statistically non-significant differences were found between the three different extractions. Furthermore, principal component analysis was used as an unsupervised pattern recognition method in order to obtain a general overview. The results provided by this study show that the presence of white grape seed copigments do not affect extractable anthocyanin content released from grape skin and may stabilise wine colour and therefore could represent a good wine-making procedure.

Acknowledgements

The Spanish MINECO is thanked for J. Nogales-Bueno FPI grant (BES-2012-060192) and project AGL2014-58486-C2. Universidad de Sevilla is thanked for J.M. Hernández-Hierro extension of Juan de la Cierva postdoctoral contract, for M.J. Jara-Palacios postdoctoral grant and also for B. Baca-Bocanegra predoctoral grant (VPPI-Universidad de Sevilla). The authors also thank the technical staff of Biology Service [Servicios Generales de Investigación (SGI), Universidad de Sevilla].

References

- [1] A. L. Waterhouse. Wine phenolics. New York Acad Sciences: New York, 2002.
- [2] M. García-Marino, M. L. Escudero-Gilete, F. J. Heredia, M. T. Escribano-Bailón and J. C. Rivas-Gonzalo, *Food Res. Int.*, **51**, 123 – 131. (2013).
- [3] J. Nogales-Bueno, B. Baca-Bocanegra, F. J. Rodríguez-Pulido, F. J. Heredia and J. M. Hernández-Hierro, *Food Chem.*, **172**, 559 – 564. (2015).
- [4] M. J. Jara-Palacios, B. Gordillo, M. L. González-Miret, D. Hernanz, M. L. Escudero-Gilete and F. J. Heredia, *J. Agric. Food Chem.*, **62**, 6975 – 6983. (2014).

Comparative study on the use of anthocyanin profile, colour image analysis and near-infrared hyperspectral imaging as tools to discriminate between four autochthonous red grape cultivars from La Rioja (Spain)

J. Nogales-Bueno^{1,*}, F. J. Rodríguez-Pulido¹, F. J. Heredia¹, J. M. Hernández-Hierro¹.

¹Food Colour & Quality Laboratory, Department of Nutrition & Food Science, Universidad de Sevilla.

*julionogales@us.es

In a cellar it is really important to know the characteristics of grapes that are taken by the vine growers (e.g. grape variety, maturity or sugar content). Maturity and sugar content are typically determined using analytical methods recommended by the O.I.V. [1], which are simple and reliable methods. However, in cellars, grape variety is typically determined by means of visual methods based on the staff experience and knowledge which are neither accurate nor repetitive. It would be appropriate to have rapid and inexpensive analytical methods to classify grapes according to their variety.

In this regard, the aim of this work is to compare the use of anthocyanin profile, colour image analysis and near infrared hyperspectral imaging as tools to discriminate between *Vitis vinifera* L. cv. Tempranillo, Graciano, Garnacha and Mazuelo grapes in order to select the more useful and accurate discrimination tool. In a second strand, a second level data fusion has also been tried to improve the results.

Tempranillo, Graciano, Garnacha and Mazuelo are four important autochthonous red grape cultivars from La Rioja (Spain). A representative set of grapes were collected from five vineyards (one vineyard per cultivar and two different vineyards for Tempranillo since it is the most widespread cultivar) at two different developmental stages during berry maturity in the 2012 vintage. A total of 50 individual grapes were selected and image and hyperspectral analyses were carried out for the whole grapes. Then grape skins were separated manually from the whole grapes and anthocyanin profiles were obtained from the methanolic grape skin extractions. Next, supervised pattern recognition methods were carried out to indicate whether samples fall into pre-defined groups, how well, and what causes these separations [2].

Therefore, in order to discriminate between these four red grape cultivars different stepwise linear discriminant analyses (SLDA) were performed using image analysis data, hyperspectral imaging data and anthocyanin profiles. Respectively for these data sets, 54%, 100% and 88% were correctly classified in the internal validation process and 52%, 86% and 86% were correctly classified in the leave-one-out cross-validation process. Finally, to improve the results obtained using the hyperspectral data set a second level data fusion was carried out. Colour and hyperspectral data were fused. A SLDA was performed with this new data set. Only near infrared hyperspectral image variables were retained and, as a result, the same discriminant functions were obtained with the same discriminant power.

In conclusion, chromatographic analysis is a widespread and reliable tool to classify red grapes according to grape variety and the results obtained in this study confirm it. Colour image analysis is a rapid and inexpensive tool that has lower discriminant power for red grape samples than chromatographic analysis. Near-infrared hyperspectral analysis is a non-destructive and fast technique that shows better or similar results than the chromatographic technique. A second level data fusion between colour data and near infrared hyperspectral data does not improve the results obtained from near infrared hyperspectral image.

Acknowledgements

The Spanish MINECO is thanked for J. Nogales-Bueno FPI grant (BES-2012-060192) and project AGL2014-58486-C2. Universidad de Sevilla is thanked for J.M. Hernández-Hierro extension of Juan de la Cierva postdoctoral contract (VPPI-Universidad de Sevilla). The authors also thank the technical staff of Biology Service [Servicios Generales de Investigación (SGI), Universidad de Sevilla].

References

- [1] OIV. Recueil de methods internationaux d'Analyse des vins. Caracteristiques chromatiques, OIV: Paris, 1990.
- [2] J. Nogales-Bueno, F. J. Rodríguez-Pulido, F. J. Heredia and J. M. Hernández-Hierro, *Talanta*, **131**, 412 – 416 (2015).

Screening of anthocyanins in single red grapes using a non-destructive method based on the near infrared hyperspectral technology and chemometrics

J. R. Martínez-Sandoval¹, J. Nogales-Bueno², F. J. Rodríguez-Pulido²,
J. M. Hernández-Hierro^{2,*}, M. A. Segovia-Quintero³, M. E. Martínez-Rosas¹,
F. J. Heredia².

¹Autonomous University of Baja California (UABC), Faculty of Engineering, Architecture and Design (FIAD), Kilometro 106 carretera Tijuana-Ensenada, Ensenada, Baja California, Mexico.

²Food Colour & Quality Laboratory, Department of Nutrition & Food Science, Universidad de Sevilla.

³Center of Scientific Research and Higher Education of Ensenada (CICESE), Carretera Ensenada-Tijuana No. 3918 Zona Playitas, CP: 22860, Ensenada, Baja California, Mexico.

*jmhhierro@us.es

Anthocyanins are the main compounds responsible for the colour of red wines and therefore it may be important to evaluate the content of the aforesaid secondary metabolites during grape ripening due to the crucial importance to determine wine colour [1]. Furthermore, heterogeneity of grapes at each ripening stage may influence the final wine composition and quality and therefore it should be considered at harvest [2]. Nowadays, there is a growing demand of rapid and non-destructive analytical tools for analysing grapes, such as the emerging hyperspectral analysis [3].

The hyperspectral images of intact grapes (*Vitis vinifera* L. cv. Tempranillo, Graciano, Garnacha and Mazuelo red grape from vineyards located in the D.O.Ca. Rioja at two different developmental stages) were recorded using a near infrared hyperspectral imaging device (900–1700 nm). Reference values of anthocyanins were obtained by HPLC-DAD. Calibrations were performed by modified partial least squares regression and present a good potential (coefficient of determination of 0.72 and standard error of cross-validation values of 0.78 and 0.70 mg per grape for total and non-acylated anthocyanins respectively) [4].

The procedure reported here presents a good potential for a fast and reasonably inexpensive screening of these compounds in intact single berries. Moreover, the heterogeneity of anthocyanins within the same ripeness stage could be evaluated using this non-destructive tool. This methodology allows the berries to be sorted according to their anthocyanins content and then the same samples could be used in further studies for other destructive analyses or purposes.

Acknowledgements

The Spanish MINECO is thanked for J. Nogales-Bueno FPI grant (BES-2012-060192) and project AGL2014-58486-C2. Universidad de Sevilla is thanked for J.M. Hernández-Hierro extension of Juan de la Cierva postdoctoral contract (VPPI-Universidad de Sevilla). Junta de Andalucía is also thanked for financial support (project P10-AGR6331). The authors also thank Bodegas RODA (La Rioja, Spain) for supplying the grape samples. R. Martínez thanks the National Council for Science and Technology (CONACyT) and also all the people that were involved with this research at the UABC, CICESE and University of Seville. The authors also thank the technical staff of Biology Service [Servicios Generales de Investigación (SGI), Universidad de Sevilla].

References

- [1] A. Crozier, M. N. Clifford, and H. Ashihara. Plant Secondary Metabolites. Occurrence, Structure and Role in the Human Diet, Blackwell Publishing: Oxford, 2006.
- [2] N. Kontoudakis, M. Esteruelas, F. Fort, J.M. Canals, V. De Freitas and F. Zamora, *Food Chem*, **124**, 767-774 (2011).
- [3] A. A. Gowen, C. P. O'Donnell, P. J. Cullen, G. Downey and J. M. Frias, *Trends Food Sci Tech*, **18**, 590-598 (2007).
- [4] J. R. Martinez-Sandoval, J. Nogales-Bueno, F. J Rodriguez-Pulido, J. M. Hernandez-Hierro, M. A Segovia-Quintero, M. E. Martinez-Rosas and F. J. Heredia, *J Sci Food Agric*, **96**, 1643-1647 (2016).

Determination of flonicamid and its metabolites in orange and bell pepper samples by ultra high performance liquid chromatography - high resolution mass spectrometry

R. López-Ruiz^{1,*}, R. Romero-González¹, J. L. Martínez-Vidal¹, J. Marín-Sáez¹, A. Garrido-Frenich¹.

¹University of Almería, Department of Chemistry and Physics, Research Centre for Agricultural and Food Biotechnology (BITAL), University of Almería, Agrifood Campus of International Excellence, ceiA3, Carretera de Sacramento s/n, E-04120 Almería, Spain.

*rlr468@inlumine.ual.es

Pesticides are widely used throughout the world to increase production and quality of crops. In consequence, pesticide and/or metabolite residues should be analysed in all agricultural commodities to improve food safety. Among these pesticides flonicamid, N-cyanomethyl-4-trifluoromethylnicotinamide, is a relatively novel selective systemic pesticide, highly effective against aphids and other insects. This compound has a minimal cross-resistance with no negative impact on beneficial arthropods. Thus, flonicamid is widely used in pest management programs. However, the maximum residue limits (MRLs) of flonicamid in food have been regulated by government authorities such as the European Commission [1]. These MRLs have been set as the sum of residues of the parent compound (flonicamid) plus its two major metabolites, TFNA (4-trifluoromethylnicotinic acid) and TFNG (N-(4-trifluoromethylnicotinoyl) glycine). Besides these two metabolites, TFNA-AM (4-trifluoromethylnicotinamide) is another metabolite that has recently been discovered in fruit and vegetables [2]. Therefore, specific and sensitive methods for the identification and quantification of flonicamid and its metabolites, TFNA, TFNG and TFNA-AM, in foods are required.

The aim of this study is the development and validation of a method to determine flonicamid, TFNA, TFNG and TFNA-AM in orange and bell pepper samples, using a simple and efficient extraction procedure and reliable quantification applying ultra high performance liquid chromatography coupled to high resolution mass spectrometry (UHPLC-ORBITRAP-MS). Thus, two fast and simple extraction procedures, one for each matrix, with acidified acetonitrile and salts (magnesium sulfate and sodium chloride) have been used. The methodology was validated, checking specificity, recoveries, precision, limits of detection (LODs) and limits of identification (LIs). The recoveries were between 70 and 105%, while precision values were lower than 17%. Finally, LODs were 1 µg kg⁻¹ for flonicamid and TFNG, 5 µg kg⁻¹ for TFNA and 6 µg kg⁻¹ for TFNA-AM. LIs were 10 µg kg⁻¹ for flonicamid and TFNG, 20 µg kg⁻¹ for TFNA and 30 µg kg⁻¹ for TFNA-AM.

References

[1] European Union: http://ec.europa.eu/food/plant/protection/pesticides/index_en.htm.

[2] EURL-SRM, Analysis of Flonicamid-Metabolites TFNA and TFNG using acidified QuEChERS method, EURL-SRM, Version 2 (2015).

Dissipation studies of flonicamid in its metabolites in orange samples by liquid chromatography - Orbitrap mass spectrometry

R. López-Ruiz^{1,*}, R. Romero-González¹, J. L. Martínez Vidal¹, J. Marín-Sáez¹, A. Garrido-Frenich¹.

¹University of Almería, Department of Chemistry and Physics, Research Centre for Agricultural and Food Biotechnology (BITAL), University of Almería, Agrifood Campus of International Excellence, ceiA3, Carretera de Sacramento s/n, E-04120 Almería, Spain.

*rlr468@inlumine.ual.es

The need for comprehensive understanding of metabolization and degradation of food contaminants is indicated by the toxicity associated with them. In most of the cases, pesticides can be transformed into metabolites, which are intermediate products of metabolism smaller than 1 kDa. Metabolites are often much more toxic than the parent compound itself, as they are typically more polar and persistent. These are generated by metabolic and simple reactions, which in many cases are common among several families of pesticides. Therefore, pesticide and/or metabolite residues should be monitored to improve food safety. Thus, the maximum residue level (MRL) of many pesticides in fruits and vegetables is the total sum of the parent compound and its metabolites. This is the case of flonicamid (N-(cyanomethyl)-4-(trifluoromethyl)nicotinic acid), whose MRL is the total sum of flonicamid and its metabolites 4-(Trifluoromethyl)nicotinol glycine (TFNG) and 4-trifluoromethylnicotinic acid (TFNA). Flonicamid is a systemic insecticide that kills aphid pest (aphids) and Aleyrodidae (Whiteflies). Flonicamid's degradation in its metabolites usually occurs at standard conditions [1], and the main metabolites in fruits and vegetables are TFNG, TFNA and 4-trifluoromethylnicotinamide (TFNA-AM). Flonicamid monitoring has been carried out because there are scarce studies related to its behaviour in plants and how this compound is degraded into its metabolites. This study has been carried out by Orbitrap because of the excellent resolution power that this mass analyzer can provide to detect individual molecular species as well as it is a powerful tool for the identification of non-target compounds.

The aim of this study is know the behavior of flonicamid and its metabolites in orange, after foliar application in orange's tree. This work has been carried out by analyzing oranges for a period of 50 days after foliar application using a simple and efficient extraction procedure and reliable quantification applying ultra high performance liquid chromatography coupled to high resolution mass spectrometry (UHPLC-ORBITRAP-MS). The results show that flonicamid degrades very quickly over time into its metabolites. The metabolite detected at higher concentrations is TFNG, meanwhile TFNA y TFNA-AM are present at low concentrations.

References

[1] EFSA, Conclusion on the peer review of the pesticide risk assessment of the active substance flonicamid, *EFSA Journal*, 8, 1445 (2010).

Determination of atropine and scopolamine in buckwheat and related products using modified QuEChERS and liquid chromatography tandem mass spectrometry

J. Marín-Sáez^{1,*}, H. Chen^{1,2}, R. Romero-González¹, R. López-Ruiz, A. Garrido-Frenich¹.

¹University of Almería, Department of Chemistry and Physics, Research Centre for Agricultural and Food Biotechnology (BITAL), Agrifood Campus of International Excellence ceiA3, Carretera de Sacramento s/n, E-04120 Almería, Spain.

²Chinese Academy of Agricultural Sciences (TRICAAS), Tea Research Institute, 9 South Meiling Road, 310008, Hangzhou, China.

*jms485@inlumine.ual.es

Buckwheat is a healthy pseudo cereal widely cultivated over the world as an important raw material used for functional food because it becomes a dietary source of bioactive compounds, such as nutritionally valuable protein, phenolic compounds, starch and dietary fiber, essential minerals and trace elements. However, buckwheat can be contaminated by stramonium (*Datura stramonium*), which can contain high concentration of tropane alkaloids, and therefore, these toxic substances can be found in buckwheat and related matrices at concentrations higher than 100 µg/kg [1].

Tropane alkaloids are synthesized by the plants of the genus from *Solanaceae* family, although they are also produced in smaller quantities in plants from other families. They can affect human and animal health due to their high toxicity and intake of contaminated food or animal feed. Although there are more than 200 tropane alkaloids, atropine and scopolamine are the most common compounds of this family.

According to the EFSA scientific opinion in 2013 [1] more attention must be focused on the contaminated levels of atropine and scopolamine in food from plant origin, such as cereal products, grain-based baby-food, buckwheat grain and food products and tea and herbal infusions. Therefore, the development of quick, easy and reliable analytical methods for the trace analysis of tropane alkaloids at trace levels in food from plant origin is desirable.

Therefore the aim of this study is the development and validation of an analytical method for the simultaneous determination of atropine and scopolamine residues in buckwheat and related products. A modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction method with acidified acetonitrile (1% formic acid v/v) followed by a clean-up step using graphitized black carbon (GBC) and primary secondary amine (PSA) was applied. For the separation and quantification of the target compounds, ultra high performance liquid chromatography (UHPLC)-coupled to tandem mass spectrometry (MS/MS), utilizing triple quadrupole (QqQ) as analyzer, was used. The method was validated, determining linearity, trueness, precision, limits of detection (LODs) and quantification (LOQs). Recoveries ranged from 75 to 92% with precision below 17% (RSD values) for all the compounds studied. Both LODs and LOQs were below 2 µg kg⁻¹.

The validated method was tested in eight commercial samples (buckwheat, wheat, soy, buckwheat flour, buckwheat noodle, amaranth grain, chia seeds and peeled millet). Target compounds were not found above the detection limits of the method.

Acknowledgements

The authors gratefully acknowledge the Spanish Ministry of Economy and Competitiveness (MINECO) and FEDER (project ref. CTQ2015-69899-R).

References

[1] EFSA. Scientific opinion on tropane alkaloids in food and feed, EFSA panel on contaminants in the food chain (CONTAM). *The EFSA Journal*, **11**, 3386-3499 (2013).

Enzymatic determination of biogenic amines. Do you want an optical sensor or do you want a strip test? You choose

I. Sanz-Vicente*, S. de Marcos, J. Navarro, R. Lozano, J. Galbán.

Analytical Biosensor Group (GBA), Institute of Nanoscience of Aragón (INA), Analytical Chemistry Department, University of Zaragoza.

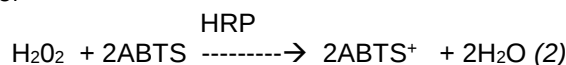
*isasanz@unizar.es

The determination of biogenic amines in food is interesting due to two reasons. On one hand due to their possible toxicological effect on health and on the other because they can be used as an indicator of the quality of the food. The food industry itself requires quick and economic methods for quality control.

In this work we have developed a method to determine biogenic amines: putrescine, cadaverine, histamine, phenylethylamine, tryptamine and tyramine. The method is based on the reaction of the amines with the enzyme Diamine oxidase (DAO):



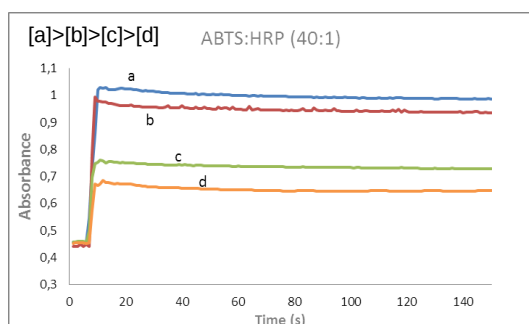
which is then coupled to:



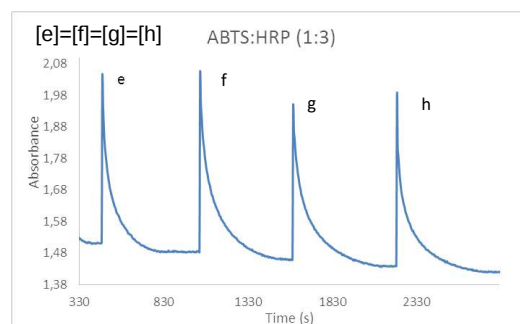
The reaction between H_2O_2 and ABTS catalized by the enzyme Peroxidase (HRP) is well known and widely used. The radical ABTS^+ is a coloured product of the reaction and therefore H_2O_2 can be spectrophotometrically determined. We have used the variation in the absorbance of ABTS to determine the biogenic amines.

From the study of the reaction mechanism and from the optimization of the conditions of the reaction it has been found that depending on the ratio of ABTS/HRP, the radical ABTS^+ is kept oxidized a long time or returns quickly to its initial state. Taking this into account, there are two interesting possibilities:

a.- Working with high concentrations of ABTS it is possible to obtain a stable colour (signal) for a long time (fig 1).



b.- Working with low concentrations of ABTS it is possible to obtain a transient signal (fig 2).



The option a is the basis to design strip test and the option b is the basis to design an optical sensor to biogenic amines.

Both alternatives are explored in this work.

Acknowledgements

This work has been supported by the MINECO of Spain (project CTQ2012-34774) and by the founding to research group of the DGA-FEDER (E74). J. Navarro thanks to the DGA for the grant.

POSTER PRESENTATIONS Sensors

New fluorescent pH indicators for optical sensing of CO₂

M. L. Aguayo-López^a, M. D. Fernández-Ramos^a, F. Santoyo-González^b,
E. de los Reyes-Berbel^b, L. F. Capitán-Vallvey^a.

^aDepartment of Analytical Chemistry; ^bDepartment of Organic Chemistry, Faculty of Sciences, University of Granada E-18071 Granada, Spain.

*mlagulop@correo.ugr.es

The so-called luminescent “solid sensor membranes” for gaseous CO₂ are based on changes on emission characteristics of a luminophore immobilized on oxygen-insensitive membranes, due to the displacement of acid-base equilibrium with CO₂ [1]. The main problems with this type of sensors are the limited number of existing fluorescent indicators and their usual poor photostability. Boron-dipyrromethene-type fluorophores (BODIPYS) fall into a class of compounds widely used as biomolecule markers, fluorescent switches, chemosensors and laser dyes although scarcely used CO₂ sensing [2,3]. We have synthesized some new BODIPYS to be used in CO₂ sensing schemes (Figure 1).

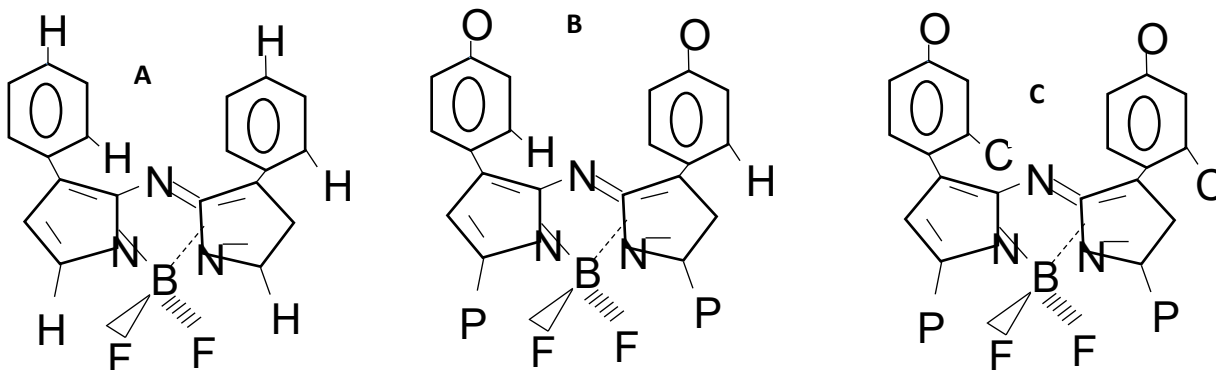


Figure 1. Structure of BODIPYS studied.

Into the polymeric sensing membrane the BODIPY dye acts as a fluorescent acid-base indicator, along with transfer phase agents (tetraoctyl or tetramethyl ammonium hydroxide) and plasticizer or surfactant to improve the membrane permeability to gaseous carbon dioxide.

Some characteristics of sensing membranes for CO₂ are:

Parameter	BODIPY A sensing membrane	BODIPY B sensing membrane	BODIPY C sensing membrane
$I_{100}-I_0$	308±3.7	137±2.1	43±1.5
Slope	16.3±1.0	159±3.4	49±2.5
Intercept	1.01±0.01	1.10±0.02	0.11±0.01
R ²	0.989	0.997	0.958

References

- [1] M.L. Aguayo-López, L.F. Capitán-Vallvey, M.D. Fernández-Ramos. *Talanta*, **126**, 196–201 (2014).
- [2] T. Jokic, S.M. Borisov, R. Saf, D.A. Nielsen, M. Kühn, I. Klimant. *Anal. Chem.*, **84**, 6723–6730 (2012).
- [3] Zhong-Hua Pan, Geng-Geng Luo, Jing-Wei Zhou, Jiu-Xu Xia, Kai Fang and Rui-Bo Wu. *Dalton Trans*, **43**, 8499-8507 (2014).

Microfluidic paper-based analytical device for colorimetric determination of glucose based on metal-organic framework as peroxidase mimetic

I. Ortiz-Gómez¹, A. Salinas-Castillo^{1,*}, B. Fernández², I. de Orbe-Payá¹, A. Rodríguez-Diéguez², L. F. Capitán-Vallvey¹.

¹Ecsens, Department of Analytical Chemistry, ²Department of Inorganic Chemistry. Campus Fuentenueva, Faculty of Sciences, University of Granada, 18071 Granada.

* alfonso@ugr.es

Metal-organic framework (MOF) are extended crystalline structures wherein metal cations or clusters of cations are connected by multitopic organic ions or molecules. They have many applications in different fields like biomedicine, electronics, optics and analytical among others due to properties such as porosity, high specific surface area, catalytic efficiency, high stability and outer-surface modification[1]. Recently, the intrinsic enzyme-like activity of materials such as MOFs has become a growing area of interest in sensing recognition area.

In this work, we developed a microfluidic paper-based analytical device (μ PAD) for glucose determination using a MOF as catalytic element of peroxidase-mimetic type for peroxidase substrates sensing in the presence of glucose oxidase (GOx). This Fe-MOF was characterized by X-ray diffraction, scanning electron microscopy (Figure 1) and infrared spectroscopy.

The μ PAD developed for glucose determination consists of three zones well differentiated: sampling zone, transport channel and detection zone. The system is based on MOF as peroxidase mimetics immobilized in the detection zone of cellulosic substrate coupled to 3,3',5,5'-tetramethylbenzidine (TMB) as colorimetric probe. The rest of reagents employed were deposited in transport channel (Figure 2). A digital camera is used for the quantitative analysis of glucose with the S coordinate of the HSV color space as the analytical parameter.

Different parameters such as the concentration of TMB, GOx and MOF, pH and buffer, volume of sample, time of reaction and position of reagents were optimized. Under optimal operational conditions, linearity was observed for glucose concentrations up to 150 μ M. The μ PAD is selective towards the common species in biological fluids (serum and urine).

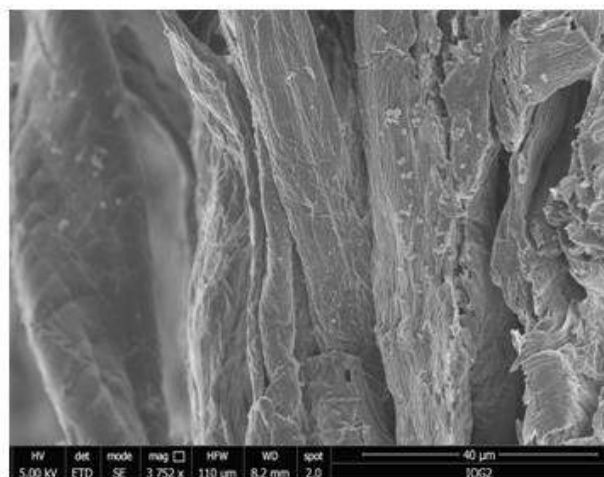


Figure 1. SEM

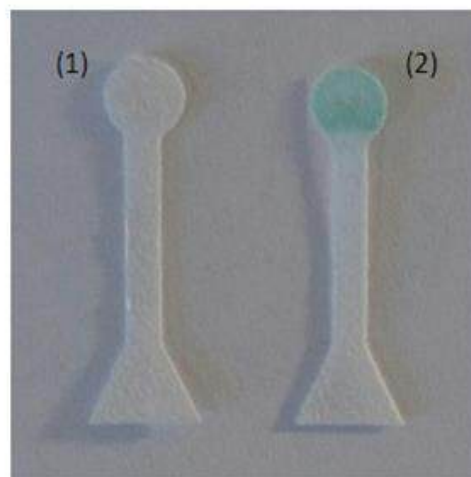


Figure 2. (1) μ PAD before and (2) after reaction with glucose.

Acknowledgements

This study was supported by projects from the Spanish MINECO (CTQ2013-44545-R), Junta de Andalucía (P10-FQM-5974), and the "Fortalecimiento de Grupos" and "Reincorporación de Doctores" Programs.

References

[1] P. Kumar, A. Deep, K.H. Kim, TrAC - Trends in Analytical Chemistry, 73, pp. 39-53 (2015).

Paper-based microfluidic device for colorimetric detection of glutathione based on Ag(I)-TMB

I. Ortiz-Gómez¹, A. Salinas-Castillo¹, I. de Orbe-Payá¹ and L. F. Capitán-Vallvey^{1,*}.

¹Ecsens, Department of Analytical Chemistry. Campus Fuentenueva, Faculty of Sciences, University of Granada, 18071 Granada.

*lcapitan@ugr.es

Glutathione (GSH) is a biothiol that is involved in various biological functions among which its detoxifying role against reactive oxygen species with an unpaired electron. The levels of GSH in human serum and urine are linked to different disease diagnostic and clinical therapies [1].

In this work, we developed a simple colorimetric method for determination of GSH based on a microfluidic paper-based analytical device (μ PAD). The proposed method is based on the fact that Ag(I) serves as an oxidant for 3,3',5,5'-tetramethylbenzidine (TMB) changing its color to blue. However, the presence of GSH could cause the reduction of oxidized TMB along with a reaction with Ag(I) which resulting blue color fading. Based on this finding, we propose a method to detect GSH by naked eyes (Figure 1). Additionally, we used a digital camera for the quantitative analysis of GSH with the S coordinate of the HSV color space as the analytical parameter.

The developed μ PAD is fabricated by a laser cutter machine and consists of three zones: sampling zone, transport channel and detection zone and it is laminated to protect the reagents.

Different parameters such as the concentration of TMB and Ag(I), reaction pH, volume of sample, time of reaction and position of reagents were optimized. Under optimal operational conditions, linearity was observed for glutathione concentrations up to 350 μ M.

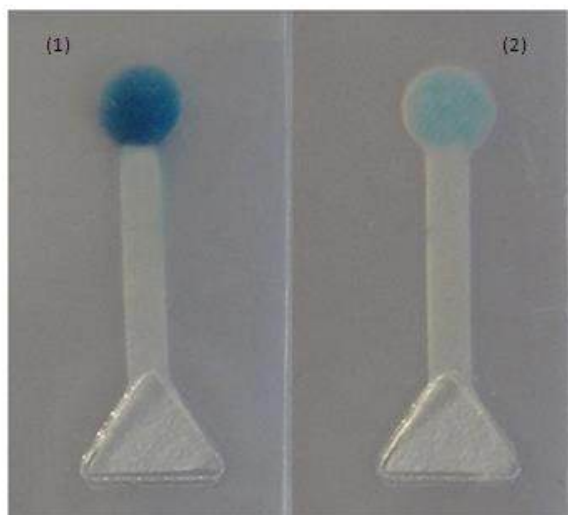


Figure 1. (1) μ PAD before and (2) after reaction with GSH.

Acknowledgements

This study was supported by projects from the Spanish MINECO (CTQ2013-44545-R), Junta de Andalucía (P10-FQM-5974), and the "Fortalecimiento de Grupos" and "Reincorporación de Doctores" Programs.

References

[1] S.C. Lu, Mol. Aspects Med. 30 42-59 (2009).

Fluorescent assay based on carbon dots synthesized by photochemistry for the detection of hydrogen peroxide and antioxidants

C. Bendicho, I. Costas-Mora, V. Romero, I. Lavilla.

Departamento de Química Analítica y Alimentaria, Facultad de Química, Universidad de Vigo, As Lagoas-Marcosende s/n 36310, Vigo, Spain.

Email: bendicho@uvigo.es

Fluorescent nanoparticles have been increasingly applied as optical probes for the detection of a wide variety of chemical species [1]. Over the last few years, carbon dots (CDs) have emerged as novel and highly interesting optical nanoprobes taking advantage of their high aqueous stability, easy functionalization and low toxicity [2]. In general, conventional methods for synthesis of CDs involve long operation time and harsh conditions (e.g. high temperature) [2], so new strategies have been investigated in order to develop easier and greener approaches.

In this work, a new synthesis of fluorescent CDs based on UV irradiation of carbohydrates (e.g. sucrose) for optical sensing of H₂O₂ and antioxidants was developed [3].

In order to reach optimal conditions for analytical applications, different parameters such as the type of carbon source, stabilizing agent, pH of the medium and irradiation time were assessed. CDs were synthesized using different carbohydrates as precursors including glucose, fructose, sucrose and starch. The analytical response depends on both the carbon source and irradiation time. On the other hand, fluorescence of CDs and analytical response increased when using stabilizing agents, e.g., poly(ethyleneglycol), Triton X-100, polyvinylpyrrolidone, sodium dodecyl sulfate, etc. The best results were obtained using sucrose (20-25 mg) as carbon precursor, PEG 35% v/v as stabilizing agent, NaOH 3 M to ensure alkaline medium and an irradiation time of 1 min. The photogenerated CDs were characterized by transmission electron microscopy (TEM) and Fourier transform infrared (FT-IR), thus demonstrating that CDs are monodisperse with an average size of 8 nm and they are surrounded by hydrophilic groups (i.e. hydroxyl and carbonyl), which leads to high aqueous solubility.

H₂O₂ is a highly reactive oxidant that can promote a decrease in CDs size and thus higher quantum confinement of emissive energy traps. When H₂O₂ is added to the reaction medium, a dramatic increase in the CDs fluorescence is observed (turn-on strategy). OH radicals formed upon photolysis of H₂O₂ could cause photochemical etching of CDs yielding surface defects, which results in fluorescence enhancement. On the other hand, antioxidants, e.g. ascorbic acid and glutathione, inhibit the photochemical reaction, causing a quenching effect in the fluorescence of the CDs/H₂O₂ system, so they can be detected through a turn on-off sensing strategy.

Under optimal conditions, detection limits of 5 µM H₂O₂ and 6 µM ascorbic acid were obtained. The repeatability and reproducibility, expressed as relative standard deviation, were 3.8% and 6%, respectively. The proposed method was applied to H₂O₂ detection in contact lens cleaning solutions, which typically contain H₂O₂ concentrations lower than 0.9 M. Besides, the effect of several compounds, e.g. sodium citrate, sodium chloride, sodium tetraborate, etc. were evaluated as potential matrix interferences. In all cases the tolerance limits found were higher than the concentrations usually present in the analyzed samples.

The main advantages of new approach are the reduced time for synthesis and sensing using CDs due to the integration of both process, which allows reaching high sample throughput along with minimal consumption of sample and reagents. Finally, the use of a portable microfluorespectrometer allows the ability of *in situ* analysis.

References

- [1] I. Costas-Mora, V. Romero, I. Lavilla, C. Bendicho, *TrAC Trends in Anal. Chem.*, **57**, 64 – 72 (2014).
- [2] S.N. Baker, G.A. Baker, *Angew. Chem. Int. Ed.*, **49**, 6726 – 6744 (2010).
- [3] I. Costas-Mora, V. Romero, I. Lavilla, C. Bendicho, *Talanta*, **144**, 1308 – 1315 (2015).

Effect of graphene substrate on the SERS Spectra of Aromatic bifunctional molecules on metal nanoparticles

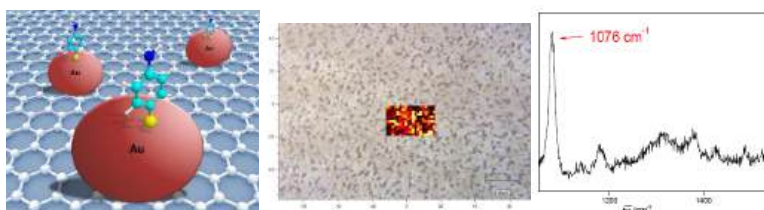
M. R. López-Ramírez*, J. L. Castro, J. F. Arenas, J. Soto, J. C. Otero.

Department of Physical Chemistry, Faculty of Science, University of Málaga, E-29071-Málaga, Spain.

*mrlopez@uma.es

The design of molecular sensors plays a very important role within nanotechnology and especially in the development of different devices for biomedical applications. Biosensors can be classified according to various criteria such as the type of interaction established between the recognition element and the analyte or the type of signal detection from the analyte (transduction). When Raman spectroscopy is used as an optical transduction technique the variations in the Raman signal due to the physical or chemical interaction between the analyte and the recognition element has to be detected. Therefore any significant improvement in the amplification of the optical sensor signal represents a breakthrough in the design of molecular sensors. In this sense, *Surface-Enhanced Raman Spectroscopy* (SERS) involves an enormous enhancement of the Raman signal from a molecule in the vicinity of a metal surface.

The main objective of this work is to evaluate the effect of a monolayer of graphene oxide (GO) on the distribution of metal nanoparticles (NPs) and on the global SERS enhancement of p-aminothiophenol (pATP) and 4-mercaptobenzoic acid (4MBA) adsorbed on this substrate. These aromatic bifunctional molecules are able to interact to metal NPs and also they offer the possibility to link with biomolecules. Additionally by decorating Au or Ag NPs on graphene sheets, a coupled EM effect caused by the aggregation of the NPs and strong electronic interactions between Au or Ag NPs and the graphene sheets are considered to be responsible for the significantly enhanced Raman signal of the analytes [1-2]. Since there are increasing needs for methods to conduct reproducible and sensitive Raman measurements, *Graphene-enhanced Raman Scattering* (GERS) is emerging as an important method [3].



The Raman mapping analysis of the GO substrate has revealed a more homogeneous distribution of metal NPs with respect to the direct deposition of metal NPs on glass which favours the reproducibility of the SERS substrates. On the other hand, pATP and 4MBA have been shown a weaker adsorption capability on the graphene sheets and only bind to Au or Ag NPs, so the whole Raman spectra can be considered an overlapping of the spectra of these molecules and those of graphene enhanced by the Au or Ag NPs individually.

Acknowledgements

This research has been supported by the Spanish Ministerio de Economía y Competitividad (Projects: CTQ2012-31846 and CTQ2015-65816-R).

References

- [1] J. Huang, L. M. Zhang, B. A. Chen, N. Ji, F. H. Chen, Y. Zhang, Z. J. Zhang, *Nanoscale* **2**, 2733–2738 (2010).
- [2] Y. Wang, Z. Ni, H. Hu, Y. Hao, C. P. Wong, T. Yu, J. T. L. Thong, Z. X. Shen, *Appl. Phys. Lett.* **97**, 163111–163113 (2010).
- [3] F. Yaghobian, T. Korn, C. Schiller, *Chem. Phys. Chem.* **13**, 4271 – 4275 (2012).

Mercury (II) off-on luminescence chemosensing based on the incorporation of Rhodamine 6G spirocyclic phenylthiosemicarbazide derivative in Au nanoparticles

A. Muñoz de la Peña¹, M. J. Culzoni², M. C. Onaindia², R. Brasca², H. C. Goicoechea².

¹Department of Analytical Chemistry and IACYS, University of Extremadura, 06006, Badajoz, Spain.

²Laboratorio de Desarrollo Analítico y Quimiometría, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional de Litoral, 3000, Santa Fe, Argentina.
arsenio@unex.es

On the basis of the maximum level (2 ng mL⁻¹) of mercury in drinking water permitted by the United States Environmental Protection Agency (EPA), an ideal probe should display a very low detection limit and a high selectivity towards Hg²⁺ in the presence of other metals [1]. Considerable attention has been recently focused on the design of luminescence chemodosimeters for mercuric ion due to the highly sensitive, quick, and nondestructive advantages of luminescence methods. The chemodosimeter strategy is based on the use of a selective reaction that is induced by the target species and gives rise to an observable luminescence signal (preferably off-on). Rhodamine and BODIPY derivatives are two families of small molecular probes reported for selective Hg²⁺ detection [2].

Rhodamine 6G spirocyclic phenylthiosemicarbazide derivative (FC1) has been previously proposed for Hg²⁺ determination in diverse matrices, as water and fish samples [3,4], and the procedure improved by immobilization of the reagent in nylon membranes [5], hydrophilic water-insoluble poly(2-hydroxyethylmethacrylate-co-methylmethacrylate) co-polymer [6] and electrospinning generated microfibre nonwoven mats [7].

The purpose of this work is to enhance the sensitivity of the reaction of FC1 towards Hg²⁺ by coupling the probe to Au nanoparticles, maintaining its high selectivity.

Au nanoparticles were synthesized by reduction of AuCl₃ with sodium citrate and characterized by DLS (Dynamic Light Scattering). In the presence of the Au nanoparticles FC1 exhibits lower fluorescence than in its absence due to an intense resonance energy transfer (RET) effect. This fact improves the sensitivity of the reaction towards Hg²⁺, allowing its determination at concentrations below 2 ng mL⁻¹. The chemodosimeter has been applied to the determination of traces of Hg²⁺ in different aqueous samples. The proposed method allows the determination of Hg²⁺ in a variety of aqueous samples, in a fast, economical and simple way. The emission of fluorescence was measured at 555 nm, by exciting at 528nm, in the interval between 0.5 and 10 ng mL⁻¹, with a detection limit of 0.15 ng mL⁻¹, being 5 times more sensitive than the original method in absence of the Au nanoparticles [4].

Acknowledgements

Universidad Nacional del Litoral (Argentina) (CAI+D 2012 No. 11-11 and 11-7), CONICET (PIP 455), ANPCyT (PICT 2014-0347), MEyC of Spain (CTQ2014-52309P) and Gobierno de Extremadura (GR15090-FQM003), co-financed by European FEDER funds.

References

- [1] T. Syversen and, P. Kaur, *J. of Trace Elem. in Medicine and Biol.*, **26**, 215-226 (2012).
- [2] M.J. Culzoni, A. Muñoz de la Peña, A. Machuca, H. C. Goicoechea and R. Babiano, *Anal. Meth.*, **5**, 30-49 (2013).
- [3] Y.-K. Yang, K.-J. Yook and J. Tae, *J. Am. Chem. Soc.*, **127**, 16760-16761 (2005).
- [4] D. Bohoyo, M.I. Rodríguez, M.C. Hurtado and A. Muñoz de la Peña, *Appl. Spec.*, **64**, 520-527 (2010).
- [5] V. A. Lozano, G. M. Escandar, M. C. Mahedero and A. Muñoz de la Peña, *Anal. Methods*, **4**, 2002-2008 (2012).
- [6] F.J. Orriach-Fernández, A. Medina-Castillo, J.F. Fernández-Sánchez, A. Muñoz de la Peña and A. Fernández-Gutiérrez, *Anal. Methods*, **5**, 6642-6648 (2013).
- [7] F.J. Orriach-Fernández, A. L. Medina-Castillo, J.E. Díaz-Gómez, A. Muñoz de la Peña, J.F. Fernández-Sánchez, A. Fernández-Gutiérrez, *Sensors and Actuators B:Chemical*, **195**, 8-14 (2014).

Carbon dots-based phosphorescent probe for biothiols sensing

F. Murru, A. Salinas-Castillo*, L. F. Capitán-Vallvey.

Ecsens, Department of Analytical Chemistry, Faculty of Science, Campus Fuentenueva, 18071 Granada (Spain).

**alfonsos@ugr.es*

Recently, some approaches for the development of Carbon dots (Cdots) based fluorescent sensing systems have been described. However, little has been reported to date on room temperature phosphorescence (RTP)-based sensor systems even though the phosphorescent detection provides a number of important advantages including low emission background and increased selectivity by delaying the measurement time. It is possible to achieve much higher selectivity with phosphorimetry than with spectrophotometry and fluorimetry because only a few compounds are phosphorescent compared to the much larger number which absorb radiation or show fluorescence [1].

Biothiols such as glutathione, cysteine and homocysteine are biomarkers that play role in many biological process. Therefore, it is great importance to development sensitive, selective and fast method for determination of biothiols in biological samples [2].

Cdots suffer attenuation of their phosphorescence (Figure 1) in the presence of various metal ions so can be used for the determination of these metal ions as well as species that interact with them (turn off-turn on systems). In this study the attenuation of luminescence Cdots by Hg (II) (turn-off) for the development of a method for determining biothiols (turn-on) is used. We studied different influencing parameters such as the concentration of Cdots, the concentration of Hg (II), pH, time, and the presence of various potential interfering (amino acids and carbohydrates). The analytical method developed present good analytical characteristics.

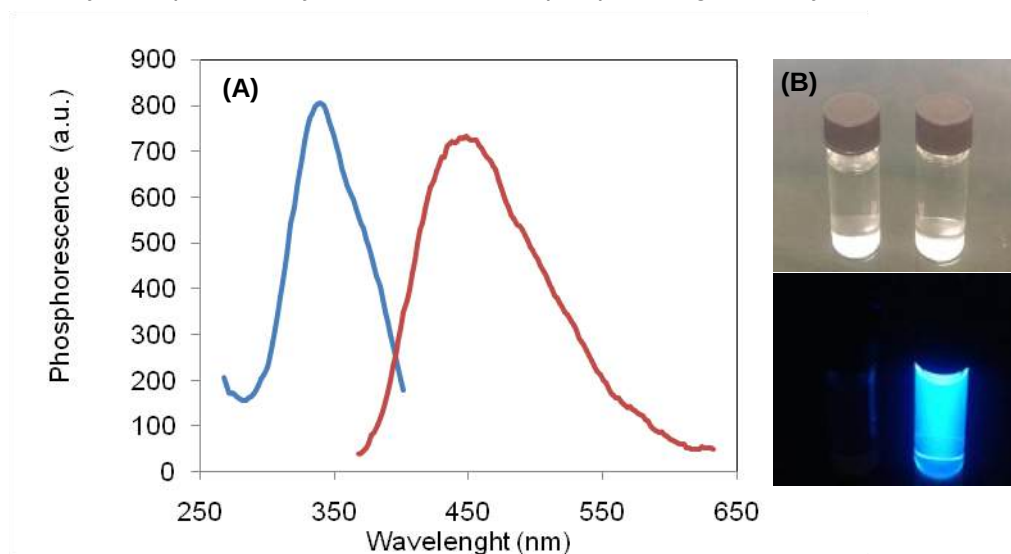


Figure 1. (A) Phosphorescence emission and excitation spectrum. (B) Photographs of Cdots solutions under visible light (right) and UV beam of 365 nm (left) in comparison with only water

Acknowledgements

This study was supported by projects from the Spanish MINECO (CTQ2013-44545-R), Junta de Andalucía (P10-FQM-5974), and the "Fortalecimiento de Grupos" and "Reincorporación de Doctores" programs.

References

- [1] R.J. Hurtubise, *Phosphorimetry: Theory, instrumentation and applications*, VCH, New York (1990).
- [2] J. Houet *et al.*, *Anal. Chim. Acta*, **859**, 72–78 (2015).

Abiotic Degradation of Triazine Pesticides Analyzed with Surface-enhanced Raman Scattering

S. Sánchez-Cortés^{1*}, R. J. G. Rubira², C. J. L. Constantino¹, M. V. Cañamares¹, J. V. García-Ramos¹.

¹*Instituto de Estructura de la Materia, IEM-CSIC, Serrano 121, E-28006-Madrid, Spain.*

²*FCT, Univ. Estadual Paulista, Presidente Prudente, SP, Brazil.*

**s.sanchez.cortes@csic.es*

Triazine herbicides are considered among the most important class of agricultural chemicals ever developed. During their 50 years of use they have contributed to the huge increment of the crop production. Atrazine is by far the most employed triazine pesticides because of its high flexibility in combined treatments [1]. This herbicide continues to be widely used today in USA, but it was banned in EU from 2003 [2]. One of the most interesting chemical characteristics of triazine pesticides is its high tendency to degradation by several factors: hydration, photolysis, temperature. In general, it is widely considered that these degradation processes are enhanced at extreme pH's, mainly at acidic one. The most frequently detected atrazine degradation products are DEA (des-ethyl atrazine) and DIA (des-isopropyl atrazine), but also the hydroxylated degradation products are considered the major degradation products. These products are also common to other triazines structurally related to atrazine such as simazine or prometryne.

Therefore, the possible detection of triazine herbicides in water (lakes, rivers, etc.), related to the agricultural practices, by SERS (Surface-enhanced Raman scattering) must take into account all these effects. Of course, always considering the possible catalytic effect caused on the pesticide structure by the metal surface.

In this work we present the preliminary results obtained in our laboratory on the detection of atrazine and prometryne by using different SERS substrates: Ag and Au nanoparticles (spherical and nanostars), as well as Ag films obtained by pulsed-laser deposition (PLD).

The results obtained from these experiments revealed several important facts: a) the commercial triazine compounds presented a partial degradation; b) the SERS cross section of the degraded triazines is ca. two orders of magnitude higher than the precursor original triazine; and c) these herbicides display a high tendency to the degradation at relatively mild conditions, such as the increase of temperature under aqueous solution. A comparison of the SERS spectra of the degradation compounds with the theoretical spectra and the commercial products was carried out in order to properly assign the corresponding compounds detected by SERS.

Acknowledgements

This work has been supported by the *Ministerio de Economía y Competitividad* (FIS2014-52212-R).

References

- [1] M. Homer, H.M. LeBaron and G. Müller, *The Triazine Herbicides: 50 years Revolutioning Agriculture*, H.M. LeBaron, J.E. McFarland, O.C. Burnside, Eds., Elsevier, San Diego, 2008, 63-73.
- [2] H. Barchanska and E. Jodo, *Atrazine Degradation Processes and Their Influence on Living Organisms*, Nova Science Publishers, Inc. New York, 2011.

Bioavailability of anticancer drugs: Study of the interaction with kinase-inhibitor and Human Serum Albumin

J. Fernández¹, E. Niza¹, P. J. Pacheco¹, A. Garzón¹, **I. Bravo¹**, C. Alonso², J. Albaladejo¹.

¹Dpto. Química Física, Facultad Farmacia (Albacete) y Facultad de Ciencias y Tecnologías Químicas (Ciudad Real), Universidad de Castilla-La Mancha (UCLM), Spain.

²Área de Química Inorgánica, Facultad Farmacia (Albacete) y Facultad de Ciencias y Tecnologías Químicas (Ciudad Real), UCLM, Spain.

Ivan.bravo@uclm.es

Drug efficiency is related to LADME processes (Liberation, Absorption, Distribution, Metabolism and Excretion). In our group, we have studied the distribution and liberation processes of different kinase-inhibitor anticancer drugs. Bearing this goal in mind, we have focused on the following research topics.

(1) *Studies with proteins.* The interactions between different anticancer drugs and human serum albumin (HSA) have been studied in our laboratory by Steady State Fluorescence Spectroscopy (SSFS) and Time Resolved Fluorescence Spectroscopy (TRFS) using the native fluorescence of the protein. Binding constants (K_a) were obtained from those studies. Thermodynamic parameters of the protein - ligand interaction (ΔH , ΔS y ΔG) were determined by varying the working temperature. The changes in the secondary structure of the protein were analyzed by UV-Vis absorption spectroscopy and FTIR. Moreover, Density Functional Theory (DFT) calculations were used to analyze the recorded spectra.

(2) *Drug delivery studies by using biodegradable polymers.* Interest on drug controlled-release systems for keeping efficient levels of drugs during an optimum period of time is currently growing. Different kinds of biodegradable polymers, harmless for both humans and environment, such as polyglycolic acid (PGA), polylactic acid (PLA), poly (lactid-co-glycolic acid), polycaprolactone (PCL) have been synthesized [1]. These polymers could be suitable for future drug delivery applications. In our laboratory, we have studied the drug delivery kinetics of several antitumor drugs, previously loaded in PLA and PCL polymers, by means of UV-Vis absorption spectroscopy, SSFS and TRFS [2].

Acknowledgements

The authors would like to thank the Consejería de Educación y Ciencia de la Junta de Comunidades de Castilla-La Mancha (FEDER projects and PEII-2014-043-P (FOTOCINE)), for supporting this research.

References

- [1] M. Vert; Y. Doi; K. H. Hellwich; M. Hess; P. Hodge; P. Kubisa; M. Rinaudo; F. Schué, Terminology for biorelated polymers and applications (IUPAC Recommendations 2012). *Pure Appl. Chem*, **84**, 377–410 (2012).
- [2] H. Fan, and Z. Jin. Hierarchical porous polycaprolactone microspheres generated via a simple pathway combining nanoprecipitation and hydrolysis. *Chem. Commun*, **51**, 15114 (2015).

Lifetime pH sensors for Fluorescent Lifetime Imaging Microscopy (FLIM) based on perylene-diimide compounds

P. J. Pacheco^{1,*}, J. Fernández¹, E. Niza¹, A. Garzón¹, I. Bravo¹, J. Albaladejo², M. L. Nueda³, J. Laborda³, M. Melguizo⁴.

¹Dpto. Química Física, Facultad de Farmacia, Universidad de Castilla-La Mancha (UCLM). Cronista Fco. Ballesteros Gómez, 1, 02071, Albacete, Spain.

²Dpto. Química Física, Facultad Ciencias Químicas, UCLM. Camilo José Cela, 10, 13071, Ciudad Real, Spain.

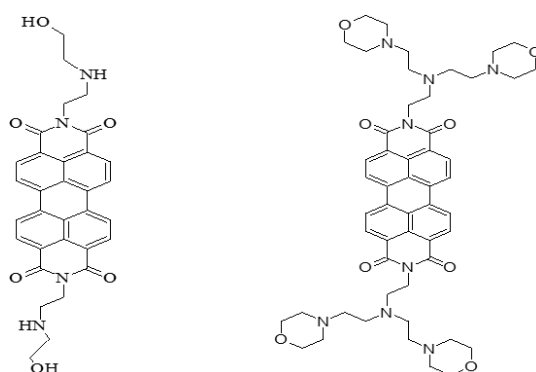
³Dpto. Química Orgánica, Inorgánica y Bioquímica, Facultad de Medicina, UCLM. Almansa, 14, 02071, Albacete, Spain.

⁴Dpto. Química Inorgánica y Orgánica, Facultad de CC. Experimentales, Universidad de Jaén. Campus las Lagunillas, 23071, Jaén, Spain.

*pedro.pacheco@uclm.es

Different diseases such as Alzheimer or cancer produce modifications in the intracellular environment. For instance, the intracellular pH in cancer cells is more acid than in healthy cells [1,2]. Different kinds of fluorescent probes are been extensively investigated as intensity indicators [3] and lifetime sensors for intracellular pH. Lifetime sensors have many advantages since fluorescence lifetime (τ_F) does not depend on the excitation wavelength and intensity, duration of light exposure and fluorophore concentration [4].

Two types of π -conjugated compounds have been studied as lifetime pH sensors. They are formed by a core of perylene-diimide with pendant chains including different kinds of amine groups. In both compounds, an influence of pH on the τ_F was observed. The dependence of τ_F with respect to pH was studied in buffered solutions which mimic the intracellular environment. τ_F was measured in cancer cells (SK-MEL₂) and healthy cells (C3H10T_{1/2}) by using Fluorescence Lifetime Imaging Microscopy (FLIM). Higher values of τ_F were observed in cancer cells than in healthy cells.



Acknowledgements

The authors would like to thank the Consejería de Educación y Ciencia de la Junta de Comunidades de Castilla-La Mancha (FEDER projects and PEI11-0279-8538) for supporting the research described in this article. One of us (P.J. Pacheco) thanks the Universidad de Castilla-La Mancha for a pre-doctoral grant (2014/10340).

References

- [1] Davies, T.A.; Fine, R.E.; Johnson, R.J.; Levesque, C.A.; Rathbun, W.H.; Seetoo, K.F.; Smith, S.J.; Strohmeier, G.; Volicer, L.; et al. *Biochem. Biophys. Res. Commun.*, **194**, 537-543. 1993.
- [2] Izumi, H.; Torigoe, T.; Ishiguchi, H.; Uramoto, H.; Yoshida, Y.; Tanabe, M.; Ise, T.; Murakami, T.; Yoshida, T.; Nomoto, M.; Kohno, K. *Cancer Treatment Rev.* 2003, **29**, 541-549.
- [3] J. Han and K. Burgess, *Chem. Rev.*, 2010, **110**, 2709-2728.
- [4] M. Y. Berezin and S. Achilefu, *Chem. Rev.*, 2010, **110**, 2641-2684.

Electrochemiluminescence sensors for the determination of enrofloxacin in aqueous samples based on $\text{Ru}(\text{phen})_3^{2+}$ modified carbon screen-printed electrodes and molecularly imprinted solid phase extraction

B. Jiménez-Lafitez, A. Canal-Martín, J. Urraca, A. M. Gutiérrez, C. Pérez-Conde*, M. C. Moreno-Bondi.

Facultad de Ciencias Químicas. Departamento de Química Analítica, Universidad Complutense, 28040 Madrid.

**cpconde@ucm.es*

Fluoroquinolones (FQ) are a group of antimicrobials broadly used to treat humans and food-producing animals. These antimicrobials have been detected in aquatic systems around the world, and recent studies have revealed the presence of FQ -resistant bacterial strains near hospitals and antibiotic production plants. Current methods of FQ analysis in food or environmental samples are based on liquid chromatography (LC) techniques coupled with either tandem mass spectrometry (MS/MS), or with optical (fluorescence, ultraviolet (UV)) detectors. These methods are usually cost-effective, require skilled operators and are scarcely available in developing countries. In this way, electrochemiluminescent (ECL) sensors are particularly useful for the determination of these analytes due to their high sensitivity, wide dynamic range and simple instrumentation. Ruthenium(II) chelates, such as tris(1,10-phenanthroline)ruthenium(II), $[\text{Ru}(\text{phen})_3^{2+}]$, have attracted much attention for the development of sensitive ECL sensors because of its excellent stability and ability to produce ECL with a good quantum yield.

In this presentation, we report on the development of ECL sensors based on the immobilization of $[\text{Ru}(\text{phen})_3^{2+}]$ by direct deposition, or entrapped into silica nanoparticles (NPs), on carbon screen-printed electrodes. The NPs were prepared by a modification of Stöber method [1] and several parameters including, silica composition, dye concentration, reaction time have been optimized to obtain spherical, monodisperse dyed-NPs with a mean diameter of 160 ± 13 nm. The particles were modified to contain amino functional groups for further covalent binding onto the electrode surface and were characterized by scanning electron microscopy (SEM), microelemental analysis and infrared spectroscopy.

Various parameters influencing the ECL sensors performance for ENRO monitoring including buffer composition, pH, and reagents concentration have been optimized using cyclic voltamperometry and luminescence detection.

Due to the presence of potentially interfering substances in the samples a preliminary solid phase extraction step using a FQ selective molecularly imprinted polymer [3] has been optimized to improve sensor selectivity. The method has been applied to the analysis of ENRO in aqueous samples.

Acknowledgements

This study was supported by MINECO (Ref: CTQ2012-37573-C02 and CTQ2015-69278-C2-1-R).

References

- [1] J. H. Zhang, P. Zhan, Z. L. Wang, W.Y. Zhang, N.B. Ming, *J. Mater. Res.* **18**, 649-653 (2003).
- [2] J. L. Urraca, M. Castellari, C. A. Barrios, M. C. Moreno-Bondi, *Journal of Chromatography A*, **1343**, 1-9 (2014).

Direct Electron Transfer between Cytochrome c and modified gold electrodes

S. López-Bernabeu^{1,*}, F. Huerta², F. Montilla¹, E. Morallón¹.

¹Departamento de Química Física e Instituto Universitario de Materiales, Universidad de Alicante, Apartado 99, E-03080 Alicante, Spain.

²Departamento de Ingeniería Textil y Papelera. Universitat Politècnica de València. Pza- Ferrándiz y Carbonell, E-03801- Alcoy (Alicante), Spain.

*sara.lopez@ua.es

Cytochrome c (Cyt c), which is a heme-protein model in the present work, is a very basic redox protein, playing an important role in the biological respiratory chain [1]. Direct Electron Transfer (DET) between Cyt c and electrodes is usually difficult, probably due to protein denaturation at the metal electrode surface, leading to slow electron-transfer kinetics [2,3]. Moreover, studies of the electrochemistry of Cyt c have been hampered because it adsorbs strongly on Pt, Hg, Au, Ag, and other electrodes [4].

The DET from conducting materials to biomolecules, particularly proteins, has become a central issue in the development of certain biotechnological devices such as biofuel cells or chemical biosensors. A particularly and widely used conductive p-type polymer is poly(3,4-ethylenedioxythiophene) (PEDOT) whose previous studies have shown that their electronic behavior is complex and appears to depend on the structure of the polymer, which in turn depends on the synthetic conditions, doping level, charge balancing ions, and other factors, such as the nature of the contacts. Thus, the DET between Cyt c and PEDOT-modified gold electrode has been studied in this work. It has been demonstrated that the thickness of PEDOT layer is an important factor in the electrochemical response of the Cyt c.

In situ IR spectroscopy has been used to characterize the surface of solid species and to assess reactants and products have been sorbed on the electrode. In this work, it has been utilized to study structural changes of Cyt c with potential. Using in situ FTIR spectroscopy, it has also been verified that a thin PEDOT layer allow the DET between Cyt c and the electrode surface. This study would contribute to the development of modified electrodes in order to promote a third generation of biosensors.

Acknowledgements

The authors thank financial support from the Spanish Ministerio de Economía y Competitividad (MAT2010-15273) and Generalitat Valenciana (PROMETEO 2013/038).

References

- [1] G.A. Mines, T. Pascher, S.C. Lee, J.R. Winkler, H.B. Gray. *Chem Biol*, **3**, 491-497 (1996).
- [2] X. Chen, H-Y. Long, W-L- Wu, Z-S. Yang. *Thin Solid Films*, **517**, 2787-2791 (2009).
- [3] Y. Zhou, J. Zhi, Y. Zou, W. Zhang, S-T. Lee. *Anal Chem*, **80**, 4141-4146 (2008).
- [4] L. Wang, E. Wang. *Electrochem Commun*, **6**, 49-54 (2004).

**POSTER
PRESENTATIONS**

**Advances in
instrumental and
methodological
analysis**

LLDPE Characterization in High-Strength Flexible Multilayer Packaging by Nuclear Magnetic Resonance

M. T. Tena*, M. Ezquerro, J. Cordón, M. Monge, J. M. López de Luzuriaga, M. E. Olmos.

Department of Chemistry. Faculty of Science and Technology. University of La Rioja. C/ Madre de Dios 53, E-26006 Logroño (La Rioja), Spain.

**maria-teresa.tena@unirioja.es*

Flexible multilayer packaging consists of a series of layers of different nature, each of which confers specific properties to the complex. The inner layer is usually made of linear low density polyethylene (LLDPE) which allows packaging closure by thermal sealing. The composition of this material is of crucial importance as it is in contact with the content and certain content ingredients may interact with it and produce an undesirable delamination process. Therefore, when the product to be packaged contains these "aggressive" ingredients it is necessary to design a special high-strength packaging.

The formulation of this kind of packaging usually includes several layers of polyethylene of different nature and thickness. The final LLDPE macroscopic properties (such as barrier and mechanical properties) depend on the molecular structure of the individual polyethylene chains and its assembled nanostructure, which finally depends on how polyethylene has been synthesized.

The LLDPE is obtained by copolymerization of ethylene and α -olefins such as 1-butene, 1-hexene and 1-octene, with Ziegler-Natta or metallocene type catalyst. Variables that primarily determine the molecular structure of the polymer (distribution of linear chain length, degree of lateral branches and length thereof) are the proportion and nature of α -olefin used as a co-monomer and the type of catalyst employed.

From the standpoint of quality control and design of new laminates, the determination of the LLDPE molecular structure is essential to produce high-strength multilayer packaging that does not undergo delamination. This communication presents the results obtained applying ^{13}C $\{^1\text{H}\}$ NMR technique to characterize different polyethylene resins used in the multilayer packaging manufacture.

Acknowledgements

Constantia Tobepal is thanked for providing LLDPE samples.

Feasible supercontinuum source with an optimized spectral width (1140 nm to 2282 nm)

J. E. Saldaña^{1,2}, S. Jarabo¹, F. J. Salgado-Remacha¹.

¹Departamento de Física Aplicada, Facultad de Ciencias, Universidad de Zaragoza, España.

²Departamento Académico de Ciencias Básicas, Universidad Nacional de Huancavelica, Perú.

*703872@unizar.es

The phenomenon of supercontinuum generation (SCG) consists of a wide spectral broadening of light signal during its propagation through a nonlinear medium [1, 2]. Supercontinuum has several applications in some interesting fields, including spectroscopy and remote chemical sensing [3-5]. In this work we present a supercontinuum source based on a simple and feasible setup (Figure 1), since only commercial Erbium-Doped Fiber Amplifiers (EDFAs) are required.

A ring-cavity laser pulsed by passive mode-locking based on the nonlinear polarization rotation effect is used as optical seed. In order to control the dispersion, positive and negative dispersion monomode fibers are placed inside and outside the ring, respectively. Finally, the pulses amplified with a second EDFA are coupled on a Highly Nonlinear Fiber (HNLF) to generate supercontinuum. Thus, it represents a good opportunity for spectroscopy as attainable and ultra-wide (around an octave) light source.

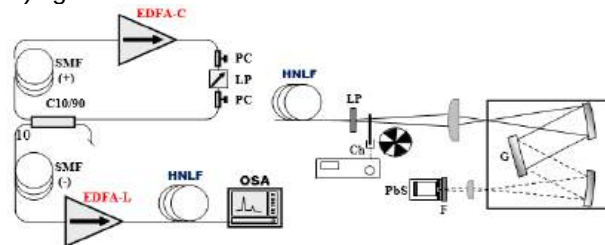


Figure 1. Experimental setup for the supercontinuum generation.

We have optimized the HNLF fiber length, maximizing the spectral width. Figure 2 shows supercontinuum spectra for different HNLF lengths. The maximum spectral width was obtained for 63 m, with spectrum spanning from around 1140 nm up to 2282 nm (1142 nm), an octave of spectral width.

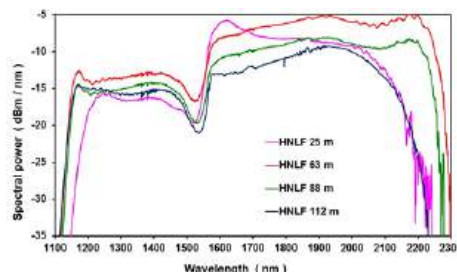


Figure 2. Supercontinuum spectral generated by various lengths of HNLF.

We have presented a novel supercontinuum source, easily attainable with commercial devices. This ultra-wide spectral source may find useful applications in the field of spectroscopy.

Acknowledgements

This work was supported by the project FIS2013-44174-P (Ministerio de Economía y Competitividad) and a grant of the Banco de Santander and the Universidad de Zaragoza.

References

- [1] R Alfano., The Supercontinuum Laser Source, Second edition, Springer, New York, 2006.
- [2] J. Swidersky, *Prog. Quantum Electron.*, **38**, 189-235 (2014).
- [3] Xia Ch., Mid-Infrared Supercontinuum Laser System and its Biomedical Applications, Thesis, Michigan, 1-147 (2009).
- [4] A. M Heidt, J.H.V Price, C Baskiotis, J S Feehan, Z Li; S.U. Alam, D. J. Richardson, *Opt. Express* **21**, 24281–24287 (2013).
- [5] R. Buczynsk, H. Bookey, M. Klimczak, D. Pysz, R. Stepien, T. Martynkien, J. E. McCarthy, A. J. Waddie, A. K. Kar, M. R. Taghizadeh, *Materials*, **7**, 4658-4668 (2014).

Magnetic graphene oxide as sorbent for headspace sorptive extraction of some chlorobenzenes prior to thermal desorption-gas chromatography mass spectrometry

M. Ahmadi¹, L. Vidal^{2,*}, T. Madrakian¹, A. Canals^{2,*}.

¹ Faculty of Chemistry, Bu-Ali Sina University, Hamedan, Iran.

² Departamento de Química Analítica, Nutrición y Bromatología e Instituto Universitario de Materiales, Universidad de Alicante, P.O. Box 99, E-03080, Alicante, Spain.

*lorena.vidal@ua.es (L. Vidal), a.canals@ua.es (A. Canals).

Analytical chemists have been always benefited from various available instruments such as chromatography, spectroscopy and microscopy, as well as sensors and microdevices. But these instruments cannot fully satisfied analytical chemist appetite for trace analysis of analytes of interest in complex matrices. In this regard, more often than not, one or more pretreatment steps are necessary to increase sensitivity and selectivity of the instruments. These are referred to "Sample Preparation", whose goal is enrichment, cleanup, and signal enhancement [1].

Since solid phase microextraction (SPME) was innovated and proposed as a sample preparation technique [2], plenty efforts have been made to improve this technique drawbacks. These efforts led to introduce some alternatives such as micro-solid phase extraction, stir-bar sorptive extraction, microextraction in a packed syringe and etc. Analytical chemistry researchers are always looking for simpler sample preparation techniques to increase selectivity and sensitivity of their determinations with lower total analysis cost. One of these efforts, in this work, led to introduce a new method based on headspace extraction, so-called Magnetic Headspace Adsorptive Extraction (Mag-HSAE), with improved simplicity, sensitivity and cost effectiveness. In the proposed method, the conventional SPME fibers were replaced with neodymium supermagnets which was modified with magnetic nanomaterials. In order to evaluate the Mag-HSAE technique feasibility, as model application, it was used for headspace extraction of some chlorobenzenes from aqueous samples using magnetic graphene oxide as adsorbent prior to GC-MS determination. The results showed that very sensitive (pg L⁻¹ detection limits), repeatable (1.5-9.5 RSD% values), accurate (86.2-110.1 recoveries percent), and at the same time cost-effective determination of the investigated model compounds was achievable using the newly developed technique.

References

- [1] S. Mitra, Sample Preparation Techniques in Analytical Chemistry, John Wiley & Sons, United States, 2004.
[2] C.L. Arthur and J. Pawliszyn, *Anal. Chem.*, **62**, 2145-2148 (1990).

Spectral characterization of optical retarders

A. Messaadi^{1,*}, M. M. Sánchez-López^{2,*}, P. García-Martínez³, I. Moreno¹.

¹*Departamento de Ciencia de Materiales, Óptica y Tecnología Electrónica, Universidad Miguel Hernández, 03202 Elche.*

²*Instituto de Bioingeniería, y Departamento de Física y Arquitectura de Computadores, Universidad Miguel Hernández, 03202 Elche.*

³*Departamento de Óptica, Universidad de Valencia, 46100 Burjassot, Valencia.*

**e-mail address: abdelghafor.messaadi@gmail.com*

In this communication we present our recent advances in the application of spectroscopic measurements for the characterization of optical linear retarders. These elements are useful components for any optical application requiring control of the state of polarization. Their spectral characterization is required to be able to use them in different wavelength ranges, as well as for the production of spectral birefringent filters.

We present here the calibration laboratory facility we have developed [1], that incorporates either a thermal broadband light source or a supercontinuum laser, two broadband beam-splitter polarizers, and two spectrometers, operative in the visible and in the near IR band regions respectively. As a result, we can precisely determine the spectral retardance function of different retarders in a wide spectral range from 400 nm to 1600 nm. We include results with static waveplates as well as liquid-crystal retarders (LCR).

Spectral measurements make it possible for us to easily identify multiple-order and zero-order retarders. We present results with quartz retarders, and show a simple technique to determine the order of the retarder from the spectroscopic information. The results will be compared to more sophisticated retarders such as achromatic retarders made of a combination of two different materials, or a Fresnel rhomb, that provides a constant retardance.

Finally, we present results with liquid-crystal retarders (LCR) [2]. These are very useful elements since they are low-order linear wave-plates with a variable retardance, tunable upon a small applied voltage. They can be fabricated in the form of a single LCR element, or in the form of one or two-dimensional arrays, as in a spatial light modulator (SLM) device. These devices are becoming very useful in all kind of applications that require programmable control of the intensity, the phase, the state of polarization, and the spectral content of an input light beam, thus becoming key components in advanced optical instruments for optical microscopy, interferometry, polarimetry or spectral filtering.

In all cases, the precise determination of the optical modulation, as a function of both the applied voltage and the wavelength, is very important to achieve accurate and reliable results with the instruments where they are incorporated. The presented spectroscopic facility and the related techniques allow the complete optical modulation characterization of all these kinds of retarder elements, including possible Fabry-Perot interference effects [3]. This communication presents the facility, the methods, and examples of the spectroscopic characterization of all the above-mentioned different optical devices.

Acknowledgements

We acknowledge support from Ministerio de Economía y Competividad from Spain (ref.: FIS2015-66328-C3-3-R).

References

- [1] P. Velásquez, M. M. Sánchez-López, I. Moreno, D. Puerto, F. Mateos, "Interference birefringent filters fabricated with low cost commercial polymers", *Am. J. Phys.*, **73** (4), 357-361 (2005).
- [2] A. Vargas, R. Donoso, M. Ramírez, J. Carrión, M. M. Sánchez-López, I. Moreno, "Liquid crystal retarder spectral retardance characterization based on a Cauchy dispersion relation and a voltage transfer function", *Opt. Rev.*, **20** (5), 378-384 (2013).
- [3] A. Vargas, M. M. Sánchez-López, P. García-Martínez, J. Arias, I. Moreno, "Highly accurate spectral retardance characterization of a liquid crystal retarder including Fabry-Perot interference effects", *J. Appl. Phys.*, **115** (3), 033101 (2014).

Selective determination of chloramphenicol residues at ultra-trace level by ion mobility spectrometry

S. Armenta¹, M. de la Guardia¹, A. Abad-Fuentes², A. Abad-Somovilla³, F. A. Esteve-Turrillas^{1,*}.

¹Department of Analytical Chemistry, University of Valencia, 50th Dr. Moliner St., 46100 Burjassot, Spain.

²Department of Biotechnology, Institute of Agrochemistry and Food Technology, Consejo Superior de Investigaciones Científicas (IATA-CSIC), 7th Agustín Escardino Av., 46980 Paterna, Spain.

³Department of Organic Chemistry, University of Valencia, 50th Dr. Moliner St., 46100 Burjassot, Spain.

*e-mail address of corresponding author. Sergio.Armenta@uv.es

Different sample pre-treatments have been evaluated for the analysis of chloramphenicol residues in food and biological fluids by ion mobility spectrometry (IMS). A combination of a selective solid-phase extraction (SPE) and dispersive liquid-liquid microextraction (DLLME) allowed the high sensitive determination of chloramphenicol in water, milk, honey, and urine samples. The performance of selective SPE supports such as immunoaffinity chromatography (IAC) and molecular imprinted polymers (MIP) have been compared in terms of selectivity, sensitivity, trueness, precision, and reusability. Quantitative recoveries were obtained for chloramphenicol residues, ranging from 91 to 123 % for water, from 99 to 131 % for skimmed milk, and from 95 to 137 % for urine using IAC-DLLME-IMS and MIP-DLLME-IMS methods. Quantitative recoveries (from 88 to 104%) were also achieved for honey samples using IAC-DLLME-IMS, but low recoveries were obtained using MIP-DLLME-IMS. The limit of quantification was set at $0.1 \mu\text{g L}^{-1}$ which is lower than the minimum required performance limit established by the EU. The proposed methodology is an attractive strategy for the relatively fast, sensitive, and selective analysis of chloramphenicol residues.

Figure 1 shows the IMS plasmagrams of urine, skimmed milk and honey samples spiked with chloramphenicol at $50 \mu\text{g L}^{-1}$ using solid-phase extraction by using IAC coupled to DLLME and MIP coupled to DLLME, as sample preparation step before IMS measurement.

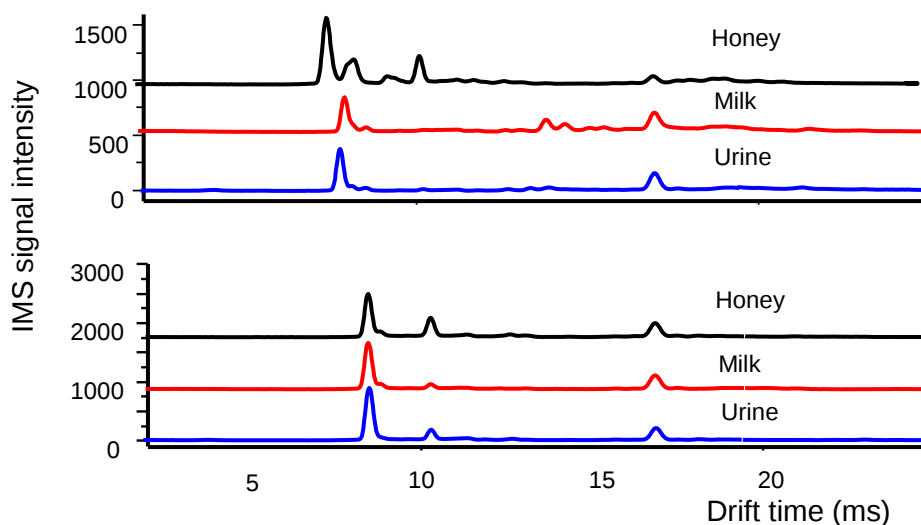


Figure 1. IMS plasmagrams of urine (blue line), skimmed milk (red line), and honey (black line) samples spiked with chloramphenicol at $50 \mu\text{g L}^{-1}$ using IAC-DLLME and MIP-DLLME before IMS measurement.

Acknowledgements

Authors gratefully acknowledge the financial support of the Ministerio de Economía y Competitividad (AGL2012-39965-C02-01-02/ALI, CTQ-2012-38635, and CTQ-2014-52841) and Generalitat Valenciana (PROMETEO-II 2014-077).

Photophysical Properties of a Triptycene-based ligand and its use as chemical sensor for Zinc

J. Sanmartín-Matalobos¹, M. Fondo¹, A. M. García-Deibe¹, M. Amoza¹,
P. Bermejo-Barrera^{2,*}, M. R. Domínguez-González², A. J. Mota³, J. L. Pérez-Lustres⁴,
S. Bhowmick⁵, N. Das⁵.

¹ Dpto. of Inorganic Chemistry, Faculty of Chemistry; Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain.

² GETEE Group, Dpto. of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry; Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain.

³ Universidad de Granada, Departamento de Química Inorgánica, Facultad de Ciencias Universidad de Granada. 18002 Granada, Spain.

⁴ CIQUS, Departamento de Química Física, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain.

⁵ Indian Institute of Technology Patna, Department of Chemistry, Patna 800 013, Bihar, India.

*pilar.bermejo@usc.es

The new triptycene-based ditopic Schiff base ligand H₂L was synthesized and satisfactorily characterized. H₂L is able to adopt the adequate topology required for optimal binding to naked metal ions leading to a neutral trinuclear circular helicate of the type M₃L₃·nH₂O. Helicate results from the assembly of three naked metal ions and three *anti* conformers of the ligand.

A novel trinuclear circular species have been isolated and unambiguously characterized by elemental analysis, mass spectrometry, infrared and NMR spectroscopies. We have synthesized the circular helicate Zn₃L₃·8H₂O that results from the zinc-assisted diastereoselective assembly of three units of the *anti-s-cis* helican H₂L, as (*R,R*)- or (*S,S*)-enantiomers. H₂L can adopt three different conformations, and DFT calculations suggest that these conformers have similar energy. The NMR characterization of H₂L shows that it adopts a syn configuration in DMSO-d₆ solution. The reaction of H₂L with Zn(II) in a basic medium in acetonitrile as solvent leads to the formation of pure Zn₃L₃(H₂O)₈. The NMR characterization of Zn₃L₃·8H₂O in DMSO-d₆ as solvent shows that the Schiff base in the metal complex adopts a 3_2R,R'-*anti-s-cis* configuration.

In order to confirm that the triptycene-based N,O-donor ligand can be an optical sensor for Zn²⁺, the correlation between analytical signal (Absorbance) and concentration of Zn²⁺ has been studied. The calibration curves obtained for Zn²⁺ at 267 and 348 nm shown a good correlation. The limit of detection (LOD) and quantification (LOQ) were calculated according to LOD=(3SD)/m and LOQ=(10SD)/m, where SD is the standard deviation of eleven measurements of a blank and m is the slope of the calibration graph. The detection and quantification limits obtained with the proposed method at 267 nm were 4.2 and 4.1 µg/l for Zn. Because of the results for LOD and LOQ were slightly better at 348 nm this is the wavelength chosen to perform the Zn determination.

Acknowledgements

N.D. thanks the Indian Institute of Technology (IIT) Patna, for financial support. S.B thank IIT Patna for an Institute Research Fellowship
Xunta de Galicia (Grupo de Referencia Competitiva 6RC2014/2016).

3D mapping by laser-induced breakdown spectroscopy combined with linear correlation

M. P. Mateo*, R. Cerrato, A. Casal, G. Nicolás.

Universidad de A Coruña, Laboratorio de Aplicaciones Industriales del Láser, Campus de Ferrol, Spain.

**paz.mateo@udc.es*

In a previous work [1], the benefits of using linear correlation combined with LIBS depth profiling, especially for the characterization of thick layers, were shown. In this work, the combination of linear correlation with three dimensional (3D) mapping by laser-induced breakdown spectroscopy (LIBS) has been studied. At this regard, a previous software that allowed the calculus of linear correlation coefficients and its representation in the format of depth profiles was improved to calculate those linear correlation coefficients and display them in form of lateral distributions, depth-profiles and 3D maps.

With the aim of testing the improvements of 3D mapping by LIBS due to the application of linear correlation, brass specimens with an oxide film covering fully or partially the metal surface have been prepared and analyzed. The results of the analysis demonstrate that the application of linear correlation to the generation of 3D LIBS maps can be a very useful tool to identify oxidized regions in different depths and areas of a brass sample. Moreover, the use of this statistical tool provides supplementary information since it improves the sensitivity and the accuracy of LIBS technique to discriminate regions with different characteristics, opening a new range of possibilities for the application of this new methodology to the quality control in the industry sector.

References

[1] M.P. Mateo, G. Nicolas, V. Piñon and A. Yañez, *Surf. Interface Anal.*, **38**, 941–948 (2006).

Electrochemical modification of boron-doped diamond with gold nanoparticles: Monitoring the deposition via SEIRA effect

J. Izquierdo^{1,*}, B. Mizaikoff¹, C. Kranz¹.

¹Institute of Analytical and Bioanalytical Chemistry, Ulm University, 89081 Ulm, Germany.

*javier.izquierdo@uni-ulm.de

Among infrared transparent electrodes (ITEs), boron-doped diamond (BDD) has recently gained considerable attention due to its advantages in electrochemistry and spectroscopy. The advantages include (i) a large potential window in aqueous environments (ca. 3 V), (ii) low background and noise current levels, (iii) transparency in a wide range of the electromagnetic spectrum, ranging from the UV-Vis to the far-IR regions, and (iv) resistance to fouling and biofouling due to its nonpolar character [1]. These properties, along with the superb physical and chemical stability of diamond, make BDD an ideal material for spectroelectrochemical measurements in the mid-IR region [2], to discern mechanisms of heterogeneous electrochemical reactions of organic species. Besides, enhancement of the optical and electrochemical sensitivity may be achieved through the decoration with gold nanoparticles (NPs) and nanostructures, thus promoting surface enhanced infrared absorption (SEIRA) effect of adsorbed IR-active molecules [3]. According to literature, BDD surfaces have been modified via electrochemical synthesis of AuNPs with accurate control of the NPs size and shape [4]. In addition, it was shown that the deposition of AuNPs on insulating IR-ATR crystals has been monitored *in situ* via the SEIRA effect induced in the water molecules [5].

In the current contribution, the electrochemical modification of BDD electrodes with AuNPs is presented for the enhancement of the optical and electrochemical sensitivity of the ITE. The synthesis of the AuNPs was *in situ* monitored by the enhancement effect induced on the IR absorption band related to the bending mode of adsorbed water molecules. The observed SEIRA effect allowed the optimization of the AuNPs in terms of density, size and shape, which was also characterized by atomic force microscopy with a combined AFM-IR-ATR setup [2]. The analytical merits of the developed system for spectroscopic measurements are demonstrated by SEIRA measurements of urea and KSCN, obtaining enhancement effects of more than one order of magnitude. The advancements for spectroelectrochemical measurements are illustrated for the redox couple hexacyanoferrate (II/III), evidencing the presence of the ferric or ferrous complex upon anodic or cathodic polarization, respectively. Results suggest that the AuNPs-modified BDD is a promising electrode material for spectroelectrochemistry as the elucidation of mechanisms of electron exchange processes and the investigation of the structural changes in the functional groups of the involved species and intermediates becomes accessible.

Acknowledgements

J. Izquierdo thanks the Alexander von Humboldt Foundation for the award of a Postdoctoral Fellowship. The Boehringer Ingelheim Ulm University BioCenter (BIU) is acknowledged for financial support.

References

- [1] J.V. Macpherson, *Phys. Chem. Chem. Phys.*, **17**, 2935-2949 (2015).
- [2] D. Neubauer, J. Scharpf, A. Pasquarelli, B. Mizaikoff, C. Kranz, *Analyst*, **13**, 6746-6752 (2013).
- [3] A. Hartstein, J.R. Kirtley, J.C. Tsang, *Phys. Rev. Lett.*, **45**, 201-204 (1980).
- [4] M.-J. Song, S.-K. Lee, J.-Y. Lee, J.-H. Kim, D.-S. Lim, *J. Electroanal. Chem.*, **677–680**, 139–144 (2012).
- [5] A.I. López-Lorente, M. Sieger, M. Valcárcel, B. Mizaikoff, *Anal. Chem.*, **86**, 783-789 (2014).

Laser induced breakdown spectroscopy for the analysis of Candida strains and their discrimination

S. Manzoor^{1,*}, L. Ugena¹, J. Tornero³, H. Martín², M. Molina², J. J. Camacho⁴, J. O. Cáceres^{1,*}.

¹*Departament of Analytical Chemistry, Faculty of Chemical Sciences, Complutense University, 28040 Madrid, Spain.*

²*Department of Microbiology II. Faculty of Pharmacy, Complutense University, 28040 Madrid, Spain.*

³*Hospital "Los Madroños", 28690 Brunete, Madrid, Spain.*

⁴*Departament of Applied Physical Chemistry, Autonoma University of Madrid, 28049 Madrid, Spain.*

jcaceres@ucm.es, smanzoor@ucm.es

The use of Laser Induced Breakdown Spectroscopy (LIBS) for biomedical applications has gained much interest since the last decade. The studies on microorganisms have mainly focused on bacterial samples with particular relevance to infectious diseases in humans [1- [2],[3],[4] but not much attention has been paid to the analysis of fungal samples by LIBS. Therefore, in this work LIBS has been employed for the analysis of different strains of *Candida* species. Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM-EDS) was also used as a complementary technique to reveal the cellular structure, three-dimensional visualization and provide information about elemental composition of *Candida* cells. These strains were selected for their medical importance as they are the commonly found in human fungal infections. Strains belonging to different species of *Candida* were included in the study. The potential of LIBS technique in discriminating between different *Candida* strains based on the spectral information and composition has been evaluated. The use of LIBS-NN combination provided reliable discrimination among the distinct *Candida* strains with a high spectral correlation index for the samples analysed, without any false positive or false negative. The results obtained in this study demonstrated the potential of LIBS-NN based methodology to be used as fast fungal diagnostic method.

References

- [1] D. Marcos-Martinez, J.A. Ayala, R.C. Izquierdo-Hornillos, F.J.M. de Villena, J.O. Cáceres, Identification and discrimination of bacterial strains by laser induced breakdown spectroscopy and neural networks, *Talanta*. 84 (2011) 730–737. doi:10.1016/j.talanta.2011.01.069.
- [2] J. Diedrich, S.J. Rehse, S. Palchaudhuri, *Escherichia coli* identification and strain discrimination using nanosecond laser-induced breakdown spectroscopy, *Appl. Phys. Lett.* 90 (2007) 163901–3. doi:10.1063/1.2723659.
- [3] S.J. Rehse, Q.I. Mohaidat, S. Palchaudhuri, Towards the clinical application of laser-induced breakdown spectroscopy for rapid pathogen diagnosis: the effect of mixed cultures and sample dilution on bacterial identification, *Appl. Opt.* 49 (2010) C27–C35. doi:10.1364/AO.49.000C27.
- [4] M. Baudelet, J. Yu, M. Bossu, J. Jovelet, J.-P. Wolf, T. Amodeo, et al., Discrimination of microbiological samples using femtosecond laser-induced breakdown spectroscopy, *Appl. Phys. Lett.* 89 (2006) 163903–3. doi:10.1063/1.2361270.

Nutrient assessment in olive leaves through NIR spectroscopy

F. Comino¹, A. Domínguez-Vidal¹, V. Aranda², M. J. Ayora-Cañada¹.

¹Department of Physical and Analytical Chemistry, University of Jaén, Campus Las Lagunillas s/n, 23071-Jaén, Spain.

²Department of Geology, University of Jaén, Campus Las Lagunillas s/n, 23071-Jaén, Spain.

*fcr00011@red.ujaen.es

Olive cultivation and olive oil production are a worldwide important business, which has been typically concentrated in the Mediterranean countries, especially south of European Union countries. Olive growing has become more intensive over the last three decades, and is using an increasing amount of agricultural land and resources [1]. Nutrient deficiencies can cause major losses to farmers due to decrease in fruit quality and yield. Standard routine treatments against nutritional disorders are commonly carried out by application of fertilisers, often ignoring the real nutritional status of trees. Thereby, the use of fertilisers on a regular basis can lead to an excess of available nutrients in regard to the real nutrient demand of crops. Such nutrient surplus can be either immobilised in the soil or leached, and can consequently contaminate superficial and underground waters. Thus, to maximize olive production with the minimum environmental cost, it is necessary to realize a correct fertilisation.

Suitable fertilisation decisions require proper characterization of plant nutritional status. Currently, this is carried out by chemical analyses of leaf samples performed in the laboratory. These analyses involve different analytical methods for various nutrients and usually include extensive sample preparation steps, like sample digestion, prior to analysis by atomic or molecular absorption spectroscopy or Kjeldahl method, in the case of nitrogen. These methods are expensive and time-consuming; therefore the situation calls for the introduction of an efficient analytical strategy that can effectively replace the traditional laboratory routine analysis [2]. Near infrared spectroscopy emerge as an interesting alternative, which has been applied for nutrition diagnose of different plant species, with dissimilar results [3]. For characterization of leaf chemical composition of olive trees, few studies are available. They are limited to nitrogen concentration [2] and other parameters like oleuropein [4].

In the present study we propose the use of FT-NIR spectroscopy for the prediction of several nutrients in olive leaves. One hundred olive leave samples from different olives varieties have been collected from around 30 different municipalities throughout the provinces of Jaén, Granada, Córdoba and Gerona. They correspond to two different seasons (winter and summer) collected in the period from 2013 to 2015. Using NIR spectra and applying different pre-processing tools in order to improve models accuracy, predictive models were constructed using a partial least squares regression (PLSR) with random subsets cross validation. Results obtained were excellent for nitrogen and calcium prediction (RPD >2), good for phosphorus and potassium prediction (RPD >1.5), and acceptable for magnesium, manganese and boron prediction (RPD >1). Predictive model for zinc gave inaccurate results with RPD <1. Results show that NIR spectroscopy could be an alternative for nutrient prediction in olive leaf, although it would be necessary to build more robust models expanding the number and variety of samples.

Acknowledgements

This research was performed in collaboration with Agronutrientes Jaén S.C.A. and supported by the Instituto de Estudios Giennenses (IEG), Diputación de Jaén, Spain.

References

- [1] G. Camarsa, S. Gardner, W. Jones, J. Eldridge, T. Hudson, E. Thorpe and E. O'Hara, LIFE among the olives: Good practice in improving environmental, Office for Official Publications of the European Union: Luxembourg, 2010.
- [2] N. Rotbart, Z. Schmilovitch, Y. Cohen, V. Alchanatis, R. Erel, T. Ignat, C. Shenderoy, A. Dag U. Yermiyahu, *Biosystems Engineering*, **114**(4), 426–434 (2013).
- [3] F. Aouidi, N. Dupuy, J. Artaud, S. Roussos, M. Msallem, I. Perraud Gaime and M. Hamdi, *Industrial Crops and Products*, **37** (1), 292–297 (2012).
- [4] H. Liao, J. Wu, W. Chen, W. Guo and C. Shi, *Journal of Plant Nutrition*, **35** (11), 1725–1734 (2012).

Enantiomeric separation of (-) and (+)-hyoscyamine by High Performance Liquid Chromatography-Tandem Mass Spectrometry

J. Marín-Sáez^{1*}, R. Romero-González¹, J. L. Martínez-Vidal¹, R. López-Ruiz, A. Garrido-Frenich¹.

¹ University of Almería, Department of Chemistry and Physics, Research Centre for Agricultural and Food Biotechnology (BITAL), Agrifood Campus of International Excellence ceiA3, Carretera de Sacramento s/n, E-04120 Almería, Spain.

*jms485@inlumine.ual.es, Tel: 670351603

Interest in natural toxins produced by fungi or plants has grown in recent years due to its toxicity and its impact on feeding and animal feed and human food safety. Among these toxins, alkaloids and more specifically tropane alkaloids, are very significant and they are characterized by presenting anticholinergic activity. Tropane alkaloids are synthesized by the plants from *Solanaceae* family and in smaller quantities from plants from other families. This family of compounds involves more than 200 compounds, although the compounds most studied are scopolamine, atropine and (-)-hyoscyamine [1].

In the case of atropine, which is a racemic mixture of (-) and (+)-hyoscyamine, it is well known that (-)-hyoscyamine exhibit stronger anticholinergic effects than the (+)-hyoscyamine. Moreover, racemization occurs during the treatment process of the raw material and cooking procedures due to pH and temperature modifications, since only the (-)-hyoscyamine is naturally formed. For this reason, according to the EFSA interest in 2013 [1] it is necessary to develop methods which allows the separation of these two chiral enantiomers to elucidate the conditions that favor the transformation between both enantiomers, because there are not many studies that evaluate these processes.

Therefore the aim of this study is the optimization of the enantioselective separation of (-) and (+)-hyoscyamine with a Chiralpak-AY3 (150x4.6 mm, 3 µm) analytical column in combination with high performance liquid chromatography (HPLC)-coupled to tandem mass spectrometry (MS/MS) has been used. The developed method allowed the separation of the two target compounds in 10 min, using ethanol 0.1% diethanolamine as mobile phase. In addition, the influence of different parameters, such as pH apparent or temperature, in the signal of the (-) and (+)-hyoscyamine was studied. Finally the method was applied to the determination of the target compounds in buckwheat samples. Performance characteristics, such as trueness (in terms of recovery), precision, linearity, detection limits (LODs) and quantification limits (LOQs) were studied. Suitable performance characteristics were obtained for a reliable determination of both enantiomers in the selected samples.

Acknowledgements

The authors gratefully acknowledge the Spanish Ministry of Economy and Competitiveness (MINECO) and FEDER (project ref. CTQ2015-69899-R).

References

[1] EFSA. Scientific opinion on tropane alkaloids in food and feed, EFSA panel on contaminants in the food chain (CONTAM). *The EFSA Journal*, **11**, 3386-3499 (2013).

Effect of Laser Pulse Width (fs/ns) and Fluence in the Direct Ionization Time-of-Flight Mass Spectrometry of Soil Particles

S. Medina, J. M. Vadillo, J. J. Laserna.

UMALASERLAB, Universidad de Málaga, C/ Jimenez Fraud 4, 29010 Málaga.
samara@uma.es

Direct determination of particulated matter is challenging due to the inherent lack of reproducibility derived from the size distribution and non-regular geometries of the sizes. In conditions where the probe size is larger than that of the particle, the heating of the particle may be assumed complete and the vaporization/excitation yield approaches 100%. As long as the particle size is larger, such value decreases. On the other hand, even under a successful hitting of the particle, the sample composition may affect the results as the amount of refractory oxides alter the heating dynamic of the particle. In this sense, the conventional analytical strategy demands a controlled optimization of the energy per pulse required to overcome the enthalpy barrier. Another strategy focuses on the taking advantage of the better energy coupling of ultrashort lasers to increase the excitation yield.

The present communication compares data obtained with the same Time-of-Flight Mass Spectrometry where two optical lines have been implemented to allow the excitation with a nanosecond laser (5 ns pulse width) and a femtosecond laser (35 fs pulse width).

Acknowledgements

This work is funded by project CTQ2014-56058P.

Solid phase microextraction (SPME) combined with laser induced breakdown spectroscopy (LIBS) for sensitive elemental analysis of aqueous samples

F. J. Ruiz, L. Ripoll, M. Hidalgo*, A. Canals.

Department of Analytical Chemistry and Food Sciences and University Materials Institute, University of Alicante, E-03080, Alicante, Spain.

**montserrat.hidalgo@ua.es*

Recent trends in analytical chemistry are aimed to automatize and miniaturize both devices and procedures, with the purpose to achieve portable systems capable of performing *in-situ* analysis. Laser induced breakdown spectroscopy (LIBS) is one of the few analytical techniques satisfying this need. The ability to analyse practically any kind of sample is an inherent advantage of LIBS. However, its application to elemental analysis of liquid samples is still scarce, due to the low competitiveness in sensitivity and precision compared to other well-known instrumental techniques (e.g. ICP-OES, ICP-MS or GFAAS). A substantial sensitivity enhancement can be achieved with the aid of microextraction methodologies, such as Solid Phase Microextraction (SPME). In SPME, analytes from the liquid samples are concentrated into a solid sorbent, allowing the direct analysis of the resulting solid matrix by LIBS with all the advantages that it implies.

The aim of the present work has been the development and evaluation of a novel methodology for trace elemental analysis of liquid samples based on the abovementioned SPME-LIBS combination. To this end, two different materials (*i.e.*, activated carbon and graphene oxide) were tested as possible sorbents for SPME. After optimization of the microextraction and detection procedures, analytical figures of merit of the developed SPME-LIBS methodologies were evaluated and compared. The obtained results indicate graphene oxide to be the best choice as sorbent material for the target analytes, providing increased sensitivity and experimental simplicity compared to activated carbon.

Acknowledgements:

The authors are grateful to the Regional Government of Valencia (Spain) (PROMETEO/2013/038) for the financial support.

New concentric atmospheric pressure glow discharge for ambient the analysis of gas samples: electrical and optical characterization of discharge

J. Fandiño¹, J. Orejas¹, D. Blanco², A. Sanz-Medel³, N. Borden¹, J. Pisonero¹.

¹University of Oviedo, Department of Physics, Calvo Sotelo s/n, 33007, Oviedo.

²University of Oviedo, Department of Construction and Manufacturing, Pedro Puig Adam s/n, 3303 Gijón.

³University of Oviedo, Department of Physical and Analytical Chemistry, Julián Clavería 8, 33006 Oviedo.

*pisonerojorge@uniovi.es

Introduction. Plasmas based on electrical discharges have had great interest, both in research and in practical applications. There is a great number of electrical discharges that are used as ionization sources for Mass Spectrometry (MS), including inductively coupled plasma (ICP), corona discharge (CD) or glow discharge (GD). Nevertheless, the introduction of a new group of Desorption/Ionization sources operating at Atmospheric pressure (ADI) has motivated a revolution in the so called Ambient Mass Spectrometry field.

Ambient Desorption/Ionization sources for Mass Spectrometry. Atmospheric desorption and ionization sources have enormously progressed in the last years [1]. The main advantage of ADI sources is their capability to analyse many samples in their natural form. These sources allow direct analysis of solid, liquid or gas samples with minimum sample preparation preventing contact between the sample and the vacuum of the analyser, and achieving correct sampling, desorption and ionization at room conditions.[2]. Moreover, ADI ionization sources are considered soft ionization sources that produce low fragmentation. Therefore, they are very suitable to provide molecular information. These analytical features provide a huge applicability of ADI-MS (e.g. fast detection of drugs, explosives and other substances or even direct analysis of biological tissues). In this particular case, an electrical plasma is generated in a helium atmosphere. The molecules present in the sample are first desorbed (by thermal process in the most cases). Then the discharge have interaction with the ambient space and, due to reactions between the excited species of He and atmospheric components (N₂, O₂ and H₂O), a number of reagent ions are generated: N₂⁺, O₂⁺, NO⁺, H₂O⁺ also including the protonated water clusters [(H₂O)_n + H]⁺, involved in protonation, the most important ionization reaction [3].

Atmospheric pressure glow discharge characterization (APGD). Our laboratory has recently developed an experimental system for direct solid/liquid/gas analysis, using an ADI source coupled with a quadrupole mass spectrometer. The developed ADI source was based in a concentric-electrode-APGD, using helium as a discharge gas. In this study, we present a new concentric-electrodes-APGD with a central channel that enables gas samples introduction. In particular, an electrical and optical characterization of the source has been carried out to investigate the influence of gas sample introduction, electrodes dimensions, current and helium flow on the discharge.

Acknowledgements

Authors acknowledge the financial support through the national project reference "MINECO-13-CTQ2013-49032-C2-2-R". Jaime Orejas appreciates the financial support for the completion of his doctoral thesis through the FPU grant reference "FPU-AP2009-1372".

References

- [1] J. H. Gross, "Ambient Mass Spectrometry," in *Mass Spectrometry: A Textbook*, 2nd ed., Springer, p. 754, (2010).
- [2] G. A. Harris, A. S. Galhena, and F. M. Fernández, *Anal. Chem.*, **83**, 4508–4538 (2011).
- [3] J. Orejas, K. P. Pfeuffer, S. J. Ray, J. Pisonero, A. Sanz-Medel, and G. M. Hieftje, *Anal Bioanal Chem*, **406**, 7511–7521 (2014).

Optimization of a methodology for selenium determination in complex solid samples using graphite furnace atomic absorption spectrometry

B. Gómez-Nieto*, M. J. Gismera, M. T. Sevilla, J. R. Procopio.

Universidad Autónoma de Madrid. Dpto. Química Analítica y Análisis Instrumental. Avda. Francisco Tomás y Valiente, 7, 28049, Madrid.

*bea.gomez@titulado.uam.es

Selenium is a non-metal element with properties that are intermediate between the chalcogen elements sulfur and tellurium. Selenium salts are toxic in large amounts. In fact, the most of the early researches on this element were carried out with the goal of addressing selenium toxicity. In the middle 20th century, selenium was recognized as a micronutrient and its biological function was studied with regard to its importance in human nutrition. Trace amounts of this element are necessary for cellular function in many organisms, including mammals [1]. Although selenium is considered an essential element, the variation between deficiency and toxicity of this element is the smallest of all the essential elements. For this reason, analytical methodologies for the sensitive and selective determination of this element are necessary. Methods based on atomic absorption spectrometry with a graphite furnace atomizer (GF AAS) are adequate for this purpose. However, some difficulties are associated with the determination of this element by GF AAS such as the presence of spectral interferences and the loss of selenium by volatilization. The main absorption line for selenium is located at a very short wavelength (196.026 nm). In the vicinity of this resonance line three iron lines are located and besides in this region it is more frequent to find spectral overlaps with diatomic molecules such as PO, CS, NO or SiO and radiation scattering effects become significantly more pronounced [2]. Zeeman correction systems are usually used for background correction but for samples with complex matrixes, such as in direct solid analysis, the correction of these spectral interferences is very difficult and sometimes it is not possible their complete elimination. In addition, selenium may be lost even at low temperatures due to the volatility of organoselenium compounds and also during pyrolysis and atomization due to the formation of decomposition products which are not dissociated before leaving the graphite tube [3]. Although diverse modifiers have been added to reduce volatilization losses, their effectiveness as stabilizing agents depends on the oxidation state of selenium species.

In this work, we have evaluated different types of chemical modifiers to prevent the loss of selenium in GF AAS measurements regardless of the oxidation state of this element in the sample. Palladium nitrate, magnesium nitrate, ruthenium and palladium nanoparticles synthesized by following a straightforward procedure have been assayed as chemical modifiers. The measurements were performed with a high-resolution continuum source atomic absorption spectrometer. With this instrument, the spectral interferences are easily eliminated due to its better capabilities for background correction compared to conventional atomic absorption spectrometers. The pyrolysis and atomization temperatures have been also optimized to control the volatilization processes. Standard reference materials have been used to evaluate the applicability of this methodology for the direct analysis of solid samples.

References

- [1] S. Kurokawa, M.J. Berry in: Interrelations between Essential Metal Ions and Human Diseases, in Metal Ions in Life Science. A. Sigel, H. Sigel, R.K.O. Sigel (Ed.), Springer: **13**, 499-534, (2013).
- [2] M. Resano, M. R. Flórez, E. García-Ruiz, *Anal. Bioanal. Chem.*, **406**, 2239- 2259 (2014).
- [3] R.A. Zanao, F. Barbosa, S.S. Souza, F.J. Krug, A.L. Abdalla, *Spectrochim. Acta B*, **57**, 291-301 (2002).

Laser-Induced Breakdown Spectroscopy for Chloride Determination in Mortars

J. Mateo^{1*}, A. Rodero¹, M. C. Quintero¹.

¹Department of Physics. School of Engineering Sciences of Belmez. University of Cordoba. Avenida de la Universidad s/n. E-14240 Belmez (Cordoba-SPAIN).

*sc3macaj@uco.es

Laser-induced breakdown spectroscopy (LIBS) is a laser diagnostic technique, where a laser beam focused onto a material generates a plasma when laser intensity exceeds the breakdown threshold of the material. LIBS has many advantages as an analytical technique, that no need of sample preparation, which avoids deterioration and further contamination of the material to be analyzed. LIBS is also applicable to the analysis of extremely hard materials that are difficult to digest or dissolve, such as ceramics and semi or super-conductors.

The objective of the present work is centered on the application of LIBS technique in the determination of concrete contamination by chloride. This element is the main cause of deterioration of concrete structures in marine environment. Determination of their presence in these structures is very important to know the status of them. The LIBS will allow the development of field-portable instruments capable of in-situ and real time analysis of structures without their deterioration.

In this work, a laser (Litron Nd:YAG Nano series) is coupled to a Czerny-Turner monochromator (Andor Shamrock 303) with three dispersion gratting: 600 groves/mm, 1200 groves/mm and 2400 groves/mm, and a sensitive optical detector, the intensified charge-coupled device (Andor iStar ICCD DH374 18mm). The measure was made in a vacuum chamber where the pressure can be controlled. The optimization of the method has been previously carried out by the use of the mineral chlorides halite (NaCl) and sylvite (KCl). Cl lines in the range of 800-900 nm have been found to be the best option for this study by its low influence of other spectrum lines. The intensity of these lines is compared with lines from other elements present in the sample, e.g. Ca from cement.

A group of mortar samples previously contaminated with different concentrations of chloride ion have been studied. Cement mortar samples have been made according to rule UNE-EN 196-1:2005, adding different amounts of sodium chloride to the water used in the mix. As a contrasting method, the Volhard chemical method, as it is described in rule UNE-EN 196-2:2006, has been used.

The final result is that there is a linear dependence between the ratio of lines Cl/Ca and the chlorine concentration that indicates that, though being a semi-quantitative technique, Laser Induced Breakdown Spectroscopy is a valid method to carry out quick and non-destructive analysis of mortar and concrete samples affected by deteriorative elements.

Acknowledgements

The authors are grateful to *Física de Plasmas: Diagnósis, Modelos y Aplicaciones* (FQM 136) research group of Andalusia Regional Government from the University of Córdoba for their technical and financial support. The authors also wish to express their gratitude to *Escuela Politécnica Superior de Belmez* from the University of Córdoba for providing the necessary resources to carry out this research.

References

- [1] G. Wilsch, F. Weritz, D. Schaurich, H. Wiggenhauser, *Constr. Build. Mater.*, **19**, 724 – 730 (2005).
- [2] O. Poupard, V. L'Hostis, S. Catinaud, I. Petre-Lazar, *Cement Concrete Res.*, **36**, 504 – 520 (2006).
- [3] D. Body, B.L. Chadwick, *Spectrochim. Acta B*, **56**, 725 – 736 (2001).

Development and characterization of a new multicapillary nebulizer for sample introduction in plasma-based spectrometric techniques

C. Lúñez¹, L. Kaynarova², M. A. Aguirre^{1,*}, A. Canals¹.

¹*Departamento de Química Analítica, Nutrición y Bromatología e Instituto Universitario de Materiales, Universidad de Alicante, P.O. BOX: 99, Alicante 03080, Spain.*

²*Department of Analytical Chemistry and Computer Chemistry, University of Plovdiv, 24 Tzar Asen St., Plovdiv 4000, Bulgaria.*

**aguirre.pastor@ua.es*

Plasma-based spectrometric techniques are considered the state of the art for the elemental analysis, offering excellent sensitivity that permits trace level quantification in different sample matrices. Despite these advances, continuous research is going on in order to enhance the analytical performance and to extend their applicability. One active research field is dealing with the sample introduction systems, aiming to improve the transport efficiency and to reduce the sample consumption.

In this work, a new multicapillary nebulizer for sample introduction in microwave plasma atomic emission spectrometry (MP-AES) and inductively coupled plasma optical emission spectrometry (ICP-OES) has been developed and aerosols generated have been characterized by Phase Doppler Particle Analyzer (PDPA). The new pneumatic nebulizer incorporates one nebulization nozzle with two independent liquid capillaries and a common gas inlet [1].

Aerosols generated under different nebulizer gas flow rate and liquid flow rate have been evaluated. Total solvent and analyte transport efficiency have been also investigated. The nebulizer experimental conditions have been optimized in MP-AES and ICP-OES.

Under optimum conditions, the analytical figures of merit (i.e., sensitivity and limit of detection) have been evaluated and compared with those obtained with two conventional pneumatic nebulizers (i.e., Conikal and SeaSpray). Finally, the analytical capabilities have been demonstrated by performing on-line calibration methodologies and on-line chemical vapour generation.

Acknowledgements

The authors kindly acknowledge the financial support from the Government of Spain (CTQ2011-23968) and Agilent Technologies for the donation of spectrometers (i.e., MP-AES and ICP-OES).

References

[1] A. Canals, M. Hidalgo, N. Kovachev and M. A. Aguirre, Multicapillary nebulizer for simultaneous nebulization of two or more liquids, 4 December 2014, Spanish Patent No. ES 2529965 B2.

POSTER PRESENTATIONS Environment

Assessment of tin distribution by a sequential extraction procedure in surface sediments from the Bahía Blanca estuary, Argentina

P. Y. Quintas¹, M. Garrido², M. B. Álvarez^{2,*}, C. E. Domini².

¹Instituto Argentino de Oceanografía (IADO-CONICET/UNS), Florida 7000, (8000), Bahía Blanca, Argentina.

²Instituto de Química del Sur (INQUISUR – CONICET/UNS), Avenida Alem 1253, Bahía Blanca (Argentina).

*malvarez@ciba.edu.ar

Tin inputs on the marine environment are mainly through antifouling paints which are generally applied in the hulls of ships to prevent fouling. Once released from an antifouling coating, is rapidly absorbed by organisms such as bacteria and algae or adsorbed onto the surface of particles suspended in the water [1,2]. Due to the different chemical forms in which metals can accumulate, total concentrations are frequently not sufficient to obtain information relevant to environmental studies.

Sequential chemical extraction with solutions of increasing dissolving power allows a broad operational classification of the most important binding forms of metals, which can, in principle, be associated with the constituent solid phases in sediments. The need of adequate quality control of the sequential procedures to allow the comparison of results is widely recognized. A fractionation method has been proposed by the European Standards, Measurements and Testing Program (formerly BCR) aiming to improve the comparability while retaining the most significant empirical information generated [3, 5].

Sampling was carried out in seasonal campaigns during 2013, at ten sites along the estuary. Atomic spectrometric techniques were particularly suited for the analysis of the relatively low volumes of the liquid phases resulting from the successive extraction steps. The sum of tin concentrations associated to each fraction obtained by the extraction scheme (acid soluble + reducible + oxidizable + residual) is in good agreement (89 - 106%) with the total metal content obtained by a conventional digestion method. From the environmental point of view, it was observed that those sites with the highest pollution relate not only to human activities, but with tidal currents within the estuary (**Figure 1**).

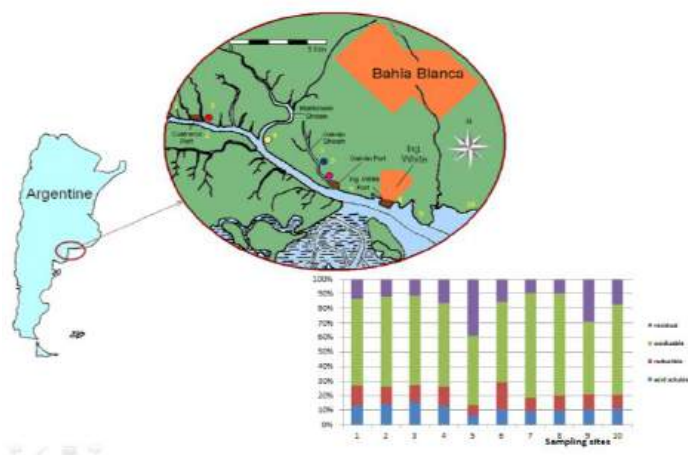


Figure 1 Sampling sites in the Bahía Blanca estuary and tin distribution pattern

Acknowledgements

This research was supported by PGI 24/Q054 - PGI 24/Q050 from the Universidad Nacional del Sur

References

- [1] ED. Burton, IR. Phillips and DW. Hawker. *Environ Sci Technol*, **38**, 6694–6700 (2004).
- [2] TG. Luan, J. Jin, SMN. Chan, YS. Wong and Tam NFY. *Process Biochem*, **41**, 1560–1565 (2006).
- [3] M. A. Ashraf, M. J. Maah, I. Yusoff. *Iranian Journal of Science & Technology*, **A2**, 163-180 (2012).
- [4] Chun-gang Yuan, Jian-bo Shi, Bin He, Jing-fu Liu, Li-na Liang, Gui-bin Jiang. *Environment International* **30** 769– 783 (2004).
- [5] Abdel Boughriet, Nicolas Proix, Gabriel Billon, Philippe Recourt, Baghdad Ouddane. *Water Air Soil Pollut*, **180**, 83–95 (2007).

Determination of chemical oxygen demand by UV/H₂O₂ oxidation treatment followed by in-drop turbidimetric analysis

I. Lavilla^{1,*}, J. Akhoundzadeh², M. Costas¹, M. Chamsaz², F. J. Pena-Pereira¹, C. Bendicho¹.

¹Departamento de Química Analítica, Área de Química Analítica, Facultad de Química, Universidad de Vigo, Campus As Lagoas-Marcosende s/n, 36310 Vigo, Spain.

²Department of Chemistry, Faculty of Sciences, Ferdowsi University of Mashhad, 0511-8794086, Mashhad, Iran.

*isela@uvigo.es

Chemical oxygen demand (COD) is defined as “the amount of a specified oxidant that reacts with the sample under controlled conditions” [1]. Standard methods for determination of COD involve the use of large volumes of a strong chemical oxidant (e.g., dichromate or permanganate) as well as highly corrosive and/or toxic chemicals (e.g., sulphuric acid or mercury sulphate). In addition, they require the use of large volumes and extended analysis times. This work reports the development of a greener analytical methodology for determination of low concentrations of COD in natural waters and wastewaters. The proposed method is based on the photochemical degradation of organic matter by means of an optimized UV/H₂O₂ oxidation treatment, in-drop precipitation of the generated CO₂, and microvolume turbidimetric analysis with no dilution of the BaCO₃ generated *in situ* [2]. A number of experimental parameters were evaluated for optimal performance, including UV irradiation time, H₂O₂ concentration, sample pH, microextraction time, drop composition (namely, Ba(OH)₂ and glycerol concentration) and drop volume. Under optimal conditions, the method demonstrated linearity over the concentration range of 3.4-20 mg/L oxygen. The limits of detection and quantification, calculated following the 3 σ and 10 σ criteria, were found to be 1.2 and 4 mg O₂/L, respectively. The repeatability, expressed as relative standard deviation, was 5.1% (n=6). The accuracy of the method was evaluated by analysing a certified reference material (COD Demand-Constant Value, RT Corportion Ltd, Salisbury, UK), showing no significant differences at the 95% confidence level (t-test, $t_{exp} < t_{crit}$, $t_{exp} = 1.491$, $t_{crit} = 2.132$). The analytical characteristics of the proposed method are, in general, comparable to those reported in the literature. Remarkably, the present methodology can be considered as an advantageous alternative to conventional procedures reported for COD determination in accordance with the “greenness” criteria proposed by the American Chemical Society Green Chemistry Institute [3].

References

- [1] A.D. Eaton, L.S. Clesceri, E.W. Rice and A.E. Greenberg, in: Standard methods for the examination of water and wastewater, 5220 Chemical oxygen demand, 21st ed., American Public Health Association, Washington DC, 2005.
- [2] J. Akhoundzadeh, M. Costas, M. Chamsaz, I. Lavilla and C. Bendicho, *Microchim. Acta*, **180**, 1029 – 1036 (2013).
- [3] L.H. Keith, L.U. Gron, J.L. Young, *Chem. Rev*, **107**, 2695 – 2708 (2007).

Microvolume UV-vis spectrophotometric determination of total phenol index in aqueous matrices after dispersive liquid-liquid microextraction

I. Lavilla, F. J. Pena-Pereira, S. Gil, M. Costas, C. Bendicho.

Departamento de Química Analítica, Área de Química Analítica, Facultad de Química, Universidad de Vigo, Campus As Lagoas-Marcosende s/n, 36310 Vigo, Spain.

**isela@uvigo.es*

The present work reports the development of an alternative methodology to the 5530 American Public Health Association (APHA) standard method for determination of phenols in aqueous samples [1]. The method involves the chemical conversion of phenols in the aqueous phase by oxidative coupling of phenolic compounds with 4-aminoantipyrine, followed by the enrichment of the formed antipyrine dye into a microvolume of trichloromethane, and final determination of the extract by a commercial microvolume UV-vis spectrophotometric system at 460 nm. Appropriate conditions for the enrichment of phenols were assessed. Experimental variables affecting the colorimetric reaction included concentration of reagents (4-aminoantipyrine, potassium peroxodisulfate and potassium ferricyanide), sample pH and temperature, whereas experimental parameters affecting the preconcentration of the antipyrine dye included different types of extraction and disperser solvents, volume and ratio of the extractant/disperser mixture. Under optimal conditions, an enrichment factor as high as 700 was attained for phenols. The limits of detection (LOD) and quantification (LOQ) of the proposed method, calculated following the 3σ and 10σ criteria, were 0.8 and $2.5 \mu\text{g L}^{-1}$, respectively. The linear dynamic range covered concentrations from the LOQ to $150 \mu\text{g L}^{-1}$, and the precision of the method, expressed as relative standard deviation (RSD), was 5.2% ($n=6$). Remarkably, the proposed methodology showed excellent analytical characteristics and a much greener profile than the 5530 APHA standard method [2] in accordance with the suggestions of the National Environmental Methods Index (NEMI) [3,4]. Specifically, corrosive substances are not required, the amount of waste generated is lower than 50g, and persistent, bioaccumulative and toxic chemicals (PBT) are not used in the proposed method. Finally, the method was successfully applied to the determination of total phenol index in five different water and wastewater samples, yielding recovery values in the range of 90-99%.

References

- [1] I. Lavilla, S. Gil, M. Costas, C. Bendicho, *Talanta*, **98**, 197 – 202 (2012).
- [2] A.D. Eaton, L.S. Clesceri, E.W. Rice and A.E. Greenberg, in: Standard methods for the examination of water and wastewater, 5530 Phenols, 21st ed., American Public Health Association, Washington DC, 2005.
- [3] L.H. Keith, L.U. Gron, J.L. Young, *Chem. Rev.*, **107**, 2695 – 2708 (2007).
- [4] NEMI: National Environmental Methods Index <http://acwi.gov/methods/nemi/index.html> (accessed 14 Feb 2016).

Comparison of methods of analysis of oils by ICP-OES

J. M. Suárez-Muñoz^{1,2,*}, R. Pascual-Juez¹.

¹Rey Juan Carlos University. C/Tulipan s/n, Móstoles, Madrid (Spain).

²Degree in Energy Engineering. Rey Juan Carlos University. C/Tulipan s/n, Móstoles, Madrid (Spain).

*juanmanuel.suarez@urjc.es

There are different rules for determining metals in oils by ICP-OES technique. Depending on the source and the use of the oil in which it was used, the methods are different. In this research the standard ASTM D7151-13^[1] and the ASTM D5185-13 E1^[2] are compared, although the measuring ranges for the metals are different. They can be used for the determination of metals at the same concentration. Different measures have been carried out for each of the methods to a concentration of 10 mg/kg, being the maximum concentration referred to the standard ASTM D7151. The study was performed with an optical ICP in the axial configuration and a multielement pattern of 21 elements.

After performing the measurements, in figure 1 it can be seen that the recovery for the different elements is better for the case of ASTM D7151 standard, than for the ASTM D5185 standard. The largest variation is observed with Ca, Na, P and Si elements, whose values differ from the nominal value of the standard by more than 30% (Fig.1). This is due to the difference in the concentration of the internal standard and the measurement uncertainty associated with each of these elements in the different methods.

The concentration of the internal standard in the ASTM D5185 is higher than in ASTM D7151, so the ASTM D5185 presents greater interferences^[3] than those listed in ASTM D7151. This gives rise to a greater variability in the spectra obtained by the equipment as the concentration is increased, because the ratio of internal standard concentration and multielement pattern is higher. Thus, the amount of standard used to prepare ASTM D7151 curve is higher, which makes, along with the concentration of the internal standard, that the measurement stability is greater decreasing its associated uncertainty. Considering that the initial pattern for the preparation of the calibration samples for the determination of the calibration lines was the same, it is concluded that for low concentrations is advisable to use the ASTM D7151 standard, while for concentrations above 10 mg/kg is necessary the ASTM D5185 standard, due to the recoverability enhances with increasing the concentration.

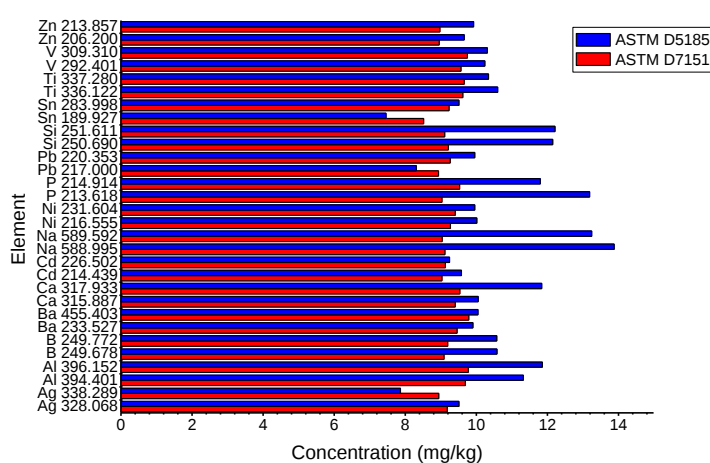


Fig1. Recuperability

References

- [1] ASTM D7151-13: Standard Test Method for Determination of Elements in Insulating Oils by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). 2013.
- [2] ASTM D5185-13 E1: Standard Test Method for Multielement Determination of Used and Unused Lubricating Oils and Base Oils by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). 2015.
- [3] R. K. Winge. Inductively Coupled Plasma-Atomic Emission Spectroscopy. An Atlas of Spectral Information. Sixth impression. Ed: Elsevier. Amsterdam. 1993.

Spectroscopic Raman characterization of Rutherfordine: a combined DFT and experimental study

F. Colmenero^{1,*}, V. Timón¹, L. J. Bonales², J. Cobos².

¹Instituto de Estructura de la Materia (CSIC). C/ Serrano, 113. 28006 Madrid (Spain).

²Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas, CIEMAT. Avda/ Complutense, 40. 28040 Madrid (Spain).

*francisco.colmenero@iem.cfmec.csic.es

Uranyl minerals, containing the uranyl ion $U^{VI}O_2^{2+}$, have an increasing interest in environmental, nuclear and energetic disciplines because they are expected to be the main phases as a result of alteration of irradiated $U^{IV}O_2$ reactor spent nuclear fuel (SNF).

Quantum theoretical methods are free of the difficulties associated to experimental methods, but their application to uranium-containing solids is difficult due to the necessity of using effective potentials for core electrons and relativistic corrections. There is very few published theoretical work about these mineral phases, mainly due lack of appropriate pseudopotentials. At first in this work a norm-conserving relativistic pseudopotential of uranium atom was generated and evaluated for a series of uranium-containing minerals: oxides, hydroxides, oxihydroxides, carbonates and silicates in this work.

Uranyl carbonate minerals as rutherfordine, UO_2CO_3 , are important in the SNF context, because carbonate and bicarbonate, present in many natural waters, are exceptionally strong complexing agents for actinide ions. In this work, rutherfordine natural sample obtained from Sierra Albarrana (Córdoba, Spain) was studied by means of Raman spectroscopy and theoretically described by first principle calculations based on density functional theory (DFT) using CASTEP software [1].

The two different crystal orthorhombic structures were studied: Pmmn and Immm. Theoretically optimized structures were determined and the resulting lattice parameters, bond lengths, bond angles, and X-Ray powder spectrum were found in very good agreement with experimental values (Fig.1b). The experimental Raman spectrum and the calculated one are also in good agreement (Fig.1a). The energies and spectra associated to both structures were very close so that both may be simultaneously present in nature. For most of the bands in the experimental Raman spectrum we found a correspondence with bands in the theoretical spectrum and, thus, they were related to specific atomic vibrational motions. Only three weak bands were left without assignments, and they were related to other mineral phases: schoepite and uranophane.

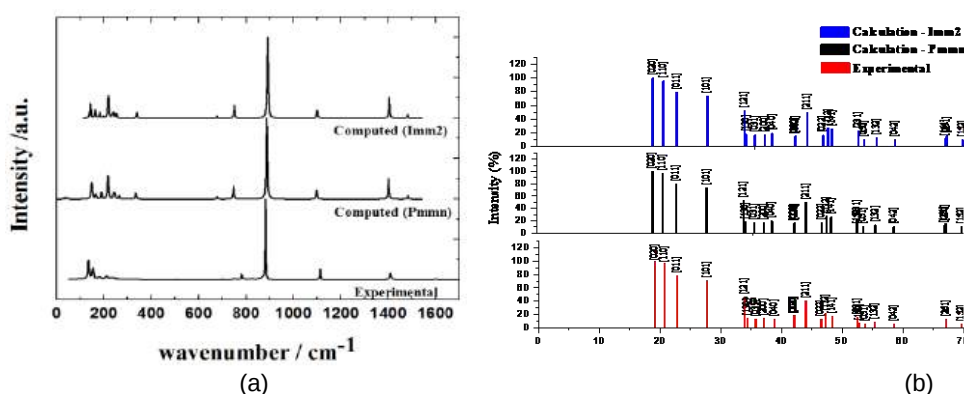


Fig. 1: Calculated and measured rutherfordine Raman spectra and XRD pattern.

Acknowledgements

This work was supported by ENRESA in the project: N° 079000189 and Spanish MINECO project FIS2013- 48087-C2-1-P. Supercomputer time by the CETA-CIEMAT and CESGA centres are also acknowledged

References

- [1] S.J. Clark, M.D. Segall, C.J. Pickard, P.J. Hasnip, M.I.J. Probert, K. Refson, M.C. Payne, *Z. Kristallogr.*, **220**, 567-570 (2005).
- [2] L.J. Bonales, C. Menor-Salván, J. Cobos, *J. Nucl. Mater.*, **462**, 296-303 (2015).

POSTER PRESENTATIONS

Historic and artistic heritage

SERS analysis of red lakes paints: coated glass slides and cross sections

M. S. Zalaffi¹, M. V. Cañamares^{2,*}, S. Prati¹, S. Sánchez-Cortés².

¹Microchemistry and Microscopy Art Diagnostic Laboratory, University of Bologna, Via Guaccimanni 42, 48121 Ravenna, Italy.

²Instituto de Estructura de la Materia, IEM-CSIC, Serrano 121, 28006, Madrid, Spain.

*mvca@iem.cfmac.csic.es

The SERS technique has already been applied in the field of Cultural Heritage to study organic dyes using different samples (solid micro-samples, fibers) taken from several artistic objects (paintings, pottery, statues, textiles). However, little has been studied about the application on cross sections mounted on different supports (resins or inorganic salts) [1]. It is well known that only the cross section can reveal the precise stratigraphy and the spatial collocations of all the layers composing a work of art.

This work focuses on the formulation of a protocol for the application of the SERS technique directly on cross sections using metal nanoparticles (NPs). Analysed organic colorants are anthraquinone-based red lakes: pure purpurin, carminio estratto (mainly composed by carminic acid complexed with aluminum) and lacca rubia (made of alizarin complexed with Al³⁺). Samples were studied as paint layers on glass slides and cross sections.

Various types of silver NPs were employed as SERS substrates: Ag spherical NPs prepared by chemical reduction with citrate (AgCt) [2] and hydroxylamine (AgHx) [3], Ag nanostars (AgNS) [4], Au NPs with different sizes [5] and Ag NPs prepared by photo reduction [6].

Regarding the analysed dye, purpurin is the easiest to detect because it is not a lake. Between the two lakes, carminium and lacca rubia, the first one is more detectable. Considering the type of SERS substrate, AgCt colloid shows the best performance. AgNS, in spite of being slightly less effective, are valuable as well because they do not need to be aggregated, decreasing the level of the interfering species on the sample and increasing the reproducibility of the measurements. The AuCt colloid has the great advantage that its analysis is performed at 785 nm, laser that lowers consistently the fluorescence but the size of its NPs must be at least of 70 nm to be effective. Concluding, the AgHx one, although free from the highly interfering citrate, does not work well in the detection of colorants.

Acknowledgements

This work was supported by CSIC (project PI 201560I029), Comunidad de Madrid/EU (project GEOMATERIALES 2) and MINECO (project FIS2014-52212-R).

References

- [1] Idone A., Aceto M., Diana E., Appolonia L. and Gulminic M., *J. Raman Spectrosc.*, (2009).
- [2] Lee P.C., Meisel D. *J. Phys. Chem*, **86**, 3391- 3395 (1982).
- [3] Cañamares M.V., Garcia-Ramos J.V., Gomez-Varga J.D., Domingo C. and Sánchez-Cortés, S., *Langmuir*, **21**, 8546–8553 (2005).
- [4] Garcia-Leis A., García-Ramos J.V. and Sánchez-Cortés S. *J. Phys. Chem. C*, **117**, 7791-7795 (2013)
- [5] Frens G. *Natural Physical Science*, **241**, 20-22 (1973)
- [6] Cañamares M.V., Garcia-Ramos J.V., Gomez-Varga J.D., Domingo C. and Sánchez-Cortés S., *Langmuir*, **23**, 5210-5215 (2007).

Raman microspectroscopy and Attenuated Total Reflection Far Infrared Spectroscopy as complementary techniques for identification of mineral pigments in work of arts

A. Domínguez-Vidal*, L. Pulido-Moriana, M. J. Ayora-Cañada.

Department of Physical and Analytical Chemistry, Universidad de Jaén, Campus las Lagunillas E-23071, Jaén Spain.

**adovidal@ujaen.es*

Raman spectroscopy, specially coupled to a microscope, is a well-established technique for the study of works of art. Its main advantages to this field are related to the absence of sample preparation in most of the cases and the possibility of the in-situ measurements [1]. The molecular information that can be obtained can give us knowledge about the original materials employed and also about the degradation products [2]. This information can be useful in the restoration and conservation works. However the most common drawbacks are the fluorescence background and the low signal of some compounds. Here we propose the use of far infrared spectroscopy (FIR) as a complementary technique that could improve the information obtained from the samples. The use of FIR has not been fully exploited in the field of cultural heritage. Far Infrared can be a useful spectral region for the study of mineral pigments (inactive in the mid-infrared region) [3]. The use of attenuated total reflectance (ATR) was selected for the measurements due to its ease to register the spectra without any need of sample preparation. Furthermore, ATR measurements at low wavenumbers are more sensitive because of the longer penetration depth of the evanescent wave. However, there is a lack of deep studies on the use of this approach considering interacting effects such as the refraction index of the samples. Here, we have developed an easy methodology based on the use of paraffin mixed with the pigment and constructed a database including a wide range of commercial pigments. Combining the information provided by both techniques, the pigments of two spaces in the Alhambra were characterized. In the plasterwork of the stalactite vaulting in the Hall of the Kings, typical pigments of the Nasrid period were found. More recent pigments appeared in the Hall of the Beds (Comares Bath) due the redecorations and restoration works in the XIXth century.

Acknowledgements

This work was carried out in collaboration with Alhambra and Generalife Council and it has been financed by the research projects BIA2013-41686-R (Spanish Ministry of Economy and competitiveness and FEDER funds) and UJA2014/06/12 (Universidad de Jaén, Caja Rural de Jaén).

References

- [1] P. Vandenabeele, H. G. M. Edwards and L. Moens, *Chem. Rev.*, **107** (3), 675–686, 2007.
- [2] A. Domínguez-Vidal, M. J. de la Torre-López, M. J. Campos-Suñol, R. Rubio-Domene and M. J. Ayora-Cañada, *J. Raman Spectros.* **45**, 1006-1012, 2014.
- [3] S. Vahur, A. Teearu, I. Leito, *Spectrochim Acta A*, **75**, 1061-1072, 2010.

Raman and FT-IR microspectroscopies for characterization of Nasrid polychrome wood ceilings

A. Domínguez-Vidal^{1,*}, P. Arjonilla¹, E. Correa-Gómez², R. Rubio-Domene², M. J. de la Torre-López³, U. Schade⁴, M. J. Ayora-Cañada¹.

¹Department of Physical and Analytical Chemistry, Universidad de Jaén, Campus las Lagunillas E-23071, Jaén Spain.

²Department of Conservation, Alhambra and Generalife Council, Granada, Spain.

³Department of Geology, Campus Científico Tecnológico, Linares, Spain.

⁴IRIS Beamline, Helmholtz Zentrum für Materialien und Energie Berlin, Germany.

*adovidal@ujaen.es

The polychrome decorations of the wood ceilings in two spaces of the Alhambra complex, the Hall of the Two Sisters and the Hall of the Abencerrages have been investigated. These wood structures were constructed by joining small, geometric pieces shaped as, square, star, pentagon, and hexagon. During the conservation works the ceiling was disassembled revealing drawings and sketches also in the reverse of the wooden pieces. The pieces were studied in a completely non-destructive manner by means of Raman microspectroscopy employing a portable instrument (innoRam, B&W TEK) equipped with a 785-nm laser and a back thinned two dimensional CCD detector and fiber optics probe. The experimental configuration was adapted to suit the artifact under study by mounting the probe head either horizontally or vertically on an extension arm. Furthermore, to get information about inner layers, small samples were carefully taken in selected locations. These samples were investigated by Raman microspectroscopy using bench top instrument equipped with excitation laser at 514 and 785 nm. In addition FTIR spectra were obtained using both a conventional IR source and synchrotron radiation. In both cases FTIR transmission spectra were collected from the samples placed on a diamond cell viewed through the microscope system.

The pigments identified were lead white, cinnabar, lapis lazuli, orpiment and carbon black. Furthermore, these studies revealed the execution technique of these decorations. The different colors were applied over a priming layer of minium, which probably played a protective role for the wood. This is in agreement with some findings of a previous work in a different Hall of the Alhambra, although in that case unambiguous identification of the lead compound was not achieved¹. On the top of the decoration there was a crackled layer identified as beeswax. This was probably a protective layer, not original, since it is documented that in the intervention carried out in the first half of the 20th century woodworks in the Alhambra were treated with a mixture of linseed oil, turpentine and beeswax to protect them against deterioration². Finally, it was possible to detect the presence of proteinaceous material, probably employed as binder to fix the pigments.

Acknowledgements

This work was carried out in collaboration with Alhambra and Generalife Council and it has been financed by the research projects BIA2013-41686-R (Spanish Ministry of Economy and competitiveness and FEDER funds) and UJA2014/06/12 (Universidad de Jaén, Caja Rural de Jaén).

References

- [1] C. Cardell, L. Rodríguez Simon, I. Guerra, A. Sanchez-Navas, *Archaeometry* **51**, 637-657, 2009.
- [2] L. Torres-Balbás, L., *Cuadernos de la Alhambra* **1**, 1–34, 1965.

POSTER PRESENTATIONS

Sample preparation

Magnetic solid-phase extraction using ZSM-5 zeolite for BTEX determination in water samples

E. Fernández, L. Vidal*, A. Canals.

Departamento de Química Analítica, Nutrición y Bromatología e Instituto Universitario de Materiales, Universidad de Alicante, P.O. Box 99, E-03080 Alicante, Spain.

**lorena.vidal@ua.es*

Magnetic solid-phase extraction (MSPE) has recently received great interest since it reduces sample preparation time and facilitates sorbent manipulation. In MSPE, the magnetic sorbent is dispersed into the aqueous phase and after extraction, it is easily separated from sample solution by applying an external magnetic field, and thus avoids time-consuming filtration or centrifugation steps for phases separation. Next, target analytes can be desorbed using a proper eluent solvent or thermally for further determination by liquid or gas chromatography, respectively.

The purpose of this work is to present a new composite based on ZSM-5 zeolite decorated with iron oxide magnetic nanoparticles as a valuable sorbent for MSPE. Zeolites possess unique sorbent properties that enable molecules to be discriminated based on morphology (i.e., pores and channels size and shape), and is thus advantageous for selective extractions. In addition, the proposed composite presents remarkable advantages such as low cost, rapid and simple synthesis, easy manipulation under a magnetic field and reuse options.

A proposal is made to determine benzene, toluene, ethylbenzene and xylenes (BTEX) in water samples as an analytical approach using gas chromatography-mass spectrometry detection. A two-step multivariate optimization strategy, using Plackett–Burman and Circumscribed Central Composite designs, has been employed to optimize experimental parameters affecting MSPE. The method has been evaluated under MSPE optimized conditions obtaining a linear response from 1 to 100 $\mu\text{g L}^{-1}$ ($N=6$) for benzene; from 10 to 100 $\mu\text{g L}^{-1}$ ($N=6$) for toluene, ethylbenzene and o-xylene; and from 10 to 75 $\mu\text{g L}^{-1}$ ($N=5$) for m,p-xylene. The repeatability of the proposed method has been evaluated at 40 $\mu\text{g L}^{-1}$ spiking level and coefficients of variation ranged between 8 and 11% ($n=5$). Limits of detection range from 0.3 $\mu\text{g L}^{-1}$ for benzene to 3 $\mu\text{g L}^{-1}$ for the other analytes. These values satisfy the current normative of the Environmental Protection Agency and European Union for BTEX content in waters for human consumption, especially for benzene, which presents the most restrictive levels. Finally, drinking water, wastewater and river water have been selected as real-world water samples to assess the applicability of the method. Relative recoveries vary between 85% and 114% showing negligible matrix effects.

Acknowledgements

The authors would like to thank the Spanish Ministry of Science and Innovation (project n. CTQ2011-23968), Generalitat Valenciana (Spain) (projects n. GVA/2014/096 and PROMETEO/2013/038) for the financial support. E. Fernández also thanks Ministry of Education for her FPU grant.

Ultrasound-assisted ionic liquid dispersive liquid-liquid microextraction for Na, K, Ca, Mg and P determination in biodiesel by ICP-OES

F. D. Ávila-Orozco¹, M. A. Aguirre², N. Kovachev³, I. P. Román², A. Canals²,
C. E. Domini^{1,*}.

¹Sección Química Analítica, INQUISUR (UNS-CONICET), Universidad Nacional del Sur, Avenida Alem 1253, B8000CPB, Bahía Blanca, Argentina.

²Dpto. Química Analítica, Nutrición y Bromatología e Instituto Universitario de Materiales, Facultad de Ciencias, Universidad de Alicante, Apdo. 99, Alicante, Spain.

³Agilent Technologies Inc., World Trade Center, Moll de Barcelona, 08039 Barcelona, Spain.

*cdomini@criba.edu.ar

Biodiesel is a composed mixture of fatty acid alkyl esters derived from vegetable oils (i.e. soybean, sunflower, palm, etc.) or animal fats. Because of their properties, biodiesel is completely miscible with diesel [1]. This biofuel is a renewable substitute (biodegradable and non toxic) that can be used in diesel engines without the need of modifications or adaptations [2]. Throughout biodiesel production, contamination may occur with metals such as Na, K, Ca, Mg, sulfur and phosphorus compounds. These contaminants could influence the stability and other physical-chemical properties [3,4].

The aim of this work was to develop a green sample preparation method based on an ultrasound-assisted ionic liquid dispersive liquid-liquid microextraction technique (USA-IL-DLLME) for Na, K, Ca, Mg and P determination in biodiesel samples by ICP-OES. The extraction was optimized using a two steps approach: (i) Plackett-Burman design, and (ii) central composite design. The optimized extraction condition values were: 0,900 g of biodiesel, 220 μ L of 10% HNO₃ solution, 75 μ L of [C10MIM][Cl] and sonication power and time, 45 W and 1 min, respectively. Under the optimized conditions biodiesel samples from different feedstock such as sunflower, corn, soybean and grape seed oils, via a base catalyst transesterification, were analyzed and relative standard deviation values were lower than 10% and recovery values ranged between 95% and 106%.

Acknowledgements

The authors gratefully acknowledge Universidad Nacional del Sur, INQUISUR-UNS-CONICET, Argentina, Ministerio de Asuntos Exteriores y de Cooperación (MAEC) and Dirección de Relaciones Culturales y Científicas (AECID) for the financial support. The authors wish to thank the Spanish Government (CTQ2011-23968) for the financial support, and also Ingeniatics Tecnologías S.L. and Agilent Technologies for the FBMN prototype and ICP-OES spectrometer provided, respectively.

References

- [1] A. Bulent Koc, *Bioresour. Technol.*, **100**, 19–24 (2009).
- [2] E.M. Shahid, Y. Jamal, *Renew. Sustain Energ. Rev.* **12**, 2484–2494 (2008).
- [3] M. Edlund, H. Visser, P.J. Heitland, *J. Anal. At. Spectrom.* **17**, 232–235 (2002).
- [4] T. Nogueira, C. Lucio do Lago, *Microchem. J.* **99**, 267–272 (2011).

Synthesis and evaluation of molecularly imprinted membranes for the selective determination of estrogens in water

R. M. Garcinuño-Martínez*, P. Fernández-Hernando, G. Paniagua-González, E. Collado-Esteban, A. Mayes.

Universidad Nacional de Educación a Distancia (UNED), Analytical Sciences Department, Faculty of Sciences, 28040 Madrid, Spain.

**rmgarcinuno@ccia.uned.es*

Nowadays, the growing attention dedicated to molecular imprinting (MIP) technology has led to the development of a wide range of applications in different ambits, including chromatography, extraction and the field of chemicals sensors. Recently, the contribution of molecular imprinting technique in the membrane preparations has allowed to different innovative research lines for many scientists in the field of separation and extraction.

This work is focused on the synthesis and characterization of novel molecularly imprinted polymer membranes (MIMs) for the selective determination of the estrogenic compounds from polluted water sources.

Glass-fibre membranes were functionalised using a grafting method to deposit molecularly imprinted polymers. Initially, the glass microfiber filters were subjected to a surface functionalization treatment for the incorporation of the UV-initiator sodium diethyldithiocarbamate (DTC) onto the glass substrate surface. As a second step, the surface-modified glass filters were incubated with the polymerisation mixture containing the molecule template (Estradiol, E2), the functional monomer (MAA) and the cross-linker (EDGMA) in acetonitrile. The polymerisation was carried out under different polymerisation process, UV lamp (365 nm at room temperature, 7 h) or thermal polymerization (60°C, 6h). Non-imprinted polymer membranes (NINs), as control, were also prepared and treated using an identical procedure without adding E2. The weight of grafted polymer membrane was determined by gravimetry, and the morphology of the membranes was examined by scanning electron microscopy (SEM). The binding capacity of the synthesized MIP-glass membranes toward the template molecules was tested in continuous dead-end filtration experiments. A series of experiments were carried out in order to optimize the specificity, the ability for extraction and preconcentration of E2 from water samples and also the regeneration of the membranes. The template molecule was monitored by spectrofluorimetric analysis. The results showed that the synthesized MIMs were useful to extract E2 from water samples at very low concentrations (under LMR established). The prepared MIP membranes presented a high stability and reusability features in a continuous filtration process without loss of their binding capacity and "memory effect" after reuse.

This study shows the suitability of the glass-fiber membranes by MIP grafting for extraction applications with respect to other polymers used in MISPE and commercially available materials.

Acknowledgements

This research work was supported by Comunidad Autónoma de Madrid (Spain) and European funding from FEDER program (project S2013/ABI-3028, AVANSECAL).

Magnetic dispersive solid-phase microextraction using zeolite/ Fe_2O_3 as a sorbent for determination of cadmium, mercury and lead prior to inductively coupled plasma optical emission spectrometry

P. Baile, L. Vidal*, M. A. Aguirre, A. Canals.

Departamento de Química Analítica, Nutrición y Bromatología e Instituto Universitario de Materiales, Universidad de Alicante, P.O. Box 99, E-03080 Alicante, Spain.

**lorena.vidal@ua.es*

Magnetic solid-phase extraction (MSPE) has recently received great interest since it reduces sample preparation time and facilitates sorbent manipulation. In MSPE, the magnetic sorbent is dispersed into the aqueous phase and after extraction, it is easily separated from sample solution by applying an external magnetic field, and thus avoids time-consuming filtration or centrifugation steps for phases separation. Next, target analytes can be desorbed using a proper eluent solvent for further determination.

Zeolites are aluminosilicate materials that exhibit well-defined highly porous structures. The general zeolite structure is a three-dimensional network of repeating isomorphous SiO_4 and AlO_4^- tetrahedra linked by oxygen atoms. Their anionic framework makes zeolites natural cationic exchangers while their well-defined pores lend some degree of preference to the ions adsorbed. Recently, a new composite based on ZSM-5 zeolite decorated with iron oxide magnetic nanoparticles (i.e., ZSM-5 zeolite/ Fe_2O_3) has been presented as a valuable sorbent for MSPE. In addition, the proposed composite presents remarkable advantages such as high extraction efficiency, low cost, rapid and simple synthesis, easy manipulation under a magnetic field and reuse options.

The aim of this work is to present a simple MSPE method for the simultaneous separation and preconcentration of trace amounts of toxic elements (i.e., lead, mercury and cadmium) in biological samples (e.g., urine) for subsequent measurement by inductively coupled plasma optical emission spectrometry (ICP-OES). The ZSM-5 zeolite/ Fe_2O_3 sorbent is modified with hexadecyltrimethylammoniumbromide (HDTMABr) surfactant and sodium diethyldithiocarbamate trihydrate (DDTC) chelating agent. In the extraction procedure, the modified sorbent is dispersed in the sample, that contains the toxic elements, and after the proper contact time is separated from the liquid phase by applying an external magnetic field. Later, the analytes are eluted from the sorbent with nitric acid, which is analysed by ICP-OES. The main factors that affect the extraction process such as the amount of adsorbent, sample pH value, extraction time, elution time, elution solvent and volume of eluent are optimized using a multivariate analysis. Finally, the suggested method is validated and successfully applied to analyze real urine samples.

Acknowledgements

The authors would like to thank the Spanish Ministry of Science and Innovation (project n. CTQ2011-23968), Generalitat Valenciana (Spain) (projects n. GVA/2014/096 and PROMETEO/2013/038) for the financial support. P. Baile also thanks Ministry of Education for her FPU grant.

POSTER PRESENTATIONS

Process control

Characterization of laser cladding coatings with graded composition by laser-induced breakdown spectroscopy

M. P. Mateo*, J. M. Amado, M. J. Tobar, A. Yañez, G. Nicolás.

Universidad de A Coruña, Laboratorio de Aplicaciones Industriales del Láser, Campus de Ferrol, Spain.

**paz.mateo@udc.es*

Laser cladding is a method of depositing material by which a powdered or wire feedstock material is melted and consolidated by use of a laser in order to coat part of a substrate to improve its mechanical properties or to extend the service life of some industrial components. In a previous paper [1], we have characterized clad layers composed by NiCrBSi alloy powder mixed with WC powder on stainless steel substrates by laser-induced breakdown spectroscopy (LIBS) technique. In the present study, new laser clads have been produced using the same metal matrix and reinforcement phase but with horizontal or vertical concentration gradients with the aim of improving the final properties of the coatings. The spatial distribution of the elements in the graded laser clads has been analyzed by generating LIBS lateral distributions and maps from the cross-sections of the coatings. The results of the analysis demonstrate that LIBS technique can be used for detecting gradients of concentration and production defects in the laser clads and evidence that its use for on-line quality control of the produced clads with concentration gradient is promising.

References

[1] J.A. Varela, J.M. Amado, M.J. Tobar, M.P. Mateo, A. Yañez and G. Nicolas, *Appl. Surf. Sci.*, **336**, 396 – 400 (2015).

POSTER PRESENTATIONS Chemometrics

Reliable quality control of pharmaceutical preparations by Digital Image colorimetry

A. López-Molinero^{1,*}, M. Csontos.

¹University of Zaragoza, Department of Analytical Chemistry, 50009 Zaragoza.

*anlopez@unizar.es

Digital Image based methods have been implemented as an efficient control tool of products as different such as water or plastics[1]. In this work a new procedure was proposed to the control of pharmaceutical formulations. Iron in pills may be determined by a compact digital camera. The chromogenic study was based on direct measurements of RGB basic reflected colors and also HSV and CIE Lab chromatic parameters. They were studied and discussed in order to find optimum conditions and the principles of the image method.

The Chromogenic compound was derived from the standard red complex formed between Fe(III) with thiocyanate in acid media, which was stabilized with Ce(IV) as oxidant. This alternative produced higher reproducibility and also wider range of linear response, up to levels from LQ to 25.0 mg/l Fe(III).

The apparent analytical potential and also the latent principles between colorimetric parameters were studied by Chemometric techniques of Analysis of Variance and Multivariate Statistical. Experimental measurements were obtained in different conditions. Set-ups based on easy and available multi-well plates of ELISA tests produced the highest Chromatic variance. RGB basic color parameters exhibited the highest variance and great differences between the three color components. However, HSV and CIE Lab parameters reduced the effect between large and small components. They produced measurements with more homogeneous values.

The variance study evidenced that 92% of chromaticity might be justified by the analyte influence, while the camera, as an instrumental effect, and the preparation method, accounted for less than 10%. The interpretation in basis to individual basic colors demonstrated that the B basic color accounted for the 83.5% of the chromaticity and produced the highest variance of the basic colors. The G contributed up to 15% while the R color contribution was reduced to 1.5%. The study with CIE Lab coordinates demonstrated a similar tendency. Thus b parameter retained the highest variance with 70%, and L and a retained 27 and 3% respectively.

The chromaticity was function of the illuminant. White and r, g, b lamps were applied. It was showed that influences of reflected colors might be significantly modified and even drastically reduced. The study opens the potential of DIC to obtain multiple measurements, as a multivariable technique, with a simple change in the illuminance. Also, we emphasize the potential of the color change as new signals. In this context, interference experiments might be enfaced in order to promote/ reduce and/or inhibit the influence of certain effects.

Finally a new method was developed for the ready Fe determination in commercial products. The reproducibility was lower than 3.0% at levels of 10 mg L⁻¹. Statistical comparison with standard procedures by ICP-AES demonstrated the absence of systematic errors.

Acknowledgements

These works were funded by MINECO in the project CTQ20012- 34774 and by DGA in the DGA-FEDER project. Matte Csontos thanks the Erasmus Scholarship grant.

References

[1] A. Lopez-Molinero, D. Liñan, D. Sipiera and R. Falcon, *Microchem. J.*, **96**, 380-385 (2010).

Sampling for Raman spectroscopy: Influence of particle size in the analysis of mixtures

D. Gómez, J. Coello, **S. Maspoch***

Applied Chemometrics Research Group, Departament de Química, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain.

**santiago.maspoch@uab.cat*

One of the key points of the quality control in pharmaceutical analysis, is the determination of the active principal ingredient (API) in solid dosage forms (e.g., powders or tablets). The traditional approach consists in the processing of material, by means of a destructive extraction step, and subsequently an analytical determination by HPLC. Nevertheless, either in the frame of the classical quality control, or in the emerging analytical requirements coming from the progressive implantation of PAT (Process Analytical Technologies) in the production lines, spectroscopic techniques like Raman spectroscopy have proved to be real alternatives to the traditional HPLC [1]. These spectroscopic techniques are faster, without reagents consumption and non-destructive. The number of reports regarding the use of Raman spectroscopy to the pharmaceutical analysis has increased significantly in the last years. However, there are still some fundamentals issues not completely solved. One of these issues is related to its limit of applicability. In fact, when the API mass proportion decreases, the precision attainable becomes a function of two factors: first, the intrinsic analytical precision, function of the signal-to-noise ratio; and second, the increment of the sub-sampling error. All solid pharmaceutical samples are intrinsically heterogeneous, formed by particles of API and excipients. As the number of API particles reduces, the question arises if the sampled area can be considered representative of the whole sample. This is probably the main limitation in the application of classical Raman spectroscopy, and has fostered the development of new optical systems where the sampled area is considerably improved, being current Raman instruments able to use a laser spot with a diameter from 100 μm up to 3 mm. But even in the best of the situations, the irradiated area is considerably lower than the tablet surface. The sub-sampling error has been reduced, but not eliminated.

The main aim of this study is to contribute to the fundamental knowledge of the limits of application of Raman spectroscopy in the determination of API in a pharmaceutical tablet. Our goal is to decompose the precision obtained in its two main components: repeatability of the signal and sub-sampling error. The classical analytical concept of the minimal size of sample required for the analysis (more specifically, to obtain a defined magnitude of sampling error), can be translated in the minimal number of measurement positions to be sampled in the material. Several mixtures containing a low percentage of a considerably Raman active component with a structure containing an aromatic ring (which mimics an active ingredient of a pharmaceutical formulation), diluted in a not-so active component, microcrystalline cellulose (MCC), were prepared. Two different sets of mixtures were considered, depending on the similarity in the particle size of the components: in the first one, potassium hydrogen phthalate (20 μm) was mixed with MCC (20 μm), on the other, poly(styrene-co-divinylbenzene) (8 μm) was mixed with MCC (20 μm). Tablets with an API content from 1 % -10 % will be prepared. For the acquisition of the spectra, two instruments were used: a macro-Raman system (500 μm spot size), and a Raman microscope with a 10X objective (50 μm spot size). Having two different laser spot size we are able to apply the sampling theory to our mixtures: if the goal is to quantify a component of the sample, it is necessary to be able to measure the bulk composition of the material. This is related to the diameter of the laser, as the resulting spectrum reflects the volume sampled along the acquisition time, and an estimation of the number of points to be measured is necessary.

References

[1] Sasic, S, Pharmaceutical Applications of Raman Spectroscopy, Wiley-Interscience, USA, 2008.

POSTER PRESENTATIONS

Imaging techniques

Infrared Microspectroscopy for characterization of olive fruit changes during maturation

M. J. Ayora-Cañada*, M. González-Cabrera, A. Domínguez-Vidal.

Department of Physical and Analytical Chemistry, University of Jaén, Campus Las Lagunillas, 23071-Jaén, Spain.

*mjayora@ujaen.es

Infrared spectroscopy is a tool that has been used extensively to study the chemistry and functional properties of plants. Thus, IR spectroscopy has allowed the characterization of different components during the growth of different plant organs [1]. It has been used in the chemical and structural analysis of plant tissues, for identification of functional groups and conformations and qualitative measurements of the cutin polymerization [2]. The possibility of coupling the infrared spectrometer to a microscope (microspectroscopy) opens new interesting possibilities because it allows the study of structures in specific regions of a histological section and the chemical imaging of specific components [3].

Mid-infrared microspectroscopy allows spectral data to be collected from a sample with spatial resolution, generating a hyperspectral image, that is, a three-dimensional data cube, with x- and y-axes representing units of μm , and with the z-axis consisting of spectra collected at each coordinate. The results of analysis of the collected spectra can therefore be mapped in two dimensions to produce an image of the spatial distribution of chemical components across a sample.

Here we present the use of FTIR microspectroscopy to investigate chemical and structural changes happening in the olive fruit during maturation. Special attention has been paid to the preparation of samples in order to achieve the appropriate sample thickness to avoid saturation. Different methods of sample preparation have been evaluated using fresh and frozen olive samples that have been cut on either a vibratome or a cryostat. The tissue sections were placed on calcium fluoride (CaF_2) slides for transmission-mode imaging.

Initially the hyperspectral image cubes were converted into false-colour images using either areas of well-isolated spectral bands or relative peak intensities. In this way, different tissue types could be easily distinguished. Thus, the exocarp for example, was characterized by spectra with strong absorption bands at around 2900 cm^{-1} (methylene symmetric and anti-symmetric stretching modes) and 1730 cm^{-1} ($\text{C}=\text{O}$ bond), indicative of the large quantity of long-chain lipids that is present in the waxy cuticle. At the moment, we are working on the use of multivariate data analysis, like principal component analysis (PCA) to better discriminate major tissue types and to track biochemical changes happening during fruit maturation.

Acknowledgements

M. González-Cabrera acknowledges to Universidad de Jaén for the grant entitled "Ayuda de Iniciación a la Investigación para estudiantes de Máster Oficial de la Universidad de Jaén". The authors also are grateful to the research group FQM 363 and to the CICT (Universidad de Jaén) for financial support.

References

- [1] M. López-Sánchez, M.J. Ayora-Cañada, A. Molina-Díaz, *J Agric Food Chem.*, **58**, 82-87 (2010).
- [2] J. A. Heredia-Guerrero, J. J. Benítez, E. Domínguez, S. Ilker, R.C. Bayer, A. Athanassiou and A. Heredia, *Front Plant Sci.*, **5**, 305, 1-14 (2014).
- [3] F.J., Warren, B.B. Perston, S.P. Galindez-Najera, C.H. Edwards, P.O. Powell, G. Mandalari, G.M. Campbell, P.J., Butterworth, P.R. Ellis. *The Plant Journal*, **84**, 634–646 (2015)

POSTER PRESENTATIONS Forensic analysis

Determination of Gunshot Residue Patterns (GSR) using Laser-Induced Breakdown Spectroscopy (LIBS)

C. Álvarez-Llamas¹, M. López-López², C. García-Ruiz², J. Pisonero¹, N. Bordel^{1,*}.

¹Department of Physics, University of Oviedo, c/ Gonzalo Gutiérrez Quirós, Campus de Mieres, 33600 Mieres, Spain.

²Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering & University Institute of Research in Police Sciences, Edificio Polivalente de Química, University of Alcalá, Ctra. Madrid-Barcelona Km. 33.600, 28871 Alcalá de Henares (Madrid) Spain.

*bordel@uniovi.es

A commonly asked question to the forensic analyst is the estimation of the shooting distance in a firearm-related crime scene. Gunshot residues (GSR) are particles produced during the discharge of a firearm [1]. GSR are composed by a mixture of organic compounds, mainly from the propellant, and inorganic compounds from the primer. They are launched with the projectile resulting in a conical plume of particles that finally creates a pattern on the target (e.g. victim). This pattern depends on different factors, including the type of ammunition, type of firearm, and especially, the firing distance [3]. This range provides additional information to clarify incidents committed with firearms and can be estimated by the inspection of the distribution of the GSR in the surrounding of the entrance hole on the victim's skin or clothing [2]. Nowadays, color tests are used in the forensic laboratories to visualize the GSR patterns, which show important drawbacks like non-specificity to GSR or the application using long and tedious proceedings.[1] Laser Induced Breakdown Spectroscopy (LIBS) is an analytical technique based on the use of a short duration laser pulse focused on a small area of the sample. The high fluence over the sample surface produces the formation of a laser-induced plasma. The species present in the plasma suffer processes of atomization, ionization and excitation; and the radiation coming from the excited species can be detected by an spectroscopic device [3].

In this work, the capabilities of LIBS to perform spatial distribution of the GSR particles over different clothing target shot from three different distances, were investigated. LIBS mapping was performed to show the spatial distribution of Pb, Sb and Ba, which are the most representative element of the GSR. The use of LIBS to reveal the patterns show great advantages over the tests based on color chemical reactions such as the possibility of multi-elemental analysis, which increases the specificity and prevents potential false-positives.

Acknowledgements

We thank the Ballistics Department of the General Department for Forensic Science Police (Madrid, Spain) for the help provided and for conducting the shootings. M. López-López thanks the University of Alcalá for her postdoctoral grant. C. Álvarez Llamas, J. Pisonero, and N. Bordel acknowledge the financial support from the Government of the Principality of Asturias through the project FC-15-GRUPIN14-040. M. López-López and C. García-Ruiz thanks the Ministry of Economy and Competitiveness (MINECO) of Spain for the project CTQ2014-58688-R.

References

[1] O. Dalby, D. Butler, and J. W. Birkett, J. Forensic Sci., vol. 55, no. 4, pp. 924–43, Jul. 2010.

[2] M. López-López and C. García-Ruiz, Forensic Sci. Int., vol. 239, pp. 79–85, Jun. 2014.

[3] D. W. Hahn and N. Omenetto, Appl. Spectrosc., vol. 64, no. 12, pp. 335–366, Dec. 2010.

Determination of 3,4-methylenedioxypyrovalerone (MDPV) in oral and nasal fluids by ion mobility spectrometry

M. N. Peiró, S. Armenta*, S. Garrigues*, M. de la Guardia.

Department of Analytical Chemistry, Research Building, University of Valencia, Dr Moliner 50, E-46100 Burjassot, Valencia, Spain.

*salvador.garrigues@uv.es, sergio.armenta@uv.es

The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) informed on the availability and consume of new psychoactive substances (NPS) in Europe in the last years. Synthetic cathinones, which are sold as legal replacements for stimulants such as amphetamine and MDMA, were the second largest group seized in the last years. 3,4-methylenedioxypyrovalerone (MDPV) is a synthetic derivative of the naturally occurring substance cathinone, one of the psychoactive principles in khat (*Catha edulis* Forsk). Reports from seizures and collected samples have noted the presence of MDPV in: powders, powder-filled capsules, tablets and blotters (small paper doses for sublingual/buccal administration).

The aim of this work was the combined use of two types of biological fluids for the detection of MDPV abuse by IMS using nasal and oral fluids as target samples. There are several precedents of the analysis of MDPV by IMS in seized samples, but, as far as we know, there is no previous precedent of the MDPV analysis in biological samples by IMS. Recently, IMS has been proposed for the analysis of cocaine in nasal mucus [1] and MDMA in oral fluid samples [2]. In the present study the aforementioned strategies were applied to assess the drug abuse, using MDPV as target molecule.

In this communication a fast and sensitive methodology developed for the evaluation of the MDPV consume is presented. Based on the ion mobility spectrometry (IMS), MDPV was directly determined in nasal fluids with a limit of detection (LOD) of the order of 22 ng mL⁻¹, which corresponds to an absolute amount of 33 ng of MDPV per swab. MDPV was also determined after liquid-liquid microextraction (LLME) in oral fluids to avoid matrix effects, obtaining a LOD value of 4.4 ng mL⁻¹ in oral fluid samples. The IMS spectrum for MDPV exhibited a peak with $K_0 = 1.210 \pm 0.005 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ at a drift time of 14.62 ms, being the total analysis time 4.5 min per oral fluid and 1.5 min per nasal fluid sample. Samples must be analysed within 24 hours following collection and dissolution in 2-propanol, based on the complementary stability studies. Cocaine interference in nasal fluids has been avoided using second derivative.

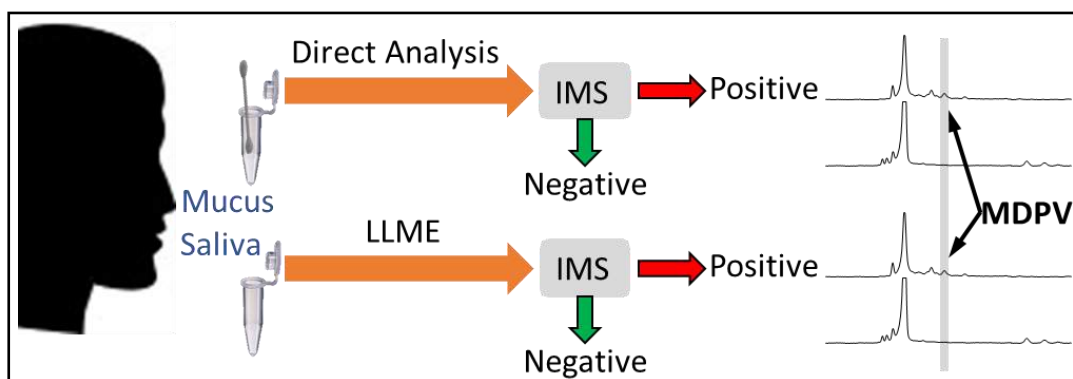


Figure: IMS based strategy for MDPV determination in mucus and saliva samples.

Acknowledgements

Authors gratefully acknowledge the financial support of the Ministerio de Economía y Competitividad (Project CTQ2014-52841), Universidad de Valencia (Project UV-INV-AE14-268280) and Generalitat Valenciana (project PROMETEO-II 2014-077).

References

- [1] S. Armenta, M. de la Guardia, M. Alcalà and M. Blanco, *Anal. Chem.*, **85**, 11382–11390 (2013).
- [2] C. Andreou, M.R. Hoonejani, M.R. Barmi, M. Moskovits and C.D. Meinhardt, *ACS Nano*, **7**, 7157–7164 (2013).

FT-NIR and chemometric tools for predicting alkaline earth and diagenesis screening in buried bones

M. M. Cascant*, S. Rubio, G. Gallelo, A. Pastor, S. Garrigues, M. de la Guardia.

Department of Analytical Chemistry, University of Valencia, 50th Dr. Moliner St., 46100 Burjassot, Valencia, Spain.

*casmamer@uv.es

A fast, cheap and green analytical method has been developed for the direct determination of calcium, magnesium and strontium, and to understand post-mortem degradation in bones remains caused by the environment using FT-NIR and chemometric tools. Two hundred forty three samples obtained from at least 78 individuals from the Late Roman Necropolis of Virgen de la Misericordia Street and en Gil Street, both located in Valencia (Spain) were analyzed. Bone samples have been collected mainly from femur, tibia, humerus, radius and parietal bones and were classified as cortical bones and ribs as spongy bones. Furthermore, bone samples from the outer bone layer and soil samples have been analyzed.

Principal Component Analysis (PCA) was applied to identify bone samples with a well preserved elemental composition. Results indicate that external part of bones suffer an increased degradation produced by diagenetic factors than internal part of bones, especially spongy external part of bones due to their high porosity.

Partial Least Square (PLS) regression model was applied to NIR spectral data to develop an innovative analytical method for determination of alkaline earth in skeletal remains. PLS-NIR model has a good potential to predict calcium, magnesium and strontium content in bone remains, being many advantageous over the conventional employed analytical techniques because it is quick, inexpensive, non-destructive, and does not require the use of chemical solvents.

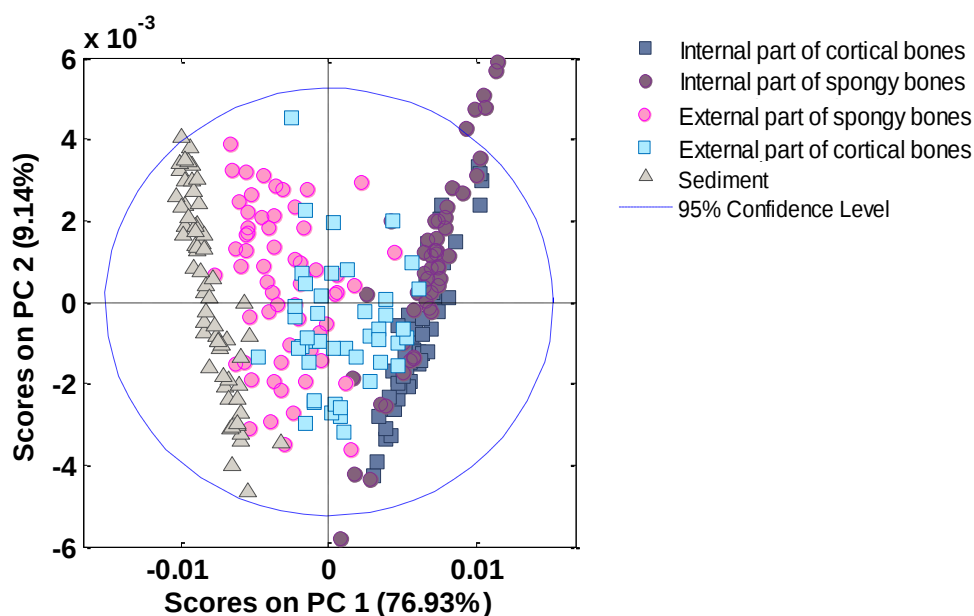


Figure 1. Scores plot for PC1 and PC2 after FD and MC pretreatment for Misericordia and En Gil Necropolises using differentiated internal and external part of bones in spongy and cortical remains.

Acknowledgements

Authors acknowledge the financial support of Generalitat Valenciana (Project PROMETEO II/2014/077) and Ministerio de Economía y Competitividad-Feder (Projects CTQ 2014-52841-P and CTQ 2012-38635). M.C acknowledges the FPI grant (BES-2012-055404) provided by the Ministerio de Economía y Competitividad of the Spanish government.

POSTER PRESENTATIONS

Speciation

Speciation of very low amounts of antimony in waters using magnetic-core modified silver nanoparticles

M. Hernández-Córdoba^{1,*}, I. López-García¹, M. J. Muñoz-Sandoval¹, S. Rengevicova².

¹Department of Analytical Chemistry, Faculty of Chemistry, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, E-30100 Murcia, Spain.

²Department of Analytical Chemistry, P.J. Šafárik University at Košice, Slovak Republic.

*hcordoba@um.es

Recent advances in microextraction techniques aimed to the determination of metals and metalloids have demonstrated that advantages are obtained when they are coupled with electrothermal atomic absorption spectrometry (ETAAS) as the final measurement technique [1]. The volume of the liquid phase obtained after a microextraction process is low and, since ETAAS operates with low injection volumes, aliquots can be taken and injected into the electrothermal atomizer without the need for the dilution stage that is mandatory for other analytical techniques. In this way, high preconcentration factors (PFs) can be achieved. A number of adsorbents have been proposed for the solid phase extraction (SPE) of metals but, in most cases, their physical characteristics and the subsequent leaching of the analytes do not permit high PFs to be obtained. One way to increase PFs is to use nanoparticles with a high adsorbent capacity so that the amount of solid required for the separation stage is minimal. Magnetic particles (MPs) are particularly interesting for this purpose. They can be used directly as the adsorbent material or as the core of appropriate composites obtained by covering them with other nanoparticles or chemical reagents. When used for SPE microextraction processes, these materials are easily separated by a magnet without the need for centrifugation [2].

In this communication, new procedures for the speciation of antimony at the sub-ppb level in waters are reported. Magnetic-core nanoparticles are first covered with silver nanoparticles and then functionalized with sodium mercaptoethanesulfonate (MESNa). The nanocomposite in this way obtained (Fe₃O₄@Ag@MESNa) is next used in a μ -solid phase dispersion procedure to retain antimony species. Since the retention of the analytes (Sb(III) and Sb(V)) is pH-dependent, speciation can be obtained by carrying out two experiments in solutions with different acidity. High PFs are obtained if, instead of releasing the analytes by means of a change in acidity and then measuring the element by ETAAS, the small amount of nanocomposite recovered with the aid of the magnet is first dried and then resuspended in a low volume of solution. Even a better performance is achieved provided the solid is not slurried but directly introduced into an electrothermal atomizer equipped with an accessory unit for the introduction of solids, as is the case of the ContraAA 700 spectrometer. A PF as high as 300, that means an extreme sensitivity (detection limit below 0.01 μ g/L) is obtained.

Acknowledgements

This work has been financed by the Comunidad Autónoma de la Región de Murcia (Fundación Séneca, 19888/GERM/15), the Spanish MINECO (Project CTQ2015-68049-R) and the European Commission (FEDER/ERDF)

References

- [1] I. López-García, Y. Vicente-Martínez and M. Hernández-Córdoba, *J. Anal. At. Spectrom.* **30**, 1980-1987 (2015).
- [2] A. Ríos, M. Zougagh and M. Bouri, *Anal. Methods* **5**, 4558-4573 (2013).

Kinetics and stability studies on the binding of cisplatin to S- and N-donor groups in peptides

G. Artiaga*, A. Iglesias-Jiménez, E. Moreno-Gordaliza, M. L. Mena, M. Gómez-Gómez.

Department of Analytical Chemistry, Faculty of Chemistry, Universidad Complutense de Madrid, Avenida Complutense s/n 28040 Madrid, Spain.

*gmaayuso@ucm.es

The binding of cisplatin to the nitrogen atom at position 7 of two neighboring guanines in DNA is considered to be the main responsible for its antitumor activity [1]. However, upon its administration, cisplatin is able to bind to nucleophilic groups of proteins, making difficult that platinum species reach the DNA. Some studies have suggested that a Pt moiety that is bound to a methionine residue could be transferred to the DNA [2]. However, nowadays, the mechanism of action of Pt cancer drugs is still not completely understood. Therefore, it is necessary to obtain more information on their interaction with S- and N- donor biomolecules that play a key role in the process of drug distribution in the cell, as well as the stability of the Pt-peptide complexes along all the steps involved in the analytical process.

In this work, the reactivity and the stability of cisplatin towards two synthetic peptides that contain the same peptide sequence, except for one amino acid residue, methionine (S-donor) or histidine (N-donor), was evaluated. This model was employed as an approach to study the differences in the binding kinetics and the strength between Pt-S and Pt-N bonds formed in biological systems. For this purpose, incubations of both peptides with cisplatin, in a peptide molar excess of 2:1, were carried out at physiological conditions at different incubation times (90 min-15 days). The results obtained by SEC-ICP-MS showed that the methionine-containing peptide was 6-fold more reactive than the histidine-containing peptide, reaching the steady state after 7 days, while it was achieved after 2 days in the latter. Furthermore, analysis by nESI-LTQ-MS/MS confirmed the coordination of the cisplatin mono-aqua and diaqua-complexes through methionine and histidine amino acid residues as preferential Pt binding sites.

In order to study the stability of Pt-peptide bonds, platinated peptides were incubated separately with the different chemical compounds used under usual electrophoretic conditions for SDS-PAGE and OFFGEL-IEF separations: 5% β -Mercaptoethanol (BME) or Dithiothreitol (DTT) 350 mM for 5 minutes at 95°C for SDS-PAGE, and Urea 6.7 M, Thiourea 1.9 M and DTT 62 mM for 48 hours at 37°C for OFFGEL-IEF. The Pt content that remained bound to peptides after treatment with these reagents was determined by SEC-ICP-MS and RP-ICP-MS. The results showed that the use of urea was compatible with the preservation of the Pt-complexes. However, the presence of denaturing or reducing agents with S-donor groups such as thiourea, BME and DTT produced deleterious effects in the Pt-peptides binding, either at high temperatures or during long incubation times, being the latter the most critical. Also, it was found that thiourea was the most aggressive agent producing loss of up to 90% for the methionine-containing peptide and 50% for the histidine-containing peptide. For all the reagents and conditions tested, the histidine-containing peptide presented higher bond strength with Pt-losses around 16-50%, being more pronounced for the methionine-containing peptide (35-90%).

According to the results obtained in this study, there is no doubt about the strong reactivity of cisplatin towards S-donor biomolecules. However, it seems that Pt-S (Met) interactions are kinetically preferred, while the binding of Pt-N (His) would be thermodynamically favored, resulting in more stable bonds. These facts could support the hypothesis that Pt-Met species could act as a Pt reservoir.

Acknowledgements

This work was financially supported by MINECO, Grant numbers: CTQ 2011/24585 and CTQ 2014-55711-R. G.Artiaga also acknowledges MECO, for her FPU predoctoral fellowship (13/01693).

References

- [1] Guo Z, Sadler PJ, *Angew Chem Int Ed*, **38**, 1513 – 1531 (1999).
- [2] Reedijk J, *Chem Rev*, **99**, 2499 – 2510 (1999).

POSTER PRESENTATIONS Others

Potential Chemopreventive Compounds Against Melanoma - New Perspectives using Raman Microspectroscopy

P. S. C. Medeiros¹, A. L. M. Batista de Carvalho¹, M. M. Dias¹, C. Ruano², J. C. Otero²,
M. P. M. Marques^{*1,3}.

¹"Molecular Physical Chemistry" R&D Unit, Fac. Science and Technology, Univ. Coimbra, Portugal

²Dep. Physical-Chemistry, Fac. Science, Univ. Malaga, Unidad Asociada SCAI, Spain.

³Dep. Life Sciences, Fac. Science and Technology, Univ. Coimbra, Portugal.

*pmc@ci.uc.pt

Melanoma contributes to 85% of the deaths due to skin cancer (although it represents only 4-5% of these), which is threatening to reach epidemic proportions. The rising rates of melanoma are attributed to several combined factors, sunlight exposure being the main cause of melanoma development since UV radiation affects cells and tissue homeostasis via DNA damage and ROS generation.

The assessment of the antitumour activity and cellular impact of three chromone derivatives present in the diet – fisetin, quercetin and luteolin – previously found to display high antioxidant capacity [1], was carried out in the present study, through *in vitro* assays in a human melanoma cell line (A375). Biological methods for evaluation of antitumour and anti-invasive abilities were combined with state-of-the-art vibrational microspectroscopy techniques.

The compounds under study were found to exhibit cytotoxic activity against the A375 cells, upon a 48 h exposure (LD50 values of 16.21, 16.51 and 17.90 μ M for fisetin, quercetin and luteolin respectively). MicroRaman analysis of formalin-fixed cells after incubation with these antioxidant phytochemicals clearly allowed to unveil their impact on the cellular metabolic profile. Multivariate data analysis (unsupervised PCA) led to a clear discrimination between control and treated cells, and evidenced distinctive effects on their main biochemical components.

The results thus gathered will hopefully allow to develop natural-based agents capable of fighting cancer both through their chemopreventive (antioxidant) ability and via their use in adjuvant chemotherapy (in combination with conventional antineoplastic drugs).

Acknowledgements

The authors acknowledge financial support from the Portuguese Foundation for Science and Technology – UID/MULTI/00070/2013; SFRH/BD/72851/2010.

References

[1] M.M. Dias, N.F.L. Machado, M.P.M. Marques, *Food&Function*, **2**(10), 595-602 (2011).

The impact of Dietary Antioxidants in Cancer Cells Probed by Raman Microspectroscopy. Effect of Daidzein on Human Breast Cancer

C. Ruano^{1,*}, P. S. C. Medeiros², A. L. M. Batista de Carvalho², J. C. Otero¹, M. P. M. Marques².

¹Univ. Málaga, Dep. Physical-Chemistry, Fac. Science, Univ. Málaga, Avenida Cervantes, 2, Campus de Teatinos, 29071, Málaga, Spain.

²Molecular Physycal Chemistry R&D Unit, Fac. Science and Technology, Univ. Coimbra, 3004-535 Coimbra, Portugal.

*cruano@uma.es

Breast cancer is the second most common type of cancer worldwide and the most frequent among women, being the fifth cause of death from neoplastic disease. Since this is an oxidative-stress related neoplasia, it is largely preventable. A dietary isoflavone – daidzein [1,2] was currently investigated as to its chemopreventive and/or chemotherapeutic properties towards the human MDA-MB-231 (metastatic, estrogen-unresponsive) and MCF-7 (estrogen-responsive) breast cancer cell lines. Biological assays for evaluation of antitumour and anti-invasive activities were combined with state-of-the-art vibrational microspectroscopy techniques. At 50 and 100 μ M concentrations and 48 h incubation time, daidzein was found to induce a marked decrease in cell viability (ca. 50%) for MDA-MB-231 and MCF-7 cells (respectively ca. 50% and 42%) and 40% inhibition of cell migration 48 h. MicroRaman analysis of fixed cells upon exposure to this isoflavone unveiled its metabolic impact on both cell lines. Multivariate data analysis (unsupervised PCA) led to a clear discrimination between control and DA-exposed cells, with distinctive effects on their biochemical profile, particularly regarding DNA, lipids and protein components, in a cell-dependent way. This is the first reported study on the impact of dietary antioxidants on cancer cells by microRaman techniques.

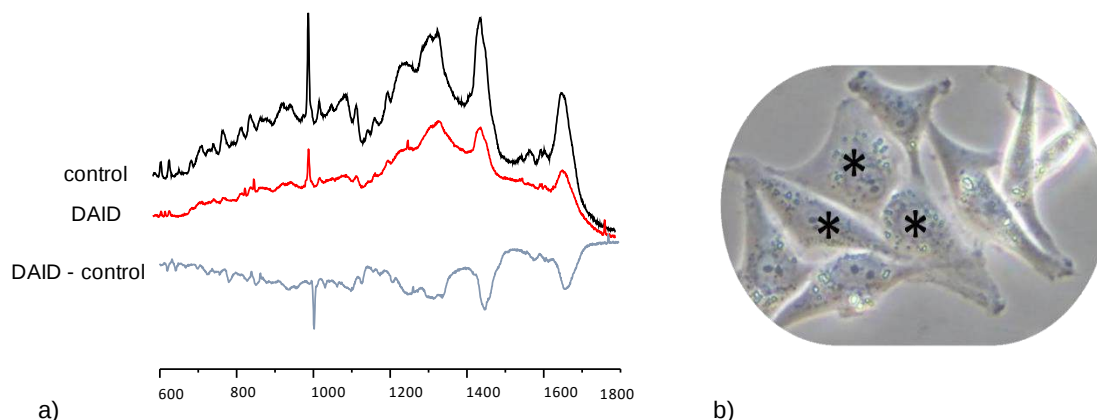


Figure 1: Mean Raman and difference spectra to the control a) and microscopic image of a cell (x100 magnification) showing some points at which data were captured b).

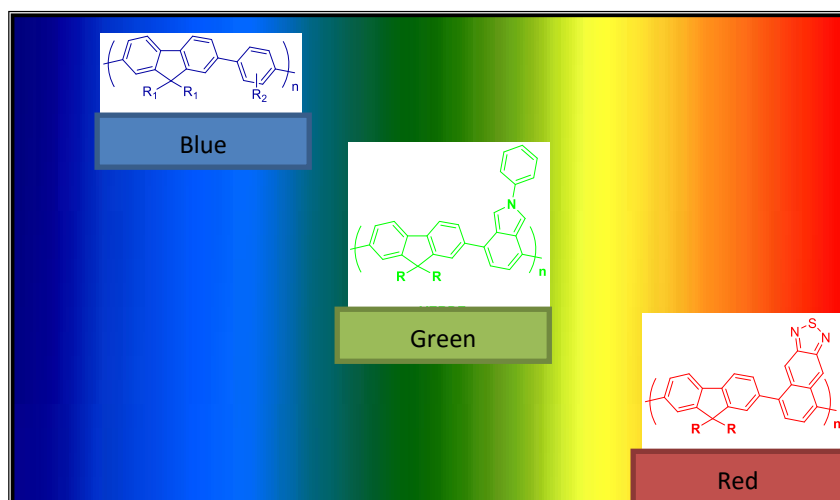
References

- [1] N.F.L. Machado and L.A.E. Batista de Carvalho and J.C. Otero and M.P.M. Marques, et al., *Vib Spectrosc*, **68**, 257 – 265 (2013).
- [2] M.M. Dias and N.F.L. Machado and M.P.M. Marques, *Food Funct*, **2**, 595 (2011).

Spectroscopic study of blue, green and red-emitting polyfluorene derivatives in solution

R. Vázquez-Guilló, Z. Kahveci, A. Mira, M. J. Martínez-Tomé, C. R. Mateo, R. Mallavia*.
Instituto de Biología Molecular y Celular, Universidad Miguel Hernández, Elche.
 *r.mallavia@umh.es

Polyfluorene derivatives have a high interest because of their high photoluminescence quantum efficiency, high thermal stability, good solubility and their color tunability. These polymers have great interest for bioimaging, biosensing and also in solar cells and photoelectronic devices, because of their interesting and useful properties, including strong absorption and high efficiencies in both photoluminescence and electroluminescence. The design and development of fluorescent conjugated polyelectrolytes (CPEs) emitting in different region of the visible spectrum is at present of great interest for bioimaging studies due to their relative solubility in aqueous solutions. Polyfluorenes can be modified in the side chain, modulating their solubility (obtaining polyelectrolytes), or in the main chain to form copolymers with different rings, this modifications modulate its optical properties (for example wavelength emission), In this work, we describe the synthesis and photophysical characterization in solution of new polyfluorenes derivatives (neutral and ionic) at different region of the visible spectrum. With this aim, we have incorporated different chromophores into the polymer backbone (benzene, aniline, benzotriazole and naphthothiadiazole) and also with changes in pendant chain: neutral to cationic [1,2,3]. We describe the spectroscopic characterization (maximum absorption and emission and quantum yield) of these polymers in different solvents, and we analyse the behaviours in different environments. We report the important role played by the polarity of the solvent in the excited transition states and association effects of these polymers, which is indicative of interchain aggregations or preferential conformations.



Acknowledgements

To the Spanish Economy and Competitivity ministry for the projects: MAT-2014-53282; MAT-2011-23007 and BES-2012-051900.

References

- [1] R. Mallavia, D. Martínez-Pérez, B. F. Chmelka, and G. C. Bazan, *Bol. Soc. Esp. Ceram. V.*, **43** (2) 327-330 (2004)
- [2] Z. Kahveci, M. J. Martínez-Tomé, R. Mallavia and C. R. Mateo *Biomacromolecules*, **14**, 1990–1998 (2013)
- [3] Z. Kahveci, R. Vázquez-Guilló, M. J. Martínez-Tomé, R. Mallavia and C. R. Mateo, *ACS Applied Materials & Interfaces*, 2015.

Diffusion measurements of methane in amorphous water ice

B. Maté*, G. Molpeceres, J. Ortigoso, I. Tanarro, V. J. Herrero.

²*Instituto de Estructura de la Materia, IEM-CSIC, Serrano 121, 28006 Madrid, Spain.*

**belen.mate@csic.es*

Icy mantles covering dust grains in dense clouds of the interstellar medium are known to be responsible of the large molecular complexity of our universe. Within those mantles atoms and molecules can meet and react with larger probability than in the gas phase. The chemical reactivity of interstellar ice is limited by the diffusion of reacting atoms or molecules in water ice, its major component. For this reason the knowledge of diffusion coefficients of different molecules in water ice is of great astrophysical interest.

In this work we have designed an experiment to determine the thermal diffusion of CH₄ in amorphous water ice at 50 K. In a high vacuum chamber methane ice layers covered by water ice layers were grown by vapor deposition at 30 K. Then, the two-layered system was warmed to 50 K and kept at that temperature while the diffusion of CH₄ molecules was monitored by means of FTIR spectroscopy. The decay of intensity with time of the strong IR absorption associated to the ν_3 mode of methane (see Figure1) is a measure of the number of methane molecules that have moved through the whole water ice layer and left the sample. Diffusion coefficients were extracted from the isothermal experiments using Flick's second law of diffusion [1,2].

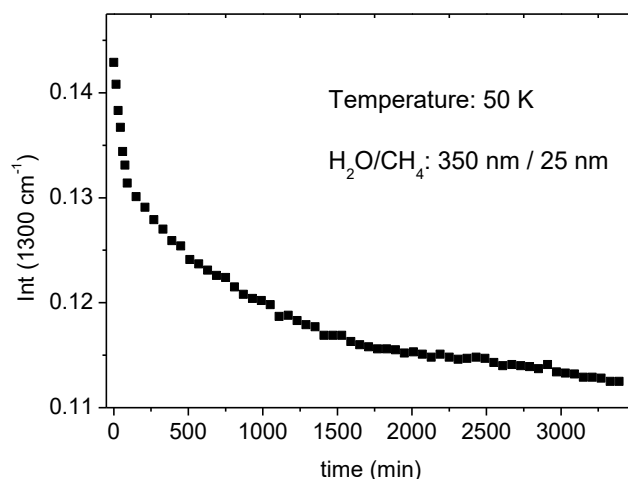


Fig.1. Integrated intensity of the 1300 cm⁻¹ CH₄ IR band of methane versus elapsed time at 50 K. The system consists on a 350 nm layer of amorphous water ice grown on top of a 25 nm methane layer.

Acknowledgements

This research has been supported by the Spanish MINECO, Projects FIS2013-48087-C2-1-P and by the European Research Council, project ERC-2013-Syg-610256. G.M. acknowledges MINECO PhD grant BES-2014-069355.

References

- [1] F. Mispelaer, P. Theulé, H. Aouididi, et al., *A&A*, **555**, A13-11 (2013).
- [2] L. J. Karssemeijer, S. Ioppolo, M. C. van Hemert, A. van der Avoird, et al., *ApJ*, **781**, 16-15 (2014).

Double Pulse Laser-Induced Breakdown Spectroscopy of metallic samples submerged at oceanic pressures

M. López-Claros, F. J. Fortes, J. J. Laserna.

Department of Analytical Chemistry, Faculty of Sciences, University of Malaga, Campus de Teatinos s/n, 29071, Malaga. <http://www.laser.uma.es>.

**laserna@uma.es*

Deep sea exploration involves the investigation of physical, chemical, and biological conditions on the seafloor. From the scientific and commercial points of view, the impact of this application is remarkably significant. From this perspective, the development of new chemical sensors for oceanography takes a significant relevance. Nowadays, laser-induced breakdown spectroscopy (LIBS) has emerged as a new solution for oceanography since it combines many of the required features for this application (multielemental information, no sample preparation, unlimited range of material capability and real time analysis). LIBS has no limits on the horizon and the continuous advances in reducing the size and weight while increasing the capabilities of lasers, spectrographs and detectors make possible the development of compact and rugged instrumentation. Thus, seafloor exploration at deep-ocean (2000-3000 meters) could be accomplished by the integration of LIBS technology in a remotely operated vehicle (ROV).

The main motivation of this work is to gain fundamental and basic knowledge regarding underwater LIBS analysis and the effect of pressure on the spectral fingerprint of different materials. For this purpose, a dual pulse laser system was used to irradiate the sample surface inside a high-pressure chamber. Results have been reported exploring the feasibility of LIBS for pressures beyond 100 bares. Thus, the effect of pressure on the analytical LIBS response and its interrelationship within experimental parameters (laser pulse energy, temporal acquisition conditions...) as well as the kinetics of plasmas generated underwater at oceanic pressures was also studied. Additionally, a variety of DP-LIBS configurations and several options for plasma collection geometry have been evaluated.

Acknowledgements

The authors acknowledge the funds from projects P11-FQM-7193. One of the authors (MLC) thanks the Junta de Andalucía for providing a fellowship. Furthermore, they thank the collaboration with the Subaquatic Archaeological Centre (CAS).

Study of the dual character of charged metal-molecule hybrids in SERS-CT processes

J. Román-Pérez, C. Ruano, S. P. Centeno, M. R. López-Ramírez, J. F. Arenas, J. Soto, I. López-Tocón, J. C. Otero¹.

¹Universidad de Málaga, Facultad de Ciencias, Departamento de Química Física.

E-mail: jessica.roman@uma.es, jc_otero@uma.es; Fax y Tel: +34 952132019

In SERS electrochemical experiences signal come mainly from very special surface sites of nanoparticles usually called “hots spots” [1] where the enormous enhancement of the electromagnetic field is concentrated. The gain (G) or efficiency of the electrode potential in tuning the energy of charge transfer (CT) states is due to two different contributions $G=SC$. Firstly, the sensibility of the CT states of the metal-molecule system on the effective charge excess of the metal (S), and, secondly, the effectiveness of the metal for accumulating the excess of charge in the “hot spots” of SERS (C) [2].

DFT calculations predict the existence of two different kinds of hybrids complexes formed by metals bonded to charged molecules corresponding to different electronic structures which are selected depending on the surface charge. Therefore, the electrode potential is able to select two kinds of surface hybrids: a P-hybrid characterized by a weak surface complex with properties tunable in a continuous way ($q_{\text{eff}} \leq 0$) that results in metal-to-molecule CT states very sensitive to the charge, and a C-hybrid, a strongly bonded system ($q_{\text{eff}} > 0$) whose electronic structure is hardly modified by E_V and with reverse molecule-to-metal CT states insensitive to the charge [3].

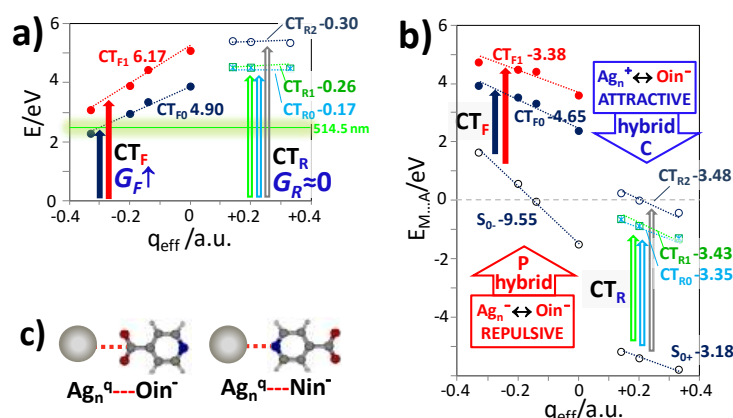
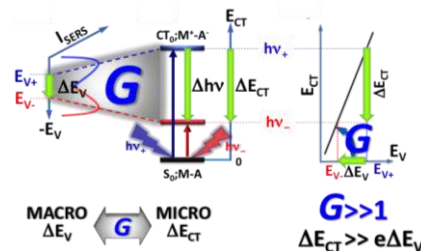


Figure 1. Dependence on q_{eff} of: (a) TD-M06-HF/LanL2DZ vertical energies (eV) of the forward ($CT_{Fi=0,1}$) and reverse ($CT_{Ri=0-2}$) charge transfer states of silver-isonicotinate complexes $[(Ag_n^q-Oin)^{q-1}]$, (b) TD-M06-HF/LanL2DZ energies of the same states but referred to the energy of the metallic clusters (Ag_n^q) and the adsorbate (in^-) at infinite separation ($E_{M...A}$). (c) C_{2v} complexes of isonicotinate bonded to silver through the aromatic nitrogen ($Ag-Nin^-$) or the carboxylate ($Ag-Oin^-$).

References

- [1] ShalaeV, V. M.; Sarychev, A. K. *Phys. Rev. B*, **57**, 13265-13288 (1998).
- [2] J. Roman-Perez, C. Ruano, S. P. Centeno, I. López-Tocón, J. F. Arenas, J. Soto, J. C. Otero. *J. Phys. Chem. C*, **118**, 2718-2725 (2014).
- [3] J. Roman-Perez, S. P. Centeno, M. R. Lopez-Ramirez, J. F. Arenas, J. Soto, I. López-Tocón, J. C. Otero. *Phys. Chem. Chem. Phys.*, **16**, 22958-22961 (2014).

Vibrational Spectroscopic Study of Seaweed Polysaccharides

P. Ribeiro-Claro^{1,*}, L. Pereira².

¹CICECO-Aveiro Institute of Materials / Department of Chemistry, Campus de Santiago, University of Aveiro, P-3810-193 Aveiro, Portugal.

²MARE (Marine and Environmental Sciences Centre) and IMAR (Institute of Marine Research), Department of Life Sciences, Faculty of Sciences and Technology, University of Coimbra, 3000-456 Coimbra, Portugal.

*prc@ua.pt

Seaweeds are a source of renewable biopolymer-based materials and seaweed polysaccharides are found to have a wide range of applications [1-3]. These polysaccharides present a regular chemical structure related with the taxonomic position of the algae from which they are extracted: agar is produced by agarophytes (red seaweeds belonging mainly to the genera *Gelidium*, *Pterocladia* and *Gracilaria*), carrageenans are produced by carrageenophytes (red algae belonging mainly to the genera *Kappaphycus*, *Euclima*, *Chondrus*, *Gigartina* and *Chondracanthus*) and alginates are produced by alginophytes (brown algae belonging to the order Fucales and Laminariales).

The combined use of infrared absorption spectroscopy and Raman spectroscopy has proven to be useful in the analysis of polysaccharides content of seaweed samples [4,5]. The limitations of sample preparation and sample sensitivity (e.g., laser-induced fluorescence and degradation) are easily overcome by infrared ATR (from Attenuated Total Reflectance) spectroscopy and FT-Raman spectroscopy, allowing for the determination of the composition of the different phycocolloids from dried ground seaweed.

In this work, polysaccharides present in several seaweeds are analysed by spectroscopic techniques, based on the previous identification of the principal phycocolloids (namely, alginate, agar, kappa-, mu-, iota-, nu-, lambda-, tetha- and ksi-carrageenan [4,5]). The nature of the polysaccharides (with extraction and without any type of extraction) present in these seaweeds was determined with FTIR-ATR and FT-Raman analysis of extracted phycocolloids and ground dry seaweed.

Acknowledgements

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID /CTM /50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement

References

- [1] L. Pereira, P. Ribeiro-Claro, in *Marine Algae: Biodiversity, Taxonomy, Environmental Assessment, and Biotechnology*, L. Pereira (Ed.), J.M. Neto Neto (Ed.), CRC Press, Boca Raton (USA), 228-250, 2015.
- [2] L. Pereira, F. Meireles, H. T. Abreu, and P. Ribeiro-Claro, in *Marine Algae Extracts: Processes, Products, and Applications*, Wiley, N.Y., 277-294, 2015.
- [3] S. RudhZIAHA, M.S.A. Rani, A. Ahmad, N.S. Mohamed, H. Kaddami, *Ind. Crops Prod.*, **72**, 133–141, 2015.
- [4] Pereira L., Sousa A., Coelho A. H., Amado A. M., Ribeiro-Claro P.: *Biomol. Engin.*, **20**, 223-228, 2003.
- [5] L. Pereira, A. M. Amado, A. T. Critchley, F. van de Velde, P. Ribeiro-Claro, *Food Hydrocoll.* **23**, 1903–1909, 2009.

Inelastic neutron scattering study for the structural characterization of cellulosic nanostructured materials

M. Nolasco^{1,*}, P. Ribeiro-Claro², P. D. Vaz^{3,4}, S. Rudic⁴.

¹Departamento de Física e CICECO, Universidade de Aveiro, 3810-193 Aveiro, Portugal.

²Departamento de Química e CICECO, Universidade de Aveiro, 3810-193 Aveiro, Portugal.

³CQB, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal.

⁴ISIS Neutron & Muon Source, Rutherford Appleton Laboratory, Chilton, Didcot, Oxfordshire OX11 0QX, United Kingdom.

*mnolasco@ua.pt

As a response to the limited availability of fossil fuels and a globally growing concern on the future of our environment, the demand for sustainable alternatives to oil and oil-based products is constantly increasing. Alternative resources to replace current raw materials are sought by the industry and, at the same time, sustainable materials are gaining more and more interest among consumers around the world. Potential raw materials to meet these challenges can be found in cellulosic biomass, which forms the feedstock for second-generation biorefineries.

In order to utilize the full potential of the available resources, to improve the current processes and to develop new ones, a better understanding on the cellulose fundamental structural aspects, which is relatively limited should be attained [1,2].

Cellulose has a complex, multi-level supermolecular architecture. This natural polymer is built from superfine fibrils having diameters in the nano-scale and each such nanofibril contains ordered nanocrystallites and low-ordered nano-domains [3].

Accessibility of cellulose plays a crucial role in many kinds of chemical and physical reactions. Especially for molecules or enzymes of nanometer dimensions, the physical structures formed by cellulose microfibrils may have a considerable limiting effect on this accessibility. Therefore, methods to characterize the nanometer scale morphology and packing of cellulose microfibrils and their bundles are highly desired. Cellulose from various sources is all the same at the molecular level but they differ in the crystalline structures and bindings by other biochemicals. It is this difference that makes possible a persistent research on cellulose.

In this context, inelastic neutron scattering (INS) will be applied to gain a deeper insight at the molecular level by characterizing the nanoscale structure of cellulose from a variety of sources (bacterial and natural cellulose). Model compounds such as the cellulose derivatives - carboxymethylcellulose sodium salt and acetylcellulose will be used for comparison. Special attention will be devoted to the analysis of low frequency modes that should probe differences in the structural packing of cellulose.

Acknowledgements

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID /CTM /50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement.

References

[1] H. Chanzy, *Cellulose*, **18**, 853-856 (2011).

[2] S.P.S. Chundawat, G.T. Beckham, M.E. Himmel, B.E. Dale, *Annu. Rev. Chem. Biomol. Eng.*, **2**, 121-145 (2011).

[3] M. Ioelovich, *BioResources* **3**, 1403-1418(2008).

Chemotherapy Response of Quercetin Combined with a Cisplatin-like Agent Probed by Microvibrational Spectroscopy in Triple Negative Breast Cancer

A. L. M. Batista de Carvalho¹, M. Pilling², P. Gardner², J. Doherty^{2,3}, G. Cinque³, K. Wehbe³, C. Kelley³, **L. A. E. Batista de Carvalho**^{*1} M. P. M. Marques^{1,4}.

¹"Química-Física Molecular", University of Coimbra, 3004-535 Coimbra, Portugal.

²Manchester Institute of Biotechnology, University of Manchester, Manchester, M1 7DN, UK.

³Diamond Light Source, Didcot, Oxfordshire OX11 0DE, UK.

⁴Dep. Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal.

*labc@ci.uc.pt

Despite all efforts, cancer is still a growing health problem worldwide, the triple negative breast carcinoma being the second most lethal cancer among women. About 30% of cancer mortality is attributable to an inappropriate diet (with suspected carcinogens and insufficient beneficial components). Indeed, there seems to be an inverse relationship between a decreased risk of cancer and a regular consumption of fruits and vegetables, which is ascribed to phytochemicals with potential chemopreventive properties – polyamines, phenolic acids and flavonoids. Cancer being a multifactorial disease that involves numerous pathways, its prevention may be achieved by these pharmacologically safe compounds from natural sources, capable of modulating multiple targets and cell signalling routes.¹⁻³ Additionally, this type of non-toxic agents can be used alone as cancer chemopreventives, or in combination with chemotherapy (e.g. in chemosensitisation strategies or for downregulating chemoresistance processes).

The present study reports the combined effect of quercetin (Q), an abundant dietary flavonol with a recognised antioxidant capacity⁴, with cisplatin (*cis*-Pt(NH₃)₂Cl₂) and a new generation cisplatin-like Pd(II) chelate (Pd₂Spm, Spm=spermine)⁵⁻⁷, aiming at an improved activity and selectivity. The influence of pre-sensitisation (for 24 h) of a human triple negative breast cancer cell line (MDA-MB-231) with the antioxidant agent (50 µM)⁴ on the pharmacodynamic behaviour of the drugs (4 µM)⁵ was assessed, with a view to understand the influence of the daily diet on chemotherapeutic intervention. MicroRaman and synchrotron-radiation infrared microspectroscopy (SR-IRMS) of formalin-fixed cells allowed to determine the metabolic impact of the [Q/drug] combinations, for a 48 h incubation time (after a prior 24 h cell sensitisation with the antioxidant). The data thus obtained was compared with that measured for incubation with quercetin alone, for the same cell line and experimental conditions.⁸

Upon unsupervised PCA of the data (after RMieS-correction of the IR results), a clear separation was obtained between the quercetin-treated cells and those pre-sensitised with the antioxidant and then exposed to either cisplatin or Pd₂Spm. For both the Raman and SR-IR results this discrimination occurred through PC-1, which represents mainly the DNA and protein cellular content. This is in accordance with the mode of action of Pt- and Pd-based anticancer agents, known to exert their cytotoxic effect *via* covalent binding to DNA, combined to coordination to specific proteins thus altering their structure and leading to unfolding and exposure of side chains (evidenced by intensity changes in the Phe band at ca. 1000 cm⁻¹). The SR-IR results, in turn, unveil a significant impact of the [Q/drug] combination on the lipids.

Acknowledgements

Portuguese FCT – UID/MULTI/00070/2013, SFRH/BD/72851/2010, Project PTDC/QEQ-MED/1890/2014. Diamond Light Source (B22/SM10065), European Funding Calypso FP7.

References

- [1] A. Crozier *et al.* *Nat. Prod. Rep.*, **26**, 1001 (2009).
- [2] K. Sak *Pharmacogn Rev.*, **8**, 122 (2014).
- [3] S. A. Heleno *et al.* *Food Chem.*, **173**, 501 (2015).
- [4] M.M. Dias *et al.* *Food&Function*, **2**, 595 (2011).
- [5] S.M. Fiuza *et al.* *Chem.Biol.&Drug Design*, **77**, 477 (2011).
- [6] M.P.M. Marques *ISRN Spectroscopy* 2013, **1** (2013).
- [7] S. M. Fiuza *et al.* *New J. Chem.*, **39**, 6274 (2015).
- [8] A.L.M. Batista de Carvalho *et al.* *Faraday Discussions* (2016).

Theoretical models for the spectroscopy of methane/ethane ice mixtures

R. Escribano^{1,*}, G. Molpeceres¹, M. A. Satorre², C. Millán², J. Ortigoso¹, B. Maté¹.

¹Instituto de Estructura de la Materia, IEM-CSIC, Serrano 123, 28006 Madrid, Spain.

²Centro de Tecnologías Físicas, EPS Alcoi, Universitat Politècnica de València, Alicante, Spain.

*rafael.escribano@csic.es

In a recent work [1], we have determined the optical constants and band strengths of methane/ethane ice mixtures in the near- and mid-infrared ranges. We present here recent calculations on models for these mixtures. Methane and ethane are constituents of planetary ices in our Solar System. Methane has been detected in outer solar system bodies like Titan, Pluto, Charon, Triton, or other TNO's [2]. Ethane has also been identified in some of those objects [2]. The motivation of this work was to provide new laboratory data that may contribute to the understanding of those systems, in the new era of TNO's knowledge opened up by the New Horizons mission [3].

The models are designed to match the composition and density of the mixtures studied in our laboratories. The calculations include several steps: first, the amorphous samples are generated, via a Metropolis Montecarlo procedure (see Fig. 1); next, the amorphous structures are relaxed to reach a minimum in the potential energy surface; at this point, the harmonic vibrational spectrum is predicted. Finally, the relaxed structures are processed by molecular dynamics with the final aim of obtaining an anharmonic prediction of the spectra, which includes the near-infrared region. Both the harmonic and anharmonic spectra are compared to the experimental measurements in the mid- and near-infrared regions (see Fig.2), respectively.

All calculations are carried out by means of Materials Studio software, using the Density Functional Theory method, with GGA-PBE approximations.

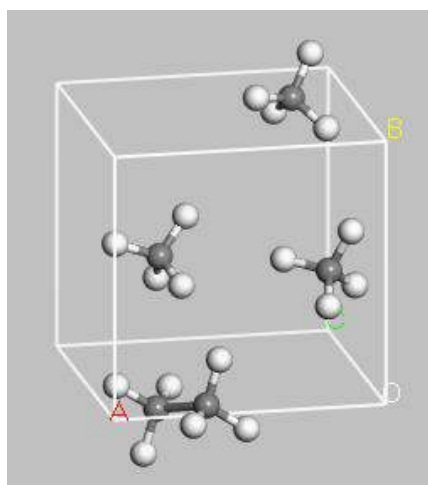


Fig.1. Schematic representation of an amorphous mixture with three methane and one ethane molecules.

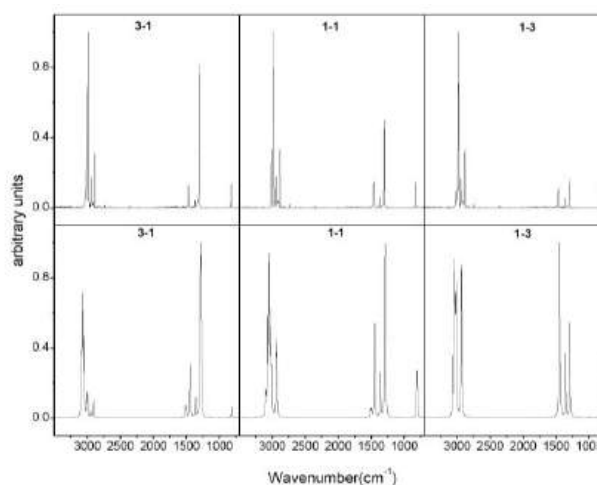


Fig. 2. Comparison of experimental (top) and calculated spectra in the mid-IR for three of the mixtures studied in this work with the indicated CH₄:C₂H₆ ratio

Acknowledgements

This research has been supported by the Spanish MINECO, Projects FIS2013-48087-C2-1-P and FIS2013-48087-C2-2-P. G.M. acknowledges MINECO PhD grant BES-2014-069355. We are grateful to M.A. Moreno, J. Rodríguez, and I. Tanarro for technical help and to V. J. Herrero and I. Tanarro for discussions. Calculations have been carried out at Trueno-CSIC.

References

- [1] G. Molpeceres et al., *Ap. J*, submitted (2016).
- [2] D.P. Cruikshank et al., *Icarus*, **246**, 82–92, 2015.
- [3] A. Stern et al., *Science*, **350**, 260–292, 2015.

Spectral and Hyperspectral Approaches for the Understanding of the Dynamics of Molecular Bands in Laser-Induced Plasmas of Organic Compounds

P. Purohit, **J. M. Vadillo**, J. J. Laserna.

UMALASERLAB, Universidad de Málaga, C/ Jimenez Fraud 4, 29010 Málaga.
samara@uma.es

Spectroscopy of laser-produced plasmas (LIBS) is one of the most powerful analytical techniques that can be used to obtain basic information directly from solid samples due to its versatility. Despite its exclusive use as elemental analysis technique, it is possible to extract molecular information from di- or triatomic molecules of interest to determine the kinetics of decomposition of certain compounds (explosives, for example) or even for additional information to differentiate structural isomers based on the intensity ratios of their molecular emissions (C₂, CN, OH, NH, ...).

However, most of these analyses are carried out at atmospheric pressure, where air may also ionize and becomes part of the composition of the plasma studied. Under such frame, there are always doubts about the sources of the bands studied. Thus, it is critical the performing of fundamental studies of plasmas formed to define the significance of the surrounding media in the spectra recorded.

In this communication, spectral and hyperspectral experiments have been used to understand the dynamic of the expanding plume. Variable conditions from atmospheric pressure to 10⁻³ mbar were used to demonstrate the variation in the composition according to each situation. In addition, the study is completed by adding spatial resolution, which enables finely sectioning the plasma to meet the timely distribution of the species within it. Finally, a comparison between the results obtained using nanosecond and femtosecond lasers is shown, since the different mechanisms followed in both cases may cause significant differences in the characteristics of the plasma such as morphology and plume formation.

Acknowledgements

This work is funded by project CTQ2014-56058P.

LIST OF AUTHORS

List of authors

A

Abad-Álvaro, I.	30
Abad-Fuentes, A.	119
Abad-Somovilla, A.	119
Abidi, M.	63
Aboal-Somoza, M.	72, 73
Abujetas, D. R.	42
Aguayo-López, M. L.	101
Aguirre, M. A.	32, 131, 150, 152
Ahmadi, M.	117
Akhoundzadeh, J.	136
Albaladejo, J.	74, 109, 110
Alonso, C.	109
Alonso-Chamarro, J.	43
Álvarez, M. B.	135
Álvarez-Llamas, C.	31, 167
Amado, J. M.	155
Amaro-López, M. A.	87
Amigo, J. M.	79, 80
Amoza, M.	120
Anwar, J.	37
Anzano, J.	37
Aranda, V.	124
Arenas, J. F.	105, 182
Arjonilla, P.	145
Armenta, S.	29, 54, 119, 168
Arrifano, G. P. F.	53
Artiaga, G.	174
Ávila-Orozco, F. D.	150
Ayora-Cañada, M. J.	124, 144, 145, 163

B

Baca-Bocanegra, B.	56, 89, 90, 91
Baile, P.	152
Baonza, V. G.	20, 50
Barciela-Alonso, M.C.	83
Barros, A.	86
Bassani, D. M.	22
Batista de Carvalho, A. L. M.	177, 178, 185
Batista de Carvalho, L. A. E.	185
Bayo, F.	37
Bello, C.	37
BenAzouz, A.	19
Bendicho, C.	67, 104, 136, 137
Benito-Peña, E.	39
Bento, A.	25
Berenguel-Alonso, M.	43
Berlin-Larque, P.	40
Bermejo-Barrera, P.	30, 72, 73, 83, 84, 85, 120
Besada-Romero, B.	72
Bhowmick, S.	120
Biel, B.	68
Blanco, D.	128
Blanco-Liaño, I.	73
Böhme, K.	24

Bolea, E.	30
Bonales, L.	50
Bonales, L. J.	139
Bordel, N.	31, 128, 167
Bouzas-Ramos, D.	44, 46
Brasca, R.	106
Bravo, I.	74, 109, 110
Brenny, B. J. M.	42
Bruen, D.	19

C

Cáceres, J.	37
Cáceres, J. O.	123
Cáceres, M.	50
Cajal, J.	37
Callejón, R. M.	79, 80, 82
Calvo, A.	37
Camacho, J. J.	123
Cámara-Martos, F.	87, 88
Campillo, N.	77
Canales-Vázquez, J.	74
Canal-Martín, A.	111
Canals, A.	32, 33, 117, 127, 131, 149, 150, 152
Canilho, N.	59
Cañamares, M. V.	108, 143
Capitán-Vallvey, L. F.	38, 43, 68, 78, 101, 102, 103, 107
Carrasco-García, I. M.	35
Carrillo-Carrion, C.	47
Casal, A.	121
Casas, J.	37
Cascant, M. M.	169
Castellanos, S.	68
Castillo, J. R.	30
Castro, J. L.	105
Centeno, S. P.	182
Cerrato, R.	121
Chamsaz, M.	136
Chan, W.	24
Chemnitzer, R.	51
Chen, H.	96
Cinque, G.	185
Cobos, J.	139
Coello, J.	160
Coleman, S.	19
Collado-Esteban, E.	151
Colmenero, F.	139
Comino, F.	124
Constantino, C. J. L.	108
Corda, E.	70
Cordón, J.	115
Corps-Ricardo, A. I.	55
Correa-Gómez, E.	145
Costa-Fernández, J. M.	44, 46, 47, 71
Costas, M.	136, 137
Costas-Mora, I.	104
Crespo-López, M. E.	53
Csontos, M.	159
Culzoni, M. J.	106

D

Dar, A.	37
Das, N.	120
De La Calle, I.	67
de la Guardia, M.	29, 54, 119, 168, 169
de la Torre-López, M. J.	145
de los Reyes-Berbel, E.	101
de Marcos, S.	97
de Orbe-Payá, I.	38
del Valle, J.	37
Descalzo, A. B.	39
Diamond, D.	19, 78
Dias, M. M.	177
Diéguez, L.	24
Doherty, J.	185
Domínguez-González, M. R.	83, 84, 120
Dominguez-Manzano, J.	81
Dominguez-Perles, R.	86
Domínguez-Vidal, A.	124, 144, 145, 163
Domini, C. E.	135, 150
Dunne, A.	19
Durán-Merás, I.	81

E

Eiró, N.	49
Elbal, L.	29
Encinar, J. R.	46, 47, 71
Erenas, M. M.	38
Escribano, R.	60, 186
Escudero, M.	37
Escudero-Gilete, M. L.	90
Espiña, B.	24
Esteve-Turrillas, F. A.	54, 119
Ezquerro, M.	115

F

Fandiño, J.	128
Feldmann, J.	46
Fernandes, C. I.	25
Fernández, B.	43, 68, 102
Fernández, E.	149
Fernández, J.	74, 109, 110
Fernández-Argüelles, M. T.	24
Fernández-Hernando, P.	151
Fernández-Lien cres, M. P.	74
Fernández-Ramos, M. D.	101
Fernández-Sánchez, M. L.	49
Florea, L.	19
Fondo, M.	120
Fortes, F. J.	34, 58, 181
Francis, W.	19

G

Gajdosechova, Z.	46
Galbán, J.	97
Gallart-Mateu, D.	29
Gallelo, G.	169
García-Cortés, M.	71
García-Deibe, A. M.	120
García-González, D. L.	79, 80
García-Martínez, J. C.	74
García-Martínez, M.	32
García-Martínez, P.	118
García-Ramos, J. V.	70
García-Ramos, J. V.	108
García-Ruiz, C.	59, 167
Garcinuño-Martínez, R. M.	151
Gardner, P.	185
Garrido, M.	135
Garrido-Frenich, A.	94, 95, 96, 125
Garrigues, S.	168, 169
Garzón, A.	74, 109, 110
Gaspar, E.	37
Gil, S.	137
Gismera, M. J.	129
Gleisner, H.	51
Goenaga-Infante, H.	52
Goicoechea, H. C.	106
Gómez, D.	160
Gómez-Gómez, M.	174
Gómez-Gómez, M. M.	45
Gómez-Nieto, B.	129
Gómez-Rivas, J.	42
González de Vega, R.	49
González-Blasco, P.	37
González-Cabrera, M.	163
Granadino-Roldán, J. M.	74
Gryglicka, M.	67
Guirado, S.	58
Gutiérrez, A. M.	111
Guzmán-Bernardo, F. J.	55

H

Hamida, S.	37
Hentati, S. B.	63
Herbello-Hermelo, P.	84, 85
Heredia, F. J.	56, 89, 90, 91, 92, 93
Hernandez, M.	70
Hernández-Córdoba, M.	77, 173
Hernández-Hierro, J. M.	56, 89, 90, 91, 92, 93
Herrero, V. J.	60, 180
Hidalgo, M.	33, 127
Huerta, F.	63

I

Iglesias-Jiménez, A.	174
Itagaki, T.	52
Izquierdo, J.	122

List of authors

J

Jarabo, S.	116
Jara-Palacios, M. J.	91
Jiménez Rodríguez, A.	83
Jiménez-Lafítez, B.	111
Jiménez-Moreno, M.	53
Jiménez-Redondo, M.	60

K

Kahveci, Z.	179
Kaynarova, L.	131
Kelley, C.	185
Kovachev, N.	52, 150
Kranz, C.	122
Krupp, E.	46

L

Laborda, F.	30
Laborda, J.	110
Lamas, J. P.	84
Laserna, J. J.	34, 35, 36, 58, 126, 181, 187
Lasheras, R. J.	37
Latorre, M.	85
Lavilla, I.	67, 104, 136, 137
Lobato, A.	50
López de Luzuriaga, J. M.	115
López-Bernabeu, S.	63
López-Claros, M.	35, 58, 181
López-García, I.	173
López-López, M.	167
López-Lorente, A. I.	41
López-Molinero, A.	40, 159
López-Navarrete, J. T.	62
López-Ramírez, M. R.	105, 182
López-Ruiz, N.	38
López-Ruiz, R.	94, 95, 96, 125
López-Sanz, S.	48
López-Tocón, I.	69, 182
Lores, M.	84
Lozano, R.	97
Lúñez, C.	131

M

Machado, N.	86
Madrakian, T.	117
Mallavia, R.	179
Manzoor, S.	123
Marín-Sáez, J.	94, 95, 96, 125
Marques, M. P. M.	177, 178, 185
Martín, H.	123
Martín-Doménech, R.	26
Martínez Vidal, J. L.	95
Martínez, A.	57

Martínez-Cisneros, C. S.	43
Martínez-Máñez, R.	23
Martínez-Olmos, A.	38
Martínez-Ramírez, S.	61
Martínez-Rosas, M. E.	93
Martínez-Sandoval, J. R.	93
Martínez-Tomé, M. J.	179
Martínez-Vidal, J. L.	94, 125
Martín-Garrido, M.	61
Maspoch, S.	160
Maté, B.	60, 180, 186
Mateo, C. R.	179
Mateo, J.	130
Mateo, M. P.	121, 155
Mayes, A.	151
Medeiros, P. S. C.	177, 178
Medina, S.	36, 126
Melguizo, M.	110
Mena, M. L.	174
Menéndez-Miranda, M.	44, 46, 47
Messaadi, A.	118
Millán, C.	186
Mira, A.	179
Mizaikoff, B.	41, 122
Molina, M.	123
Molpeceres, G.	60, 180, 186
Moncayo, S.	37
Monge, M.	115
Montalvo, G.	59
Montejo, M.	57
Montilla, F.	63
Montoro, O. R.	50
Moral, M.	74
Morales, M. L.	79, 82
Morallón, E.	63
Moreda-Piñeiro, A.	84, 85
Moreno, E.	26
Moreno, I.	118
Moreno, M. A.	60
Moreno-Bondi, M. C.	39, 111
Moreno-Gordaliza, E.	174
Moreno-Oliva, M.	62
Moreno-Rojas, R.	87, 88
Mota, A. J.	120
Muñoz de la Peña, A.	106
Muñoz-Caro, G. M.	26
Muñoz-Sandoval, M. J.	173
Mur, R.	37
Murru, F.	107

N

Navarro, A.	74
Navarro, J.	97
Neira, Y.	85
Nicolás, G.	121, 155
Niza, E.	74, 109, 110
Nogales-Bueno, J.	56, 89, 90, 91, 92, 93
Nolasco, M.	184
Nueda, M. L.	110
Nunes, C. D.	25

List of authors

Nunez, S. 52

O

Ocaña, J. A. 79
 Oddershede, L. B. 21
 Oliver-Pozo, C. 80
 Olmos, M. E. 115
 Onaindia, M. C. 106
 Orbe-Payá, I. 102, 103
 Orejas, J. 128
 Orellana, G. 39
 Ortega, F. 59
 Ortigoso, J. 180, 186
 Ortiz-Gómez, I. 102, 103
 Otero, J. C. 69, 105, 177, 178, 182

P

Pacheco, P. J. 74, 109, 110
 Palma, A. J. 38
 Paniagua-Domínguez, R. 42
 Paniagua-González, G. 151
 Parak, W. J. 47
 Parras, D. J. 57
 Pasc, A. 59
 Pascual-Juez, R. 138
 Pastor, A. 169
 Pastor, M. 77
 Paules, D. 37
 Pavel, I. A. 59
 Peiró, M. N. 168
 Pena-Pereira, F. J. 136, 137
 Peña-Farfal, C. 85
 Peña-Ruiz, T. 74
 Peña-Vázquez, E. 30
 Pereira, L. 183
 Pérez de Vargas-Sansalvador, I. M. 78
 Pérez-Bernal, J. L. 82
 Pérez-Conde, C. 111
 Pérez-Lustres, J. L. 120
 Pilling, M. 185
 Pisonero, J. 31, 49, 128, 167
 Polman, A. 42
 Prado, M. 24
 Prati, S. 143
 Prazeres, S. F. 59
 Procopio, J. R. 129
 Pulido-Moriana, L. 144
 Purohit, P. 34, 187
 Puyol, M. 43

Q

Quintas, P. Y. 135
 Quintero, M. C. 130
 Quispe, F. 88

R

Ramos, A. 86
 Ramos, K. 45
 Ramos, L. 45
 Rengevicova, S. 173
 Ribeiro-Claro, P. 183, 184
 Ríos-Castro, A. 48, 55
 Ríos-Reina, R. 79, 80
 Ripoll, L. 33, 127
 Rísquez, C. 57
 Rodero, A. 130
 Rodríguez, M. O. 57
 Rodríguez, M. P. G. 57
 Rodríguez-Diéguez, A. 68, 102
 Rodríguez-Fariñas, N. 48
 Rodríguez-López, J. 74
 Rodríguez-Martín-Doimeadios, R. C. 48, 53, 55
 Rodríguez-Pulido, F. J. 56, 89, 91, 92, 93
 Román, I. P. 150
 Román-Pérez, J. 182
 Romero, V. 67, 104
 Romero-González, R. 94, 95, 96, 125
 Romero-Molina, L. 90
 Rosa, E. 86
 Ruano, C. 177, 178, 182
 Rubio, S. 169
 Rubio-Domene, R. 145
 Rubira, R. J. G. 108
 Rudic, S. 184
 Rudić, S. 25
 Ruiz, F. J. 127

S

Saldaña, J. E. 116
 Salgado-Remacha, F. J. 116
 Salinas, A. 68
 Salinas-Castillo, A. 43, 102, 103, 107
 Salis, F. 39
 Sánchez, A. 57
 Sánchez-Cortés, S. 108, 143
 Sánchez-Gil, J. A. 42
 Sánchez-López, M. M. 118
 Sangüesa, S. 37
 Sanmartín-Matalobos, J. 72, 120
 Santos, R. M. 51
 Santoyo-González, F. 101
 Sanz-Medel, A. 44, 46, 47, 49, 71, 128
 Sanz-Vicente, I. 97
 Satorre, M. A. 186
 Schade, U. 145
 Segovia-Quintero, M. A. 93
 Serrano-Vargas, R. 48
 Sevilla, M. T. 129
 Sevilla, P. 70
 Sevillano-Morales, J. 87
 Soto, J. 69, 105, 182
 Suárez-Muñoz, J. M. 138

List of authors

T

Tanarro, I.	60, 180
Tapia, O.	37
Taravillo, M.	50
Tena, M. T.	115
Timón, V.	60, 139
Tobar, M. J.	155
Tolosa, J.	74
Tornero, J.	123
Torres, A.	77
Tuñón, J.	57

U

Ubeda, C.	82
Ugena, L.	123
Urraca, J.	111

V

Vadillo, J. M.	35, 36, 126, 187
Valencia-Reyes, Z. L.	88
Van-Dam, A. D.	42
Vandenabeele, P.	57
Vasimalai, N.	24

Vaz, P. D.	25, 184
Vázquez-Guilló, R.	179
Vidal, L.	117, 149, 152
Vilas, V.	24
Villar-Navarro, M.	82
Viñas, P.	77
Vizoso, F. J.	49

W

Wang, P.	41
Wehbe, K.	185
Wilbur, S.	52
Wünscher, S.	51

Y

Yamanaka, M.	52
Yañez, A.	155

Z

Zagorovsky, K.	24
Zalaffi, M. S.	143
Zougagh, M.	55