SOCIEDADE PORTUGUESA DE QUÍMICA

ANALÍTICA 2018

9th Meeting of Division of Analytical Chemistry

26-27 MARCH, PORTO-PORTUGAL

FFUP/ICBAS – UNIVERSITY OF PORTO

www.analitica2018.eventos.chemistry.pt

BOOK OF ABSTRACTS

TÍTULO
Book of Abstracts Analítica 2018 - 9th Meeting
of Division of Analytical Chemistry

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ANALÍTICA 2018

9th Meeting of Division of Analytical Chemistry

Organized under the auspices of Sociedade Portuguesa de Química

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ORGANIZING ENTITIES

SPONSORING ENTITIES

GENERAL INFORMATION

CONFERENCE VENUE

The Analítica 2018 - 9th Meeting of Division of Analytical Chemistry conference will be held in the FFUP/ICBAS building, University of Porto.

REGISTRATION

All attendees must register at the registration/information desk located at the foyer of the Main Auditorium that will be open from 9H30 and throughout the course of the scientific program.

AUDIO-VISUAL EQUIPMENT

Each conference room is equipped with a computer and standard audio-visual equipment namely a data projector, projection screen, a laser pointer, and a microphone. Each speaker should upload and review his/her presentation as far in advance as possible, being advised for it to be placed in the previous session. Please take note of the following:

All presentations must use a PowerPoint (Microsoft) or PDF format.

A USB memory stick should be used for uploading the presentation.

Personal laptops cannot be used for presentations.

ORAL PRESENTATIONS

The Plenary Lectures (PL) will last 50 minutes and the Keynote Lectures (KL) will last 30 minutes, including the discussion. Oral Communications (OC) will last 20 minutes. The session moderator will introduce each speaker, monitor time, and assist in facilitating an interactive, educational experience during the question and answer period. All speakers are requested to carefully plan the presentation in order to respect the schedule avoiding delays in the program.

POSTER SESSIONS

Posters should be posted only on the day of the session where they were allocated, preferably before the beginning of the scientific program of the respective day. Members of the organizing committee will be available to assist attendees during the time of poster set-up. Presenting authors are expected to stand by their poster during the poster session to answer questions and discuss their research. Posters must be removed from the poster board at the end of the day of each session by the authors of the communication.

POSTER PITCH SESSIONS

'Poster Pitch' will be presented within the parallel sessions program, and it involves a 2 minute oral presentation. Please note that the presentation will be strictly timed and will be stopped at 2 minutes. 'Poster Pitch' sessions will not include any additional time for questions or discussion within the parallel sessions. Instead, the audience will be encouraged to meet and discuss the work with the author in the poster discussion session or another mutually agreed time.

LUNCHES AND COFFEE BREAKS

There are several restaurants near the FFUP/ICBAS building, as indicated on the additional documentation present in participants' bag. There is also a canteen and a cafeteria inside the FFUP/ICBAS building (building 2, ground floor). Do not hesitate in contacting committee members for more details and advice.

SOCIAL EVENT

The Conference dinner will be held at Restaurante Terrella (Rua Ofélia Diogo da Costa 105 A, 4149-071 Porto) on March 26th at 20H00.

WORKSHOP ON SCIENTIFIC WRITING AND PUBLISHING TRAINING

Learn how to get published in top journals by attending the workshop on scientific writing and publishing training. The workshop will be held on the afternoon of March 27th, from 17H10. All participants en-rolled in the Analítica 2018 have free access to the workshop.

POSTER PRIZES

Two poster prizes are offered by the publishers of the journal Ana-lytical and Bioanalytical Chemistry for the best poster presentations of young researchers. These prizes are vouchers for books from the product range of the Springer publishing house. The Analitica 2018 scientific committee will establish a panel of judges to assess post-ers. Prizes will be presented in public at the Analítica 2018 closing ceremony.

SCIENTIFIC PROGRAM

26 MARÇO 2018
Monday - Salão Nobre FFUP

9H30-10H40 **REGISTRATION IS OPEN**

10H40-11H10 **OPENING CEREMONY**

11H10-12H00 **PL1 - M. Miró**

12H00-12H30 **KL1 - J.P. Conde**

12H30-14H00 **LUNCH BREAK**

14H00-14H50 **PL2 - J.A. Lopes**

14H50-15H20 **KL2 - I.M. Valente**

15H20-15H40 **OC1 - M.N. Sánchez**

15H20-15H40 - Anfiteatro 8
OC2 - M.F. Camões

15H40-16H20 **POSTER PITCH**

15H40-16H00 - Anfiteatro 8
OC3 - O. Pellegrino

16H00-16H20 - Anfiteatro 8
OC4 - V.M. Morgado

16H20-17H20 **COFFEE BREAK + POSTERS**

17H20-17H40 **OC5 - S.J. Mazivila**

17H20-17H40 - Anfiteatro 8
OC8 - I.H. Srámková

17H40-18H00 **OC6 - R.N.M.J. Páscoa**

17H40-18H00 - Anfiteatro 8
OC9 - N. Neng

18H00-18H20 **OC7 - S.M. Ahmad**

18H00-18H20 - Anfiteatro 8
OC10 - C.E. Monteiro

18H30-19H30 **MEETING DAC-SPQ**

20H00 **CONFERENCE DINNER**

27 MARÇO 2018
Tuesday - Salão Nobre FFUP

9H00-9H50 **PL3 - A.M.OLIVEIRA-BRETT**

9H50-10H20 **KL3 - J.M.F. NOGUEIRA**

10H20-10H40 **OC11 - J.L.M. Santos**

10H20-10H40 - Anfiteatro 8
OC13 - W.B.S. Machini

10H40-11H00 **OC12 - R.B.R. Mesquita**

10H40-11H00 - Anfiteatro 8
OC14 - M. David

11H00-11H50 **COFFEE BREAK + POSTERS**

11H50-12H10 **OC15 - C.M.A. Brett**

11H50-12H10 - Anfiteatro 8
OC17 - L. Zelená

12H10-12H30 **OC16 - M.F. Barroso**

12H10-12H30 - Anfiteatro 8
OC18 - S.A.P. Pereira

12H30-14H00 **LUNCH BREAK**

14H00-14H50 **PL4 - R.J. Schneider**

14H50-15H20 **KL4 - M.E. Pereira**

15H20-15H40 **OC19 - R. Cesário**

15H20-15H40 - Anfiteatro 8
OC20 - P. Carl

15H40-16H20 **POSTER PITCH**

15H40-16H00 - Anfiteatro 8
OC21 - J. Narciso

16H00-16H20 - Anfiteatro 8
OC22 - L. Carvalho

16H20-17H10 **COFFEE BREAK + POSTERS**

17H10-17H40 **WORKSHOP (M. MIRÓ)**

17H40-18H00 **CLOSING CEREMONY**

SCIENTIFIC PROGRAM
DETAILED ORAL PRESENTATION SCHEDULE

MONDAY, MARCH 26TH, 2018

9H30	REGISTRATION	
	MAIN AUDITORIUM	
10H40	OPENING SESSION	
11H10	PL1	Novel approaches for in-vitro bioaccessibility & bio-availability of nutrients and contaminants in solids using flow approaches: pros and cons ***Manuel Miró***
12H00	KL1	Lab-on-chip platforms for chemical and biological analysis ***João Pedro Conde***
12H30	LUNCH BREAK	
	MAIN AUDITORIUM	
14H00	PL2	Chemometrics for (bio)pharma process analytics ***João Almeida Lopes***
14H50	KL2	An analytical chemistry perspective over animal science ***Inês M. Valente***
15H20	OC1	Quantitative and qualitative analysis of polycyclic aromatic hydrocarbons in urine samples using a non-separative method based on mass spectrometry *Miguel del Nogal Sánchez, Patricia Martín Santos, José Luis Pérez Pavón,* ***Bernardo Moreno Cordero***
15H40	POSTER PITCH	
	ROOM ANFITEATRO 8	
15H20	OC2	The kilogram and the mole - redefinition and metrological traceability ***Maria Filomena Camões***
15H40	OC3	What is the new SI and what does it change for analytical chemistry? *Andreia Furtado, Raquel Quendera, Florbela A. Dias,* ***Olivier Pellegrino***
16H00	OC4	Monte Carlo method for uncertainty evaluation: a simple & reliable method for complex measurements ***Vanessa M. Morgado,*** *Carla Palma, Ricardo B. Silva*

16H20 POSTER SESSION + COFFEE BREAK

MAIN AUDITORIUM

17H20 OC5 In-line monitoring of green synthesis of novel lamivudine-theophylline co-crystal using FT-IR spectroscopy and MCR-ALS
Sarmento J. Mazivila, *Ricardo A.E. Castro, João M.M. Leitão, Joaquim C.G. Esteves da Silva*

17H40 OC6 Application of near- and mid-infrared spectroscopy coupled with chemometrics for the discrimination of Humulus lupulus varieties
Julio C. Machado Jr., Miguel A. Faria, Isabel M.P.L.V.O. Ferreira, ***Ricardo N.M.J. Páscoa,*** *João A. Lopes*

18H00 OC7 High throughput bar adsorptive microextraction (HT-BAμE) - a new effective approach to screen benzodiazepines in biological matrices
Samir M. Ahmad, *J.M.F. Nogueira*

ROOM ANFITEATRO 8

17H20 OC8 Sequential injection analysis in screening of extraction properties of nanofibers
Ivana H. Šrámková, *Laura Carbonell Rozas, Burkhard Horstkotte, Dalibor Šatínský*

17H40 OC9 Determination of benzophenone & related compounds by tape adsorptive microextraction
Nuno Neng, *J.M.F. Nogueira*

18H00 OC10 Environmental relevance of platinum and rhodium in tagus estuary
Carlos E. Monteiro, *Margarida C. dos Santos, Antonio Cobelo-García, Pedro Brito, Miguel Caetano*

MAIN AUDITORIUM

18H30 GENERAL ASSEMBLY Division of Analytical Chemistry - SPQ

20H00 CONFERENCE DINNER

TUESDAY, MARCH 27TH, 2018

	MAIN AUDITORIUM	
9H00	PL3	Electrochemical approaches to analytical sensing of biomolecules ***Ana Maria Oliveira-Brett***
9H50	KL3	Back to basics: recent trends in microextraction techniques ***José Manuel F. Nogueira***
10H20	OC11	Ternary semiconductor quantum dots for chemical analysis ***João L. M. Santos,*** *José X. Soares, S. Sofia M. Rodrigues, Rafael C. Castro, David S. M. Ribeiro*
10H40	OC12	Microfluidic paper-based devices as disposable, easy- to-use, real-time quantification methods ***Raquel B.R. Mesquita,*** *António O.S.S. Rangel*
	ROOM ANFITEATRO 8	
10H20	OC13	In situ sensing of DNA-antileishmanial drug miltefosine interaction using a DNA-electrochemical biosensor ***Wesley B.S. Machini,*** *Ana M. Oliveira-Brett*
10H40	OC14	Layer-by-layer label-free biosensor for improved glucose sensing using poly(3,4-ethylene-dioxythiophene) conducting polymer ***Melinda David,*** *Madalina M. Barsan, Monica Florescu, Christopher M.A. Brett*
11H00	POSTER SESSION + COFFEE BREAK	
	MAIN AUDITORIUM	
11H50	OC15	Electrochemical enzyme inhibition biosensor platforms for the detection of toxic species ***Christopher M.A. Brett,*** *Mariana Emilia Ghica*
12H10	OC16	Electrochemical genosensing of the maize endogenous gene using Fe3O4@Au nanoparticles as magnetoplatform ***M. Fátima Barroso,*** *Juliana Sousa, J. Ramos-Jesus, C. Pereira, C. Freire, N. de-los-Santos-Álvarez, R. Fonseca, J. Ribeiro Junior, C. Delerue-Matos*

ROOM ANFITEATRO 8

11H50	OC17	Automated approach for the sequential injection determination of the herbicide 2,4-D based on online preconcentration using a polymer inclusion membrane ***Lucie Zelená,*** *M. Inês G. S. Almeida, Robert W. Cattrall, Hana Sklenářová, Petr Solich, Spas D. Kolev*
12H10	OC18	Antipsychotics' degradation obtained through chemical and biochemical oxygen demand levels ***Sarah A.P. Pereira,*** *Susana P.F. Costa, Edite Cunha, Marieta L.C. Passos, André R.S.T. Araújo, M. Lúcia M.F.S. Saraiva*
12H30	LUNCH BREAK	

MAIN AUDITORIUM

14H00	PL4	Immunoanalytical methods for screening and monitoring in environmental analysis ***Rudolf J. Schneider***
14H50	KL4	Mercury monitoring in waters: is it possible to measure easily at trace levels? ***Maria Eduarda Pereira,*** *Daniela S. Tavares, Cláudia B. Lopes, Tito Trindade, Carlos Vale*
15H20	OC19	Tidal effect on dissolved gaseous mercury formation and mercury volatilization in intertidal sediments ***Rute Cesário,*** *Laurier Poissant, Martin Pilote, Nelson J. O´Driscoll, Ana M. Mota, João Canário*
15H40	POSTER PITCH	

ROOM ANFITEATRO 8

15H20	OC20	Environmental screenings of bioactive compounds in wastewater using a novel multiplex suspension array fluorescence immunoassay ***Peter Carl,*** *Dominik Sarma, Inês I. Ramos, Ana Machado, Andreas Lehmann, Kristin Hoffman, Knut Rurack, Marcela A. Segundo, Adriano A. Bordalo, Rudolf J. Schneider*
15H40	OC21	Validation and uncertainty evaluation of the identification of doping agents in sport by GC-MS/MS ***José Narciso,*** *Susana Luz, Ricardo B. Silva*
16H00	OC22	Method validation for metals quantification in water intended for human consumption ***Lina Carvalho,*** *Eugénio Soares, Paula Figueira, Eduarda Pereira*

16H20	POSTER SESSION + COFFEE BREAK	
	MAIN AUDITORIUM	
17H10	**WORKSHOP**	How to write a scientifically sound article: tips & tricks ***Manuel Miró***
17H40	CLOSING CEREMONY	

SCIENTIFIC PROGRAM
POSTER PRESENTATION SCHEDULE

Poster areas:

A) Biosensors

B) Biotechnological Applications

C) Chemometrics

D) Electroanalysis

E) Examinology and metrology

F) Flow Analysis

G) Method Validation

H) Modern Analytical Methodologies

I) Separation Processes

Posters from areas A, B, C, D, E, F, and I will be presented on Monday, March 26th.
Posters from areas G and H will be presented on Tuesday, March 27th.

A) BIOSENSORS

PA 1. Evaluation of the molecular architecture of fluorescent calix[4]arene-based sensors in detection of toxic metals
Alexandra I. Costa, Patrícia D. Barata, Carina B. Fialho, José V. Prata

PA 2. Characterization of glucose biosensor comprising glucose oxidase immobilized in a pencil graphite electrode
Álvaro Torrinha, Maria C. B. S. M. Montenegro, Alberto N. Araújo

PA 3. Metal ion recognition-induced by calix[4]arene-carbazole-containing polymers
Patrícia D. Barata, Alexandra I. Costa, Carina B. Fialho, José V. Prata

PA 4. Amperometric biosensor for pyruvate determination based on carbon nanotubes and prussian blue-chitosan nanoparticles
Ricardo J. B. Leote, Mariana Emilia Ghica, Christopher M. A. Brett

PA 5. Development of a thyroxine immunosensor using screen-printed electrodes
Rosa A. S. Couto, Luís Moreira Gonçalves, Marcio Sousa Góes, Cecília M. P. Rodrigues, M. Beatriz Quinaz, José A. Rodrigues

B) BIOTECHNOLOGICAL APPLICATIONS

PB 1. Optimization and characterization of 2D lipid models for the assessment of acetylcholinesterase inhibitors – membrane interactions
Nuno Guedes, Ana F. Pinheiro, Cláudia Nunes, Salette Reis

PB 2. Development and characterization of nanoagents loaded with carbon monoxide to treat rheumatoid arthritis
Andreia Marinho, Cláudia Nunes, Teresa Santos-Silva

PB 3. Evaluation of the stability and phototoxicity of deet, a common active ingredient of commercial insect repellents
Emmely Gelaude, E. Manuela Garrido, Patrícia Moreira, Helena Carmo, Isabel F. Almeida, Jorge Garrido

PB 4. Evaluation of the antioxidant potential of ascorbic acid derivatives used in skin care products
Cristina Correia, E. Manuela Garrido, Jorge Garrido, Paulo C. Costa, Isabel F. Almeida

PB 5. Polyphenolic composition and in vitro antioxidant activity of *Chamaerops humilis L.*
José Coelho, Jerson Veiga, Ruben Elvas-Leitão, Manuel Matos, M. Conceição Oliveira, Amadeu Brigas

PB 6. Antioxidant activities and antioxidant components of aqueous extracts of mushrooms strains
Magda C. Semedo, Sónia Martins, Amin Karmali

PB 7. Nanostructured lipid carriers as a versatil oral delivery vehicle of streptomycin
Soraia Pinto, Sara Pinheiro, Joana Magalhães, Alexandre Vieira, Marina Pinheiro, Salette Reis

PB 8. Protein-phenolic interaction as a strategy to reduce the precursors of volatile phenols in wine
Susana S. M. P. Vidigal, Francisco M. Campos, José A. Couto, António O. S. S. Rangel, Tim Hogg

PB 9. Ultrasound assisted extraction of triterpenoids from *Ganoderma lucidum*
Oludemi Taofiq, Lillian Barros, M. A. Prieto, M. Filomena Barreiro, Isabel C. F. R. Ferreira

PB 10. Cosmeceutical potential of ergosterol and use of microencapsulation to ensure controlled release
Oludemi Taofiq, Sandrina A. Heleno, Ricardo C. Calhelha, Isabel P. Fernandes, Maria José Alves, Ana M. González-Paramás, Lillian Barros, Isabel C. F. R. Ferreira, M. Filomena Barreiro

C) CHEMOMETRICS

PC 1. Coupling of on-column trypsin digestion-peptide mapping and principal component analysis for stability and biosimilarity assessment of recombinant human growth hormone
Basma M. Eltanany, Sara M. Shatat, Abeer A. Mohamed, Medhat A. Al-Ghobashy, Faten A. Fathalla, Samah S. Abbas

PC 2. Monitoring grapevine leaves variability with infrared spectroscopy
E. Martín-Tornero, R. N. M. J. Páscoa, A. R. Graça, J. A. Lopes

D) ELECTROANALYSIS

PD 1. Electrochemical characterization of cefadroxil and amoxicillin in aqueous media
Caroline G. Sanz, Silvia H. P. Serrano, Christopher M. A. Brett

PD 2. Development of a novel redox polymer-film modified electrochemical sensor using deep eutectic solvents
Hugo C. Novais, Melinda David, Vanessa Baião, Luciana I. N. Tomé, Christopher M. A. Brett

PD 3. Electrochemical behaviour of lercanidipine at carbon black modified electrodes
Isabel P. G. Fernandes, Ana Maria Oliveira-Brett

PD 4. Antidiabetic drug metformin voltammetric evaluation in the presence of Cu(II)
Isabel P. G. Fernandes, Ana Maria Oliveira-Brett

PD 5. A two year survey on fluoride concentration in mineral waters and teas consumed by the Portuguese population
Nelson A. F. Silva, Hugo F. F. A. Silva, Manuel J. Matos

PD 6. Impedimetric sensor based on gold nanoparticle doped - poly-(8-anilino-1-naphthalene sulphonic acid) modified gold electrodes for tyramine detection
Wanderson da Silva, M. Emilia Ghica, Rachel F. Ngece, Emmanuel Iwuoha, Christopher M. A. Brett

E) EXAMINOLOGY AND METROLOGY

PE 1. Total hardness in seawater samples of the Portuguese coast
Bárbara Anes, Cristina Oliveira, Ricardo B. Silva, Maria Filomena Camões

PE 2. Comparative analysis of fatty acid composition of wild vs. farmed salmon
Liliana Grazina, Maria A. Nunes, Isabel Mafra, M. Beatriz P. P. Oliveira, Joana S. Amaral

PE 3. Examinology for analytical chemistry?
Olivier Pellegrino

PE 4. Cumulative standard addition method for electrochemical measurements of biological fluids
Tony R. L. Dadadmos, Airton J. Damaceno, Fernando L. Fertonani, Ricardo J. N. Bettencourt da Silva

F) FLOW ANALYSIS

PF 1. Development of a sequential injection method for bromate determination in soil leachates
Ana S. G. Cerqueira, Raquel B. R. Mesquita, António O. S. S. Rangel

PF 2. Monitoring glucose, calcium, and magnesium levels in saliva as a non-invasive analysis by sequential injection multiparametric determination
Ana Machado, Rui Maneiras, Adriano A. Bordalo, Raquel B. R. Mesquita

PF 3. Automated bead-based immunoassay for the determination of carbamazepine in wastewater
Inês I. Ramos, Peter Carl, Rudolf J. Schneider, Marcela A. Segundo

PF 4. Study of 3,4–hydroxypyridinones functionalized beads for iron(III) determination in a microsequential injection solid phase spectrometry mode
Joana L. A. Miranda, Raquel B. R. Mesquita, Andreia Leite, André Silva, Maria Rangel, António O. S. S. Rangel

PF 5. Monitoring soil/water interface: development of an integrated sequential injection system applied to laboratory scale soil core column and micro soil column
Letícia S. Mesquita, Raquel B. R. Mesquita, Maria Rangel, Andreia Leite, Tânia Moniz, António O. S. S. Rangel

PF 6. Bioacessibility of zinc in pet food determined by a dynamic leaching method
Sara R. Fernandes, Ana Margarida Pereira, Elisabete Matos, Francisco Castanheira, Cláudia S. Baptista, Ana Rita J. Cabrita, Marcela A. Segundo

PF 7. Polymer inclusion membranes (PIMS) as an alternative for on-line solid phase extraction (SPE) in flow analysis
Tânia C. F. Ribas, Charles F. Croft, Raquel B. R. Mesquita, M. Inês G. S. Almeida, Spas D. Kolev, António O. S. S. Rangel

G) METHOD VALIDATION

PG 1. Measurement uncertainties in platinum and rhodium quantification by adsorptive cathodic stripping voltammetry
Carlos E. Monteiro, Miguel Caetano, Antonio Cobelo-García, Margarida C. dos Santos

PG 2. Lipid oxidation assessment in lotions using the thiobarbituric acid reaction
Verónica Costa, Cristina Soares, Maria João Ramalhosa, Valentina F. Domingues, Cristina Delerue-Matos

PG 3. Quantification of tranexamic acid in human plasma: development and validation of UHPLC-MS/MS method
Luisa Barreiros, Júlia L. Amoreira, Sandia Machado, Sara R. Fernandes, Eduarda M. P. Silva, Paula Sá, Sibylle Kietaibl, Marcela A. Segundo

PG 4. HPLC analytical method development for apomorphine hydrochloride quantification
João Campos, João Cunha, Domingos Ferreira, Paulo Costa

PG 5. Validation of HPLC method for quantification of bosentan on two different oral vehicles
João Cunha, João Campos, Domingos Ferreira, Paulo Costa

PG 6. HPLC-MS/MS method for quantification of the neuropeptide Y Y1 receptor antagonist BIBP 3226 in cell extracts
Luisa Barreiros, Eduarda M. P. Silva, Inês S. Alencastre, Meriem Lamghari, Marcela A. Segundo

PG 7. Inclusion complex of *p*-chloro-thio-nor-β-lapachone in 2-hydroxypropyl-β-cyclodextrin and development of UV spectrophotometric analytical methodology-
Marcella de Sá Haddad Queiroz, Caroline Deckmann Nicoletti, Débora Omena Futuro, Vitor Francisco Ferreira

PG 8. Simultaneous determination of dapsone and clofazimine in nanoformulations by HPLC
Sandia Machado, Sara R. Fernandes, Luíse L. Chaves, Sofia A. C. Lima, Eduarda M. P. Silva, Luisa Barreiros, Salette Reis, Marcela A. Segundo

PG 9. Process characterization of ultrasonic welding of electrical wires
Sandra Matos, Fernando Veloso, Carlos Santos, Emanuel Carvalho, Leonardo Gonçalves

PG 10. Analytical microsystem for the spectrophotometric determination of titratable acidity in wines
Natalia Sandez, Antonio Calvo-López, Susana S. M. P. Vidigal, Julian Alonso-Chamarro, António O. S. S. Rangel

H) MODERN ANALYTICAL METHODOLOGIES

PH 1. Hollow fiber microextraction (HFμE) for ultra-trace analysis of polycyclic aromatic hydrocarbons in real matrices
A. H. Ide, J. M. F. Nogueira

PH 2. Comparison of an automatic chemiluminescence assay and closed bottle test for the determination of deep eutectic solvents' biodegradability
Ana F. D. C. Neves, André G. Vilaranda, Ana M. O. Azevedo, Susana P. F. Costa, Edite Cunha, M. Lúcia M. F. S. Saraiva

PH 3. Bioactive compounds, antioxidant activity and cell viability of *Actinidia arguta* infusions and decoctions
Ana Margarida Silva, Diana Pinto, Francisca Rodrigues, M. Beatriz P. P. Oliveira

PH 4. Toxicity assessment of low melting organic salts towards *Saccharomyces cerevisiae*
Ana M. O. Azevedo, Ana F. D. C. Neves, André G. Vilaranda, João L. M. Santos, Maria João Sousa, M. Lúcia M. F. S. Saraiva

PH 5. Metal accumulation in native flora: biodiversity prospecting for phytoremediation of contaminated aquatic environments
Cristina M. C. M. Couto, Cláudia Ribeiro, Ana Rita Ribeiro, Alexandra Maia, Mariana Santos, Maria Elizabeth Tiritan, Edgar Pinto, Agostinho A. Almeida

PH 6. Elemental impurities in lipsticks: a comparative study of Portuguese and Brazilian products
Edgar Pinto, Cristina M. C. M. Couto, Agostinho Almeida

PH 7. Photoluminescent nanohybrid probe based on distinct sized quantum dots for simultaneous determination of Hg^{2+}, Pb^{2+} and Cu^{2+} using multivariate chemometric tools
David S. M. Ribeiro, José X. Soares, S. Sofia M. Rodrigues, Ricardo N. M. J. Páscoa, Rafael C. Castro, João L. M. Santos

PH 8. Flours of melon seeds: effect of the oil extraction process on their nutritional composition and antioxidant activity
Diana Pinto, Anabela Costa, Manuel Alvarez-Orti, Adrián Rabadán, Francisca Rodrigues, José E. Pardo, M. Beatriz P. P. Oliveira

PH 9. Determination of moisture and protein content in banana "*nanica*" cultivated on Santa Cruz * Santiago island
Elba Monteiro, Sandra Freire

PH 10. Development of a UHPLC-MS/MS methodology for the determination of phenolic compounds in vine shoots
Manuela Moreira, M. Fátima Barroso, João Vasconcellos Porto, Simone Morais, Cristina Delerue-Matos

PH 11. Evaluation of enzymatic digestion conditions for determination of immunoglobulins by tandem mass spectrometry
Gabriela S. Guerra, Inês I. Ramos, Luísa Barreiros, Eduarda M. P. Silva, Marcela A. Segundo

PH 12. In vitro dissolution testing of an itraconazole amorphous solid dispersion: sink vs. non-sink-conditions
Inês Almeida, Filipe Vultos, Teresa Marta, Mafalda Paiva

PH 13. Tandem mass spectrometry characterization of nitrated cardiolipin
Javier-Fernando Montero-Bullón, Tânia Melo, M. Rosário M. Domingues, Pedro Domingues

PH 14. Green and chiral quantum dots
José X. Soares, David S. M. Ribeiro, Rafael C. Castro, S. Sofia M. Rodrigues, Ute Resch-Genger, João L. M. Santos

PH 15. Design and development of a microfluidic paper-based analytical device for calcium determination in saliva
Karina C. Acciainoli, Mafalda T. S. Silva, Raquel B. R. Mesquita, António O. S. S. Rangel

PH 16. Teaching analytical chemistry in Portugal, a new reality
Manuel Matos, Nelson A. Silva, Hugo F. Silva, José Coelho, Cristina Oliveira

PH 17. Screening of lipids by differential scanning calorimetry (DSC) for the production of lipid nanoparticles
Maria Inês Teixeira, Carla Martins Lopes, Maria Helena Amaral, Paulo Costa

PH 18. A fast and simple approach for screening sulfonamides in water
Patrícia S. Peixoto, Sandia Machado, Luisa Barreiros, Ana Machado, Adriano A. Bordalo, José L. F. C. Lima, Marcela A. Segundo

PH 19. Determination of trigonelline in coffee using low pressure ion pair chromatography with amperometric detection
Manuela Sousa, João Rodrigo Santos, Paulo Almeida, José A. Rodrigues

PH 20. Free formaldehyde determination in cosmetics containing formaldehyde donors
Pedro Francisco Brandão, Rui Miguel Ramos, José António Rodrigues

PH 21. Nanohybrid quantum dots probes for the determination of different metals in water
Rafael C. Castro, David S. M. Ribeiro, José X. Soares, S. Sofia M. Rodrigues, João L. M. Santos

PH 22. Proximal composition and vitamin E profile of germinated leguminous seeds
Rita C. Alves, Cátia Araújo, Anabela S. G. Costa, Sílvia Bessada, Clícia M. J. Benevides, Graça Soveral, M. Beatriz P. P. Oliveira

PH 23. Comparison of different plant infusions regarding total phenolics, total flavonoids and antioxidant activity
Juliana Peixoto, Anabela S. G. Costa, Rita C. Alves, M. Beatriz P. P. Oliveira

PH 24. Coffee silverskin potentialities as ingredient for diverse industries – state of the art
Rita Teixeira, Rita C. Alves, Francisca Rodrigues, M. Beatriz P. P. Oliveira

PH 25. Gas phase fragmentation confirms plastidial glycolipids in photosynthetic animals
Felisa Rey, Elisabete da Costa, Ana M. Campos, Paulo Cartaxana, Elisabete Maciel, Pedro Domingues, M. Rosário M. Domingues, Ricardo Calado, Sónia Cruz

PH 26. Comparison of biomimetic models of oxidized phosphatidylethanolamines using mass spectrometry analysis
Simone Colombo, Pedro Domingues, M. Rosário Domingues

PH 27. Fatty acids analysis to differentiate nutritional quality of similar bakery products
Tânia Gonçalves Albuquerque, Helena S. Costa, M. Beatriz P. P. Oliveira

PH 28. Contribution of nuts for the daily intake of salt, fat and fatty acids
Tânia Gonçalves Albuquerque, Helena S. Costa, M. Beatriz P. P. Oliveira

PH 29. Optimization of collision energy in tandem mass spectrometry for improving the identification of nitrated phospholipids
Bruna Neves, Tânia Melo, Pedro Domingues, Rosário Domingues

PH 30. Microfluidic paper-based analytical device (μPAD) for salivary ammonia/ammonium
Yanisa Thepchuay, Raquel B. R. Mesquita, Duangjai Nacapricha, António O. S. S. Rangel

I) SEPARATION PROCESSES

PI 1. Profiling biogenic amines in ruminal content
Liliana Cordeiro, Inês Maria Valente, Margarida R. G. Maia, António J. M. Fonseca, Ana Rita J. B. Cabrita, José António Rodrigues

PI 2. Chromatographic method for the assessment of vitamin B_1 and B_6 derivatives in whole blood
Jaroslav Jenčo, Lenka Kujovská Krčmová, Luboš Sobotka, Petr Solich

PI 3. A SALLE methodology for biogenic amines determination in food products of animal origin
Karen C. Almeida, Pedro F. Brandão, Rui Ramos, Luís M. Gonçalves, Arnaldo A. Cardoso, José A. Rodrigues

PI 4. Preparation of polysulfone membrane with α-tocopherol and α-lipoic acid to reduce oxidative stress
Michaela Kohlová, Célia Amorim, Alberto Araújo, Lucie Zelená, Petr Solich, Alice Santos-Silva, Conceição Montenegro

PI 5. Validated RP-HPLC and TLC - densitometry methods for the simultaneous determination of sulfacetamide sodium and two of its official impurities; sulfanilamide and dapsone
Nariman A. El Ragehy, Maha A. Hegazy, Ghada A. Sedik, Samia A. Tawfik

PI 6. Evaluation of encapsulation efficiency in methotrexate-loaded nanoparticles using separative methods
Sara S. Marques, Inês I. Ramos, Luísa Barreiros, Sofia A. C. Lima, Salette Reis, Maria R. Domingues, Marcela A. Segundo

PLENARY LECTURES

PL1

NOVEL APPROACHES FOR IN-VITRO BIOACCESSIBILITY & BIOAVAILABILITY OF NUTRIENTS AND CONTAMINANTS IN SOLIDS USING FLOW APPROACHES: PROS AND CONS

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This lecture is aimed at illustrating via representative examples the merits and pitfalls of flow-based approaches for miniaturization and automation of chemical bioaccessibility assays and *in-vitro* biovailability tests. The idea behind is to serve as invaluable tools to answer distinct biorelevant questions and provide insight into (i) the human exposomics of contaminants in potentially contaminated solids [1] and (ii) the nutritional value of natural products, genetically modified seeds and food supplements [2,3]. The lecture is divided in three parts: The first is devoted to pinpointing the underlying fundamentals of flow approaches against classical batchwise counterparts for in-line leaching tests as demanded in bioaccessibility testing to simulate worst-case scenarios [1]. The second part will focus on a variety of *in-vitro* oral bioaccessibility tests mimicking physiologically-based digestion conditions for investigation of the release of both elements and organic species in the gastric and gastrointestinal compartments using dynamic flow-extraction setups [3-5]. The third section is to be focused on novel biomimetic tools for automation of bioavailability assays using liposomes as cell-mimicking entities to investigate membrane permeation and the use of cell monolayers in Franz-Cell units connected to flow systems to investigate the interaction of drugs and/or contaminants with cell transporters [6,7].

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PL2

CHEMOMETRICS FOR (BIO)PHARMA PROCESS ANALYTICS

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The actual regulatory context of the pharmaceutical industry is gradually changing paving the way to the real transposition for a XXI century industry, specifically adopting the concepts of digitalization or industry 4.0 [1]. Examples of the changes operated in the last decade include the recent guidelines issued by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), Q8 to Q11 and the imminent release of Q12 (product life cycle management) [2]. Additionally, the primary and secondary manufacturing industry are gradually migrating from the more frequent batch processing to continuous processing, who ultimately requires a more scientific solid understanding of the underlying mechanisms governing manufacturing and relation with product attributes [3]. Linked with continuous manufacturing is the need for investment in real-time high-throughput process monitoring (to ensure process, drug and drug products quality) that can result on parametric release, thus reducing the burden in terms of quality control while fastening batches' approval. This area represents a challenge for analytical methods, that should gradually shift from the lab to the manufacturing areas. Process analytics are nowadays considered a commodity and therefore methodologies for appropriate data handling, management and efficient use are mandatory. Because first principles modelling approaches are typically difficult to implement as a pharmaceutical company typically processes multiple products and raw-materials, chemometric methods (data-driven) have been increasingly adopted [4]. Data becomes in this context more relevant, and consequently new challenges in terms of ensuring data integrity arise. Moreover, with the increasing introduction of biologics and structurally complex drug products (e.g., based on nanocarriers), manufacturing sites are being gradually equipped with highly selective hyphenated analytical methods that generate huge amounts of complex data that must be processed efficiently in order to allow for batch release [5]. This communication explores the challenges for chemometrics, promoted by the recent regulatory developments, from pharmaceutical development to manufacturing control and parametric release.

Acknowledgements: The author acknowledges iMed.ULisboa's grant UID/DTP/04138/2013.

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PL3

ELECTROCHEMICAL APPROACHES TO ANALYTICAL SENSING OF BIOMOLECULES

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The interaction of biomolecules, nucleic acids, peptides, proteins and pharmaceuticals, with solid electrode surfaces is not only a fundamental phenomenon but is also a key to important and novel analytical sensing applications in biosensors, biotechnology, medical devices and drug-delivery schemes.
Many compounds bind and interact with DNA causing changes in the structure of DNA and in the base sequence, leading to perturbations in DNA replication. The need for the analysis of gene sequences, the detection of oxidative damage to DNA, and the understanding of DNA interactions with molecules or ions led to the development of DNA-nanoscale electrochemical biosensors. DNA nanostructures provided a methodology for nanoscale electrode construction of patterned structures that self-assembled into periodic and aperiodic lattices and were characterised by atomic force microscopy.
The possibility of foreseeing the damage that different compounds can cause to DNA integrity arises from the pre-concentration of either the starting materials or of the redox reaction products on the DNA-electrochemical biosensor surface, thus enabling electrochemical probing of the presence of short-lived intermediates and of the oxidative damage they cause to DNA.
Peptides and proteins are essential components of organisms and are involved in a wide range of biological functions. Oxidative damage to peptides or proteins is considered to be one of the major causes of aging and age-related diseases. Electrochemical studies in qualitative and quantitative analysis of proteins, not containing a centre with fast-reversible redox reactions, are still very few.
Tumour cells, like most normal cells, have a high diversity of receptors on their surfaces. Molecules on the outside of the cell can attach to these receptors, causing changes within the cells. Immunotherapeutic drugs, monoclonal antibodies (mAbs), have been recently used in clinical oncology, as they recognise and lock on to specific antigen proteins on the surface of cancer cells, helping the body's immune system recognise the cancer cells and destroy them. The direct electrochemical oxidation mechanisms of some native and denatured anti-cancer mAbs, and *in situ* electrochemistry, enabled the detection of anti-cancer mAbs interaction with DNA, showing that they bind to the DNA but do not cause oxidative damage.
Detection of *in vitro* oxidative damage to DNA can be very useful for screening and evaluating the effect caused to DNA by carcinogens and oxidising substances, for which voltammetric methods are a miniaturisable, inexpensive and faster detection procedure, with very low detection limits.

Acknowledgments: Projects FCT (UID/EMS/00285/2013) and SUDOE Innovec'EAU (SOE1/P1/F0173).

PL4

IMMUNOANALYTICAL METHODS FOR SCREENING AND MONITORING IN ENVIRONMENTAL ANALYSIS

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A vast number of emerging pollutants has been detected in the environment over the last decades. Analytical methods suitable for trace analysis are needed that are desirably also fast, inexpensive and, if possible, robust and portable. Immunoanalytical methods which are available in a broad range of formats, can be profitably used here to analyze for the distribution and the trends of concentration levels of contaminants in the environment.
Some of these formats are single-analyte but high-throughput methods. In order to use them wisely, indicator substances, sometimes called anthropogenic markers, should be selected and used in screening approaches. Other methods are suitable to be performed on portable instrumentation in the field (*on-site*) or in facilities such as wastewater treatment plants for *on-line* monitoring. Furthermore, there are the so-called array technologies that allow for parallel analysis of several analytes of interest (multiplexing).
The microtiter-plate based ELISA (Enzyme-linked Immunosorbent Assay) is the method of choice for the analysis of a large number of samples [1]. ELISA screening data for anthropogenic markers such as the antiepileptic carbamazepine, the analgesic diclofenac, the anti-histaminic cetirizine, the steroid hormone estrone, the antimicrobial sulfamethoxazole, the stimulants caffeine and cocaine, the priority pollutant bisphenol A, and the bile acid isolithocholic acid [2] are presented.
For *on-site* screening and monitoring, simpler formats, like mix-and-read assays, e.g. the Fluorescence Polarization Immunoassay (FPIA) or Lateral-flow Immunoassays (LFIA) are more suitable tools. Electrochemical formats run on portable devices provide additional advantages as no light source is required. Some examples are presented and discussed.
The suitability of multi-analyte formats such as immunomicroarrays depends on the choice of a signal-producing system that provides small uncertainties and good reproducibility of the measurements. Biochip (“flat”) arrays read out on slide scanners and bead-based (“suspension”) arrays read out in flow cytometers are two options and show their distinct pros and cons.
Altogether these approaches show the great potential immunoanalytical methods provide for the screening for environmental contaminants in the aquatic environment.

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KEYNOTE LECTURES

KL1

LAB-ON-CHIP PLATFORMS FOR CHEMICAL AND BIOLOGICAL ANALYSIS

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Microfluidic lab-on-chip sensing platforms are currently being intensively studied for detection of bioanalytes (such as DNA, proteins, cells, metabolic products) in applications such as food safety, health monitoring and environmental control because of their compelling potential advantages, such as portability, speed, sensitivity, multiplexing, no need for highly skilled operators or laboratory infrastructure, and low cost.
I will use our work on the detection of mycotoxins (aflatoxin B1 (AFB1), deoxynivalenol (DON) and ochratoxin A (OTA)) produced by fungi that contaminate several sources of food and drink as a case study of an integrated lab-on-a-chip analytical system. I will start by describing our research involving the use of thin-film silicon photodetectors as optical transducers in integrated microfluidic biochips. These transducers are integrated with molecular recognition elements capable of specifically capturing the bioanalyte of interest. I will discuss the strategies that we use for this integration, and how the characteristics of the biosensor relate to the sensitivity of the detection. In addition, I will describe our strategies for simultaneous (multiplex) detection of various target molecules.
To take full advantage of the miniaturization of the biosensor, it is crucial in addition to address two other issues which I will discuss in some detail: (i) fluidic handling from sample to sensor and (ii) consideration of the interfering effects of the often chemically and physically complex biological sample matrix.

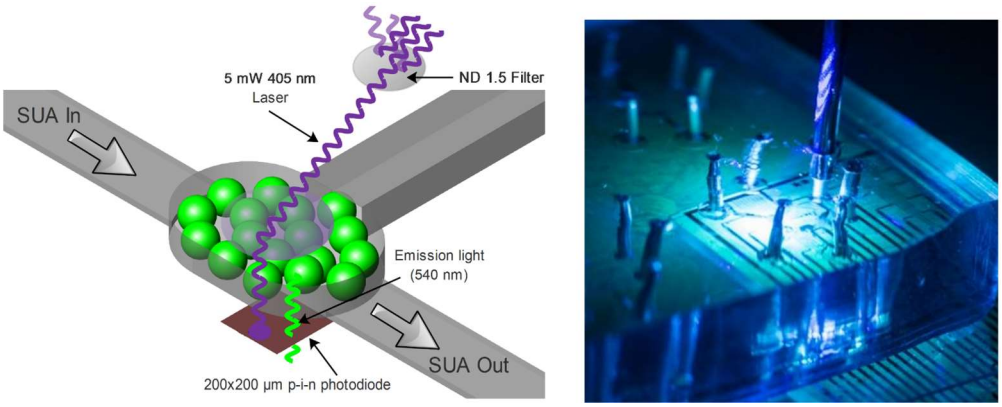

Figure 1: (left) schematic diagram of a microbead-based microfluidic biosensor with integrated optical detection; (right) photograph of a PDMS chip aligned to a microsensor array.

KL2

AN ANALYTICAL CHEMISTRY PERSPECTIVE OVER ANIMAL SCIENCE

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The world population increase rises the demand for food products, requiring the improvement on the efficiency of both agricultural and livestock systems. In the latter, efficiency have to be achieved simultaneously with the improvement of animal performance and the assurance of animal health and well-being [1]. In general, efficiency of cattle production is concerned with minimizing the amount of inputs and losses of nutrients or undesired outputs to produce a given quantity of meat or milk.
In ruminants, the end-products of rumen fermentation have distinct functions in animal metabolism. A vast group of volatile compounds is formed and can be related to the feed composition, acting as biomarkers to evaluate the feed conversion rate based on the analysis of rumen content, feces, urine and milk. Moreover, these volatile markers enable the diagnosis of digestive and metabolic upsets.
Current analytical protocols used during in vivo studies of feed conversion rate are laborious, time-consuming and require the use of animals surgically prepared with cannulas, making it unsuitable for routine and in situ analyses [2, 3]. For these reasons, innovative methods of sample collection and chemical analysis for in vivo monitoring of volatile compounds will positively impact further research in animal science.
In this presentation the role and importance of analytical chemistry and the development of new analytical solutions in the animal science field will be discussed, concerning the current state-of-the-art. Also, new developments on this area will be presented [4].

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KL3

BACK TO BASICS: RECENT TRENDS IN MICROEXTRACTION TECHNIQUES

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In the past three decades, the passive microextraction techniques has played an important role in analytical chemistry, as modern sample preparation approaches for trace analysis of emerging or priority compounds in complex matrices [1]. The main features of the passive microextraction techniques are the use of miniaturized devices, great simplification, easy manipulation, strong reduction or absence of the use of toxic organic solvents, selectivity and sensitivity enhancement, as well as low sample volume requirement, making them convenient for interfacing with the common chromatographic and hyphenated systems [2]. Some well-established examples are the passive liquid-based microextraction techniques, such as dispersive liquid-liquid microextraction (DLLME), single-drop microextraction (SDME) and hollow fibber liquid-phase microextraction (HF-LPME). On the other hand, passive solid-based microextraction or sorption-based techniques have also been proposed as effective alternatives for trace analysis, like solid phase microextraction (SPME), stir bar sorptive extraction (SBSE) and, more recently, bar adsorptive microextraction (BAμE) [3]. Despite all of this, one cannot simply use a single technique as a universal approach, but the most suitable technique should be selected according to the target analytes and matrix involved. Furthermore, some of these techniques are neither user-friendly, eco-friendly or cost-effective nor suitable for the routine work. In general, the liquid-based microextraction approaches (*i.e.* DLLME, SDME and HF-LPME) present fast kinetics, use very simple apparatus and are costly-effective. On the other hand, the solid-based microextraction techniques (*i.e.* SPME, SBSE and BAμE) are easier to manipulate, more environmental-friendly, allow automation although need a back-extraction stage, which is not attractive since requires time-consuming steps particularly if liquid desorption (LD) is implemented. Furthermore, this drawback is more pronounced if reusable devices are adopted, making the LD the limitative stage. For all these reasons, novel ideas and concepts are welcome, especially if using simple analytical strategies.
In this contribution, the main advantages and limitations of the most used microextration techniques will be discussed, as well as proposing basic concepts using eco-user-friendly and cost-effective approaches that simultaneously could be dedicated for routine analysis [4,5].

Acknowledgements: The author wishes to thank Fundação para a Ciência e a Tecnologia (Portugal) for financial support (Project: UID/MULTI/00612/2013).

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KL4

MERCURY MONITORING IN WATERS: IS IT POSSIBLE TO MEASURE EASILY AT TRACE LEVELS?

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According to the Water Framework Directive and Marine Strategy Framework Directive, EU Member States establishes monitoring programs for assessment of the environmental status of their marine waters on the basis of an indicative lists of different elements. Mercury is classified as a dangerous substance and its level in waters need to be monitored. The total mercury concentrations in seawater between a few to hundreds ng L^{-1} have been reported in several works and a broad concentration range can be found in the literature [1]. Facts such as low Hg concentration, the complexity of water matrices and mercury's high volatility, losses or contamination of the sample during sampling, storage and pre-treatment of samples or inadequate analytical techniques, may generate erroneous data. Hence reliable determinations of the analyte are a major analytical challenge. Present work proposes a simple and reliable methodology for mercury determination in natural waters using iron oxide nanoparticles coated with silica shells functionalized with dithiocarbamate groups (Fe_3O_4@SiO_2SiDTC) for extraction and pre-concentration of mercury [2-3]. 10 mg L^{-1} of Fe_3O_4@SiO_2SiDTC nanoparticles (NPs) were able to remove more than 80% of 10-500 ng L^{-1} in model Hg^{II} solutions (ultra-pure water and artificial seawater) within 24 hours. Mercury sorbed to the NPs is measured directly by thermal decomposition atomic absorption spectrometry with gold amalgamation. The detection limit of approximately 1.8 ng L^{-1} is below the values reported in the literature. Furthermore, real water samples were analysed and good recoveries were obtained from 90%.

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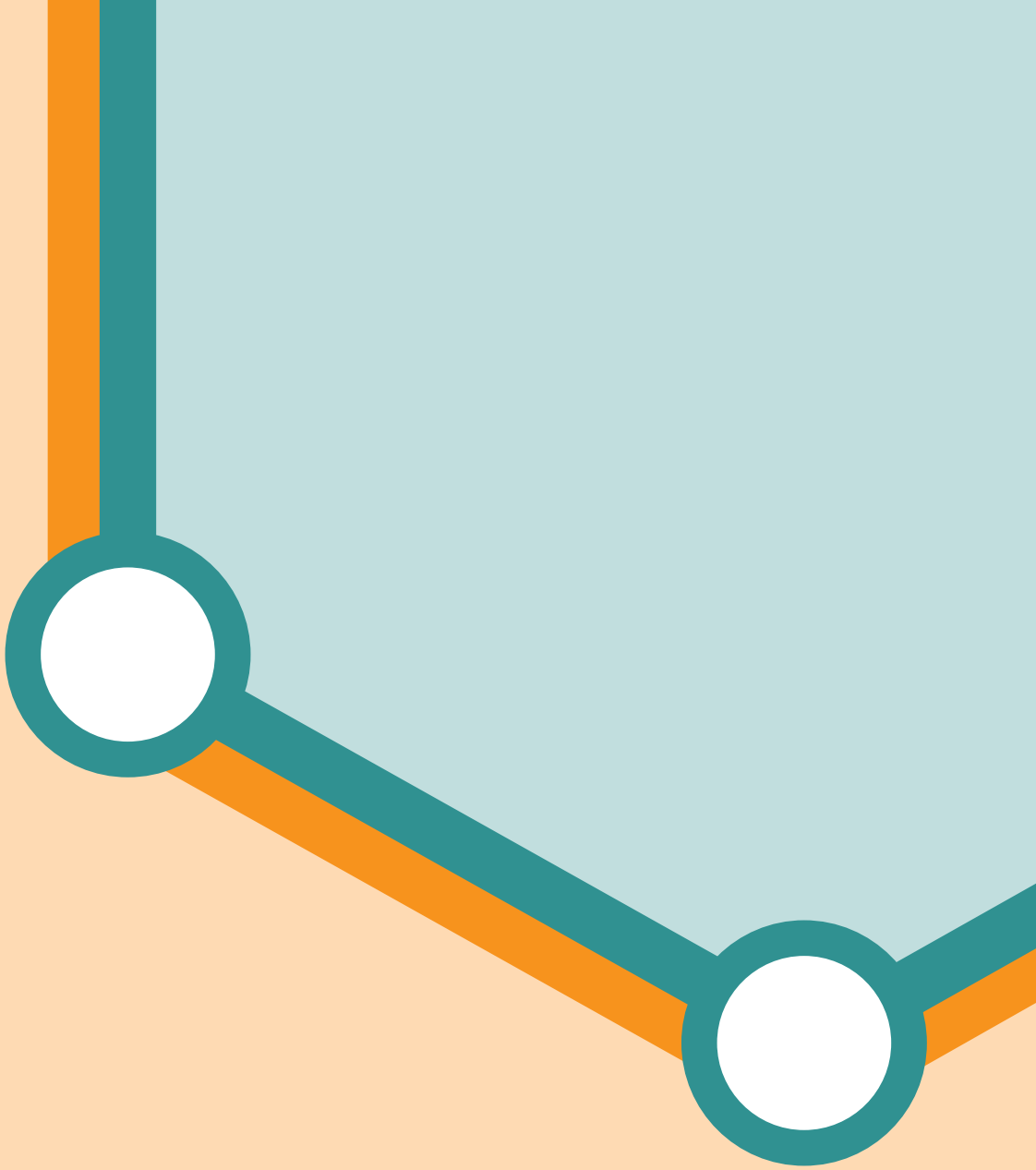

ORAL COMMUNICATIONS

OC1

QUANTITATIVE AND QUALITATIVE ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS IN URINE SAMPLES USING A NON-SEPARATIVE METHOD BASED ON MASS SPECTROMETRY

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In this work, a method for the quantitative and qualitative analysis of 11 polycyclic aromatic hydrocarbons (PAHs) in urine samples is reported. The method is based on the coupling of a programmed temperature vaporizer (PTV) with a quadrupole mass spectrometer (qMS), via a deactivated fused silica tubing. Before the PTV-qMS analysis, the samples were subjected to a liquid-liquid extraction (LLE).

The method was rapid since no chromatographic separation was performed. The samples were introduced directly into the PTV, and the analytes were trapped in the Tenax-TA® packed liner while the solvent was purged. After that, all the compounds reached the mass spectrometer, obtaining the fingerprint of the analysed samples.

Urine samples free of PAHs and the same samples spiked with the compounds were analysed. The resulting profile signals were used to quantify the analytes using multivariate calibration, and to classify the samples according to the presence or absence of the PAHs. In the latter task, non-supervised and supervised pattern recognition techniques were employed. The calibration models worked satisfactorily and errors lower or equal to 15 % were obtained, in most cases, when an external validation set was analysed. Regarding the classification of the samples, most of the supervised pattern recognition techniques provided excellent results (100 % success), where all of the samples were classified correctly.

OC2

THE KILOGRAM AND THE MOLE REDEFINITION AND METROLOGICAL TRACEABILITY

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As science advances and methods of measurement are refined, the definitions of the base units used to express the various kinds of quantities must be revised. A variety of experimental methods generally described by the Bureau International des Poids et Mesures (BIPM) Consultative Committees may be used to realize the definitions. In 2011 the General Conference on Weights and Measures (CGPM) noted the intention of the International Committee for Weights and Measures (CIPM) to revise the entire International System of Units (SI) by linking all seven base units (kilogram: mass/kg; metre: length/m; second: time/s; ampere: electric current/A; kelvin: thermodynamic temperature/K; mole: amount of substance/mol; candela: luminous intensity/cd) to seven fundamental physical constants. This is perhaps the most significant revision in the SI since its establishment and it is expected the New SI will be announced at the 26th CGPM in 2018. To be of practical use, these units also must be realized physically for dissemination, ensuring metrological traceability.

The kilogram and the mole are of special interest to chemists and the International Union of Pure and Applied Chemistry (IUPAC) has confirmed its support for the redefinition project.

The kilogram is the only base unit where the definition is still based on an artefact, the International Prototype of the Kilogram (IPK), a cylinder with diameter and height of about 39 mm, made of an alloy of 90 % platinum and 10 % iridium, conserved at the BIPM since 1889. The proposed definition fixes the numerical value of the Planck constant, *h,* to be 6.626 070 040 × 10^{-34} kg m^2 s^{-1}. A Watt balance links *h* to the mass of the IPK, $m_{IPK,}$ by measuring the ratio h/m_{IPK} with a measurement uncertainty of 2 parts in 10^8, that is 20 µg in 1 kg [1, 2]. The mole is the amount of substance of a system which contains as many elementary entities as there are atoms in 0.012 kilogram of carbon 12. The proposed definition states that one mole contains exactly 6.022 140 76 × 10^{23} elementary entities. This number is the fixed numerical value of the Avogadro constant, N_A, when expressed in mol^{-1}, and is called the Avogadro number. An elementary entity may be an atom, a molecule, an ion, an electron, any other particle or specified group of particles. While this conceptual change does not bring any immediate practical benefits to our ability to better realize the mole, it realigns the definition of the mole with the way most chemists understand it [1,2].

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OC3

WHAT IS THE NEW SI AND WHAT DOES IT CHANGE FOR ANALYTICAL CHEMISTRY?

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In November 2018, at its 26th meeting, the *Conférence Génerale des Poids et Mesures* (CGPM) will adopt the new System of Units ("new SI"), that will no longer be based on artefacts but on constants of Nature [1]. This is the biggest change that happens to the SI since its creation, in 1960. It is motivated by the observed drift of the value of the last base unit, which is still defined by an artefact, the kilogram, a divergence of 50 μg in a century. This is also motivated by the abandon of the definition of the unit of the electric current, for the benefit of using both quantum Hall effect and Josephson effect, easily enabling to reaching 10^{-8} relative uncertainties. However, it is worth noticing that defining base units from universal constants required the determination of the numerical values of these constants, within accuracy acceptable for the international scientific community: at the 25th CGPM, occurred in 2014, it was foreseen to be adopted the new SI, but the experimental results obtained by different international institutes were not robust enough. [2]
In this communication, we intend to briefly display the determination of the numerical values of the fundamental constants defining the kilogram and the mole, the most used quantities in the field of analytical chemistry, namely the Planck constant and the Avogadro constant. We will show how the results obtained by different institutes using experiences of different principles went to with results within the 10^{-8} expected stabilities values [3].
As announced in previous communications at Congresses of the Portuguese Chemical Society [4-6], the definition of the mole is no longer based on the kilogram, which has some consequences on the meaning of the unit. Indeed, it corresponds to the conception of the mole as a multiple of a number of entities, which is one of the interpretations of the quantities used before the mole, termed "atom-gram" and "molecule-gram" [7]. In fact, with the new definition of the mole, any set of entities can be gathered in moles, for instance electrons, atoms or even photons, as soon as they are in multiple numbers of $6.022\ 140\ 76 \times 10^{23}$ [8].

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OC4

MONTE CARLO METHOD FOR UNCERTAINTY EVALUATION: A SIMPLE & RELIABLE METHOD FOR COMPLEX MEASUREMENTS

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Dredging of sediments is essential to maintain the navigability of rivers and coastal waters. In Portugal, most of the dredged material is deposited in the sea. However, if sediments are not heavily contaminated, can be used for specific purposes [1]. The annex III of Portaria nº 1450/2007 regulates the uses of dredged sediments.
Statistics point to 85 % of heavy metals released into the aquatic environment are accumulated in sediments surface [2]. Taking into account the serious adverse effects caused by these chemical elements, sediments must be monitored before their use.
The Division of Marine Chemistry and Pollution of 'Instituto Hidrográfico', Designated Metrological Institute for measurements in sediments, has developed procedures to determine metals in sediments by atomic absorption spectrometry. These procedures involve a prior microwave digestion of samples using a strong or a less vigorous extraction before spectrometer quantification. The measurement procedures were validated to prove that produced measurements are fit for the intended use.
For the validation of the measurement procedure, a metrological evaluation of measurements requirements is indispensable, which gives a verdict on the procedure's suitability and on the quality of their measurements. In this work, the validation of the measurement procedure involved the assessment of several performance parameters including the evaluation of the measurement uncertainty performed by two top-down approaches proposed in the Eurachem [3] and Nordtest [4] guides, and by the differential approach [5]. The quantification and combination of the uncertainty components of the differential approach were performed using Monte Carlo simulations.
Comparing the results of both top-down approaches with the differential approach ones, it was concluded that the uncertainties estimated by the top-down approaches are larger. The top-down and differential estimates of the measurement uncertainties proved to be valid since the results, reported with uncertainty, of the analysis of Certified Reference Materials are compatible with the reference value and the relative expanded uncertainty is smaller than a target (maximum admissible) value of 25 %.
The novel Monte Carlo implementation of the Differential Approach has revealed to be a promising strategy for the evaluation the uncertainty of complex measurements.

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OC5

IN-LINE MONITORING OF GREEN SYNTHESIS OF A NOVEL LAMIVUDINE-THEOPHYLLINE CO-CRYSTAL USING FT-IR SPECTROSCOPY AND MCR-ALS

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Pharmaceutical co-crystals are crystalline complexes of active pharmaceutical ingredient (API) and at least one coformer, in order to improve tablet solubility and increase its bioavailability. This work reports a green synthesis of a novel lamivudine-theophylline co-crystal in solid-phase through of the mixture between lamivudine (API) and theophylline (coformer) in the proportion of 1:1 by grinding in a ball mill. Fourier transform-infrared (FT-IR) spectroscopy and multivariate curve resolution with alternating least-squares (MCR-ALS) were employed as non-destructive analytical method for in-line monitoring of the evolution of co-crystal synthesis [1]. FT-IR spectra were collected after 5, 10, 15, 30, 35 and 60 minutes of reaction and posterior analyzed by MCR-ALS, according to the flowchart depicted in Figure 1. The concentration profiles retrieved by MCR-ALS allow to: (1) identify of the end of the reaction and (2) understand the mechanism involved in synthesis of lamivudine-theophylline co-crystal.

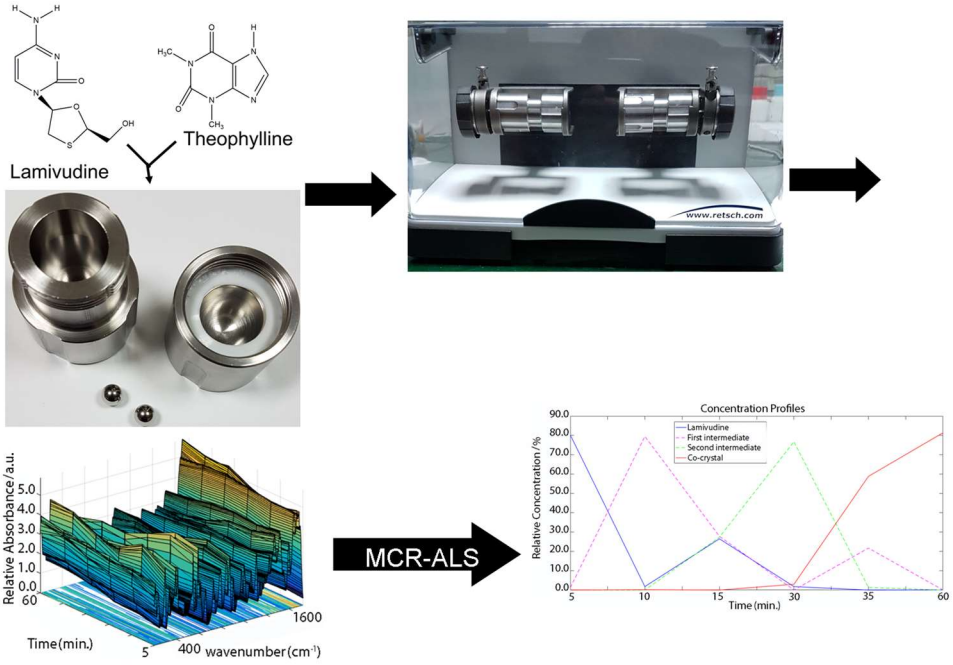

Figure 1: Flowchart of the experimental procedure for monitoring the synthesis of pharmaceutical lamivudine-theophylline co-crystal.

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OC6

APPLICATION OF NEAR- AND MID-INFRARED SPECTROSCOPY COUPLED WITH CHEMOMETRICS FOR THE DISCRIMINATION OF *Humulus lupulus VARIETIES*

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Humulus lupulus, known as hop, is an important ingredient for the production of beer and it has a strong impact in the final organoleptic properties of beer [1]. Currently, there is an increase interest of brewers in specific hops' varieties. However, each hop variety has its market value and there are several high valued varieties. Therefore, its authentication is essential to prevent fraudulent practices. In this work, near and mid-infrared spectroscopy were applied in the discrimination of 33 commercial hop varieties (which represent around 75% of the total volume commercialized worldwide). The hop samples were differentiated through DNA-based single nucleotide polymorphism (SNP) characterization. The spectra of five samples for each hop variety were acquired by both techniques. The chemometric tools used included principal component analysis (PCA), hierarchical cluster analysis (HCA) and partial least squares discriminant analysis (PLSDA). Both NIR and MIR data were pre-processed using different techniques and studied in different spectral regions. The PCA revealed the formation of clusters according to hops variety in both NIR and MIR data using the best pre-processing technique and spectral region. The PLSDA gave around 94% and 97% of correct hop varieties classifications for NIR and MIR data, respectively. Both techniques revealed a very good accuracy for the discrimination of hop varieties and compared with reference procedures are rapid, non-destructive and non-expensive alternatives.

Acknowledgements: Authors are grateful to the projects NORTE-01-0145-FEDER-000011 and UID/QUI/50006/2013-POCI/01/0145/FEDER/007265 with support from FCT/MEC through National funds and co-financed by FEDER, under the Partnership Agreement PT2020. This work received financial support from the European Union (FEDER funds through COMPETE POCI-01-0145-FEDER-016735) and National Funds (FCT) through project PTDC/AGR-PRO/6817/2014. R.N.M.J. Páscoa thanks FCT (Fundação para a Ciência e Tecnologia) and POPH (Programa Operacional Potencial Humano) for Post-Doc Grant SFRH/BPD/81384/2011. The authors thank as well "Os Três Cervejeiros, Lda" for providing the samples.

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OC7

HIGH THROUGHPUT BAR ADSORPTIVE MICROEXTRACTION (HT-BAμE) - A NEW EFFECTIVE APPROACH TO SCREEN BENZODIAZEPINES IN BIOLOGICAL MATRICES

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In the last decades public concern has emerged with regards to the chronic and/or acute consumption of benzodiazepines for medical and/or non-medical purposes, once many symptoms of toxicity due to their exacerbated consumption have been reported, including death [1, 2]. For these reasons, there is a need for innovative analytical approaches that allow a robust and effective monitoring of these substances in biological matrices such as blood, plasma, serum or urine.
In this contribution we present the development, optimization and application of high throughput bar adsorptive microextraction (HT-BAμE; figure 1) in combination with microliquid desorption and conventional high performance liquid chromatography-diode array detection, as a new effective approach to screen benzodiazepines in biological matrices. This novel methodology was dedicated for the analysis of diazepam, prazepam, bromazepam, oxazepam, lorazepam, alprazolam, temazepam and loflazepate in blood, plasma, serum and urine matrices. The device has the possibility of accommodating 100 sampling vials for the simultaneous microextraction and subsequent back-extraction stages. The data shows that average sample preparation time of around 2 min per sample was achieved (recoveries > 60 % for all target compounds), demonstrating that the proposed approach has great potential for further applications in biological matrices.

Figure 1: High throughput bar adsorptive microextraction (HT-BAμE) device operating under ultrasonic bath for the simultaneous microextraction of 100 samples.

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OC8

SEQUENTIAL INJECTION ANALYSIS IN SCREENING OF EXTRACTION PROPERTIES OF NANOFIBERS

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Electrospun nanofibers (NF) have gained a lot of attention during the last years due to their unique features such as a large surface-to-volume ratio, possibility of using natural materials, and the possibility of customized chemical modification. Apart from their novel implementation e.g. in medicine [1], NF are currently in the focus of analytical chemists for their great potential as a sorbent in solid phase extraction (SPE) techniques [2].

Sequential injection analysis (SIA) system comprising a piston pump, a switching valve, and a suitable detector is an advantageous tool to study the potential of NF as extraction sorbent offering simple operation, flow manipulation, and fast results evaluation. In this work, we studied handling, packing, and use of different geometries and devices to engage NF in a SIA system for the first time. Both planar arrangements and column format were studied. A specially designed 3D-printed holder allowing to house a single sheet of NF was chosen as the most suitable device due to the low amount of NF required, low backpressure, and minimized dead volume compared to the column format.

The extraction capacities of polyvinylidene fluoride, polycaprolacton/polyvinylidene fluoride composite, polyacrylonitrile and polyamide NF were tested with model analytes differing in their physical-chemical properties. Retention of the molecules was evaluated from peak height measurements and the results will be presented.

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OC9

DETERMINATION OF BENZOPHENONE & RELATED COMPOUNDS BY TAPE ADSORPTIVE MICROEXTRACTION

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The daily exposure to sunlight and the awareness about the risks of it, has increase the use of personal care products containing ultraviolet (UV) filters, such as, sunscreens, skin lotions, hair sprays, lipsticks, among others. The most common organic compounds used as UV-filters are benzophenone and its derivatives. Due to intensive usage, these compounds can easily reach environment and, several toxicity studies have shown that they are hazardous to plants, mutagenic in some bacteria cell systems and are linked to cancer and endocrine disruption in humans [1].
Recently, bar adsorptive microextraction (BAμE) was introduced as a novel sample enrichment approach and has already proved to be remarkable alternative for trace analysis of medium-polar to polar compounds in aqueous media, being already successfully applied for trace analysis of several classes of emerging or priority compounds with high effectiveness. BAμE that operates under the floating sampling technology present several advantages such as the possibility to choose the most suitable sorbent phase (*e.g*. activated carbons, polymers, etc.) for each particular type of application [2]. In this contribution, a novel enrichment technique, tape adsorptive microextraction (fig. 1) followed by microliquid desorption combined with high performance liquid chromatography-diode array detection (TAμE-μLD/HPLC-DAD), is proposed to monitor benzophenone and related compounds in aqueous media. By using this new approach, some improvements were introduced, including the downsizing of the analytical device, as well as the reduction of the solvent volume of the back-extraction stage. These new advances allow the elimination of the solvent switch step, making possible the back-extraction in one single step and turning the manipulation much simpler.

Figure 1: Tape adsorptive microextraction.

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OC10

ENVIRONMENTAL RELEVANCE OF PLATINUM AND RHODIUM IN TAGUS ESTUARY

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Platinum-group elements (PGEs) are considered emergent contaminants in the environment, due to their increasing usage over the past decades in a variety of technology-based activities. For this reason, PGE environmental concern has raised. Despite being well characterised in urban environments, Pt and Rh remain poorly understood in aquatic systems. The aims of this study were to identify their sources and to assess spatial distribution in sediments from Tagus estuary.

Superficial sediments were collected in 72 stations covering potential different Pt and Rh sources. Acid digested samples were analysed by Adsorptive Cathodic Stripping Voltammetry. Master environmental parameters were also determined to better understand metal variability.

Concentrations of Pt and Rh in superficial sediments ranged 0.18–5.1 ng Pt g^{-1} and 0.019–1.5 ng Rh g^{-1}. According to both elements spatial distribution, four PGE point source areas were defined: industrialised, motorway bridges, waste- (WWTP) and pluvial discharges and 'PGE-clean' areas. Two main origins of Pt and Rh to the Tagus estuary were identified: historical industrial activities and automotive catalytic converters (ACC). The highest concentrations were found in industrialised areas as in the surroundings of a long motorway bridge. Sediments from waste- (WWTP) and pluvial waters discharge sites presented concentrations similar to those found in other parts of the estuary ('PGE-clean' areas). However, a distinct signal was found in two WWTP outfalls than in upstream/downstream nearby stations. This signal was attributed to the urban runoff. Despite Pt/Rh ranged 0.48–9.7 within the estuary, in the typical range for ACC (5–16), no distribution pattern could be directly associated with ACC emissions. Additionally, two elevated values were found at the inactive industrialised area. Significant correlations were found for Pt with Al, Fe, Mg and LOI contents, while for Rh no relationships were found. This distinct behaviour suggests a differentiate reactivity of both PGE elements. Furthermore, hydrodynamics of Tagus estuary (mesotidal) coupled with other physical mechanisms may be key factors driving the transport of these emerging contaminants.

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OC11

TERNARY SEMICONDUCTOR QUANTUM DOTS FOR CHEMICAL ANALYSIS

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Binary semiconductor quantum dots, such as CdSe, CdTe or CdS nanocrystals, have gathered an indubitable reputation as excellent photoluminescence probes in a variety of (bio)chemical applications. Able to provide enduring photostability, high quantum yields, wide range tunable emission wavelengths, simplicity of conjugation with selected molecules, tailored surface reactivity and wide absorption and sharp emission bands they were applied in the analysis of pharmaceuticals, environmental pollutants, food contaminants, biomarkers, etc.

Despite their huge analytical potential, these nanomaterials were also affected by toxicological issues arising from the use of hazardous heavy metals in their binary composition, which not only restrained the prospects of a wider application but motivated at the same time the search for more environmental friendly and innocuous materials. In recent years a new family of multicomponent nanocrystals has emerged, with ternary and quaternary composition, which retain most of the unique features of binary nanocrystals while offering some new interesting potentialities.

This work seeks to discuss the synthesis and characterization of ternary quantum dots, usually composed of I-II-VI elements (I = Cu, Ag; II=In, Sn, Ga; VI = S, Se, Te, etc) like $CuInS_2$, $CuInSe_2$ and the $AgInS_2$, and to appraise their potential in bioanalytical or imaging applications.

OC12

MICROFLUIDIC PAPER-BASED DEVICES AS DISPOSABLE, EASY-TO-USE, REAL-TIME QUANTIFICATION METHODS

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The demand for faster, user friendly, ready-to-use, and still accurate monitoring techniques has been increasing. The idea of having a solution on hand for instant analysis is appealing and has been gaining relevancy.
In this context, microfluidic paper-based analytical devices (μPADs) have, in recent years, provided a novel approach for conducting inexpensive, on-site analyte determinations. This approach could become an attractive alternative to the current monitoring techniques requiring specialized skills, laborious laboratory processes, or/and expensive equipment. The μPADs small dimensions, minimal consumption of both reagents and sample, together with employing inexpensive materials and ease of operation, has made them ideally suited for unskilled operators and regular monitoring.
The use of digital scanning as detection process has enabled to maintain the accuracy and reliability of the analysis in opposition to other paper-based visual indication techniques, with a positive/negative or concentration range response. Colourimetric reactions are generally employed as the concentration of analyte can be related to the colour intensity, which can be easily measured using a flatbed scanner and computer software (i.e. ImageJ) [1].
Overall, the analytical performance of the μPADs makes them quite attractive for rapid on-site analysis in many fields, namely environmental and biological samples. In this work, an overview of the advantages and limitations of this emerging quantification method is presented. The potential and versatility of μPADs is highlighted with some applications to both natural waters and saliva samples: determination of iron(III) in natural waters; calcium and ammonia determination in saliva.

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OC13

IN SITU SENSING OF DNA-ANTILEISHMANIAL DRUG MILTEFOSINE INTERACTION USING A DNA-ELECTROCHEMICAL BIOSENSOR

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Leishmaniasis is one of the most important parasitic neglected disease and its treatment remains a challenge due to problems, such as high cost of drugs, high drug-dosage, incidence and prevalence of drug resistance, side-effects, and lack of affordable new antileishmanial drugs. Miltefosine (hexadecylphosphocholine), an alkyllysophospholipid analogue, Figure 1, is one of the drugs used in the treatment of leishmaniasis.

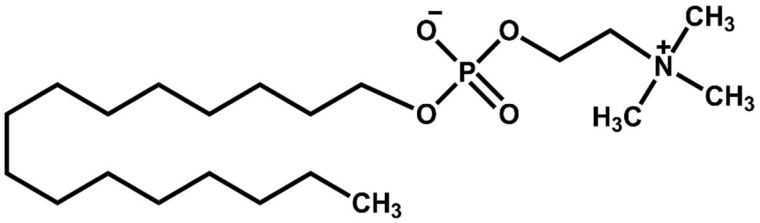

Figure 1: Chemical structure of miltefosine.

The electrochemical evaluation of the antileishmanial drug miltefosine-dsDNA interaction in incubated solutions, by differential pulse voltammetry and ultraviolet-visible spectroscopy, and *in situ* using dsDNA-, poly[G]- and poly[A]-electrochemical biosensors, for different time periods, was investigated.

The effect of the miltefosine-dsDNA interaction was electrochemically followed comparing the changes in the oxidation peaks of guanosine and adenosine residues, in the absence and presence of miltefosine, and monitoring the occurrence of free guanine and free adenine oxidation peaks, and the purine biomarkers: 8-oxoguanine, guanine oxidation product, and 2,8-dihydroxyadenine, adenine oxidation product.

The miltefosine-dsDNA interaction mechanism occurred in two ways. The miltefosine interaction independent of the dsDNA sequence, producing a rigid miltefosine-dsDNA complex structure and condensation/aggregation of DNA strands, was detected.

The preferential interaction, between miltefosine and the guanine hydrogen atoms, in the C–G base pair on the DNA strands, caused the release of guanine residues. Nevertheless, in the experimental conditions used, miltefosine did not induce oxidative damage to DNA.

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OC14

LAYER-BY-LAYER LABEL-FREE BIOSENSOR FOR IMPROVED GLUCOSE SENSING USING POLY(3,4-ETHYLENE-DIOXYTHIOPHENE) CONDUCTING POLYMER

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Poly(3,4-ethylenedioxythiophene)(PEDOT) is a conjugated, conducting polymer, here used for layer-by-layer (LbL) self-assembly in the development of a new glucose label-free biosensor. Glucose oxidase (GOx) was incorporated in a multilayer film on a gold electrode, previously modified with a porous film of PEDOT by electro-polymerization. Multilayer films, containing GOx and nitrogen-doped graphene (NG) dispersed in the biocompatible positively-charged polymer chitosan {chit$^+$(NG + GOx)}, together with the negatively charged poly(styrene sulfonate), PSS$^-$, were assembled [1].

Electrochemical voltammetric measurements and impedance spectroscopy were employed for biosensor characterization. LbL film growth was monitored by surface plasmon resonance (SPR), see Fig. 1, in order to evaluate the interactions involved in the assembly of the biomolecules. Atomic force and scanning electron microscopies confirm the adsorption of the film and reveal its morphological structure.

The analytical properties of the biosensor were determined by fixed potential amperometry and showed a substantial improvement in biosensor sensitivity in the presence of PEDOT. The biosensor operates at a low potential of −0.2 V vs. Ag/AgCl, with a detection limit of 41 μM. Biosensor applicability was evaluated by measuring the glucose content in wines obtained from Romanian grapes (*Vitis vinifera*).

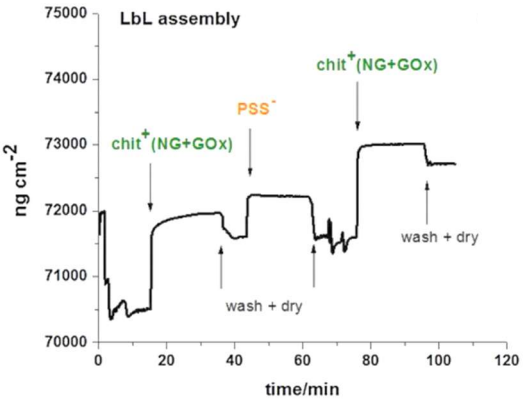

Figure 1: PEDOT thin film deposition onto Au surface and step-by-step assembly of the LbL biosensor, in the absence of NG, monitored by fixed angle SPR.

Acknowledgements: projects UID/EMS/00285/2013 and PTDC/QEQ-QAN/2201/2014 (FCT).

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OC15

ELECTROCHEMICAL ENZYME INHIBITION BIOSENSOR PLATFORMS FOR THE DETECTION OF TOXIC SPECIES

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The activity of many enzymes is inhibited by toxic species, often in a reversible way, that enables regeneration of enzyme activity. For this reason, there has been significant growth in the number of electrochemical enzyme inhibition sensors to measure the toxicity of different species in food and beverages and in environmental samples. The enzymes are immobilised on electrode surfaces either directly or after surface modification by nanostructured materials. Such materials are used to increase surface area, confer electrocatalytic effects on the reduction or oxidation of the enzymatic reaction products and enable efficient and effective immobilisation of the enzymes. Inhibition reduces biosensor response and gives rise to a toxicity index, but usually the immobilised enzyme is affected to varying extents by different toxic species. Selectivity can usually be achieved by this and because in real situations there is often predominance of one species that reduces enzyme activity.
We have developed enzyme-modified electrodes incorporating nanostructured electroactive polymers and/or carbon nanomaterials or gold nanoparticles in the sensor platform employing a layer-by-layer strategy e.g. [1-3]. These modifier combinations can lead to synergistic effects in biosensor analytical performance and to very low detection limits of the inhibitor species. Enzyme biosensors with nanomaterials developed by us have incorporated the enzymes peroxidase, catalase, glucose oxidase, xanthine oxidase and choline oxidase. They have been applied as enzyme inhibition sensors for pesticides or heavy metal ions, or for other toxic compounds such as bisphenol A. These inhibition enzyme biosensors will be compared, with emphasis on selectivity, and the inhibition mechanism and biosensor regeneration will be discussed.

Acknowledgements: FCT projects UID/EMS/00285/2013 and PTDC/QEQ-QAN/2201/2014, Marie Curie IRSES “SmartCancerSens”, and SFRH/BPD/103103/2014 (FCT).

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OC16

ELECTROCHEMICAL GENOSENSING OF THE MAIZE ENDOGENOUS GENE USING Fe_3O_4@Au NANOPARTICLES AS MAGNETOPLATFORM

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Core/shell iron oxide/gold (Fe_3O_4@Au) magnetic nanoparticles (MNPs) possess distinct physical and chemical attributes that make them excellent scaffolds for the fabrication of novel electrochemical (bio)assays [1]. Several strategies can be used to prepare monodispersed Fe_3O_4 MNPs, namely coprecipitation of Fe^{3+} and Fe^{2+} in an aqueous alkaline solution, or thermal decomposition of an iron complex precursor (such as iron(III) acetylacetonate) in high-boiling organic solvents [2]. The possibility of coating the magnetic core with a gold shell is associated to an enhancement of the chemical stability and more importantly, This MNPs exhibits a good biocompatibility and affinity to amine/thiol groups [3]. Taking advantage of the attributes of the Fe_3O_4@Au, this MNPs was used as a nanomagnetoplatform for the development of a sensitive electrochemical genoassay for the detection of a specific sequence from maize, the endogenous HMGA gene, which can serve as reference in the quantitation of genetically modified organisms.

The design of this genoassay consisted on several steps, namely i) Fe_3O_4@Au surface modification via binary self-assembled monolayers (SAM) composed by mercaptohexanol (MCH) and mercaptohexanoic acid (MHA), ii) covalent immobilization of aminated DNA capture probes, iii) hybridization of complementary DNA sequence by using a sandwich format assay with enzymatic labels, iv) electrochemical signal detection by chronoamperometry. The best analytical conditions were used to study the relationship between electrochemical signal and DNA target concentration.

This novel electrochemical paramagnetogenoassay was successfully applied for the cereal species detection by targeting the presence of maize in transgenic maize flour after PCR amplification.

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OC17

AUTOMATED APPROACH FOR THE SEQUENTIAL INJECTION DETERMINATION OF THE HERBICIDE 2,4-D BASED ON ONLINE PRECONCENTRATION USING A POLYMER INCLUSION MEMBRANE

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A highly sensitive sequential analysis system has been developed, optimized and validated for the trace determination of the herbicide for 2,4-dichlorophenoxyacetic acid (2,4-D) in natural waters, which uses a polymer inclusion membrane (PIM) containing 20% Aliquat 336 as the carrier, 70% poly(vinyl chloride) as the base polymer and 10% 1-tetradecanol as a modifier. PIMs are extracting liquid membranes which offer improved stability compared to supported liquid membranes [1,2]. They have been used successfully for on-line extractive separation in flow analysis because the extraction and back-extraction processes take place simultaneously on the two sides of a PIM [3,4].

Acknowledgements: The study was supported by the STARSS project CZ.02.1.01/0.0/0.0/15_003/0000465, co-funded by ERDF, and by the Mobility Fund of the Charles University.

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OC18

ANTIPSYCHOTICS' DEGRADATION OBTAINED THROUGH CHEMICAL AND BIOCHEMICAL OXYGEN DEMAND LEVELS

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Antipsychotic (AP) drugs consumption has seen a huge increase (171%) during the last years, but little attention has been given to their environmental fate in comparison to other pharmaceuticals micropollutants [1]. BOD_5 measures the biodegradability of wastewater but the extent of pollution can be better presented by considering the magnitude of COD. The ratio (BOD_5/COD) is used to measure the percentage (%) of degradation in a wastewater sample [2]. So, in this work, it was determined this ratio to evaluate the impact of AP drugs. The results obtained by the reference methods and the automated methods, based on sequential injection analysis (SIA), were quite similar to each other.
According to the results, it is possible to distinguish that the first generation APs are more biodegradable than the second generation APs that can be justified by the chemical structure of APs.

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OC19

TIDAL EFFECT ON DISSOLVED GASEOUS MERCURY FORMATION AND MERCURY VOLATILIZATION IN INTERTIDAL SEDIMENTS

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Intertidal sediments of Tagus estuary regularly experiences complex redistribution due to tidal forcing, which affects the cycling of mercury (Hg) between sediments and the water column. This study quantifies total mercury (Hg) and methylmercury (MMHg) concentrations and fluxes in a flooded mudflat as well as the effects on water level fluctuations on the air-surface exchange of mercury.
A fast increase in dissolved Hg and MMHg concentrations was observed in overlying water in the first 10 min of inundation and corresponded to a decrease in pore waters, suggesting a rapid export of Hg and MMHg from sediments to the water column. Estimations of daily advective transport exceeded the predicted diffusive fluxes by 5 orders of magnitude.
A fast increase in dissolved gaseous mercury (DGM) concentration was also observed in the first 20–30 min of inundation (maximum of 40 pg L^{-1} at 20 min). Suspended particulate matter (SPM) concentrations were inversely correlated with DGM concentrations, suggesting that an increase in SPM may lead to lower amounts of Hg photochemical reduction or that a large percentage of elemental mercury (Hg0) was adsorbed to SPM.
Dissolved Hg variation suggested that biotic DGM production in pore waters is a significant factor in addition to the photochemical reduction of Hg. Mercury volatilization (ranged from 1.1 to 3.3 ng m^{-2} h^{-1}; average of 2.1 ng m^{-2} h^{-1}) and DGM production exhibited the same pattern with no significant time-lag suggesting a fast release of the produced DGM. These results indicate that Hg sediment/water exchanges in the physical dominated estuaries can be underestimated when the tidal effect is not considered.

OC20

ENVIRONMENTAL SCREENINGS OF BIOACTIVE COMPOUNDS IN WASTEWATER USING A NOVEL MULTIPLEX SUSPENSION ARRAY FLUORESCENCE IMMUNOASSAY

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Monitoring water quality regarding emerging pollutants, such as drug residues, demands for selective, high-throughput and multi-target analytical methods. On the one hand, the influence of sewage on natural surface waters must be routinely monitored. On the other hand, estimation of removal efficiencies of pollutants, such as drug residues, is in the focus of industrial and public wastewater treatment. Immunoassays, such as ELISA, offer the possibility to be highly sensitive and selective due to the high target affinity and specificity of target recognition by antibodies. Batch-wise processing in microtiter plates allows for the necessary high-throughput, but only a single analyte can be determined within one measurement.

To overcome these disadvantages, we developed a four-plex **S**uspension **A**rray **F**luorescence **I**mmunoassay (SAFIA), which is adaptable for the microtiter plate format. The modular and self-prepared bead support consists of polystyrene-core silica-shell microparticles. While the polystyrene core is used for encoding, by introducing different amounts of fluorescent dyes, the silica shell creates a solid-support for the immunoassay: the target analytes, three drugs, carbamazepine, diclofenac and caffeine, and the fecal marker isolithocholic acid are covalently coupled to amino groups on the surface via NHS chemistry. Specific recognition of these haptens is accomplished by further introduction of PEG moieties, suppressing non-specific binding. A competitive immunoassay is subsequently conducted in a simple mix-and-read procedure, eliminating the demand for laborious washing steps and decreasing time-to-result. An automated flow cytometer is used for simultaneous decoding and quantification.

After optimization, the SAFIA showed limits of detection for all analytes in the low $\mu g\ L^{-1}$ range, meeting the sensitivity criteria for wastewater analysis. Then, applicability of the SAFIA was studied on real wastewater samples from three different wastewater treatment plants in Berlin. The results were in good comparability to LC-MS/MS indicating high matrix stability. Moreover, the accuracy of the assay exceeded that of the respective ELISA. Finally, we used SAFIA to assess the influent of treated and untreated wastewater on the Douro river estuary in Portugal. The obtained results of the analysis were comparable to ELISA. However, the measurements by SAFIA could be carried out in one quarter the time of analysis required for ELISA.

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OC21

VALIDATION AND UNCERTAINTY EVALUATION OF THE IDENTIFICATION OF DOPING AGENTS IN SPORT BY GC-MS/MS

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The uncertainty in qualitative analysis (examinations) is a growing concern in many analytical fields. Contrary to quantitative analysis, qualitative ones lack a harmonised form of reporting the reliability of a result. The identification of doping agents in athlete's biological fluids is one of the fields in which the uncertainty of examinations is crucial to ensure the correct interpretation of information.

Some authors have proposed several ways of estimating the examination uncertainty, being the Bayes' Theorem one of the most convenient theories for this assessment as it allows the combination of true (TP) and false positive (FP) results rates in a single metric, and the update of the uncertainty when a new evidence is made available. Bayes' Theorem can be used to report the uncertainty of the examination result as a likelihood ratio (LR=TP/FP) or as the probability of collected evidences being correct.

One of the most popular instrumentations for the identifications of doping agents in athletes' biological fluids, after adequate pre-treatment, is GC-MS/MS. Analytes are identified by the agreement between retention times and ion abundance ratios of their mass spectrum observed in samples and calibrators. The World Anti-Doping Agency (WADA) defined criteria for the identification parameters based on a general knowledge of identification performance. However, these criteria may produce high false negative rates, FN, whenever the analytes have to be screened near the Detection Limit.

In this work, eight substances were study and the performance of the examination was evaluated considering two different identification criteria.

A computational tool, implemented in an MS-Excel spreadsheet, was developed that allows the definition of statistical criteria for the parameters used in analytes identification that takes into account the observed experimental performance and, the non-normal distribution and the correlation of the input variables of the identification parameters. This tool allows estimating both TP and FP required to report the examination uncertainty and the evaluation of the adequacy of the identification criteria of analytes defined by WADA. The FP and TP estimates associated to different identification parameters were combined and presented as the uncertainty of the examination in the form of LR or P(x|e).

The obtained results allow concluding that the instrumental analysis of all the analysed substances, applying the developed and WADA's criteria, produces positive results with a high probability of being correct. However, in some cases, the identification criteria proposed by WADA produce high FN rates. The developed computational tool can be extremely useful to support the use of the regulated identification criteria defined by WADA, namely by estimating the probability of the negative result being false, which may indicate the need to perform replicate or complementary exams.

OC22

METHOD VALIDATION FOR METALS QUANTIFICATION IN WATER INTENDED FOR HUMAN CONSUMPTION

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Water is essential to the survival of every living organism but it is also an excellent solvent, allowing the dissolution of several chemical elements, being some potentially dangerous and even harmful to human health, as it is the case of trace elements such as Pb, Cd, and Cr. Industrialization, which enabled man to process and transform natural resources in a variety of ways, has led to increasing release of different contaminants into ecosystems. As such, the declining environmental has become a major issue to the general population, and thus turning the status of water resources for human consumption into one of the major concerns of the 21st century [1]. Water purity can be defined by its physical, chemical and biological characteristics, known globally as “water quality parameters” and their criteria are well defined in European legislation [2, 3]. Therefore, it is important to comply with the quality parameters established by the European Council Directive 98/83/EC of November 30, with the changes described in the new directive on the quality of water intended for human consumption (Directive 2015/1787 of the European Commission of October 6, 2015). This Directive also refers to the interest in updating information in the light of scientific and technical progress, in order to ensure consistency with European legislation [2, 3].
The current work aims to validate a method for the quantification several metals in water intended for human consumption using coupled plasma techniques. The validation method process included the study of method performance characteristics such as selectivity, linearity, limits of detection and quantification, trueness, recoveries, precision and uncertain. Both the precision and trueness of method were below 10%, verified using a certificated reference material (SRM 1643e). The developed method revealed to be efficient, complying with the legal requirements of the Directive 2015/1787 of the European Commission. This presentation will elucidate about the key aspects regarding the method development and validation work.

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BIOSENSORS

POSTER COMMUNICATIONS

PA 1

EVALUATION OF THE MOLECULAR ARCHITECTURE OF FLUORESCENT CALIX[4]ARENE-BASED SENSORS IN DETECTION OF TOXIC METALS

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Chemical sensors have been playing a crucial role in analytical chemistry, bio-medicinal science and environmental chemistry. Chemosensors offer an accurate and low-cost finding of anions, cations, enzymes and toxic metal ions with high selectivity and sensitivity. In this regard, many organic compounds have been synthesized and are being used as successful chemosensors [1, 2]. In particular, cation complexing ligands containing calix[4]arene have been used to obtain more selective metal ions receptors.
Herein, we report fluorescent calix[4]arene-based sensors with different molecular architecture [3] and their potentialities to address the detection of toxic metals (Figure 1).

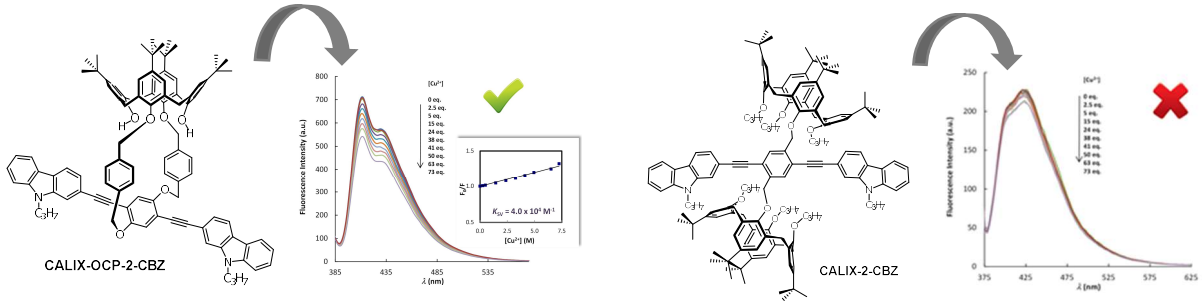

Figure 1: Calix[4]arene-based sensors (**CALIX-OCP-2-CBZ** and **CALIX-2-CBZ)** and fluorescence quenching in CH_3CN upon addition of $Cu(ClO_4)_2$ (λ_{exc} = 380 nm and λ_{exc} = 360 nm, respectively).

It was found that the bicyclic cavity of calix[4]arene (**CALIX-OCP-2-CBZ**) as compared with bis-calix[4]arene (**CALIX-2-CBZ**) has an important role in recognition event as shown by the results of fluorescent quenching by copper perchlorate salt.
These results will be also compared with those of the calix[4]arene-based polymers with carbazole segments as fluorescent signaling moieties (not shown) in order to evaluate the signal amplification in the detection event.

Acknowledgements: We thank IPL (Project IPL/2016/NoSeMeTox/ISEL) and FCT/MCTES (UID/QUI/00616/2013) for financial support.

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PA 2

CHARACTERIZATION OF GLUCOSE BIOSENSOR COMPRISING GLUCOSE OXIDASE IMMOBILIZED IN A PENCIL GHRAPHITE ELECTRODE

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The use of pencil graphite electrodes (PGE) as sensors and biosensors in analytical applications in addition to energy production through biofuel cells have been increasing in recent years. Their ubiquity and negligent cost, associated with good electrical conductance properties makes them a viable alternative to more conventional and expensive electrodes such as platinum and glassy carbon.
In the proposed biosensor, glucose oxidase (GOx) immobilization was performed in the form of enzyme precipitate coatings [1] as described next.
Prior to immobilization, PGE (2 mm, HB) was mechanically polished and the surface modified with graphene electrochemically reduced (1 mg mL^{-1}). GOx was first covalently attached to MWCNT after treatment with EDC/NHS. To promote precipitation of enzyme in the vicinity of the nanotubes and their sequent crosslinking, ammonium sulphate was added to the mixture followed by glutaraldehyde respectively. The mixture GOx-MWCNT was recovered by filtration and suspended in a nafion solution for entrapment and further deposited in the modified PGE surface.

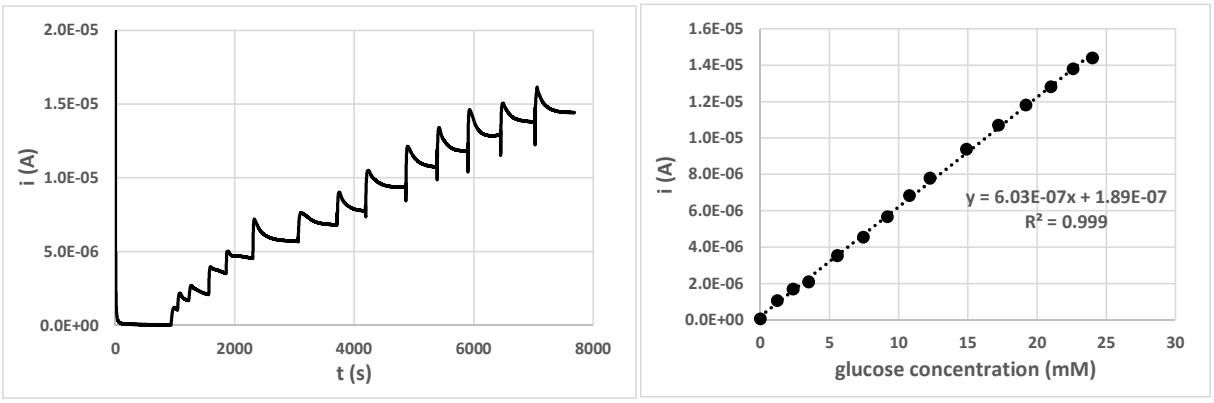

Figure 1: Amperometric detection of glucose and calibration curve.

Cyclic voltammograms confirmed the efficient immobilization of GOx when glucose was present in the solution. Accordingly, to amperometric measurements (Figure 1), the biosensor achieved a sensitivity of about 18 μA mM^{-1} cm^{-2} with a linearity range up to 39 mM and LOD of 12 μM. The biosensor also showed successful use in the amperometric determination of cadmium based on its competitive inhibitory effect over the biological catalyser, glucose oxidase.

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PA 3

METAL ION RECOGNITION-INDUCED BY CALIX[4]ARENE-CARBAZOLE-CONTAINING POLYMERS

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Sensing and recognition of ions and neutral molecules via synthetic receptors are of current interest in supramolecular chemistry because of their significant importance in several areas, such as chemistry, biology and environment. Compared with small molecules, polymers-based sensors displayed several important advantages like signal amplification. In this way, the incorporation of molecular receptors such as calixarenes with conjugated polymer backbones is expected to enhance the signalling events related to a host-guest interaction. The preorganized binding sites, easy derivatization and flexible three-dimensional steric structures make calixarenes ideal construction platforms for molecular design to generate fluorescent receptors. The use of calixarenes as supramolecular scaffolds for this type of architectures has been explored and the sensing abilities of resultant polymers toward metal and molecular ions established [1, 2].

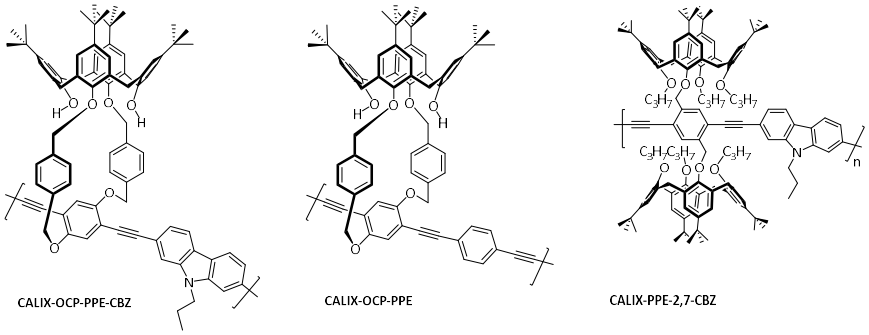

Figure 1: Calix[4]arene-PPE polymers based sensors structures.

Based on the high sensitivity shown by the non-polymeric analogue **CALIX-OCP-CBZ** (not shown) [3], to toxic metal cations, we decide to extend the sensing study to polymer materials. Herein, we report the preliminary results of the chemosensing ability of a new bicyclic calix[4]arene-carbazole-polymer (**CALIX-OCP-PPE-CBZ**) towards the detection of toxic metals in fluid phase (Figure 1). No quenching response was found for both **CALIX-OCP-PPE** and **CALIX-PPE-2,7-CBZ** in the presence of Cu(II).

Acknowledgements: We thank IPL (Project IPL/2016/NoSeMeTox/ISEL) and FCT/MCTES (UID/QUI/00616/2013) for financial support.

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PA 4

AMPEROMETRIC BIOSENSOR FOR PYRUVATE DETERMINATION BASED ON CARBON NANOTUBES AND PRUSSIAN BLUE-CHITOSAN NANOPARTICLES

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Pyruvate is a key indicator of the degree of the pungency in onions. The production of pyruvate occurs by alliinase hydrolysis of the precursor S-alk(en)yl-L-cystein sulfoxide in onion tissue when it is macerated. Pyruvate concentrations can vary from 2 to 15 μmol g^{-1}. Factors that contribute to the value of its concentration include the type of soil, the soil moisture content, the species of the onion and its maturity. Thus, the development of simple, easy and reliable analytical tools to determine the concentration of pyruvate is a necessity and portable electrochemical biosensors can be advantageous in comparison with traditional analytical methods.
A biosensor for the determination of pyruvate has been developed by immobilizing pyruvate oxidase (PyrOx) onto a glassy carbon electrode (GCE) modified with carbon nanotubes (CNT), Prussian Blue nanoparticles and FAD, in order to improve the performance compared with the literature [1]. Characterization was performed by cyclic voltammetry and fixed potential amperometry. Construction of the sensing device was carried out in three steps: first modification of the GCE surface with CNT, second the immobilization of Prussian Blue-Chitosan nanoparticles, prepared as in [2], third the immobilization of PyrOx and FAD. At each stage of assembly, the operating conditions, such as pH and applied potential, were optimized. The best loading of CNT dispersion was also evaluated. The final biosensor was investigated to obtain the most suitable conditions for applying it to the analysis of food samples. The optimum conditions were found to be pH 7.0 and an applied potential of +0.25 V vs SCE. The biosensor exhibited a linear response from 25 to 500 μM and a limit of detection of 5.2 μM. Application of the biosensor was demonstrated by measuring pyruvate in onion samples. The results are in agreement with those found in the literature and by traditional analytical methods [3, 4].

Acknowledgements: Projects UID/EMS/00285/2013 and PTDC/QEQ-QAN/2201/2014 (FCT).

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PA 5

DEVELOPMENT OF A THYROXINE IMMUNOSENSOR USING SCREEN-PRINTED ELECTRODES

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This work focuses on the construction of an electrochemical immunosensor for the quantification of thyroxine (T_4), a thyroid hormone with a key role in metabolism regulation. Indeed, both an excess and a deficiency of T_4 levels are related with several diseases, such as Graves disease, neurodevelopmental disorders and depression, being its quantification in the human body of great importance. Here, we developed a T_4 sensor based on the use of a mixed self-assembled monolayer (SAM) for the immobilization of the anti-T_4 antibody onto a gold SPE (Figure 1). 6-Mercaptohexanol and 11-mercaptoundecanoic acid were applied for the covalent binding of the antibody, creating the appropriate linker between the golden surface and the anti-T_4. Electrochemical impedance spectroscopy and cyclic voltammetry techniques were used for the modification procedure control and characterization of the modified electrodes surface. Furthermore, the analytical application of the developed biosensor was carried out by EIS after incubation of the sensor with different concentrations of T_4 hormone and using ferricyanide as redox probe [1].

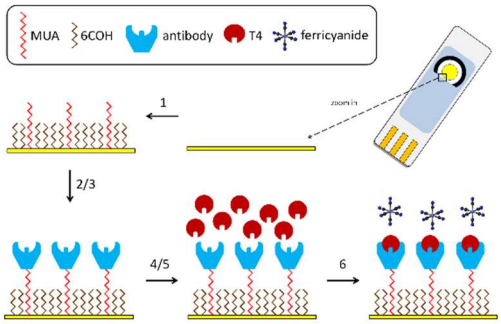

Figure 1: Schematic representation of the immunosensor preparation procedure, comprising: 1) coverage of the SPAuE surface with MUA and 6COH; 2) activation of MUA carboxylic groups with EDC and NHS, creating the appropriate link between the golden surface and the anti-body; 3) antibody incubation; 4) immersing the electrode in BSA to block non-specific sites; 5) adding T_4 thus initiating antibody-antigen reaction; 6) adding the ferricyanide redox probe.

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BIOTECHNOLOGICAL APPLICATIONS

POSTER COMMUNICATIONS

PB 1

OPTIMIZATION AND CHARACTERIZATION OF 2D LIPID MODELS FOR THE ASSESSMENT OF ACETYLCHOLINESTERASE INHIBITORS – MEMBRANE INTERACTIONS

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This work aims the study of AChE inhibitors with potential usage for the treatment of Alzheimer's disease. The study and understanding of the action that these drugs may have in acetylcholinesterase can be made using lipid membrane mimetic models. Phospholipids, such as POPC (1-palmitoyl-2-oleoyl-*sn*-glycerol-3-phosphocholine), with unsaturated and saturated chains, are a major component of neuronal cell membranes. Besides, this type of cells have enriched cholesterol domains. In this context, 2D mimetic models were developed and optimized, according with the abovementioned features, to further study the interactions with AChE inhibitors, in the presence of the enzyme. Moreover, we have used calcium to mimic the synaptic cleft where AChE is located, once it is one of the ions present in higher percentage. The models were characterized by surface pressure-area isotherms to get information about the monolayers compressibility and collapse behavior; Brewster Angle Microscopy (BAM) to assess morphological features, including size and shape of domains; Polarization-Modulation InfraRed Reflection-Absorption spectroscopy (PM-IRRAS) to acquire structural information. The effect of each component and its molecular interactions have been studied and compared.

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PB 2

DEVELOPMENT AND CHARACTERIZATION OF NANOAGENTS LOADED WITH CARBON MONOXIDE TO TREAT RHEUMATOID ARTHRITIS

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Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory and autoimmune disorder of the joints characterized by synovial inflammation that can lead to cartilage and bone destruction [1, 2]. Although notable advances have been made in the treatment of RA, long-term administration of anti-rheumatic drugs still has some disadvantages, including high dose and high frequency of administration, as well as dysfunctions in the heart, liver, kidneys, and so on [3]. Due to the above problems, new drugs continue to be needed and investigated. Nowadays, carbon monoxide (CO) is known to have anti-inflammatory effects *in situ*, however, the administration of this molecule represents a challenge and the development of a technology that controls the delivery of CO under different physiological conditions represent a major step in the use of CO-releasing molecules (CORMs) [4, 5]. Nanobased delivery systems are being developed to avert non-specific binding and upregulate the efficacy by improving the accumulation of drugs in lesion tissues [3] and can thus represent an effective approach. In this project, we intend to develop a nanosystem that is capable of releasing CORMs in a controlled and targeted way to be further used in the treatment of rheumatoid arthritis.
Customized lipid nanoparticles loaded with CORM-2 and surface functionalized, in order to direct the nanoparticles to the desired therapeutic target, have been developed. The formulations have been physic-chemically characterized regarding their size, zeta potential, payload and surface modification efficiency. Release studies were performed in order to assess whether a controlled release of the drug was achieved with the purpose of overcoming some of the drawbacks of conventional treatments. Additionally, *in vitro* cell toxicity studies have been conducted using cell line THP1. Overall, preliminary results indicate a strong potential of the designed formulation.

Acknowledgements: The authors thank FCT (Fundação para a Ciência e Tecnologia) for financial support for this project (PTDC/QEQ-MED/1902/2014).

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PB 3

EVALUATION OF THE STABILITY AND PHOTOTOXICITY OF DEET, A COMMON ACTIVE INGREDIENT OF COMMERCIAL INSECT REPELLENTS

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N,*N*-Diethyl-*m*-toluamide, commonly known as DEET, is the principle active ingredient in most personal insect repellents worldwide and is highly effective against a broad spectrum of insect pests, such as mosquitoes or biting flies [1].
DEET-containing insect repellent products, with DEET concentrations ranging from 10 to 100%, in the form of liquids, lotions, gels, sprays, sticks, and impregnated materials are sold annually [1]. Although DEET is generally considered a benign chemical, isolated reports involving heavy and excessive exposure indicate a variety of toxic side effects, including toxic encephalopathy, seizure, acute manic psychosis, cardiovascular toxicity, and dermatitis [1].
In the last few years, contamination of DEET has been widely reported and it has been detected in various aquatic environments, including rivers, groundwater, seawater, wastewaters and even in drinking water treated by conventional water-treatment systems [2]. DEET can enter the environment mainly through municipal wastewater and is considered to be persistent in hydrolysis [2]. As a result of both extensive use and unintentional discharges, DEET has become an emerging contaminant and its removal from water is of high priority.
In this study, we investigated the chemical and photochemical stability of DEET in aqueous solutions. The main objective was to elucidate and understand the reactions involved in DEET degradation in order to assess their implication on its environmental fate. Moreover, the phototoxicity of DEET was also assessed in a human keratinocyte cell line to determine whether risk minimization measures are warranted to prevent adverse events in humans. The results obtained will be presented in this communication.

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PB 4

EVALUATION OF THE ANTIOXIDANT POTENTIAL OF ASCORBIC ACID DERIVATIVES USED IN SKIN CARE PRODUCTS

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Skin is the major organ of the human body and is constantly exposed to external factors such as ultraviolet radiation, pollution, toxic chemicals, pathogenic bacteria and viruses that are to some extent responsible for the formation of free radicals [1]. Free radicals have an important role in lipid peroxidation of intercellular cement and consequently can lead to the oxidative damage of the skin structures [1]. In healthy organisms, the right balance between formation and neutralization of free radicals is maintained. However, a balance disorder associated with the excessive production of free radicals leads to oxidative stress, which is an etiopathological factor of degenerative diseases and cancer, as well as the ageing process [1].
Over the last decade, antioxidants have been proposed as one of the most effective functional ingredients to counteract the effects of oxidative damage to the skin. In modern cosmetology, antioxidants are also used to protect the raw materials and the final product.
Ascorbic acid (vitamin C) and its derivatives are known to perform various important physiological and metabolic functions in humans. In addition to dietary supplements, a number of skin care formulations containing ascorbic acid and derivatives are now available since they exert several functions on the skin such as collagen synthesis, depigmentation and antioxidant activity. Nevertheless, ascorbic acid is a very unstable vitamin and is easily oxidized in aqueous solutions and in cosmetic formulations. Thus, ascorbic acid derivatives have been tested and incorporated in formulations but unfortunately, they did not produce the same effect as that of the parent compound [2].
In this study, we investigated the antioxidant potential of different ascorbic acid derivatives used in cosmetics. The data found was used for the implementation of an in vitro method for the evaluation of skin penetration of ascorbic acid and its derivatives. The results obtained will be presented in this communication.

Acknowledgements: This project was supported by Foundation for Science and Technology (FCT) and FEDER/COMPETE (Grants UID/QUI/00081/2013, POCI-01-0145-FEDER-006980, and NORTE-01-0145-FEDER-000028).

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PB 5

POLYPHENOLIC COMPOSITION AND IN VITRO ANTIOXIDANT ACTIVITY OF *CHAMAEROPS HUMILIS L.*

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Chamaerops humilis L., a medicinal plant which belong to the Arecaceae family, is a shrub-like clumping palm, with several stems growing from a single base [1].
The leaves were dried, powdered and stored for chemical and biological studies. Plant materials were dried and milled into uniform powders using a knife crusher of the type Ika then stored carefully until use. Total phenolic content, in the extracts, was determined according to the Folin-Ciocalteu assay. Methanolic extracts of the dried leaves were examined as potential sources of phenolic compounds. Three different methods were used to test the antioxidant activity of the extracts, including colored 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid radical cation (ABTS*+), DPPH radical scavenging assay (1,1-diphenyl-2-picryl hydrazyl radical reducing power methods). The general toxicity was verified in a human mammary cell line MCF7 and enzymatic activity including the ability to inhibit AChE and TyrE was also tested. The toxicity values obtained for the extracts are very low and they do not inhibit the enzymes tested.
The phenolic compositions of the methanolic extracts were elucidated by high performance liquid chromatography coupled on line with tandem mass spectrometry (HPLC-MS/MS). The extract was mainly composed by *C*- and *O*- flavones and its *O*-methylated derivatives.
The results obtained for the various extracts are promising to future applications namely in nutraceuticals, considering its potential as antioxidants and non-toxic properties.

Acknowledgments: The authors are thankful to IPL under IDI&CA/IPL/2017/BIOPALMA/ISEL, FCT under UID/QUI/00100/2013 and REM2015.

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PB 6

ANTIOXIDANT ACTIVITIES AND ANTIOXIDANT COMPONENTS OF AQUEOUS EXTRACTS OF MUSHROOMS STRAINS

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Basidiomycete mushrooms strains are known for their wide diversity of nutritional biomolecules and medicinal value. The diversity of their bioactive components becomes increasingly attractive as functional foods due to their potential benefits on the human health. Several biological activities have been studied which include cholesterol-lowering, antibacterial, anti-allergic, antifungal, antioxidant, antitumor, immunomodulating, antidiabetic and anti-inflammatory [1, 2].
Aqueous extracts from mycelia and fruiting bodies from several mushrooms (i.e., *Coriolus versicolor*, *Ganoderma lucidum*, *Pleurotus ostreatus*, *Ganoderma carnosum*, *Hericium erinaceus*, *Lentinula edodes*, *Inonotus obliquus*, *Auricularia auricula*, *Polyporus umbellatus*, *Grifola frondosa*, *Cordyseps sinensis*, *Agaricus blazei* and *Poria cocos*) were obtained by using a sequence of several extractions with cold and boiling water, acidic and alkaline conditions. The extraction fractions were investigated for their total phenolic and flavonoid contents and were also analyzed for antioxidant activity in different systems including reducing power, free radical scavenging, superoxide anion radical scavenging, total antioxidant activity, and metal chelating activities. Those various antioxidant activities were compared to standard antioxidants such as gallic acid, BHT and ascorbic acid [3].
The antioxidant activities of the alkaline fractions/KOH were generally the highest. However, the alkaline fractions/NaOH showed the greatest superoxide anion radical scavenging activities compared to the one of ascorbic acid. The antioxidant activities of the boiling water fractions presented the strongest DPPH radical-scavenging activities, also higher than the exhibited by BHT. Concerning the total phenolic and flavonoid contents alkaline fractions/KOH exhibited the highest concentrations.
Several of the mushroom extraction fractions researched in this study have a high level of antioxidant activities and antioxidant components, which warrant investigation for their potential to improve human health.

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PB 7

NANOSTRUCTURED LIPID CARRIERS AS A VERSATIL ORAL DELIVERY VEHICLE OF STREPTOMYCIN

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Tuberculosis (TB) is one of the most prevalent worldwide infectious diseases, being caused by *Mycobacterium tuberculosis* [1]. The current treatment for TB is becoming increasingly difficult due to the long treatment duration, resulting in non-compliance to therapy and to the appearance of several adverse-effects [2, 3]. Streptomycin (STR) is a second-line anti-TB drug that is considered one of the most cost-effective drugs against *Mycobacterium tuberculosis*. The major drawbacks in the use of STR are the need of the parenteral route and its severe toxicity profile [3]. In this context, the aim of this work was to develop nanostructured lipid carriers (NLC) as an oral drug delivery system for STR. NLCs loaded with STR were synthetized by simple and double emulsion, using the hot homogenization followed by ultra-sonication techniques. The formulations with higher value of encapsulation efficiency (EE) were then selected. The selected formulations were characterized in terms of size, zeta potential, polydispersity index (PDI) and EE. In addition, the developed formulations were lyophilized using Aerosil® as cryoprotectant. The variables under study corresponded to the influence that the drug incorporation and lyophilization had in the different analysed parameters. Results showed that the nanoparticles synthetized by simple emulsion had higher EE values, size and zeta potential in modulus, and lower PDI values than the nanoparticles synthetized by double emulsion.
In the future, other studies including transmission electron microscopy, drug *in vitro* release, cell viability assays and *in vivo* studies will be performed to develop an oral alternative delivery form of STR.

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PB 8

PROTEIN-PHENOLIC INTERACTION AS A STRATEGY TO REDUCE THE PRECURSORS OF VOLATILE PHENOLS IN WINE

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In the present work, the interaction between phenolic acids (caffeic acid, *p*-coumaric acid and ferulic acid) and proteins was studied as a potential strategy to reduce the content of the precursors of volatile phenols in wine. These phenolics result from the contamination of the wine by *Brettanomyces bruxellensis* causing a wine defect often referred as the "*Brett character*" [1]. In this context, the aim of the present work is the development of a biological strategy, in which the precursors can be removed from the wine or otherwise be rendered unreactive and for that reason be unavailable for conversion into volatile phenols.

Due to their possible beneficial effects on human health, the interaction of proteins with plant-derived phenolic compounds has been an object of study [2]. This interaction can be studied using a fluorescence quenching approach [3], which has been widely employed to characterise the biochemical interactions between these types of molecules.

Bovine serum albumin (BSA) was used as a model protein and the fluorescence of the protein was measured in the absence and in the presence of the quencher - the phenolic acid. This protein-phenolic interaction can be affected by some environmental conditions, such as medium composition, pH, temperature, and protein concentration [2, 4]. The effect of the environmental conditions on the interaction between BSA and caffeic, coumaric and ferulic acids was evaluated by the determination of the binding parameters, Ka and n, where Ka is the binding constant and n is the number of binding sites per phenol/protein. Higher values were obtained for the interaction of caffeic acid with BSA.

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PB 9

ULTRASOUND ASSISTED EXTRACTION OF TRITERPENOIDS FROM *GANODERMA LUCIDUM*

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Ganodema lucidum (Curtis) P. Karst. commonly referred to as ˝reishi˝ is recognized as an important source of triterpenoids and triterpenic acids. Several health promoting benefits (anti-inflammatory, antityrosinase, antitumor and antioxidant properties) have been attributed to the contribution of these classes of compounds [1]. Based on this pharmaceutical and nutraceutical potential, the extraction of triterpenoids and triterpenic acids using novel technologies is considered interesting [2]. In the present work, *G. lucidum* dried powder (~20 mesh), was extracted in an ultrasonic device (QSonica sonicators, model CL-334, Newtown, CT, USA) using the following conditions: 40 min, 100 W and 89.5% ethanol percentage. The temperature was monitored in order to be below 30-35 °C. The triterpenoids' profile of the extract was analyzed using an HPLC system coupled to a diode array detector (DAD) and mass spectrometry (MS) with an electrospray ionization interface (ESI).
From the results obtained, the ethanolic extract of *G. lucidum* revealed the presence of 26 triterpenoids and their derivatives. Two ganoderenic acids (B and D) and seven ganoderic derivative compounds were detected. The total triterpene content was 531.26 ± 0.24 mg/g, with ganoderic acid A (43.46 ± 0.20 mg/g) and ganoderic acid H (58.13 ± 0.03 mg/g) as the most abundant triterpenoids. This study highlights the importance of *G. lucidum* as a source of added value bioactive compounds, which can be explored as ingredients for various bio-based applications.

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PB 10

COSMECEUTICAL POTENTIAL OF ERGOSTEROL AND USE OF MICROENCAPSULATION TO ENSURE CONTROLLED RELEASE

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Modern skin care formulations are designed to exert multifunctional benefits to the skin, and this has increased interest in the use of bioactive compounds as cosmeceutical ingredients. Ergosterol and some of its derivatives show antitumor, antioxidant, anti-inflammatory and antityrosinase activities, which make them interesting ingredients to be used as cosmeceutical ingredients. However, their utilization is still facing issues related with their stability under different factors such as pH and temperature [1], and microencapsulation can be used to overlap some of these limitations. The present work describes the anti-inflammatory, antityrosinase and antimicrobial activities of ergosterol. The compound was then microencapsulated using the atomization/coagulation method with sodium alginate, and the microspheres were characterized in terms of morphology, particle size distribution, FTIR and encapsulation efficiency. The individual compound and the microspheres were then separately incorporated in a semi-solid cosmetic base formulation. HPLC-DAD was used to infer the presence of the compound in the formulations. Ergosterol revealed anti-inflammatory activity by inhibiting the NO production with EC_{50} value 337.70 ± 23 µg/mL. It also displayed an antibacterial activity against *E. coli*, while up to 2 mg/mL, ant-tyrosinase activity was not achieved. The microparticles produced showed spherical morphology, various sizes with little agglomeration and a unimodal and bimodal particle size distribution in terms of number and volume respectively. The encapsulation efficiency was above 50%. After incorporation, the formulation prepared with the free ergosterol still maintained some of its bioactive properties, while the encapsulated forms preserved the bioactivity showing a slow release profile of the encapsulated compound. This encapsulation procedure is a suitable alternative to prolong retention of bioactive compounds for subsequent release.

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CHEMOMETRICS

POSTER COMMUNICATIONS

PC 1

COUPLING OF ON-COLUMN TRYPSIN DIGESTION-PEPTIDE MAPPING AND PRINCIPAL COMPONENT ANALYSIS FOR STABILITY AND BIOSIMILARITY ASSESSMENT OF RECOMBINANT HUMAN GROWTH HORMONE

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Peptide mapping (PM) is a vital technique in biopharmaceutical industry. The fingerprint obtained helps to qualitatively confirm host stability as well as verify primary structure, purity and integrity of the target protein. Yet, in-solution digestion followed by tandem mass spectrometry is not suitable as a routine quality control test. It is time consuming and requires sophisticated, expensive instruments and highly skilled operators. In an attempt to enhance the functionality of PM and extract multi-dimensional data about various critical quality attributes and comparability of biosimilars, coupling of PM generated using immobilized trypsin followed by HPLC-UV to principal component analysis (PCA) is proposed. Recombinant human growth hormone (rhGH); was selected as a model biopharmaceutical since it is available in the market from different manufacturers and its PM is a well-established pharmacopoeia test. Samples of different rhGH biosimilars as well as degraded samples: deamidated and oxidized were subjected to trypsin digestion followed by RP-HPLC-UV analysis. PCA of the entire chromatograms of test and reference samples was then conducted. Comparison of the scores of samples and investigation of the loadings plots clearly indicated the applicability of PM-PCA for: i) identity testing, ii) biosimilarity assessment and iii) stability evaluation. Hotelling's T2 and Q statistics were employed at 95% confidence level to measure the variation and to test the conformance of each sample to the PCA model, respectively.

Coupling of PM to PCA provided a novel tool to identify peptide fragments responsible for variation between the test and reference samples as well as evaluation of the extent and relative significance of this variability. Transformation of conventional PM that is largely based on subjective visual comparison into an objective statiscally-guided analysis framework should provide a simple and economic tool to help both manufacturers and regulatory authorities in quality and biosimilarity assessment of biopharmaceuticals.

PC 2

MONITORING GRAPEVINE LEAVES VARIABILITY WITH INFRARED SPECTROSCOPY

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Precision agriculture is an increasingly adopted practice for viticulture towards the maximization of control over all stages of wine production. Mastering wine properties (target product profile) and reducing year-to-year variability is a demand that can only be effectively addressed by increasing the process knowledge through a constantly evolving understanding of all wine making stages: from the grapevine to the bottle [1, 2]. The *terroir* is known to give the global characteristics of wines of one region. However, factors like plant productivity can be highly variable within blocks containing the same grapevine variety [2]. Methodologies (e.g., analytical) for high-throughput inference of plant status or grapes metabolic profiling can unveil these differences and ultimately serve as tool for a better vineyard management. This work explores near infrared spectroscopy (NIRS) and mid infrared spectroscopy (MIRS), both applicable for high-throughput and non-destructive analysis of plants and berries, in terms of the ability to profile and consistently track variability on grapevine plants, specifically by the analysis of leaves. This work encompassed analysis of leaves collected from plants of the same variety in vineyards located on two Portuguese wine regions. Leaves were collected at eight locations on each vineyard, defined on the basis of soil properties and altitude (collected from June to September 2017), transported to the lab, lyophilized and analysed by the aforementioned vibrational spectroscopy methods. Spectral data were treated with chemometric methods. Results revealed systematic differences on leaves that could be correlated with several factors. A discussion on the observed variability on infrared spectra is hereby proposed.

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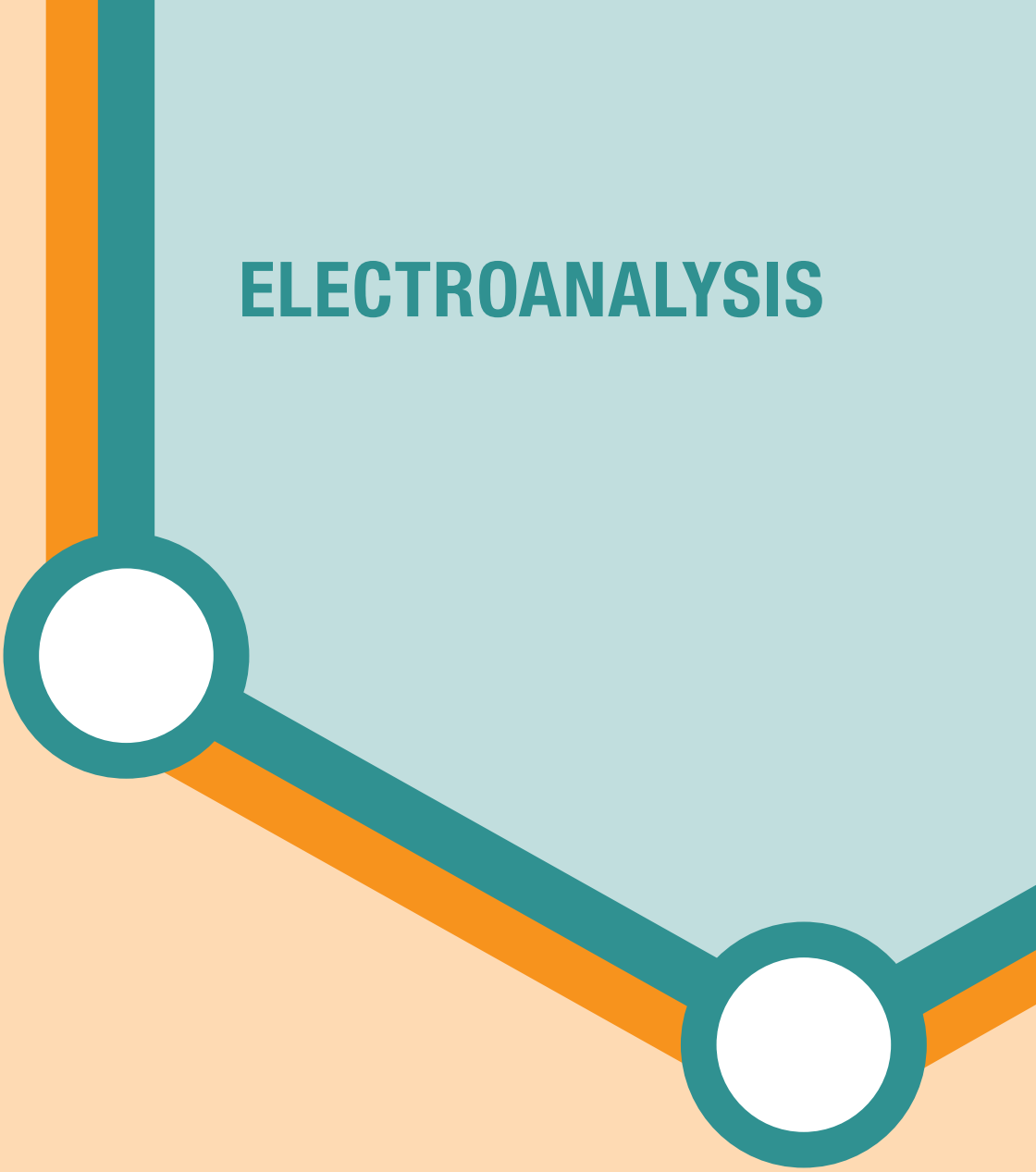
ELECTROANALYSIS



POSTER COMMUNICATIONS

PD 1

ELECTROCHEMICAL CHARACTERIZATION OF CEFADROXIL AND AMOXICILLIN IN AQUEOUS MEDIA

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Penicillins and cephalosporins are classes of β-lactam antibiotics that are highly efficient in the treatment of several types of bacterial infection. The structural differences in the thiazine/thiazolidine ring close to the β-lactam moiety (Figure 1) confers these compounds with distinct antibacterial activity, related to the inhibition of the active site of penicillin-binding proteins, in this way hindering the synthesis of peptidoglycan, a component that confers rigidity to the cell wall of prokaryotes. The electrochemical behaviour of cefadroxil and amoxicillin, both first-generation antibiotics [1], was investigated at glassy carbon electrodes using voltammetric techniques in buffer electrolytes with different pH values.

The first electrochemical oxidation (Figure1, **1a**), is obtained for both compounds in a diffusion-controlled, pH-dependent process that involves the transfer of one electron and one proton, followed by a chemical coupled reaction, generating a product that is then reversibly reduced and oxidized, visible in the subsequent scan, in a process involving two electrons and two protons (**0a** and **0a'**). This mechanism can be attributed to oxidation of the phenol moiety generating a radical, which undergoes a chemical coupled reaction with water, generating hydroquinones. The further oxidation processes (**2a**) and (**3a**) are present in the voltammograms obtained with cefadroxil and can be attributed to the nitrogen and sulphur heteroatom in the β-lactam and in the thiazine moiety, in which the oxidation products are better stabilized and are most likely to influence the interaction between the β-lactam site and PBPs and, therefore, the biological efficiency of the antibiotic. Process (**1a**) has been successfully used for the quantitative determination of both antibiotics by differential pulse voltammetry.

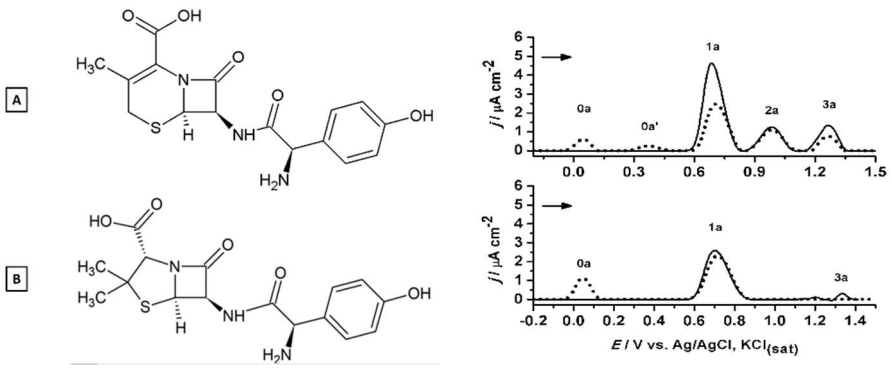

Figure 1: Molecular structures and differential pulse voltammograms in 0.1 BR buffer pH 7.0 containing 100 μM of cefadroxil (A) and amoxicillin (B). First scan (—) and second scan (●●).

Acknowledgements: PTDC/QEQ-QAN/2201/2014 (FCT), CNPq (Brazil).

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PD 2

DEVELOPMENT OF A NOVEL REDOX POLYMER-FILM MODIFIED ELECTROCHEMICAL SENSOR USING DEEP EUTECTIC SOLVENTS

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The use of deep eutectic solvents (DES) as a new class of "green" solvents have been shown to be successful in the preparation of advanced materials. DES are composed of a mixture between hydrogen bond donors and acceptors, which are able to self-associate to form a new eutectic phase [1]. Recent applications have included formation of conducting polymer films [2] and poly(methylene blue) redox polymer films [3] for sensing applications.
In this work, the azine dye neutral red (NR) was electropolymerized in DES to form a redox polymer film on a glassy carbon electrode. In order to obtain good performance, the best DES composition and polymerization conditions were established. DES were formed from a 1:2 ratio of choline chloride and ethylene glycol (ethaline), urea (reline) or glycerol (glyceline), mixing at 60 ºC and allowing to cool. A small amount of strong acid was added to improve the conductivity. The film-modified electrodes obtained with this new approach were electrochemically characterized by voltammetric measurements and impedance spectroscopy to monitor the film conductivity and stability. For comparison purposes, PNR-modified electrodes prepared in aqueous media were also prepared. The analytical parameters of the electrochemical sensor were determined by fixed potential amperometry and showed good results for acetaminophen detection.

Acknowledgements: projects UID/EMS/00285/2013 and PTDC/QEQ-QAN/2201/2014 (FCT).

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PD 3

ELECTROCHEMICAL BEHAVIOUR OF LERCANIDIPINE AT CARBON BLACK MODIFIED ELECTRODES

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Lercanidipine is a new derivative characterized with potent, long-lasting and vascular-selective calcium entry-blocking activity, used in the treatment of hypertension, angina pectoris and Raynaud's syndrome.

Lercanidipine, Figure 1, belongs to the 1,4-dihydropyridine group of compounds, that reversibly blocks voltage-dependent Ca^{2+} influx through L-type channels in cell membranes, promoting peripheral vasodilation and a reduction in blood pressure.

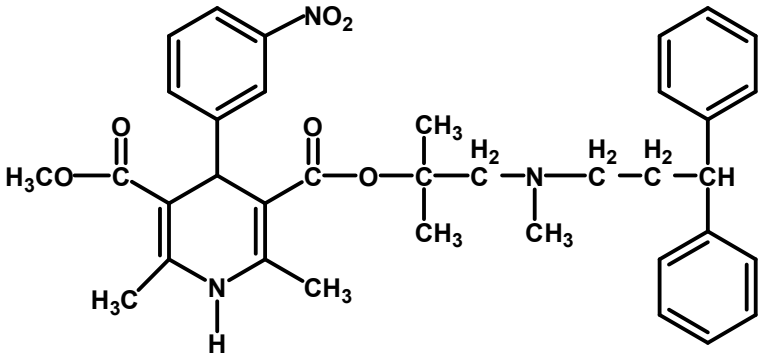

Figure 1: Chemical structure of lercanidipine.

The electrochemical behaviour of lercanidipine at carbon black (CB) modified glassy carbon electrode (GCE) and boron doped diamond electrode (BDDE), using cyclic, square-wave and differential pulse voltammetry, was investigated.

The oxidation mechanism of lercanidipine is an irreversible, pH-dependent process that occurred in two consecutive electron transfer reactions. The reduction mechanism of lercanidipine is an irreversible cathodic process. The lercanidipine reduction products are electroactive following a reversible electron transfer reaction.

The carbon black (CB) nanomaterial has been used in many electrochemical studies, due to its interesting properties, that include high conductivity, chemical stability, large surface area, and it is an extremely cheap material. Compared with the bare electrodes the GCE or BDDE modified with CB presented a considerable increase of the electrochemical peak currents and better detection limits.

Acknowledgments: Projects FCT (UID/EMS/00285/2013) and Innovec'EAU (SOE1/P1/F0173).

PD 4

ANTIDIABETIC DRUG METFORMIN VOLTAMMETRIC EVALUATION IN THE PRESENCE OF Cu(II)

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Metformin is an effective oral antidiabetic drug for the treatment of Type II diabetes, preventing the cardiovascular complications of diabetes, reducing LDL cholesterol, and triglyceride levels.

Metformin is 1,1-dimethylbiguanide hydrochloride, Figure 1, and the interaction of metformin with transition metal ions, such as Cu^{2+}, allows the formation of coordination compounds that can improve its biological and pharmacological action.

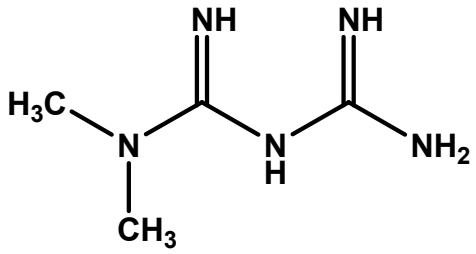

Figure 1: Chemical structure of metformin.

The electrochemical behaviour of metformin and metformin/Cu^{2+} complex, at glassy carbon electrode (GCE) and carbon black (CB) modified GCE (CB-GCE), in pH = 7.0, using cyclic, square-wave and differential pulse voltammetry, was investigated.

The carbon black (CB) nanomaterial consists in carbon nanoparticles with diameter between 3 and 100 nm, with relevant chemical, mechanical and physical properties for electrochemical investigation. The results of the present study are also important for the development of CB-based sensors used in the electrochemical determination of pharmaceuticals and other biomolecules.

As foreseen by the metformin chemical structure no electrochemical behaviour was observed. However, the oxidation of metformin/Cu^{2+} complex, both at the GCE and modified CB-GCE, occurred in an irreversible one electron transfer reaction. The dependence of peak currents and potentials on pH, concentration, buffer composition and scan rate, were examined.

The modified CB-GCE presented a considerable increase of the electrochemical metformin/Cu^{2+} complex oxidation peak currents enabling better detection limits.

Acknowledgments: Projects FCT (UID/EMS/00285/2013) and Innovec'EAU (SOE1/P1/F0173).

PD 5

A TWO YEAR SURVEY ON FLUORIDE CONCENTRATION IN MINERAL WATERS AND TEAS CONSUMED BY THE PORTUGUESE POPULATION

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In the last decades the use of fluoride exerted great impact on the control of dental caries. Fluoride is available from toothpastes and elixirs, but can also be ingested from other sources, in particular bottled water, tea, juices and other food. Low levels of fluoride don´t provide adequate protection against caries but exposure to multiple sources is associated with greater chance of developing dental fluorosis [1]. Therefore, an adequate intake of fluoride is recommended. According to the review of Venturini and Frazão [2], the worldwide consumption of bottled water increased in the last years from 178 billion to more than 231 billion of liters. This review also highlights that among US children, tap water represents 60% of water consumption, whereas bottled water reaches 40%. Since mineral water can have a significant fluoride concentration, its consumption by children can lead to dental fluorosis if the concentration of fluoride is not adequate. Most European countries recommend a sentence of caution in the bottle label, for children under seven years of age, when the water contains more than 1.5 mg F^-/L. In fact, the average value recommended by the World Health Organization (regarding fluorosis prevention) is 1.5 mg F^-/L. In ground waters, the sources of fluoride are minerals such as fluorospar (CaF_2), cryolite (Na_3AlF_6) and chiolite ($Na_5Al_3F_{14}$). One of the main factors affecting fluoride availability is pH, where acidic conditions (pH<6) favor the solubility of fluoride bearing minerals, increasing its concentration in ground waters. Another important source of fluoride intake is the consumption of tea. Tea leaves are very rich in fluoride as the tea plant (*Camellia sinensis*) takes up fluoride from the soil and accumulates it in its leaves. A substantial amount of this element is released during tea infusion becoming available to consumers. One of the main goals of this work is the comparison of the amount of fluoride determined in 15 commercial mineral waters (covering different regions of Portugal) and 15 commercial teas analyzed in March 2017 with that obtained in the same samples, purchased and analyzed in March 2018. In fact, we are interested to study if climate changes in the last year (especially bearing in mind the significant low precipitation levels) will have some influence in pH as well as other soil physicochemical parameters, thus affecting the fluoride availability in ground waters. Other contribution of this study is to identify discrepancies between the measured fluoride concentration and the levels listed on the label of bottled waters and tea packages; an irregularity that can influence people consumption of these products. The concentrations of fluoride in the studied samples were carried out by direct potentiometry using a fluoride ion selective electrode [3].

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PD 6

IMPEDIMETRIC SENSOR BASED ON GOLD NANOPARTICLE DOPED - POLY-(8-ANILINO-1-NAPHTHALENE SULPHONIC ACID) MODIFIED GOLD ELECTRODES FOR TYRAMINE DETECTION

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Tyramine (Tyr) is a well-known biogenic amine produced by the decarboxylation of the amino acid tyrosine, that occurs by degradation resulting from microbial activity. It is often found in fermented foods and beverages, meat, fish, seafood and dairy products [1]. It has been reported that Tyr-containing foods can cause unnatural and toxic effects when ingested in large quantities. Thus, a fast and accurate method to measure tyramine concentrations in foods is required [2]. Analytical methods for the determination and quantification of Tyr in food samples include high-performance liquid chromatography (HPLC), hyphenated with mass spectroscopy detection, high-performance capillary electrophoresis (HPCE), mass spectrometry. Electrochemical detection is promising due to its unique characteristics as well as rapid response, low cost, and high sensitivity and specificity [3]. Many materials have been synthesized and used as electrode modifier materials for Tyr detection, such as multiwalled carbon nanotubes, graphene, polymers and nanoparticles [4].
In the present work, a novel impedimetric sensor for the detection of Tyr was developed based on a nanocomposite: poly-(8-anilino-1-naphthalene sulphonic acid (PANSA) and gold nanoparticle) film modified gold electrode. The gold nanoparticles were synthesized by a green synthesis method employing citrus fruits that were incorporated within the PANSA network during electropolymerisation of the monomer, and which contributed significantly for the improvement of conductivity and stability. Quantification of Tyr relied on measuring changes in the charge transfer resistance at a potential where Tyr is oxidised. Under optimal conditions, the impedimetric sensor revealed a broad dynamic range and very good limit of detection, similar to more complex sensor architectures found in the literature. The reliability of the proposed sensor was investigated, as well as selectivity and stability. Application to real samples was demonstrated by analysis of foods and beverages.

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EXAMINOLOGY
AND METROLOGY

POSTER COMMUNICATIONS

PE 1

TOTAL HARDNESS IN SEAWATER SAMPLES OF THE PORTUGUESE COAST

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Accurate characterization of the composition of seawater is required to understand chemical changes occurring in the ocean and their impact on marine ecosystems [1]. Calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions are essential for biological processes in the oceans environment. Marine organisms use Ca^{2+} and carbonate ions (CO_3^{2-}), present in ocean waters to build skeletons, scales, shells and teeth. Also, Mg^{2+}, the third most abundant ion in seawater, behind sodium and chloride, is an important component of chlorophyll, essential for photosynthesis.

Water hardness is a measure of the amount of calcium and magnesium in solution, hence it is an important parameter for the development and reproduction of living marine animals and plants in seawater.

In this work, the concentration of calcium and magnesium ions dissolved in seawater were determined by Ion Chromatography with conductivity detection (IC-CD). Calibration curves were validated using least squares regressions [2].

Three different seawater samples from the Portuguese Coast, Figure 1, were analyzed and results are expressed with their respective associated uncertainties, which is of key usefulness for monitoring seawaters.

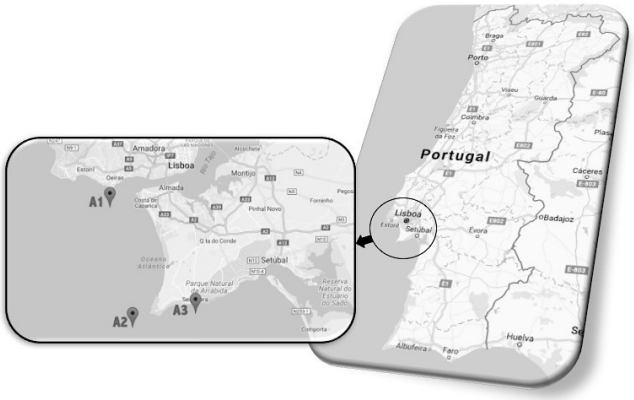

Figure 1: Location of sampling sites (A1, A2 and A3) at the Portuguese Coast.

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PE 2

COMPARATIVE ANALYSIS OF FATTY ACID COMPOSITION OF WILD VS. FARMED SALMON

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To respond to the increasing global demand for fish, nowadays, almost 50% of the global fish market comes from aquaculture production [1]. Thus, there is the need to assure a correct information, not only about the species, but also about the production method (farmed vs. wild) and the catch origin of fish. Salmon, a high-trophic-level carnivorous species with high economic value due to its popularity, is among the fish species that is frequently produced in aquaculture. Although the feed given to farm-raised salmon is designed to meet its nutritional requirements, it can present differences compared to the diet of wild salmon that can be reflected on the muscle composition of farmed *versus* wild salmons. Therefore, this work aims at comparing the fatty acid composition of salmon from aquaculture and caught in the wild.

Salmon specimens caught in the wild (n = 25) and farm-raised (n = 25) were obtained from West of Vancouver Island and Campbell River (Canada), respectively. Two lipid extraction methods (Soxhlet extraction with *n*-hexane and an adaptation of the Bligh and Dyer extraction method) and two derivatization procedures (alkaline transmethylation using KOH and acid-catalyzed transmethylation using BF_3/MEOH solution) were tested. Fatty acid methyl esters (FAME) were analyzed in a Shimadzu GC-2010 Plus gas chromatograph equipped with a Shimadzu AOC-20i auto-injector, a flame ionization detector and a CP-Sil 88 silica capillary column (50 x 0.25 mm i.d., 0.20 µm). The injector and detector temperatures were 250 and 270 °C, respectively. The compounds were identified by comparison with standards (FAME 37, Supelco).

Based on the obtained results, the modified Bligh and Dyer method was chosen for lipid extraction since it allowed obtaining higher amounts of long chain unsaturated fatty acids, particularly of docosahexaenoic acid (DHA). Similar results were obtained for both tested derivatization methodologies. In general, the two groups of salmon samples showed different profiles, with wild samples presenting significantly higher contents of omega-3 fatty acids, in particular docosahexaenoic and eicosapentaenoic acids, while farmed salmon had higher amounts of oleic and linoleic acids.

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PE 3

EXAMINOLOGY FOR ANALYTICAL CHEMISTRY?

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As already mentioned [1], the expression “qualitative measurement” is surprisingly of frequent use [2], notwithstanding not complying with the definition of measurement set by the current International Vocabulary of Metrology (VIM3) [3]. The main rationales [4] for such a use fails to rigorously corresponds to the VIM3 partitioning proprieties regarding “having magnitude” or not, namely quantities and nominal properties, respectively [5]. In line with and extending the VIM3 paradigm, the “Vocabulary for Nominal Properties” (VNP) [6], authored by the International Federation of Clinical Chemistry and Laboratory Medicine and the International Union of Pure and Applied Chemistry, suggested definitions of terms and concepts for nominal properties, introducing the term and concept of examinology, as the science of examination and its application. Indeed, VNP defines “examination” as “process of experimentally obtaining one or more property values that can reasonably be attributed to a property”, i.e. a straightforward extension of the VIM3 definition of “measurement”. Indeed, nominal examination, nominal examinand, nominal property value, etc… are defined as analogous terms and concepts for measurement, measurand, quantity value, etc.., respectively. By the same token, nominal examinational traceability and nominal examination uncertainty have also analogous definitions in VNP to metrological traceability and measurement uncertainty, in VIM3.
However, a recent work of L. Mari incites rather to consider the characteristics of nominal properties and of quantities, from the angle of the foundation of the measurement, namely an “experimental process, providing publicly trustworthy and quantitative information […] through comparison” [7]. As such, it would necessarily lead to designate such process as property evaluation. The purpose of the present communication is to review these concepts, in order to suggest a coherent approach of nominal properties and quantities, for the field of analytical chemistry. The treatment of uncertainty in property evaluations is also briefly analysed, following published works [8].

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PE 4

CUMULATIVE STANDARD ADDITION METHOD FOR ELECTROCHEMICAL MEASUREMENTS OF BIOLOGICAL FLUIDS

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The Standard Addition Method, SAM, is very popular for compensating matrix effect that vary, in an unpredictable way, between analysed items. However, the conventional SAM requires large volumes of sample to prepare the standard additions, which makes it frequently difficult to apply to the analysis of biological fluids.
The cumulative standard addition of volumes of a stock solution of the analyte to the same analytical portion, makes SAM implementation feasible, but requires the compensation of the progressive dilution of the analytical portion. The cumulative standard addition method (SAM-C) is particularly suitable for the electrochemical analysis of small volumes of biological fluids.
A new methodology for compensating the progressive dilution of the analytical portion in the SAM-C was developed and validated, including the development of detailed measurement models capable of estimating measurements uncertainty. This methodology was successfully applied to the quantification of uric acid by voltammetry using a carbon paste electrode modified with lignin and electrodeposited copper particles [1].
The calibration of the electrode involves defining the added analyte mass, *m*, has the independent variable (i.e. the stock solution concentration times the added cumulative volume) and the total solution volume, *v*, (i.e. sample volume plus cumulative added stock solution volume) times the observed signal, *I*, (i.e. *v*I*) has the dependent variable. The ratio between the intercept and the slope of the calibration curve (*v*I* vs. *m*) represents the estimated analyte mass in the analytical portion, m_S, and this value divided by the analytical portion volume, v_S, the analyte concentration, γ_S, in the sample ($\gamma_S = m_S/v_S$). If the native analyte dilution as stock solution volume is added is not considered, measurements can be affected by a large error.
A used-friendly spreadsheet was developed to validate and evaluate the uncertainty of measurements performed by the SAM-C.
The developed SAM-C and respective measurement models are applicable to any kind of non-destructive chemical measurement of a solution.

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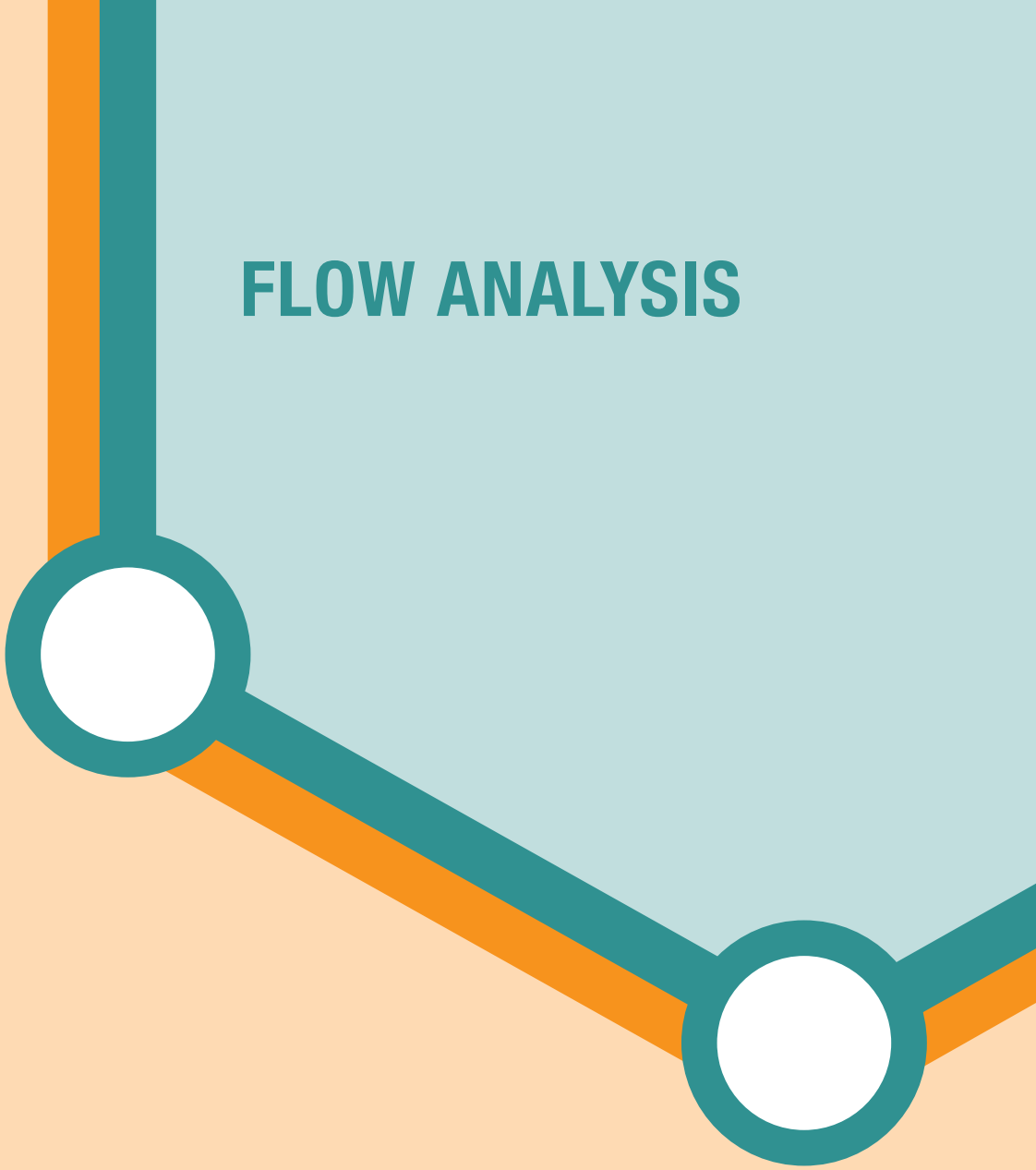

POSTER COMMUNICATIONS

PF 1

DEVELOPMENT OF A SEQUENTIAL INJECTION METHOD FOR BROMATE DETERMINATION IN SOIL LEACHATES

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Bromate, specifically potassium bromate, has been related to some adverse health effects [1] and considered as potentially carcinogenic to humans by the International Agency for Research on Cancer [2].
Although bromate is not normally present in water, it can occur as a result of industrial pollution or as consequence of soil contamination. In fact, there are several uses for potassium bromate and sodium bromate as powerful oxidizers, for example, in the textiles dyeing process that uses sulfuric dyes. In addition, water disinfection treatments like ozonation promote the oxidation of halogenides naturally present in water, namely bromide, to bromate [2]. There are several methods for the determination of bromate described such as ion chromatographic methods, gas chromatographic methods and capillarity electrophoresis [3].
Flow analysis methods, sequential injection analysis (SIA) in particularly, have been extensively used for water monitoring due to several advantages like real-time, robustness, reliability, as well as the versatility [4]. The latter is essential to incorporate additional pre-concentration and/or clean-up procedures together with in-line digestions and/or redox reactions.
The aim of this work was to develop an automatic flow analysis method for bromate determination in soil leachates. The idea was to explore the main features of flow methodologies to attain an environmental friendly and low-cost method as an alternative analytical tool for bromate monitoring. The spectrophotometric detection was based in the reaction with *o*-dianisidine (ODA) but other reagents, fuchsin and chlorpromazine, were also studied aiming for the highest sensitivity. For the application, LSSC (laboratory scale soil core) columns were used to produce leachates with and without simulation of potential contamination.

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PF 2

MONITORING GLUCOSE, CALCIUM, AND MAGNESIUM LEVELS IN SALIVA AS A NON-INVASIVE ANALYSIS BY SEQUENTIAL INJECTION MULTIPARAMETRIC DETERMINATION

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The use of saliva for diagnose and surveillance of systemic illnesses and general health has been arousing great interest worldwide being a highly desirable goal in healthcare. The main advantages of the approach are: a noninvasive sampling; stress-free; inexpensive, simple method of collection. Glucose, calcium, and magnesium concentration are three major parameters evaluated in clinical context due to their essential role in a wide range of biochemical reactions, and consequently many health disorders. In this work, a spectrophotometric sequential injection method is described for the fast screening of glucose, calcium, and magnesium in saliva samples. The glucose determination reaction involves the oxidation of the aldehyde functional group present in glucose with simultaneous reduction of 3,5-dinitrosalicylic acid (DNS) to 3-amino, 5-nitrosalicylic acid under alkaline conditions, followed by the development of color, as described by Miller [1]. The determination of both metals is an adaptation of a previous work [2] based on their reaction with cresolphtalein complexone (CPC); with the calcium interference minimized by ethylene glycol-bis[β-aminoethyl ether]-*N,N,N',N'*-tetraacetic acid (EGTA) for the determination of magnesium. The developed multiparametric method enabled the determination in the dynamic range of 50-300 mg/dL for glucose, 0.1-2 mg/dL for calcium and 0.1-0.5 mg/dL for magnesium. Determination rates of 28, 60, 52 h^{-1} were achieved for glucose, calcium and magnesium, respectively. Due to saliva viscosity and inherent necessity of dilution prior to analysis, less than 300 μL of saliva are required for the multi-parametric determination. RSDs lower than 5% were obtained, and the results agreed with those obtained by reference methods, while recovery tests confirmed its accuracy.

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PF 3

AUTOMATED BEAD-BASED IMMUNOASSAY FOR THE DETERMINATION OF CARBAMAZEPINE IN WASTEWATER

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The improvement of immunoanalytical methods for the determination of pharmaceuticals in wastewaters is a crucial yet challenging endeavor. In this work, the development of an automated miniaturized ELISA based on micro-Bead Injection Spectroscopy (μ-BIS) [1] for the determination of carbamazepine, a widely employed anti-epileptic drug and emergent pollutant [2], was pursued.
The experimental workflow comprised the offline functionalization of Sepharose beads with specific anti-CBZ antibodies via affinity immobilization using protein G, and 3 online steps inside the microfluidic analyzer lab-on-valve (LOV): I) packing of the bead column into the detection unit; II) sequential percolation of sample and a CBZ competitor- labeled with horseradish peroxidase (tracer) through the bead column; and III) on-column colorimetric detection employing the enzyme substrate 3,3',5,5'-tetramethylbenzidine. After each analysis, the bead column was discarded, and the flow cell was washed before receiving new beads.
The elimination of manual washing steps is a novel feature compared to batch-wise ELISA, making the method less error-prone and therefore more robust. The replacement of the solid support prevents memory effects and cross-contamination between runs. The use of microparticles as solid support for the molecular recognition elements accounts for high area-to-volume ratios, and low molecular diffusion distances. For that reason, time-to-result was reduced from several hours to less than 10 min. The consumption of reagents was also very low. For instance, only ca. 200 μg of solid support and 900 ng of anti-CBZ antibody were required per determination. At last, the versatility of the LOV platform offers the possibility of adapting the assay to other relevant pharmaceuticals and anthropogenic markers in water.

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PF 4

STUDY OF 3,4–HYDROXYPYRIDINONES FUNCTIONALIZED BEADS FOR IRON(III) DETERMINATION IN A MICROSEQUENTIAL INJECTION SOLID PHASE SPECTROMETRY MODE

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In a previous study [1], a bidentate 3,4-hydroxypyridinone (3,4-HPO) ligand was used as a novel colour reagent, in a sequential injection mode, to carry out the spectrophotometric determination of iron(III); the metal ion was previous retained in nitrilotriacetic acid (NTA) microbeads. In this study, the objective was to accomplish both iron(III) retention and spectrophotometric measurement, by functionalizing the beads with the bidentate 3,4-hydroxypyridinone ligand. This way, by using the sorbent/colour reagent in consecutive cycles, the ligand consumption could be minimized. The spectrophotometric reaction was carried out at the beads surface, packed in a lab-on-valve flow cell, in a solid phase spectrometry (SPS) approach. The results obtained were compared with those obtained using the NTA Superflow resin for iron (III) retention. The functionalized beads proved to be a more efficient method to quantify iron(III) at pH~7 if compared to the NTA resin, that retains iron(III) at pH~2. This is an important factor if a direct application to biological samples, namely blood serum, is envisaged.

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PF 5

MONITORING SOIL/WATER INTERFACE: DEVELOPMENT OF AN INTEGRATED SEQUENTIAL INJECTION SYSTEM APPLIED TO LABORATORY SCALE SOIL CORE COLUMN AND MICRO SOIL COLUMN

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Monitoring soil leaching of nutrients and contaminants has become essential for environmental and agricultural studies. With a growing concern on soil contamination and increasing awareness of inorganic and organic contaminants effects on soil quality, the study of soil leaching is vital. The leachates from soil have a huge impact on the quality of surface and ground waters. Conventional soil testing can hardly keep up with this ever-increasing demand of sample analysis frequency as it is based on manual or mechanical soil sampling and atomic absorption/emission spectroscopy detection, leading to costly and time-consuming assays.
In this context, the work developed aimed to tackle this issue by setting a laboratory scale soil core (LSSC) column and developing a sequential injection (SI) method for soil leachates monitoring. The LSSC and micro soil columns (μSC) were set with soil from different locations. Rain water, well water and iron complexes solutions, namely the commercially available iron fertilizer FeEDDHA and two new fertilizers from the hydroxypyridinones family of complexes $Fe(mpp)_3$, $Fe(dmpp)_3$ [1, 2], were passed through the columns, and the impact in the leachate evaluated. So, the water/iron complexes solutions were assessed before and after perfusing the LSSC and μSC.
The SI method proved to be advantageous, in terms of cost, time consumption and waste production, in comparison to conventional methods. With the developed method, an efficient monitoring of soil leachate process can be attained.

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PF 6

BIOACESSIBILITY OF ZINC IN PET FOOD DETERMINED BY A DYNAMIC LEACHING METHOD

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In dynamic leaching methods, portions of extractant reagents are continuously provided to the solid sample contained in flow-through microcolumns or chambers, enabling the renewal of extracting fluid and avoiding saturation effects from fluid stagnation. These methods are also suitable for fast measurements in real time with small extract manipulation, especially when coupled online with suitable detectors [1]. In this work, the bioaccessible fraction and kinetic leaching profile of zinc in pet food was determined using a robust flow-through device, composed by two filters placed in polypropylene holders to entrap the solid sample, designed for dynamic leaching experiments [2]. Continuous extraction flow was ensured by a peristaltic pump connecting the extraction reservoir and the extraction chamber, at a flow rate of 0.5 mL min^{-1}. Synthetic fluids simulating digestive compartments were applied as extractants. The kinetic extraction profile of fast leachable Zn was evaluated by flame atomic absorption. Operational conditions, including filters' composition and pore size, were tested. Preliminary results have shown that different extracting fluids (with and without digestive enzymes) had an influence on the total amount and on the leaching kinetic profile of Zn. In fact, higher values were obtained when enzymes were present in the extracting fluids. The proposed dynamic leaching method was suitable for evaluation of bioaccessible Zn in pet food. This information will be applied for the improvement of Zn supplementation in dog foods and for designing new products with enhanced mineral delivery.

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PF 7

POLYMER INCLUSION MEMBRANES (PIMS) AS AN ALTERNATIVE FOR ON-LINE SOLID PHASE EXTRACTION (SPE) IN FLOW ANALYSIS

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A membrane can be considered as a selective barrier between two phases; in recent years, membrane-based processes have been subject of high interest in many fields, namely biotechnology, wastewater treatment and others [1]. Membrane research has attracted considerable efforts in recent years to provide a better understanding of membrane-based separation and improve its efficiency. This line of investigation has led to the development of polymer inclusion membranes (PIMs) which are thin, flexible and stable polymeric films that can selectively separate chemical species of interest [2]. PIMs are fabricated by solvent casting a solution containing an extractant (the selective agent) and a base polymer that stably encapsulates the extractant. Some PIMs may also contain a plasticizer and/or chemical modifiers.
Solid phase extraction (SPE) is a widely used technique applied to the sample pre-treatment in analytical chemistry. This technique is associated to a significant number of advantages as it can selectively separate the analyte, or it could remove matrix interferences as a clean-up step in complex matrices. In certain conditions, SPE could also be a strategy for pre-concentration of a specific analyte present in low concentrations.
In this work, PIMs fabricated from 45 wt% di-(2-ethylhexyl)phosphoric acid (D2EHPA) and 55 wt% PVC are explored as an alternative sorbent material for SPE aiming for the separation and/or pre-concentration of Zn(II). This work aims to show that this novel SPE method can be used to separate and/or pre-concentrate Zn(II) form highly complex sample matrix.

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METHOD VALIDATION

POSTER COMMUNICATIONS

PG 1

MEASUREMENT UNCERTAINTIES IN PLATINUM AND RHODIUM QUANTIFICATION BY ADSORPTIVE CATHODIC STRIPPING VOLTAMMETRY

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Platinum-group elements (PGEs) have raised environmental and human health concern over the past years, due to relevant increase on uses in several areas of economic interest. However, suitable techniques for PGE determination at ultra-trace concentrations are still challenging and appropriate certified reference materials (CRMs) are needed for environmental matrices. Usually it is used a road dust (BCR–723), whereas for biological matrices CRMs are inexistent. The COST Action TD1407–NOTICE [1] proposed a community-wide inter-laboratory exercise to ensure analytical accuracy and seek the development of appropriate CRMs for technology-critical elements. This inter-laboratory study aimed to contribute for the development of a new CRM for Pt and Rh. An 'unknown' estuarine sediment from the Gironde Estuary, France, was collected, prepared and distributed to several laboratories. Aliquots were acid digested to be analysed simultaneously for Pt and Rh ($n > 10$) by Adsorptive Cathodic Stripping Voltammetry [2]. Procedural and digestion blanks, and BCR-723 were also analysed. Digestion blanks showed the absence of Pt and Rh cross-contaminations along digestion and analysis procedures. Limits of detection (LOD) calculated for a deposition time of 300 s were 0.012±0.002 ng Pt g^{-1} and 0.008±0.003 ng Rh g^{-1}. Recoveries of BCR-723 were 90±10% for both elements. The concentrations found in the inter-laboratory sample were 0.86±0.15 ng Pt g^{-1} and 0.36±0.12 ng Rh g^{-1}. Estimation of relative combined standard uncertainties, 16% and 8% for Pt and Rh respectively, express precision and trueness [3]. This study provides evidence of fit-for-purpose and the method adequacy for routine analysis.

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PG 2

LIPID OXIDATION ASSESSMENT IN LOTIONS USING THE THIOBARBITURIC ACID REACTION

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Lipid oxidation in products with high fat content such as creams and lotions is well known for causing changes in odor and responsible for the deterioration processes that affect product quality and safety. The secondary volatile oxidation products, particularly aldehydes, ketones and alcohols can be responsible for the development of off-odors even when present in low concentrations [1]. Since odor characteristics are important quality parameters, it is essential to control the lipid oxidation of products using an easy and practical method. Thiobarbituric acid (TBA) assay is used to quantitatively determine lipid peroxidation for aldehydic compounds in high fat content matrices. This reaction has been successfully applied in very different matrices like biological samples and foods. The thiobarbituric acid – reactive lipid aldehydes (TBARLA) can be easily measured using spectrophotometric or fluorometric detection [2]. The aim of this work was to validate and evaluate the TBARLA in an unusual matrix, body lotions, using both methods of detection. Briefly, samples were demulsified using isopropanol before extraction with trichloroacetic acid (5%). To an aliquot of this solution is added the same amount of TBA solution (0.375%) and the mixture was heated at 80 ºC during 30 min. Malonaldehyde (MDA) was used as standard [3]. Spectrophotometric analysis was performed at λ = 532 nm, whilst fluorimetric analysis was tested using λ_{ex} = 485 ± 20 nm and λ_{em} = 590 ± 35 nm. Both measurements were determined using 96-well plates, in a Synergy HT W/TRF Multimode Microplate Reader. All measurements were performed in triplicate. The spectrophotometric method presents a limit of detection (LOD) value of 4.94 μg MDA/L and 16.5 μg MDA/L for the limit of quantification (LOQ) whereas the fluorimetric method presents a LOD of 0.76 μg MDA/L and a LOQ of 2.54 μg MDA/L. Both methods were applied to four different samples of body lotions that presented different levels of lipid oxidation. When using the spectrophotometer analysis, TBARLA values varied between <LOD to 10.0 ± 0.2 μg MDA/g sample. For the fluorimetric analysis, TBARLA values for the same samples varied between 0.31 ± 0.02 to 11.5 ± 0.2 μg MDA/g sample. Therefore, TBA assay with fluorescence detection allows the analysis of lower levels of lipid oxidation products in samples due to its higher sensitivity.

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PG 3

QUANTIFICATION OF TRANEXAMIC ACID IN HUMAN PLASMA: DEVELOPMENT AND VALIDATION OF UHPLC-MS/MS METHOD

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Tranexamic acid (TXA), an antifibrinolytic drug with the ability to inhibit lysine binding at plasminogen receptors, can be used in different settings such as trauma, cardiac surgery, major orthopedic surgery, obstetric when perioperative bleeding is concerned [1]. Effective methods for determination of TXA in biological samples are still required to understand the pharmacokinetics and pharmacodynamics of this drug in variable age groups undergoing surgeries with high blood loss [2].
The development and validation of a method based on ultra-high performance liquid chromatography coupled to triple quadrupole-tandem mass spectrometry (UHPLC-MS/MS) to quantify TXA in human plasma is described herein.
A simple, inexpensive and efficient sample treatment involving protein precipitation with acetonitrile containing 0.5% (v/v) formic acid was implemented using volumes within the microliter range. Separation was achieved using a hydrophilic interaction based stationary phase and ammonium bicarbonate in the mobile phase that permitted a more efficient separation of the analyte from the matrix interferences, thus reducing matrix effects and increasing method sensitivity.
The method was validated according to the European Medicines Agency guideline [3]. Excellent linearity was achieved (r^2 > 0.997) for TXA concentrations ranging from 30 to 600 ng mL^{-1} with LOD and LOQ of 3 and 6 ng mL^{-1} in plasma extracts, respectively. The developed method proved to be selective, sensitive, accurate (96.4-105.7% of nominal concentration values) and precise (RSD ≤ 4.5%).

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PG 4

HPLC ANALYTICAL METHOD DEVELOPMENT FOR APOMORPHINE HYDROCHLORIDE QUANTIFICATION

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Raynaud's disease is a disorder related to arterial spasms due to an exacerbated activation of the sympathetic nervous system, causing blood flow restrictions of peripheral structures. No treatment is available besides preventive measures or symptom control. Drug management is often needed; however, no specific standard drug treatment has been found [1]. Topical application of apomorphine HCl may have treatment value due to its α−adrenergic antagonist effect on the endothelium. There isn't a commercial apomorphine HCl topic formulation for local application; hence, the development of an extemporaneous preparation through pharmaceutical compounding should be considered. To ensure that drug stability is maintained, an analytical method for quantification is necessary. The aim of this work was to develop and validate an in-house HPLC method for the identification and quantification of apomorphine HCl in two semisolid formulations.

Apomorphine HCl UV spectra (200-400 nm) was analyzed using a spectrophotometer. The maximum absorption value was used for the HPLC detection (275 nm). The selected chromatographic conditions were an isocratic elution of the mobile phase (methanol: an aqueous solution of 50 mM orthophosphoric acid adjusted to pH 3.5 with sodium hydroxide 20:80), the flow rate of 1 mL/min, and injection of 20 µL. A calibration curve covering 80-120% of the target concentration was obtained by analysis of standard drug solutions (0.04 to 0.12 mg/mL). The quality of the HPLC method was determined by the peak asymmetry, number of plates, retention time, and capacity factor (k') [2]. The method validation was assessed by the linearity, precision, specificity, detection limit (DL) and quantification limit (QL) [3]. The peak asymmetry of the standard apomorphine HCl solutions and samples was, respectively, 1.7033±0.0981 and 1.605±0.1372, the number of plates 4193.0±87.6 and 1489.5±186.5, and the retention time (min) 4.14±0.0085 and 3.74±0.0065. No peak overlay was observed between the drug and the sample matrix, with the k' value of 2.7417±0.0075. The linearity between the drug concentration and the signal was established by a standard curve with a determination coefficient (R^2) of 0.9995, with a sum of squared residuals (SSR) of 0.2262, with a y-interception of 0.8848, and a slope of 445.15. The graph of residuals and the Rikilt was also used to demonstrate the linearity. The DT was 0.0037 mg/mL and the QL was 0.0111 mg/mL. An HPLC in-house method for apomorphine HCl identification and quantification was achieved. The process showed satisfactory linearity, precision, and specificity, with a low detention and quantification limits.

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PG 5

VALIDATION OF HPLC METHOD FOR QUANTIFICATION OF BOSENTAN ON TWO DIFFERENT ORAL VEHICLES

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Bosentan is an endothelin receptor antagonist, useful to manage pulmonary hypertension and systemic sclerosis [1]. Currently, only 62.5 mg and 125 mg per dose solid forms are available in Portugal, containing bosentan as monohydrate [2]. These doses are unfit in various situations, such as: children under 12 years old or under 40 kg, or anyone needing a different form of administration such as patients using a feeding tube or with any kind of swallowing problems. The only way to deliver such medicines is extemporaneous compounding which raises concerns about the stability of the drug over time, and consequently, its efficacy. A reverse phase high performance liquid chromatography (HPLC) method to determine bosentan monohydrate concentrations on two different formulated liquid oral vehicles, was developed [3, 4]. The method was linear on the range between 40-100 µg/mL, with the determination coefficient (r^2) = 0.9996. The maximum coefficient of variation was 5.28% and the sum of the square residuals was 1.41, indicating the method was precise. In addition, the residuals vs concentration graphic was analyzed revealing random residuals variation. The Rikilt test was also conducted and revealed very small response variation, below 5%. The specificity was assessed by overlaying the UV spectra of bosentan monohydrate with placebo vehicles, and bosentan monohydrate compounded in each vehicle. In addition, the peak purity analysis was conducted, retrieving a peak UV match factor around 998. The detection limit (LD) and quantification limit (QL) were 2.33 and 7.07 respectively. An HPLC in-house method for bosentan monohydrate identification and quantification was achieved. The process showed satisfactory linearity, precision, and specificity, with low DL and QL.

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PG 6

HPLC-MS/MS METHOD FOR QUANTIFICATION OF THE NEUROPEPTIDE Y Y1 RECEPTOR ANTAGONIST BIBP 3226 IN CELL EXTRACTS

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Neuropeptide Y (NPY) is involved in various physiological processes, including the regulation of feeding behavior and energy homeostasis. NPY activates different receptors in several brain regions. Recently, Y1 receptor (Y1R) has arisen as a potential regulator in the local control of bone turnover suggesting that an anti-receptor strategy may be a useful therapeutic approach to prevent and/or reverse bone loss. BIBP 3226 is a potent Y1R selective antagonist that has been successfully used in *in vitro* studies showing a positive impact in bone turnover and thus providing good perspectives towards its application as a pharmacological tool for bone regeneration. Hence, the major aim of the present work was to implement a method based on high performance liquid chromatography coupled to triple quadrupole-tandem mass spectrometry for quantification of BIBP 3226 in cellular internalization assays.

Chromatographic separation was achieved using a reversed phase Kinetex® core-shell C8 column at 30 °C and elution in isocratic mode using a mixture of acetonitrile and water (30:70, v/v), containing 0.1% (v/v) formic acid, at 0.25 mL min^{-1}. Total run time was 5.0 min, with retention time of 3.7 min for the target compound. The MS/MS was operated in positive ionization mode (ESI+) and data were acquired in multiple reaction monitoring (MRM) mode (*m/z* 474>167 for quantification and *m/z* 474>107 for identity confirmation). Calibration curves were linear for concentrations ranging from 0.5 to 30 ng mL^{-1}. BIBP 3226 was quantified in cell extracts obtained from internalization assays performed with bone marrow and breast cancer cells, after solvent evaporation and resuspension in mobile phase. LOD and LOQ were 0.04 and 0.1 ng mL^{-1}, respectively, corresponding to values as low as 0.3 and 0.8 pg per well.

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PG 7

INCLUSION COMPLEX OF p-CHLORO-THIO-NOR-β-LAPACHONE IN 2-HYDROXYPROPYL-β-CYCLODEXTRIN AND DEVELOPMENT OF UV SPECTROPHOTOMETRIC ANALYTICAL METHODOLOGY

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The study of *p*-chloro-thio-nor-β-lapachone (*p*CTN-βlap) and its inclusion complex in 2-hydroxypropyl-β-cyclodextrin (2-HPβ-CD) are very important since this substance free presented, *in vitro*, promising results against the *T. cruzi* of $IC_{50/24h}$ 9.2 ± 1.4 μM and SI de 4.23 [1]. However, it has low solubility in biological fluids, which is a problem for the advancement of *in vivo* tests. Based on these considerations, this study aimed to develop an inclusion process and development of analytical method in order to quantify this substance in 2-HPβ-CD by using spectrophotometry in the UV region. The parameters evaluated were: specificity, selectivity, linearity, precision, limit of detection/quantification and robustness [2,3]. In addition, the inclusion complexes (IC) of *p*CTN-βlap in 2-HPβ-CD were obtained by three different methods: solid solution (SS), mixed solution (MS) [4] and liquid solution (LS) [5]. The three IC were evaluated for powder recovery and encapsulation efficiency (EE%). The use of spectrophotometry in the UV region was considered satisfactory according to predetermined parameters. The methods used to form the complex demonstrated a variation in the percentage of solid recovery between 79.3% and 83%. The EE% and respective variances were: 9.33% (± 0.35) to the SS, 10.96% (± 0.74) MS and 7.15% (± 0.045) LS. It is concluded that the UV spectrophotometric method is valid for the quantification of *p*CTN-βlap free and in IC. On the other hand, the methods used for the formation of IC were not suitable for improving the solubility of the compound.

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PG 8

SIMULTANEOUS DETERMINATION OF DAPSONE AND CLOFAZIMINE IN NANOFORMULATIONS BY HPLC

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The multidrug therapy with dapsone (DAP) and clofazimine (CLZ) is known as an effective treatment against *Mycobacterium leprae*. However, the low bioavailability and non-specific distribution can reduce therapy efficacy and produce side effects. The use of nanotechnological approaches was explored as a promising carrier for delivery enhancement of these drugs. Therefore, a simple and precise high-performance liquid chromatography (HPLC) method with UV/Vis detection has been developed and validated for the simultaneous determination of DAP and CLZ loaded in solid dispersion and poly(D,L-lactide-co-glycolic acid) nanoparticles, respectively, targeting therapy improvement.

A reversed phase Kinetex core-shell C18 column at room temperature followed by UV/Vis detection at 280 nm was used for chromatographic separation. The elution was performed in gradient mode using aqueous acetate buffer (50 mol L^{-1}, pH 4.8) and an increasing acetonitrile content from 27 to 63% (v/v), at a flow rate of 1.0 mL min^{-1}. The injection volume was fixed at 20 µL and total run time was 23.0 min, with a retention time of 6.0 min for DAP and 14.0 min for CLZ.

The method was validated according to EMA guideline and showed specificity, accuracy (between 99.6 and 114.0% of nominal values) and precision for intra-day (RSD ≤1.8%) and inter-day assays (RSD ≤12.5%). Calibration curves were linear (r^2 >0.9979) and LOD ≤0.03 and LOQ ≤0.06 mg L^{-1} were obtained. Stability was studied after 24 h at room temperature and over three freeze-thaw cycles, and recovery values ≥86.2% were obtained. Precipitation of CLZ was observed at low temperatures (4 °C). Entrapment efficiency in nanoformulations was evaluated as 54.8 ± 0.1% for DAP and 24.9 ± 0.2% for CLZ. The developed method was successfully validated for the simultaneous determination of DAP and CLZ in nanoparticles.

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PG 9

PROCESS CHARACTERIZATION OF ULTRASONIC WELDING OF ELECTRICAL WIRES

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The ultrasonic welding process for wires are being largely used on industry mainly in application that involve the connection between different cross sections. One of the biggest benefits of this technology is the possibility to perform the weld without addition materials, like terminals, metal rings, tapes, etc. However, it has been seen that depending on the insulation material of wires the metal bonding is not always stronger as it should be. Manufacturing of wiring harnesses demands a significant amount of joining, such as welding, crimping or soldering, to fulfill the desired layout of the harnesses and capacity requirements. However, conventional processes, such as crimping and splicing, face difficulties in joining multiple cross sections mainly to the process characteristics and equipment to be used. Ultrasonic metal welding overcomes such difficulties by using its inherent solid-state process characteristics. Despite a considerable amount of past research on ultrasonic metal welding, the fundamental mechanisms behind this process are still uncertain. Moreover, there is a lack of scientific quality guidelines for implementing ultrasonic welding in volume production due to the different characteristics of wires to use. This research develops methods for comprehensive characterization of the process and quality in ultrasonic welding of electrical wires. The research topics addressed in this paper are:
1. Characterization of joint quality in ultrasonic welding of electrical wires. Several physical weld attributes are identified by experimentally characterizing the weld formation over time using copper-to-copper wires. The weld attributes are then correlated to weld performance by examining the cross-sectioned samples of different weld quality using optical microscopy, scanning electronic microscopy, and pull/peel force measurements.
2. Analysis of weld formation using different insulation materials to determine the influence of insulation characteristics on the ultrasonic welding process.

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PG 10

ANALYTICAL MICROSYSTEM FOR THE SPECTROPHOTOMETRIC DETERMINATION OF TITRATABLE ACIDITY IN WINES

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Wine is a product of the yeast fermentation of the naturally present sugars in grape juice [1]. The chemical composition of wine is very variable and complex. The acidic compounds, besides the effect that they can have in the colour, the taste and the stability of finished wines, are responsible for keeping the wine microbiologically and chemically stable. Therefore, the determination of titratable acidity in wine has become a routine procedure determining wine character and quality [2].
In this context, a microfluidic flow-based system has been developed for the automation of a methodology for the determination of titratable acidity in wine. In the proposed system, a precise volume of sample is mixed with a buffered bromothymol blue (BTB) solution, leading to a change on the absorbance of the solution. This change in absorbance can be monitored at 607 nm by a home-made miniaturized optical detection system, and it can be related with the titratable acidity of the sample.
The hydrodynamic conditions for the microsystem were evaluated and optimized, and the system was used for the determination of the titratable acidity of different wine samples with less than 5% error when compared to the official analysis method of the Association of Official Analytical Chemists [3].

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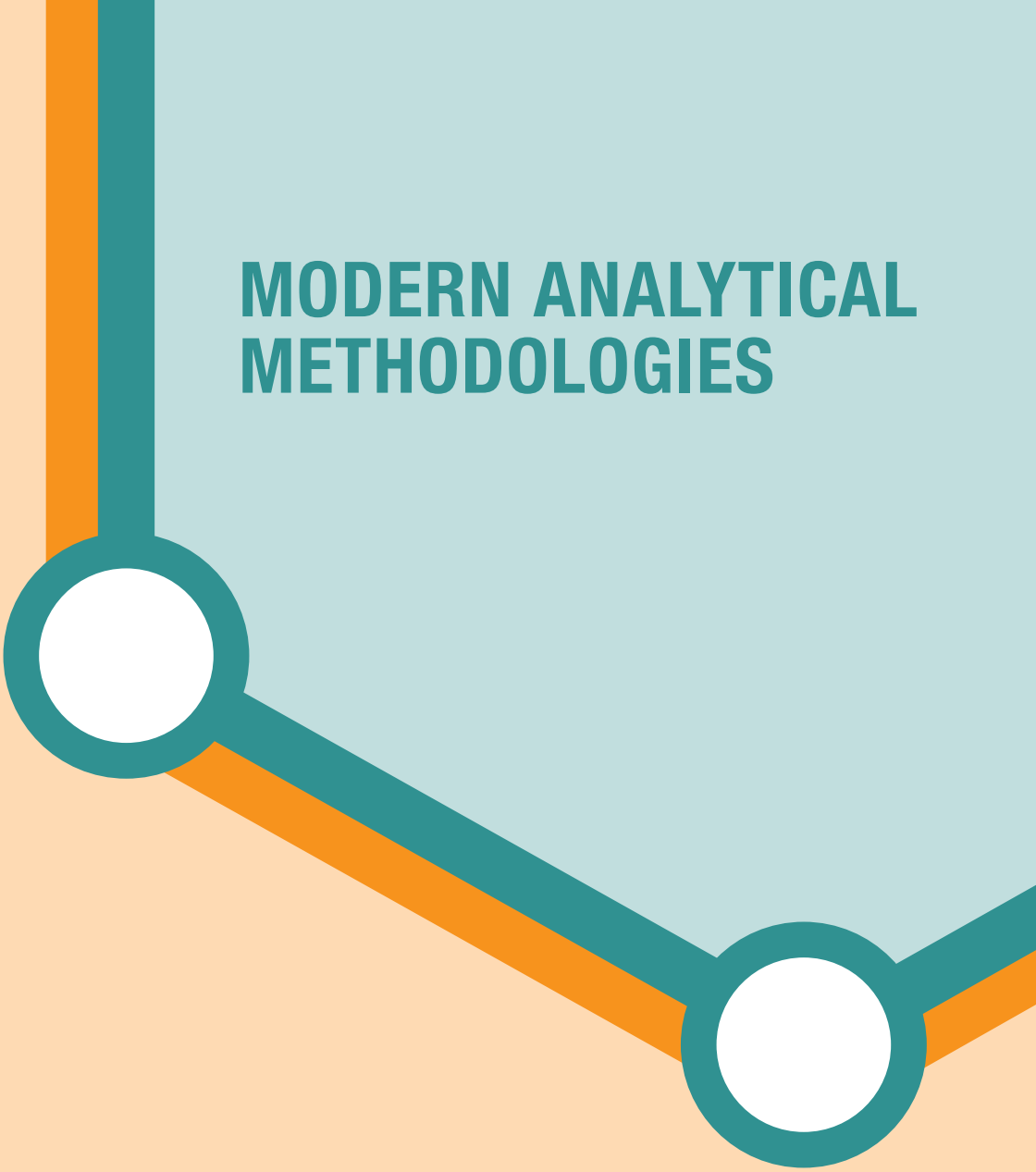

MODERN ANALYTICAL METHODOLOGIES

POSTER COMMUNICATIONS

PH 1

HOLLOW FIBER MICROEXTRACTION (HFμE) FOR ULTRA-TRACE ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS IN REAL MATRICES

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A new hybrid microextraction technique (hollow fiber microextraction; HFμE) is presented, using the main concepts and advantages of the modern miniaturized devices used for trace analysis. This novel analytical approach uses devices constituted by polypropylene membranes (10.0 mm in length and 0.6 mm internal diameter) embedded with convenient organic solvents which promote fast kinetics during the enrichment process, under the floating sampling technology. An innovative analytical cycle (Figure 1) is also introduced by using low cost disposable devices during the microextraction stage together with a user-friendly ('single liquid desorption step') back-extraction stage in compliance with the green analytical chemistry principles. To evaluate the applicability of the proposed technique, polycyclic aromatic hydrocarbons were used as model compounds in several real samples, such as environmental (wastewater and soil), food (tea) and biological (fish liver) matrices. From the data obtained, the new hybrid HFμE technique proved to be user-friendly, eco-friendly, cost-effective and very competitive for routine work. In short, the novel microextraction technique proposed herein showed to be a remarkable alternative for trace analysis of emerging compounds in real matrices over other well-established ones.

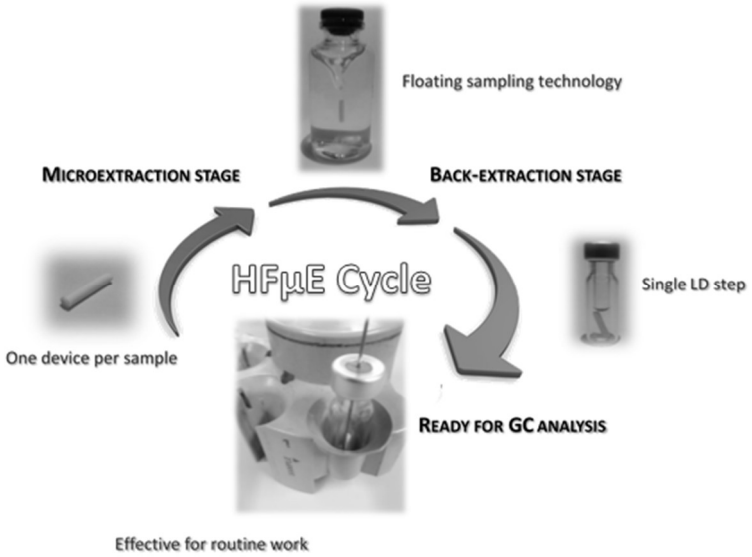

Figure 1: Scheme showing the sequential analytical procedures proposed for the innovative HFμE cycle from the microextraction stage to the instrumental analysis.

Acknowledgements: The authors wish to thank Fundação para a Ciência e a Tecnologia (Portugal) for financial support (Project: UID/MULTI/00612/2013) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Brazil) for the PhD (CAPES BEX 0394-14-9) grant.

PH 2

COMPARISON OF AN AUTOMATIC CHEMILUMINESCENCE ASSAY AND CLOSED BOTTLE TEST FOR THE DETERMINATION OF DEEP EUTECTIC SOLVENTS' BIODEGRADABILITY

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Deep eutectic solvents (DESs) have grown in popularity as a more environmentally friendly alternative to conventional organic solvents and even to ionic liquids (ILs). Despite their negligible vapour pressure and non-flammability, several studies have demonstrated that ILs are not intrinsically green, as was first believed. Actually, ILs seem to possess similar or higher toxicity than organic solvents and low biodegradability. On the contrary, the natural origin, low toxicity and biodegradability of DESs starting materials suggest that these mixtures could be greener than ILs, besides being easier to synthesize and cheaper [1]. Nevertheless, it is extremely important to assess the potential impact of DESs on the environment before their indiscriminate use.

In this context, the biodegradability of DESs, prepared with choline chloride and different hydrogen-bond donors, was determined by the conventional closed bottle test [2] and compared with data obtained using a newly developed automatic chemiluminescence assay resorting to sequential injection analysis. This assay is based on the redox reaction between quinone and *Saccharomyces cerevisiae* in the presence of organic compounds. The formed active oxygen species react with luminol under the catalysis of ferricyanide, resulting in an increase of the chemiluminescence signal [3]. The results obtained from the two methods are statistically correlated and revealed differences in biochemical oxygen demand and % biodegradation between the tested compounds, demonstrating the impact of DESs' chemical structure on its biodegradability. Furthermore, the automatic methodology enabled a reduction of reagents consumption (75-fold decrease) and improved sampling rate (from 5 days in the conventional method to 7.5 min in the automatic method). Thus, it is expected that the automatic bioassay could be used as a screening method to determine the biodegradability of DESs and predict their impact on the aquatic environment.

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PH 3

BIOACTIVE COMPOUNDS, ANTIOXIDANT ACTIVITY AND CELL VIABILITY OF *ACTINIDIA ARGUTA* INFUSIONS AND DECOCTIONS

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Herbal and fruit infusions are amongst the world's most popular and widely enjoyed beverages. These drinks offer a full range of flavours to suit every taste in any occasion. The *Actinidia arguta* (also known as hardy kiwi) is a fruit with an intense flavor, being an excellent source of vitamin C, carotenoids, chlorophylls, minerals, fibers, sugars, organic acids and phenolic compounds. The fruit is also consumed in juices, jams and ice creams. Nevertheless, to the best of our knowledge, no studies report the use of hardy kiwi in infusions and decoctions. The main aim of this study was to determine the total phenolic and flavonoid contents (TPC and TFC, respectively) as well as the antioxidant activity (through DPPH and FRAP assays) and the cell viability (in HT29-MTX and Caco-2) of infusions and decoctions of dehydrated hardy kiwi.

Hardy kiwis were harvested in September 2017 in Famalicão, Portugal. Samples were dehydrated and milled in a fine powder. For the infusion, 100 mL of boiling water was added to sample (2 g) and filtered after 5 min. For the decoction, sample (2 g) was added to 100 mL of water, heated and allowed boiling, and filtered after 5 min. For the cell culture assays the samples were lyophilized.

The TPC were 146.35 mg and 93.81 mg GAE/L, respectively, for decoction and infusion. The decoction also presented the highest TFC result (32.39 mg CEQ/L). In what concerns to the antioxidant activity, the decoction displayed the highest results (56.65% inhibition for DPPH; 1292.45 μmol $FeSO_4$/L for FRAP). Positive linear correlations were established between TFC, TPC and DPPH, FRAP. Finally, the cell viability assays demonstrated that both samples did not conduct to a decrease on Caco-2 and HT29-MTX cells viability. Thus, it is possible to conclude that the decoction presented the best results, being necessary further studies to characterize and validate this new application.

Acknowledgements: This work received financial support from the European Union (FEDER funds POCI/01/0145/FEDER/007265) and National Funds (FCT/MEC) under the Partnership Agreement PT2020 UID/QUI/50006/2013. F. Rodrigues is thankful for her post-doc grant from the project Operação NORTE-01-0145-FEDER-000011. D. Pinto is thankful for the research grant from project UID/QUI/50006.

PH 4

TOXICITY ASSESSMENT OF LOW MELTING ORGANIC SALTS TOWARDS *SACCHAROMYCES CEREVISIAE*

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Ionic liquids (ILs) chemistry has advanced greatly over the past two decades, such that these organic salts have found application in a variety of fields, namely nanotechnology, biocatalysis and analytical chemistry. However, although ILs reduce the air pollution risk due to their negligible vapor pressure, they could cause water and soil contamination through effluents discharge or leaching of landfills [1]. It is therefore essential the toxicity assessment of these low melting organic salts before their indiscriminate use with unclear long-term impact on the environment and human health.

Saccharomyces cerevisiae (*S. cerevisiae*) has proved to be a valuable eukaryotic model organism for the evaluation of xenobiotics' toxicity. The yeast offers several advantages over other toxicity models, including high homology to the mammalian systems, fully sequenced genome as well as simple and inexpensive cultivation [2]. In this work, the viability of *S. cerevisiae* cells upon exposure to low melting organic salts is evaluated using the methylene blue dye reduction test. The methodology is based on the enzymatic reduction of methylene blue by the living cells, resulting in a decrease of optical density at 664 nm [3]. Ultimately, it is expected that the obtained results could help in the identification of toxicophore substructures, contributing to the design of less hazardous compounds.

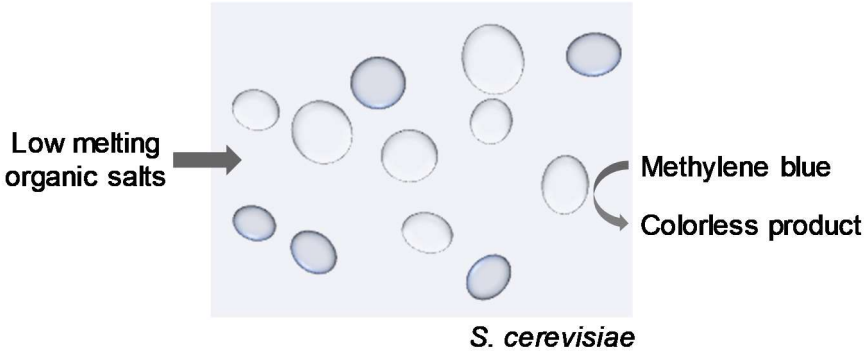

Figure 1: Evaluation of the toxic effect of low melting organic salts on *S. cerevisiae*.

Acknowledgements: Ana M.O. Azevedo thanks FCT for her PhD grant (SFRH/BD/118566/2016).

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PH 5

METAL ACCUMULATION IN NATIVE FLORA: BIODIVERSITY PROSPECTING FOR PHYTOREMEDIATION OF CONTAMINATED AQUATIC ENVIRONMENTS

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The importance of biodiversity in the cleanup of trace elements contamination in polluted ecosystems is increasingly recognized. Aquatic plants in freshwater, marine and estuarine systems may act as receptacle for several metals [1].The efficiency of phytoremediation depends on several factors including metal bioavailability, plant uptake, translocation and tolerance mechanisms.

In this study, river water, sediments and flora were studied regarding their concentration on a wide panel of trace elements (Li, Be, Al, V, Cr, Co, Ni, Cu, Zn, Se, Mo, Ag, Cd, Sb, Ba, Tl, Pb, and U) measured by ICP-MS. Two estuaries highly impacted by anthropogenic activities, Douro and Ave, were selected for this study.

Different plant organs (leaves, flowers, stems and roots) from estuarine local native flora from Douro and Ave estuaries were evaluated as possible bioindicators and/or phytoremediators of trace elements pollution. The findings of this study indicated that the extent of trace element concentrations differed among the investigated plant species, tissue bodies (root and shoot) and types of trace element. Plant communities respond differently to trace elements present depending on their ability to accumulate and detoxify [1]. No systematic pattern was observed among the distribution of the studied trace elements in the investigated species and their organ tissues though, in general, trace element levels decreased as follows: roots> leaves> flowers/fruits > stems, except for Cu, Ba, Cr and Mo. The bioaccumulation factor (BF), from soil to component parts of plants, revealed that some of the species were good accumulators of Se, Mo, Ba and Pb (BF >1). The highest BF found was 156 for Pb (found in leaves of *Plantago lanceolata*) and 112 for Se (for root and stem of *P. lanceolata.* The translocation factor (TF), ratio of concentration trace element aboveground parts/roots, was also determined. *P. lanceolata* showed the highest TF values for Cr, Co, Cu, Zn, Mo, Ba and Tl.

The results suggest that *P. lanceolata* may be considered a bioaccumulator species for some trace elements and can be used as a bioindicator of trace element pollution.

Acknowledgements: This work was supported by FCT, Ministério da Ciência, Tecnologia e Ensino Superior (MCTES), under Project PTDC/SAU-ESA/108871/2008, and CESPU, through Projects MYCO-CESPU-2016 and ChiralDrugs-CESPU-2017

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PH 6

ELEMENTAL IMPURITIES IN LIPSTICKS: A COMPARATIVE STUDY OF PORTUGUESE AND BRAZILIAN PRODUCTS

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Cosmetic products are subject to stringent regulation in most developed countries. Lipsticks, in particular, because of their potential for systemic exposure through oral ingestion, are given special attention [1, 2]. For safety reasons, the EU regulations prohibit the use of a long list of metal(loid)s as ingredients of cosmetic products. However, due to their ubiquitous and persistent nature, the presence of trace amounts of those elements in finished products is virtually unavoidable even under conditions of good manufacturing practices (GMP). According to European Regulation (EC) No. 1223/2009, Article 3, "the non-intended presence of a small quantity of a prohibited substance, stemming from impurities of natural or synthetic ingredients, the manufacturing process, storage, migration from packaging, which is technically unavoidable in good manufacturing practice, shall be permitted provided that such presence is in conformity with Article 3", i.e., it "shall be safe for human health when used under normal or reasonably foreseeable conditions of use" [1].

This study aimed at determining the elemental content of lipsticks available in the Portuguese and Brazilian markets. A total of 96 lipsticks were purchased in Brazil (n = 53; 9 brands) and Portugal (n = 43; 7 brands) and the levels of 44 elements were determined. Results ranged from <1 μg/g to several tens of μg/g (e.g., Sn, Mn, Zn). Significant differences were found between Portuguese and Brazilian products for several elements, namely for Pb. For the elements of major toxicological concern (Pb, Cd, As, Sb, Hg), mean values were always below the current limits set by the German competent authority [3]. However, a significant percentage of exceedances were observed for Pb (24%) and Cd (21%). A safety assessment was carried out for the toxicologically relevant elements. Results showed that, except for Pb, the systemic exposure resulting from lipstick use represents less than 0.2% (ca. 3% for Pb) of the respective permitted daily exposure even in the worst-case scenario (i.e., ingestion of the total amount of product applied).

The results of these surveys show that, overall, the current levels of elemental impurities in lipsticks, particularly those elements prohibited under the European Regulation (EC) No. 1223/2009, are quite low and do not pose safety concerns regarding consumers' health. However, a continuous surveillance of these products must be maintained in order to ensure the compliance with the current regulations and guidelines.

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PH 7

PHOTOLUMINESCENT NANOHYBRID PROBE BASED ON DISTINCT SIZED QUANTUM DOTS FOR SIMULTANEOUS DETERMINATION OF Hg^{2+}, Pb^{2+} AND Cu^{2+} USING MULTIVARIATE CHEMOMETRIC TOOLS

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The applications of quantum dots (QDs) as luminescent probes for the detection and quantification of metal ions have been widely employed due to their remarkable physicochemical properties namely photostability, high quantum yields and a wide range of tunable emission wavelengths. However, despite the several sensing strategies that have been proposed highlighting their great potential [1], the possible lack of selectivity to a given ion could impair the obtaining of analytical results with good accuracy. Several strategies can be used to improve the selectivity such as the selection of a specific capping ligand prone to interact with a given analyte, the adjustment of QDs size, the functionalization of the QDs surface, etc.

In this work, a combination of CdTe QDs capped with 3-mercaptopropionic acid of different sizes and thus emitting at different wavelengths (549 and 634 nm) were conjugated into a nanohybrid system being used as fluorometric probe for Hg^{2+}, Pb^{2+} and Cu^{2+} ions.

This nanohybrid probe assured a multi-point detection in which by resorting to multivariate methods (PCA and PLS) allowed the detection and quantification of the distinct tested ions in binary and ternary mixtures. The obtained results revealed that the proposed approach was able to discriminate with accuracy the ions present in a binary mixture (RMSECV and R^2CV values, from 0.01 to 0.08 mg L^{-1} and from 0.74 to 0.89, respectively). In the case of ternary mixture, an accurate detection and quantification of Pb^{2+} and Hg^{2+} was obtained while Cu^{2+} can only be detected being its quantification unattainable.

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PH 8

FLOURS OF MELON SEEDS: EFFECT OF THE OIL EXTRACTION PROCESS ON THEIR NUTRITIONAL COMPOSITION AND ANTIOXIDANT ACTIVITY

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A huge amount of undervalued by-products is generated by fruit industry. In 2016, the melon world production was around 32,000,000 t, generating approximately 920,000 t of seeds [1]. Based on a sustainable point of view, the valorization of melon seeds is a worthwhile concern. Melon seeds could be submitted to different extraction techniques, producing alternative vegetable oils and flours. Melon seed flour is the by-product resulting from oil extraction by hydraulic and screw methods and could be a good source of bioactive compounds and protein. However, their composition can be affected by the extraction methodology used. To the best of our knowledge, few studies are available describing the chemical and nutritional composition of this by-product.

Cucumis melo L. seeds from *Piel de Sapo* cultivar were extracted by hydraulic and screw presses to obtain oil. The resulting flours were evaluated in order to determine differences in what concerns nutritional composition, vitamin E profile, total phenolic and flavonoid contents (TPC and TFC, respectively) and antioxidant activity (through DPPH and FRAP assays).

Regarding the nutritional composition, the highest fat content (20%) was determined in flours obtained by hydraulic press. On the contrary, the flours obtained by screw method presented the highest carbohydrates (43%) and protein (36%) contents. In what concerns TPC, the highest values were obtained for hydraulic flours (2882.3 mg of gallic acid equivalents per gram of dry basis sample, mg GAE / g db). The TFC were 1924.5 and 1108.7 mg of catechin equivalents (CEQ)/ g db, respectively, for hydraulic and screw samples. Significant differences were found among the antioxidant capacities of the evaluated seed flours. Thus, the extraction technique used to obtain seed flours might be selected according to the final use intended.

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PH 9

DETERMINATION OF MOISTURE AND PROTEIN CONTENT IN BANANA "*NANICA*" CULTIVATED ON SANTA CRUZ * SANTIAGO ISLAND

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Banana is one of the most produced fruit in the world and the lack of knowledge of its nutritional principles can induce to its bad utilization, resulting in waste of tons of the same. In order to assist the creation of Cabo Verde banana "nanica" database on the nutritional composition, the objective of this work was the determination of the humidity levels by its influence on the banana chemical stability and protein levels taking into account its nutritional intake estimate in feeding. For this purpose, pulp and peel samples of banana "nanica" cultivated at Rocha Lama, municipality of Santa Cruz, were evaluated in different stages of ripeness. The determination of the moisture content was made by setting oven temperature at 105 ºC and the protein by formaldehyde method. The moisture analyses showed that, while pulp content increases as the banana ripens from 70.3 (± 1.6) % to 75.6 (± 2.1) %, for the peel decreases of 89.4 (± 1.5) % to 84.5 (± 4.8) %, as expected. Related to proteins, as for pulp (1.6 (± 0.3) % to 1.5 (± 0.2) %), and as well for peel (1.0 (± 0.2) % to 1.2 (± 0.3) %) the tendency was within the expected range, but different from those found in the literature. These results suggest that the centesimal composition of the fruit depends on the conditions of cultivation used, namely the climate, soil, altitude, irrigation and the seasons. The protein content in pulp and peel of banana "nanica" was variable over the stages of ripeness, which may be related to occurrence of Maillard reaction between proteins and reducing sugars. It was also possible to verify that bananas "nanica" analysed are poor in protein and high moisture content. This fact emphasis the need for maintaining the conservation conditions until the last stage of ripeness to be able for consumption. The banana "nanica" peel contains protein near the pulp, so it can be useful as a source of feed.

Acknowledgment: Prof. Eliana Furllong, Prof. Mário Lima and Domingos Araújo.

PH 10

DEVELOPMENT OF A UHPLC–MS/MS METHODOLOGY FOR THE DETERMINATION OF PHENOLIC COMPOUNDS IN VINE SHOOTS

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The cultivation of *Vitis* (Vitaceae) grape varieties is one of the most important economic activity in Portugal. According to the International Organization of Vine and Wine, in 2014 the Portuguese vitiviniculture area cultivated was 221,448 hectares [1]. Therefore, one of the biggest challenges for wine-producing is to create alternatives for processing the vast amount of grape wastes generated during harvest season. Vine-shoots constitute one of the most abundant vineyard wastes, which should be re-used with innovative applications [3]. Traditionally they are used as a heating source or left on the ground, however using this raw material as a source of bioactive compounds could increase its economic value [2].
An ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS) method using BEH C_{18} analytical column was developed for the separation and quantitation of 32 phenolic compounds. The separation was accomplished using gradient elution with a mobile phase consisting of methanol and 0.1% formic acid. Electrospray ionization (ESI) in both positive and negative ion mode was optimized to reach high sensitivity and selectivity for quantitation using multiple reaction monitoring (MRM) with the selection of proper product ions for each transition. ESI in negative ion mode was found to be more sensitive for quantitative analysis of most of the analyzed phenolic compounds. The developed method was fully validated in the terms of linearity, limit of detection and quantification and inter/intra-day precision. Work is in progress in order to apply the optimized methodology and quantify the phenolic compounds present in Portuguese vine shoot.

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PH 11

EVALUATION OF ENZYMATIC DIGESTION CONDITIONS FOR DETERMINATION OF IMMUNOGLOBULINS BY TANDEM MASS SPECTROMETRY

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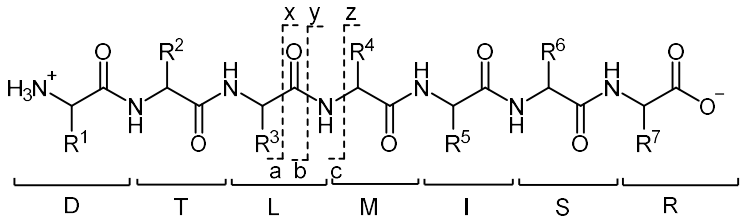
Immunoassays, namely ELISA, have been the standard method for detecting clinically significant immunoglobulins (Igs). They are based on Ig-antigen interaction, often suffering interference from matrix components. New analytical approaches using detection by tandem mass spectrometry (MS/MS) search for fundamental structure information of target Igs based on protein features. In fact, there are few examples of quantitative assays achieved by liquid chromatography coupled with triple quadrupole (QqQ) mass analyzers. Due to the limited mass range of QqQ, the use of this mass analyzer requires previous tryptic digestion of IgG for analysis of highly specific surrogate peptides. In this work, initial studies on a LC-MS/MS method for the quantitative analysis of IgG are reported. The method relies upon the detection of the generic peptide DTLMISR (Fig. 1), originated from the fraction crystallizable (Fc) region of IgG after enzymatic cleavage. The multiple reaction monitoring transitions used for quantification and identification purposes were, respectively, *m/z* 418.20 → 506.10 and 418.20 → 619.30, corresponding to the fragmentation of double-charged molecular ions. In order to investigate the influence of trypsin concentration on digestion kinetics and efficiency, the trypsin-to-protein ratios 1:20, 1:50 and 1:100 were evaluated. Moreover, the performance of the digestion process was monitored for IgG standards and plasma samples over 18 h at 37 °C. Using a 1:50 ratio, two distinct kinetic profiles were observed for standards and plasma samples with a maximum signal intensity after 6 and 18 h, respectively.



D = aspartic acid, $R^1 = CH_2COOH$
T = threonine, $R^2 = CH(OH)CH_3$
L = leucine, $R^3 = CH_2CH(CH_3)_2$
M = methionine, $R^4 = (CH_2)_2SCH_3$
I = isoleucine, $R^5 = CH(CH_3)CH_2CH_3$
S = serine, $R^6 = CH_2OH$
R = arginine, $R^7 = (CH_2)_3NHC{=}NH(NH_2)$

Figure 1: Structure of the peptide DTLMISR selected for quantification by LC-QqQ-MS/MS.

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PH 12

IN VITRO DISSOLUTION TESTING OF AN ITRACONAZOLE AMORPHOUS SOLID DISPERSION: SINK *VS.* NON-SINK-CONDITIONS

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In vitro dissolution testing of solid oral dosage forms is a very important tool in drug development for selecting and optimizing formulations. The dissolution test is particularly important for Active Pharmaceutical Ingredients (APIs) that present high lipophilicity and low aqueous solubility, such as Itraconazole (ITZ). These drugs are categorized into class II and class IV of the Biopharmaceutics Classification System (BCS). Class II drugs constitute the majority of the new API candidates [1].
An effective way to enhance the aqueous solubility of BCS class II drugs is to formulate those as Amorphous Solid Dispersions (ASDs) in which the API is molecularly dispersed in a suitable polymeric matrix. For the screening of the ASDs formulations, dissolution tests should be carefully designed to best mimic the *in vivo* conditions. Recently, working with simulated gastric (SGF) or intestinal (SIF) fluids has emerged as a popular topic within the literature [2]. Those media composed of surfactants, organic salts and lipid compounds are expected to mimic the gastric and intestinal environments under fasted or fed state conditions in order to provide a better *in vitro-in vivo* correlation [1, 2]. Furthermore, one of the most challenging issues about the dissolution testing of BCS class II drugs is the difficulty to achieve sink conditions using the standard dissolution media, which can strongly affect the obtained dissolution profiles. During the dissolution testing, “sink condition” means the use of a greater volume of solvent (*e.g.* 5 to 10 times) than the volume present in the saturated solution of the target molecule contained in the final dosage form being tested [1].
The purpose of this study was to select a suitable *in vitro* dissolution test to allow the assessment of ITZ solubility enhancement when formulated as an ASD. The ASD studied in this work was composed by ITZ and PVP-VA in a ratio of 40:60 (% w/w) and it was further compressed to tablets.
Dissolution profiles were obtained using USP Apparatus II with different dissolution biorelevant media in order to evaluate the effect of the sink *vs* non-sink conditions. Quantification of ITZ was performed by a fast and sensitive ultra-performance liquid chromatography method (UPLC). With this study, the effect of API concentration was evaluated. The suitable conditions to allow a successful ASD screening were defined. Furthermore, Dynamic Light Scattering (DLS) as well as Scanning Electronic Microscopy (SEM) were used to characterize the evolution of the tablets disintegration throughout the dissolution experiment. These data will help correlating the disintegration impact on the dissolution profile.

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PH 13

TANDEM MASS SPECTROMETRY CHARACTERIZATION OF NITRATED CARDIOLIPIN

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Cardiolipin (CL) is a phospholipid found in inner mitochondrial membrane and is a fundamental structural component, involved in oxidative phosphorylation or apoptotic signalling. It is an anionic dimeric phospholipid bearing four fatty acids. Similarly to other phospholipids, it can be modified in the unsaturated fatty acyl chains by reactive oxygen species (ROS), and oxidized cardiolipin has specific biological role in apoptotic signalling [1]. However, modifications induced by reactive nitrogen species (RNS) to CL remain unknown, although it is well-known that nitric oxide and RNS interact with mitochondrial membranes and lead to protein nitration [2]. In this work, we used gas phase fragmentation under tandem mass spectrometry (MS)-based approaches to identify nitrated CL. We used tetralinoleyl-CL, the most abundant CL in myocardium mitochondria, and nitration was performed using NO_2BF_4 in a biomimetic nitration system. The new modified CL identified by MS included CL bearing nitro (NO_2-CL), nitroso (NO-CL), hydroxy (CL-OH), peroxy (CL-OOH) and oxo (CL=O) groups. Gas phase collision induced dissociation (CID) revealed fragmentation patterns in MS/MS spectra specific of each structural modification, namely the neutral loss of the modification moiety (loss of HNO_2, for the NO_2CL and loss of HNO, for NO-CL), as can be seen in Figure 1 for the NO_2-CL. Also, product ions assigned as the loss of modified fatty acid, or modified phosphatidic acids give additional insight for the characterization of nitrated CL (Figure 1). These typical fragmentations can be used for the detection of nitrated CL in biological samples.

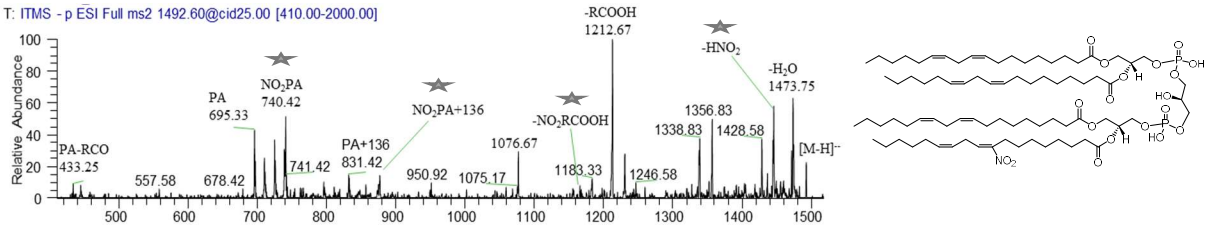

Figure 1: Tandem MS spectrum of the $[M-H]^-$ ion of NO_2-tetralinoleyl-cardiolipin.

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PH 14

GREEN AND CHIRAL QUANTUM DOTS

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Inspired by the role of chirality on molecular recognition, the development of chiral photoluminescent nanosized probes might open new avenues in the determination of biorelevant phenomena [1]. Due to their unique photophysical properties, cadmium chacolgenide quantum dots (QDs) are a good platform for the assembling of chiral sensors, since the use of stereospecific thiol capping agents confers chirality to the inorganic QD core [2]. Among the different thiol capping agents, penicillamine, an analogue of cysteine used for the treatment of Wilson's disease, has shown selective recognition of chiral analytes [1]. However, the use of penicillamine capped QDs is hampered by the toxicity inherent to cadmium.

Recently, I-III-VI ternary metal chalcogenide QDs, such as AgInS2/ZnS QDs (ZAIS), have emerged as alternatives to the cadmium-based QDs [3]. Besides he absence of heavy metals in its composition, ZAIS QDs have shown good optical properties such as high absorption coefficients, long photoluminescence (PL) lifetimes, and high PL quantum yields (QY) [4].

In this work, the synthesis of ZAIS QDs capped with the enantiomeric forms of penicillamine (D-penicillamine and L-penicillamine), is described. To retain the “greenness” of the procedure, an aqueous route using microwave-assisted heating, was followed. Precursors ratios, temperature, and pH were optimized yielding color tunable ZAIS QDs with moderate to good QY. Exploiting the penicillamine ability to chelate copper, D-penicillamine and L-penicillamine capped ZAIS QDs were used for the determination of copper. The obtained results will pave the way for the applications of L- and D-penicillamine in chiral analyte discrimination.

Acknowledgements: This work received financial support from the European Union (FEDER funds POCI/01/0145/FEDER/007265) and National Funds (FCT/MEC, Fundação para a Ciência e Tecnologia and Ministério da Educação e Ciência) under the Partnership Agreement PT2020 UID/QUI/50006/2013, and from FCT - Fundação para a Ciência e Tecnologia through the FCT PhD Programmes and by Programa Operacional Potencial Humano (POCH), specifically by the BiotechHealth Programme (Doctoral Programme on Cellular and Molecular Biotechnology Applied to Health Sciences), reference PD/00016/2012. José Soares also thanks FCT and POPH (Programa Operacional Potencial Humano) for his PhD grant (SFRH/BD/98105/2013).

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PH 15

DESIGN AND DEVELOPMENT OF A MICROFLUIDIC PAPER-BASED ANALYTICAL DEVICE FOR CALCIUM DETERMINATION IN SALIVA

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Saliva is an exocrine, aqueous and transparent fluid that contains calcium in its composition, which plays an important role in the balance of remineralization and demineralization of the teeth. The concentration of calcium in saliva depends mainly on the pH of this fluid and the salivary flow rate. The determination of salivary calcium concentration may anticipate potential problems or help to improve target therapies [1, 2]. Microfluidic paper-based analytical devices (μPAD) are devices composed of a hydrophilic zone, consisting of paper, and a hydrophobic zone that delimits them. The papers cellulose fibers enable liquids transportation by capillarity.
In this work, a μPAD was developed to determine the salivary calcium concentration in an economical and in situ manner (Figure 1). The colorimetric reaction between calcium and o-cresolphthalein complexone generates a coloured product that can be quantified through the information obtained by image capture devices [3].

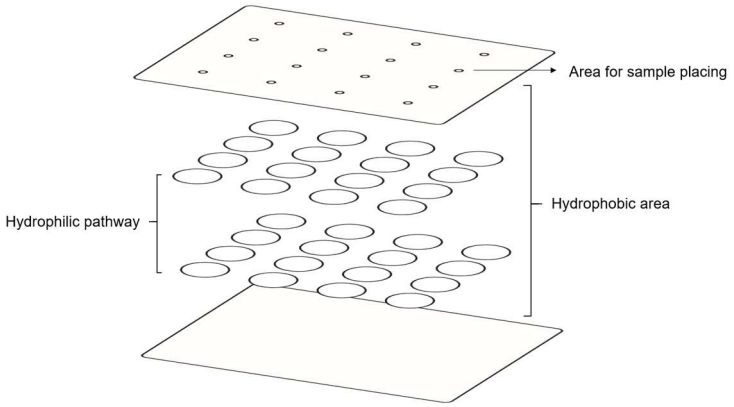

Figure 1: Exploded view of the μPAD developed.

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PH 16

TEACHING ANALYTICAL CHEMISTRY IN PORTUGAL, A NEW REALITY

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The teaching of Analytical Chemistry in Portugal has been gradually despised and replaced by a more applied content. About 20 years ago it would be common that a course of Chemistry or Chemical Engineering would present in its plan two curricular units (CU) of Analytical Chemistry accompanied by Electrochemistry, Physical Chemistry and others CU of support. This abandonment of basic science teaching and the preference for applied techniques may compromise the full understanding of the phenomena involved in the processes of analysis, now so required by quality assurance. There is an increase in the quality of chemical analysis of food, medicines, the environment and others based on increasingly demanding regulations and laws which is very good for our security. However, this requirement can be hampered by the inexistence of professionals capable of dealing with complex situations. In fact, we are witnessing an increasingly systematic lowering of levels of detection and quantification, a greater complexity of analysis techniques and a greater diversity of these techniques [1]. Also, laboratories and their technicians assume responsibility for choosing the method of analysis to apply as long as it is validated and meets the essential analytical requirements, demanding that technicians know more and more Analytical Chemistry.
Taking all these aspects into account, are we preparing the best graduates and masters for this new reality of Analytical Chemistry?
An analysis of the curricular plans of the courses in Portugal in the areas of Chemistry, Chemical Engineering, Food Chemistry, Pharmacy, Biotechnologies, Environment and related areas shows that there has been a decrease in the study of Analytical Chemistry contents [2]. The lack of graduates in the area of Analytical Chemistry has even led a company that produces pharmaceutical active ingredients in Portugal to established partnerships with various institutions to form their own analysts. This is another sign of the gap from the growing demand for graduates in Analytical Chemistry and the disinvestment in this area by the Polytechnic Schools and Universities.
With the data presented we intend to provoke (arouse) the reflection on the subject highlighting what the lack of training on Analytical Chemistry can entail to society and to the National, European and World industries.

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PH 17

SCREENING OF LIPIDS BY DIFFERENTIAL SCANNING CALORIMETRY (DSC) FOR THE PRODUCTION OF LIPID NANOPARTICLES

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A crucial parameter when developing lipid nanoparticles is the selection of the more adequate lipids to form the solid or solid/liquid lipid matrix, considering their ability to solubilize the drug. However, lipids may present different crystal structures as well as undergo polymorphic transitions, leading to unexpected and undesirable drug expulsion [1]. Therefore, it is important to take this into consideration. DSC is a useful analytical tool to assess the structural properties of lipids [2]. The aim of this work was to analyze different lipids by DSC, in order to assist in the selection of the most suitable to be used in the production of nanostructured lipid carriers (NLC).
DSC data was generated using a Model DSC 200 F3 Maia®, equipped with an automatic sample changer. Several samples of bulk lipids (solid and liquid), weighing between 4 and 7 mg, were subjected to three runs, at heating and cooling rates of 10 K/min. An empty aluminum pan was used as the reference sample. The samples were continuously flushed with nitrogen at a flow rate of 40 mL/min. Concerning the solid lipids, the samples were subjected to a heating from 20 ºC to 120 ºC, isotherm of 5 min and subsequent cooling to 0 ºC. As for the liquid lipids, the thermal program included one cooling step to -30 °C, followed by an isotherm of 5 min and then a heating from -30 °C to 60 °C.
Following the runs, the thermograms of the samples were evaluated and the fusion temperatures determined. For almost all the lipids, the melting points were coincident with those described in the literature. Nonetheless, it is interesting to note that for some of the solid lipids, in the second and third run, there was a shift to the left in the thermal profiles, as well as the appearance of smaller endothermic peaks not detected in the first run, which can be indicative that exposing those lipids to heat leads to the formation of less ordered unstable forms [3].
From this work, we can conclude that DSC is a good approach to infer about lipids' crystallinity and polymorphic transitions, thus helping to select the best ones for the development of lipid nanoparticles.

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PH 18

A FAST AND SIMPLE APPROACH FOR SCREENING SULFONAMIDES IN WATER

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Sulfonamides (SAs) are an important antimicrobial class widely used in human and veterinary medicine. These compounds are among the most consumed antimicrobials in food-producing animals [1]. Moreover, these pharmaceuticals present slow sorption to soil and leaching capacity which contribute to the contamination of the aquatic environment [2]. In fact, SAs have been found in environmental water in ng to µg per liter level.
Different approaches for quantification of SAs in water have been developed. Most of them are based on the coupling of separative techniques, mainly high-performance liquid chromatography, with tandem mass spectrometry. Besides the high sensitivity of the current laboratory based methodologies, complementary tools for fast, simple, and cost-effective screening of antimicrobials are required. In this framework, an affordable method for screening of SAs in water was implemented. For this, miniaturized solid-phase extraction using commercially available polystyrene divinylbenzene sulfonated disks was coupled to spectrophotometric microplate analysis. Spectrophotometric determination was based on the chromogenic reaction between SAs and *p*-dimethylaminocinnamaldehyde in organic medium. Different parameters were evaluated, including the chromogenic reaction conditions, sample loading rate, and eluent composition using sulfamethoxazole as model analyte. Screening of other SAs (sulfadiazine, sulfaquinoxaline, sulfamerazine, sulfanilamide, sulfamethazine, sulfametizole, sulfadimethoxine, sulfapyridine, and sulfacetamide) was also performed, providing values for limit of detection between 2 and 11 µg L^{-1} for a sample volume of 100 mL. The proposed method was successfully applied to analysis of different types of water.

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PH 19

DETERMINATION OF TRIGONELLINE IN COFFEE USING LOW PRESSURE ION PAIR CHROMATOGRAPHY WITH AMPEROMETRIC DETECTION

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In this work, the determination of trigonelline in coffee was performed, using ion pair chromatography in low pressure flow systems and an amperometric detection. The experimental apparatus included a peristaltic pump, an injection valve, a 1 cm-length C_{18} monolithic column and a boron-doped diamond electrode, used as working electrode.

Trigonelline is an important quality marker for coffee and is usually determined using HPLC-UV methodologies [1]. As this alkaloid, at pH lower than 4, is an ionic compound, it can be separated coupling ion pair chromatography and low pressure flow system. So, the developed method allowed the separation of the analyte from matrix compounds present in coffee, by adding the ion pair reagent, 1-tetradecanosulfonate sodium to the mobile phase, and the quantification of the analyte in coffee.

After optimization of experimental conditions and evaluation of the method, two coffee varieties, arabica and robusta, either green and roasted, were analyzed in this work. From the results obtained, it was observed what was expected: (1) higher trigonelline levels for arabica coffee samples in comparison with robusta green variety; (2) the decrease of trigonelline levels along the roasting process; (3) the appearance of niacin signal in dark roasted samples. This last feature highlights the potentiality of the presented strategy for multiparametric determinations.

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FREE FORMALDEHYDE DETERMINATION IN COSMETICS CONTAINING FORMALDEHYDE DONORS

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Formaldehyde is a chemical compound vastly used for the production of consumer goods, such as cosmetics or hygiene products, due to its antimicrobial and preservative properties. Despite such a widespread use, formaldehyde is also classified as toxic, harmful, corrosive, irritant, and can negatively affect human health. For this reason, the analytical control of formaldehyde in these kind of samples is of the highest importance.

Nowadays, the addition of plain formaldehyde to cosmetics is not usual and, instead, compounds called formaldehyde donors or formaldehyde releasers (FR) are used. These compounds are designed to liberate small amounts of formaldehyde overtime. The presence of these compounds makes the analytical determination of formaldehyde problematic, since common methodologies usually rely on many sample preparation steps, which can degrade the FRs, leading to an instant release, which will promote too high and non-reproducible values [1].

In this work, gas-diffusion microextraction (GDME) [2] was used for the extraction of free formaldehyde in six cosmetic and personal hygiene samples containing FRs. No sample treatment was required before the extraction. The limits of detection (1.98 mg kg^{-1}) and quantification (6.60 mg kg^{-1}) obtained are perfectly reasonable for the determination of free formaldehyde in these types of samples. A literature methodology consisting on a liquid-liquid extraction with dichlomethane [3] was used for comparison purposes. No statistically significant differences between the two methods were found (*t*-test, p=0.05) for determined concentrations between 6.9±0.3 and 365±15 mg kg^{-1}. Furthermore, mass spectrometry studies were performed in order to unbiasedly guarantee the presence of formaldehyde in every sample.

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PH 21

NANOHYBRID QUANTUM DOTS PROBES FOR THE DETERMINATION OF DIFFERENT METALS IN WATER

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Semiconductor quantum dots (QDs) have demonstrated a huge capability as reliable fluorescent probes for metals quantification. The reactivity of these quantum dots towards a given analyte is related, among other factors, with their size and the functional groups of the capping molecules, which are used as well to assure solution stability. These factors are determinant in terms of QDs sensitivity and selectivity [1].
An analyte can generate a variety of responses, either inhibiting or increasing fluorescence intensity, providing a shifting of the maximum emission wavelength, yielding differences in the QD half-life or QD quantum yield, etc. The use of nanohybrid probes and the conjugation of different metal response types may be decisive for their identification and quantification.
Despite the high analytical potential of binary QDs, used widely for varied analytical purposes, the occurrence of toxic elements in the nanoparticles core raised serious health and environmental concerns. For this reason, the synthesis of nanocrystals of less toxic elements has been a noteworthy studied alternative. Carbon dots (CDs) are a recent class of fluorescent nanomaterials that have collected a deep interest, not only due to their low toxicity and high biocompatibility, but also because they are easily prepared by using cost-effective processes [2]. Ternary and quaternary QDs have also been developed as promising cadmium-free fluorescent probes and are also considered safer materials [3].
In this work, we have investigated the reactivity of distinct types of quantum dots, of different composition and structure, and with different capping ligands and sizes, towards different metals (Cu^{2+}, Ag^{+} and Hg^{2+}) and two iron species (Fe^{2+} and Fe^{3+}).
The obtained results showed that the combination of multiple QDs in the same analysis could be exploited to assure a specific analyte-response profile, guaranteeing improved selectivity.

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PH 22

PROXIMAL COMPOSITION AND VITAMIN E PROFILE OF GERMINATED LEGUMINOUS SEEDS

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Pulses have a significant role in human nutrition, being environment friendly crops. Their ability to resist and grow in arid environments, along with the capacity to improve soil fertility, can contribute to positive effects on the environment. Indeed, they are able to capture or convert atmospheric N through a symbiotic relationship with *Rhizobium* bacteria, reducing the N to NH_3, which in turn, is incorporated in the biosynthesis of amino acids, giving rise to a high protein content [1]. Moreover, the combination of pulses with cereals (rice or corn) provides a complete amino acid profile able to replace animal protein.

In this study, the nutritional and the vitamin E profiles of germinated leguminous seeds from four different species (*Vigna unguiculata*, *Vicia faba*, *Cajanus cajan* and *Phaseolus lunatus*) was evaluated. Seeds germination was performed according to Berni and Canniatti-Brazaca [2] with minor modifications. The contents of ash, protein, fat, and fiber (total and insoluble) were analyzed by AOAC methods. The moisture content was determined using an infrared moisture analyzer. Carbohydrates and soluble fiber were indirectly calculated. Vitamin E (tocopherols and tocotrienols) profile was determined by HPLC-DAD-FLD.

In a dry weight basis, protein contents varied between 20 and 24%. The fat content ranged from 1.2 to 1.4%. All germinated seeds showed high dietary fiber contents (total: 28-32%, insoluble: 20-25%, soluble: 3-11%) and available carbohydrates ranging from 39 to 45%. Regarding vitamin E, α-tocoferol, α-tocotrienol, γ-tocoferol, δ-tocoferol were the vitamers found. The high percentage of tocopherols (greater than 90%) in relation to α-tocotrienol stands out with significant differences between the tocopherol profiles of the germinated seeds. For instance, γ-tocoferol was the main vitamer in *C. cajan*, while δ-tocoferol content was significantly higher ($p<0.05$) in *V. unguiculata*.

The results of this study show the nutritional richness of the germinated seeds, especially in insoluble fiber and protein, being an accessible raw material, relative to other protein sources.

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PH 23

COMPARISON OF DIFFERENT PLANT INFUSIONS REGARDING TOTAL PHENOLICS, TOTAL FLAVONOIDS AND ANTIOXIDANT ACTIVITY

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In the last years, an increase on the prevalence of chronic diseases associated to stress and current lifestyles (*e.g.* cardiovascular and neurodegenerative diseases) has been observed [1]. An important cause of aging and development of such diseases derives precisely from the oxidative stress resulting from the production and accumulation of reactive oxygen species.
Plant infusions are one of the simplest ways to extract and consume many phytochemicals with numerous medicinal properties, including antioxidant ones.
In this work, infusions were prepared, in triplicate, using 6 different plants (rosemary, lemon balm, lemon verbena, olive leaves, cherry stem, and fennel) using a total of 23 commercial samples. Total phenolics and total flavonoids contents, as well as antioxidant activity (DPPH$^{\bullet}$ inhibition and the Ferric Reducing Antioxidant Power) were assessed and compared. Furthermore, since, for each plant, some commercial samples were sold in bulk and others in packages, it was also possible to analyze the influence of storage on the conservation and quality of the different samples.
The lemon balm infusions presented the highest values for all assays, followed by the lemon verbena infusions, indicating the greater antioxidant ability of these samples and the highest contents in total phenolic and flavonoid compounds. The olive leaves infusions showed the lowest antioxidant power. This could be explained by the eventual presence of more lipophilic antioxidant compounds (less soluble in water), but also by the size and dense texture of the leaves that could impair the compounds extraction. Finally, for some plants, bulk samples presented lower antioxidant activity than their respective packaged samples, suggesting the higher susceptibility of some plants to oxidation by air or light exposure.

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PH 24

COFFEE SILVERSKIN POTENTIALITIES AS INGREDIENT FOR DIVERSE INDUSTRIES – STATE OF THE ART

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Food by-products recovery, besides being of utmost urgency, represents an environmental and social opportunity to obtain new products with added value and economic impact. Coffee silverskin is a by-product produced in high amounts by the coffee roasting industry, representing an environmental issue. The use of coffee silverskin has many advantages, not only in the economic field, but also socially and environmentally.

Antioxidant, antimicrobial, anti-inflammatory and anti-aging are some biological properties described and related to their chemical composition, rich in phenolic compounds (including 1-6% of chlorogenic acid), caffeine (0.8-1.25%), and melanoidins (17-23%).

In the cosmetic field, for example, the oxidative metabolism and reactive oxygen species, responsible for the increase of matrix metalloproteinases and pro-inflammatory mediators that conduct to skin aging, can be countered with coffee silverskin due to it richness in antioxidant compounds and caffeine. Besides, in the industry of functional foods, coffee silverskin has been proposed as a new potential functional ingredient based on the high content of soluble dietary fiber, the marked antioxidant activity and the potential prebiotic effect. The hyaluronidase-inhibiting action also suggested that this material is promising, not only for the development of functional foods, but also as anti-inflammatory.

The aim of this review is to highlight the importance of coffee silverskin valorization based on its chemical composition and biological effects. Indeed, a relationship between the nutritional and chemical composition of coffee silverskin and the different biological properties reported is also established through this review.

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GAS PHASE FRAGMENTATION CONFIRMS PLASTIDIAL GLYCOLIPIDS IN PHOTOSYNTHETIC ANIMALS

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Sacoglossan sea slugs are highly selective feeders that incorporate chloroplasts from specific macroalgae. These “stolen” plastids - kleptoplasts - are kept functional inside animal cells and provide a complimentary source of energy to their host. Lipid composition of chloroplast membranes contains a unique signature of glycolipids, which are characterized by the presence of galactolipids, monogalactosyl diacylglycerol (MGDG) and digalactosyl diacylglycerol (DGDG), and sulfolipids (sulfoquinovosyl diacylglycerol, SQDG). A lipidomic mass spectrometry-based analysis was performed to screen the lipidome of plastids within the sacoglossan sea slug *Elysia viridis* and its alga food, *Codium tomentosum*. The glycolipids-rich fractions were analysed using hydrophilic interaction liquid chromatography-mass spectrometry (HILIC-LC-MS). Gas phase fragmentation observed in the MS/MS was used as a fundamental tool to disclose the specific molecular composition of each glycolipid species. Galactolipids (MGDG and DGDG) were identified in positive-ion mode as $[M+NH_4]^+$ ions in MS. The MS/MS spectrum of MGDG showed product ions resulting from the combined loss of NH_3 plus the loss of hexose moiety (-180 Da, -Hex). MS/MS of DGDG showed product ions resulting from the combined loss of NH_3, loss of the hexose moiety (-180 Da, -Hex) and loss of hexose residue (-162 Da, $-Hex_{res}$). Fatty acyl composition was identified by product ions of typical acylium ions plus 74 $(RCO+74)^+$. Sulfolipids were analysed in the negative-ion mode as $[M-H]^-$ ions. MS/MS showed the ions at *m/z* 225.007 corresponding to the sulfoquinovosyl group, and the ions due to loss the fatty acyl chains [1]. This approach allowed the identification of 25 molecular species of glycolipids, namely galactolipids (8 in both organisms) and sulfolipids (17 in *C. tomentosum* and 13 in *E. viridis*). The present study revealed that these exclusive lipid classes were preserved during the process of endosymbiosis. This finding suggests the existence of a conservative mechanism that likely assists in the preservation of plastid membranes, thus favouring the retention of functional chloroplasts away from their algal host cells [2].

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COMPARISON OF BIOMIMETIC MODELS OF OXIDIZED PHOSPHATIDYLETHANOLAMINES USING MASS SPECTROMETRY ANALYSIS

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Phosphatidylethanolamines (PE) are a major class of phospholipids in cellular membranes and lipoproteins, where they display structural and signalling functions [1]. Oxidized PE (ox-PE), that can be formed *in vivo* under oxidative conditions, were found to display several biological functions among which modulating action on the immune system [2], and pro-coagulant activity [3]. Moreover, ox-PE are lately being correlated with inflammatory diseases characterized by oxidative stress [4]. The development of analytical strategies for the detection of ox-PE *in vivo* should start from a comprehensive characterization of the oxidative modifications that occur for PE. This requires *in vitro* models able to mimic the radical oxidation affecting PE upon oxidative stress (biomimetic models), and mass spectrometry (MS) platforms for the identification of the oxidized products. In this study, three biomimetic models - $H_2O_2/FeCl_2$, 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) and electrochemistry (EC) - were employed to induce the oxidation of 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphoethanolamine (PLPE) and 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphoethanolamine (PAPE). The oxidation products were analyzed by electrospray ionization-MS (ESI-MS and MS/MS). All the methods led to the identification of long- and short-chain products on the *sn-2* acyl chains. For PAPE, oxidation induced by $H_2O_2/FeCl_2$, by AAPH and by EC allowed the identification of long chain products up to 4, 5 and 10 oxygen insertions, respectively. MS/MS structural characterization unveiled a preferential formation of hydroperoxy- and hydroxy-derivatives (R-OOH and R-OH, respectively) for $H_2O_2/FeCl_2$- and AAPH-induced oxidations. Poly-hydroperoxydes ($R\text{-}(OOH)_n$) rather than poly-hydroxydes ($R\text{-}(OH)_n$), were observed after EC oxidation. The biomimetic method and the PE unsaturation level directly affect: (i) the time necessary to induce oxidation; (ii) the extent of the ox-PE formation; (iii) the structures of the ox-PE isomers.

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PH 27

FATTY ACIDS ANALYSIS TO DIFFERENTIATE NUTRITIONAL QUALITY OF SIMILAR BAKERY PRODUCTS

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Bakery products are a wide group of ready-to-eat processed foods, highly consumed, especially by young people. In the last years, increased attention has been dedicated to the intake of fat, focusing on the type of fat, namely saturated (SFA) and *trans* fatty acids. Margarine, butter, partially hydrogenated vegetable oils and shortening are the main fat sources used to produce bakery products. Therefore, it is of utmost importance to evaluate fatty acids profile of these products to deepen the knowledge on their nutritional quality.

The aim of this study was to assess the nutritional quality of similar bakery products based on their fatty acids composition. Therefore, 5 of "Maria" cookies, 8 of plain salty cookies, 9 of coated chocolate cookies, 3 of brioche filled with chocolate, 3 of brioche with chocolate chips and 2 of French croissants were acquired in the major supermarkets chains from the Portuguese market. Fatty acid transesterification was performed using a methanolic solution of potassium hydroxide and *n*-heptane. Afterwards, the samples were analysed using a gas chromatograph with a flame ionization detector. Chromatographic separation of fatty acid methyl esters was performed using a Supelco® 2560 (100 m × 0.25 mm i. d., 0.25 μm film thickness) column, a split ratio of 50:1, an injection volume of 1 μL, and the injector and detector were kept at 240 ºC.

Our results have shown, that one type of "Maria" cookies has a very different fatty acids distribution, with higher monounsaturated (MUFA) content, while all the other samples have a prevalence of SFA. For plain salty cookies group, the obtained results showed that 2 products have a clear distinct fat composition where MUFA were the major fatty acids, instead of SFA. Concerning sweet breads filled with chocolate, a higher content of polyunsaturated fatty acids was found, while the other samples had a higher content of SFA. With respect to the differences found between commercial and supermarket brands of similar foods, they were not sufficient to establish a relationship between brand/price/nutritional qualities. However, they allowed us to conclude that it is possible to produce similar foods, which are healthier with better nutritional quality.

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CONTRIBUTION OF NUTS FOR THE DAILY INTAKE OF SALT, FAT AND FATTY ACIDS

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Nuts are rich sources of unsaturated fatty acids, which link the consumption of these types of foods with decreased incidence of cardiovascular disease, due to blood cholesterol levels reduction. Besides having a favourable fatty acid profile, nowadays several nuts are processed (roasted or fried) and other ingredients are added such as salt, honey, spices and aromatic plants, which can influence their nutritional quality.

Therefore, in this study the determination of salt, fat and fatty acids composition of 16 processed nuts was used to assess their contribution for the daily intake of the above-mentioned nutrients and evaluate their impact on public health. Samples of almonds, cashews, peanuts, corn, broad beans or mixtures of different nuts, widely available in the market, were collected in supermarkets. Total fat determination was performed by acid hydrolysis method followed by Soxhlet's extraction with petroleum ether. The salt content was determined by Charpentier Volhard's titration. Preparation of fatty acids methyl esters was carried out by a combined method of methylation and transesterification, followed by gas chromatography analysis coupled with flame ionization detector. Regulation (EU) No. 1169/2011 was used to estimate the contribution of a portion (35 g) of nuts for the daily intake, for an adult, of salt (6 g/day), fat (70 g/day) and saturated fatty acids (20 g/day).

Total fat content in the analysed nuts varied between 12.2 ± 0.31 and 53.6 ± 0.49 g/100 g, while the salt content ranged from 0.24 to 2.69 g/100 g. The obtained results indicate that a portion of nuts can contribute up to 15% of the recommended daily intake for salt, while for total fat it can reach 27% of the total fat daily intake. The contribution for the daily intake of saturated fatty acids ranged from 2 to 17%, indicating a high variability.

In summary, although nuts are a source of healthy fatty acids, it is necessary to carefully evaluate the effects of processing on their nutritional quality, especially due to the addition of salt that is associated with an increase of cardiovascular diseases. Also, this study enhances the importance of nutritional quality of foods to estimate health benefits/risks and the need to develop efforts with food industry to improve the quality and safety of nuts.

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OPTIMIZATION OF COLLISION ENERGY IN TANDEM MASS SPECTROMETRY FOR IMPROVING THE IDENTIFICATION OF NITRATED PHOSPHOLIPIDS

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Nitrated and nitroxidized phospholipids (PL) were recently identified by using LC-MS-based approaches in cardiac mitochondria from type I diabetic rats and cardiomyoblasts (H9c2) subjected to starvation using an ion trap mass spectrometer [1, 2]. Identification of nitrated PL is based on the typical neutral loss of 47 Da due to loss of HNO_2, and fragmentation typical of polar head group and fatty acyl chains. This fragmentation are seen with high abundance in low energy collision induced dissociation, such as in the case of ion trap mass spectrometers. However, it was recently observed that the abundance of the reporter ions of nitrated PL could change with tandem mass spectrometry conditions, which can hinder the identification of these nitrated lipids in biological samples, where they are present in very low abundance. In this way, it is required an understanding of the fragmentation pathway of nitrated and nitroxidized PL and what parameters are best to obtain the most useful information, which are key issues for their identification.
In this work we used higher-energy collisional dissociation (HCD) in the Q-Exactive Orbitrap to evaluate the influence of collision energy on the abundance of reporter ions that allows the identification of nitroxidative modifications, namely the ones formed due to the typical neutral loss of 47 Da (HNO_2). For that, we used three different normalized collision energies (NCE 20, 25, 30) on the fragmentation pattern and in the relative abundance of the reporter ions of nitrated phosphatidylcholines (PC), synthesized through *in vitro* mimetic nitration of POPC, PLPC and PAPC with NO_2BF_4. NCE of 20 and 25 allowed the identification of reporter ions for the identification of nitrated and nitroxidized PL. After this optimization step, we have also performed LC-MS analysis of cell lipid extracts sprinkled with different amounts of nitrated POPC and cell lipid extracts pre-treated during culture phase. We have applied a stepped normalized collision energy scheme (NCE 20, 23, 25), and combining ions from these three low and medium collision energies we were able to identify the presence of nitrated POPC through the identification of typical reported ion from neutral loss of HNO_2.
We show herein that the relative abundance of reporter ions of nitrated and nitroxidized PC is significantly affected by the normalized collision energy applied, which in fact determines what fragment ions are observed.

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PH 30

MICROFLUIDIC PAPER-BASED ANALYTICAL DEVICE (μPAD) FOR SALIVARY AMMONIA/AMMONIUM

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A microfluidic paper-based analytical device (μPAD) was developed as a low cost, easy-to-use, disposable sensor for potential quantitative analysis of ammonia in saliva samples. The principle for the determination is based on the colour change of the bromothymol blue (BTB) indicator, produced by a change in pH; it shows a clear colour change from yellow to blue in the transition range of pH 6.0 - 7.6. The colour change was measured by using a desktop scanner and ImageJ software to analyze the recorded image.

Preliminary experiments were carried out using direct deployment of ammonia standards in the μPAD. The μPADs size was 75 x 105 mm and contained twenty circular hydrophilic sample zone and detection zone. The effect of indicator concentration was studied in the range from 0.13 – 0.65 mM. A 0.65 mM BTB concentration provided the highest sensitivity of ammonia detection in the linear range of 0 – 150 mg/L: $A = (0.10 \times 10^{-3} \pm 0.03 \times 10^{-3})$ x mg/L NH_3 – $(3.2\times 10^{-3} \pm 2.2 \times 10^{-3})$ ($r^2 = 0.996$). To apply this method to saliva samples, studies are now focused on isolating ammonia from the matrix, using gas-diffusion. Therefore, we are designing a μPADs with 3 layers, consisting of sample zone as the first layer, impregnated with the sodium hydroxide for conversion ammonium to ammonia. The second layer is a polytetrafluoroethylene (PTFE) hydrophobic membrane. It will allow the produced ammonia from sample zone to diffuse and react with BTB reagent at detection zone, which is the third layer. The developed μPADs approach could be useful for making point-of-care device to quantify the level of salivary ammonia/ammonium associated with some oral disease.

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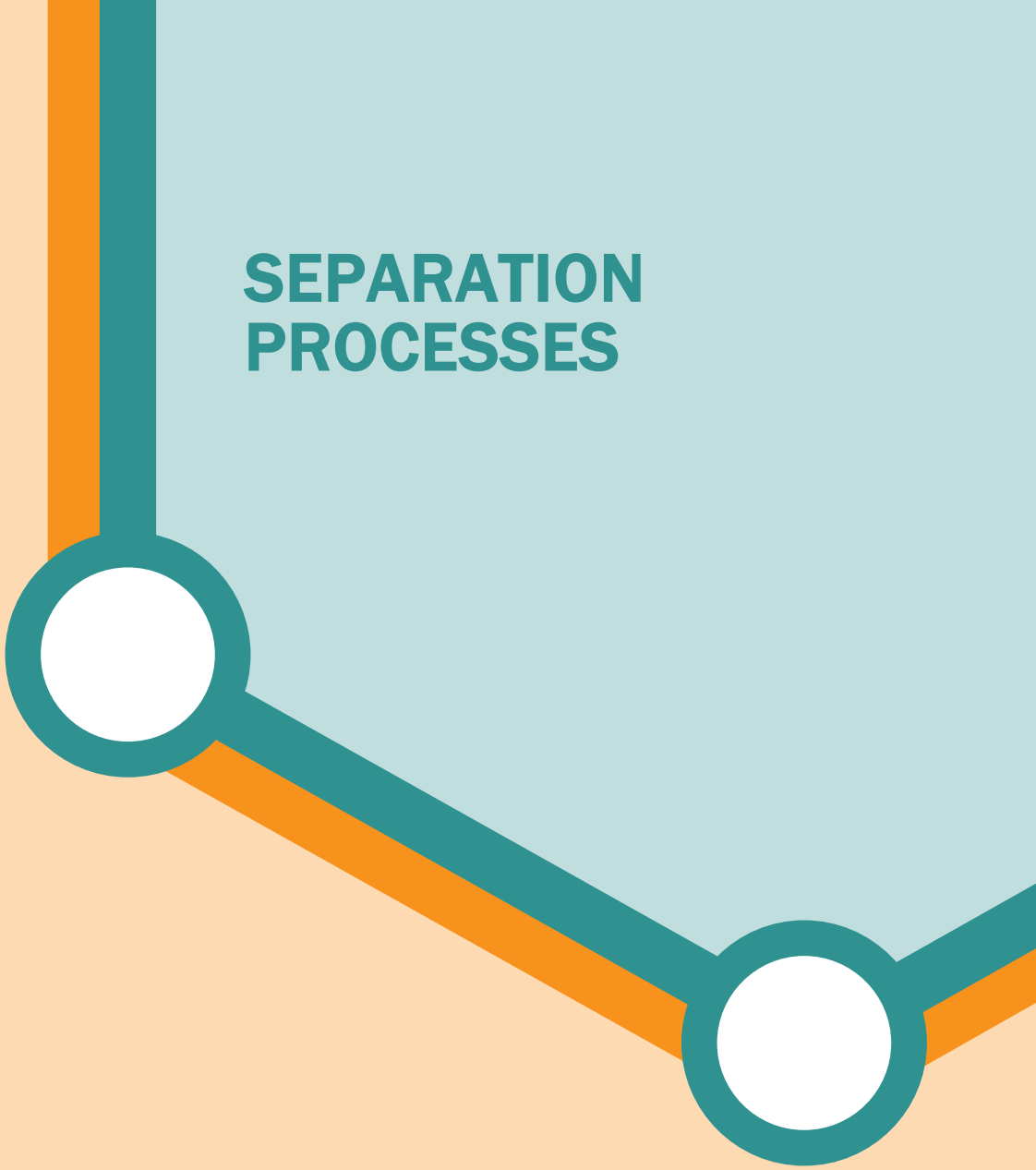

POSTER COMMUNICATIONS

PI 1

PROFILING BIOGENIC AMINES IN RUMINAL CONTENT

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The products of rumen fermentation have distinct functions in animal metabolism and consequently in the ruminants' health and nutritional status. The availability of dietary rumen-degradable nitrogen and fermentable energy and their balance have a high importance on overall feed efficiency, but the imbalance of one of the nutrients can impair animals' health [1]. High contents of amines obtained by both dietary (from forages with high protein content) and microbial (formed by microbial decarboxylation of amino acids in the rumen) sources can have various severe consequences in the animals' efficiency and health, such as the reduction of the dry matter intake, of the ruminal nitrogen degradability, and toxic effects on visceral organs [2].
This work aimed the qualitative and quantitative profiling of biogenic amines present in the ruminal content collected from rumen cannulated Holstein dry-cows fed different diets based on maize silage, hay silage and cereal straw. The biogenic amines were derivatized with dansyl chloride (DNS-Cl) and analyzed by reversed-phase high performance liquid chromatography with fluorimetric detection (HPLC-FLD) [3]. The identity of the chromatographic peaks was confirmed by mass spectrometry analysis.
The identified amines detected in the studied samples were methylamine, ethylamine, butylamine, 2-phenylethylamine, *iso*-pentylamine, putrescine, cadaverine, tyramine, spermidine and spermine. Correlations between the content of biogenic amines and the chemical composition of each diet revealed that crude fat, neutral detergent fiber and starch content significantly ($P < 0.05$) affect the amines concentration in the ruminal fluid. Spermidine content was significantly correlated ($r = -0.85$; $P < 0.01$) with crude protein.

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PI 2

CHROMATOGRAPHIC METHOD FOR THE ASSESSMENT OF VITAMIN B_1 AND B_6 DERIVATIVES IN WHOLE BLOOD

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Derivatives of thiamine and pyridoxine have crucial role in cellular metabolism. Discrepancies in their status have serious deleterious effects. Manifestation is often vague and may be easily overlooked [1]. Therefore, monitoring of vitamin status has large importance especially in patients with intensive care. Methods for simultaneous determination of thiamine, its mono- and diphosphate derivatives with an active form of vitamin B_6 pyridoxal-5-phosphate are still not widely established in diagnostics [2]. Novel HPLC-FLD method with pre-column derivatisation was developed, optimized and validated for the simultaneous analysis of thiamine and its derivatives with pyridoxal-5-phosphate in whole blood. Separation was accomplished by Meteoric Core-BIO C-18 core-shell column (100 × 4.6 mm, YMC, Germany) protected with SecurityGuard C18-WP guard column (10 × 4.6 mm, Phenomenex, USA). During gradient elution all target compounds were eluted within 15 minutes. Limits of detection are below clinically important values. Recoveries were in the range of 90 to 110% for all analytes. Bioanalytical method will be further implemented into routine practice and used primarily for the determination of thiamine and its derivatives in patients with supplementary nutrition therapy, where fluctuating level of metabolically active vitamins are associated with the occurrence of possible complications.

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PI 3

A SALLE METHODOLOGY FOR BIOGENIC AMINES DETERMINATION IN FOOD PRODUCTS OF ANIMAL ORIGIN

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Biogenic amines (BA) are formed through microbial enzymatic decarboxylation of specific free amino acids and can be found in various food products of animal origin that contain proteins or amino acids, such as cheese, meat or fish. Unhygienic conditions during food processing and storage can increase the microbial activity and favor AB accumulation. Therefore, the determination of BA concentration provides an important indication about the degree of food degradation and quality [1]. Furthermore, the ingestion of foods containing high amounts of BA may lead to severe toxicological effects on human health. The salting-out assisted liquid-liquid extraction (SALLE) uses the salting-out effect to separate water-miscible organic solvents by the addition of an electrolyte to the solution. Salting-out induces the formation of a biphasic system and changes the distribution coefficient of a given solute between the two phases [2]. The more polar of the two solvents preferentially solvates the electrolyte and thus increases the analyte extraction to the less polar solvent. Important advantages of this process in comparison with classical liquid-liquid extraction includes simplicity, low cost, lower solvent consumption and application to a wide range of analytes with different polarities [3]. In the present study a methodology based on SALLE, with a simultaneous derivatization with dansyl chloride, was developed for the extraction of BA from food products of animal origin. The analysis was performed by high-performance liquid chromatography (HPLC) equipped with a fluorescence detector. The effects of different storage conditions, such as temperature and time (after opening) were studied in canned tuna and sardine samples. The results showed an increase in cadaverine, putrescine, histamine and spermidine levels and a decrease in spermine levels in samples stored at 22 °C for a period of 7 days. This behavior can be an indication of different food degradation processes [4]. The maximum BA value was determined for histamine (64.7 ± 9.7 mg kg^{-1}) in canned tuna.

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PI 4

PREPARATION OF POLYSULFONE MEMBRANE WITH α-TOCOPHEROL AND α-LIPOIC ACID TO REDUCE OXIDATIVE STRESS

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Chronic kidney disease patients regularly undergoing hemodialysis treatment present high levels of oxidative stress and chronic inflammation biomarkers caused by treatment itself, besides the disease conditions. The long-term contact of blood with artificial material of the hemodialysis membrane causes overstimulation of inflammatory cells, leading to oxidative stress related complications in these patients. Polysulfone is nowadays the most used polymer for hemodialysis membranes, due to its improved biocompatibility. To minimize the negative oxidative stress related complications of hemodialysis procedure, the bioactive hollow-fiber polysulfone membranes modified with vitamin E are currently commercially produced and used at hemodialysis clinics [1]. Lipoic acid was also proposed and evaluated in terms of inhibition of reactive oxygen species in blood [2], although any comparison was done with vitamin E, concerning the antioxidant activity.

Our aim was to prepare bioactive polysulfone membranes (with α-tocopherol or α-lipoic acid) and compare them in terms of membrane structure, separation characteristics, as well as antioxidant capacity.

The membranes were prepared by dissolving the bioactive compounds in polysulfone solvent *N*-methyl-2-pyrrolidon and casted on silicon wafer by spin coating, followed by phase inversion process. The release of α-tocopherol or α-lipoic acid from the membranes, during the phase inversion, was quantified by fluorometry and UV spectrophotometry, respectively. The antioxidant activity of membranes was evaluated by using ferric reduction antioxidant power (FRAP) assay.

Our data showed that membranes enriched with α-lipoic acid, compared to α-tocopherol, presented better separation characteristics of biomolecules. Nevertheless, the FRAP assay showed (2 fold) lower antioxidant activity for the membranes enriched with α-lipoic acid, then with α-tocopherol, demonstrating a stronger antioxidant power. Despite that, due to favorable effect of α-lipoic acid on separation characteristics of the membranes as well as its antioxidant activity, the introduction of α-lipoic acid into polysulfone membranes looks promising. Studies concerning the reduction of oxidative stress in blood are under study in our group.

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PI 5

VALIDATED RP-HPLC AND TLC - DENSITOMETRY METHODS FOR THE SIMULTANEOUS DETERMINATION OF SULFACETAMIDE SODIUM AND TWO OF ITS OFFICIAL IMPURITIES; SULFANILAMIDE AND DAPSONE

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Sulfacetamide sodium is a widely prescribed sulfonamide drug due to its topical anti-bacterial action on eye and skin. The BP describes four impurities for this drug, among which are sulfanilamide and dapsone. This work presents two specific, accurate and precise chromatographic methods for the simultaneous determination of a tertiary mixture of sulfacetamide sodium, sulfanilamide and dapsone. The first method is an isocratic RP-HPLC, where the separation of the three components was achieved on X-bridge C_{18} column (250 mm × 4.6 mm, 5.0 µm) at ambient temperature. The mobile phase used was methanol-water (60:40, v/v), with pH adjusted to 5.0 using orthophosphoric acid. Quantitation was achieved with UV detection at 273 nm based on peak area. The retention times (t_R) were 3.7, 3.1 and 6.4 min for sulfacetamide sodium, sulfanilamide and dapsone, respectively. The second method is a TLC- densitometric one where a good separation was achieved by using silica gel plates and a simple mobile phase of chloroform-dichloromethane-acetic acid (6: 2.5:1.5, by volume). Determination was done by densitometry in the absorbance mode at 273 nm. The retardation factors (R_f) were 0.60, 0.41 and 0.75 for sulfacetamide sodium, sulfanilamide and dapsone, respectively. Both methods were validated based on linearity, range, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), specificity and robustness in compliance with ICH guidelines. Both methods were successfully applied for the determination of sulfacetamide and its impurities in Ocusol ® ophthalmic solution where no impurities were detected in the pharmaceutical preparation. The obtained results were statistically compared to the results obtained by applying the official methods of analysis and no significant difference was found with respect to both accuracy and precision.

PI 6

EVALUATION OF ENCAPSULATION EFFICIENCY IN METHOTREXATE-LOADED NANOPARTICLES USING SEPARATIVE METHODS

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Nanotechnology-based drug delivery systems have received a great deal of attention as alternative therapies for several diseases. Indeed, the potential advantages of active ingredients' encapsulation, such as higher bioavailability at target tissues, have led to a significant increase in nanoparticles' production. However, their use as therapeutic agents depends on the assessment of several quality parameters during production. One of them is the determination of active ingredients' encapsulation efficiency and the quality assurance of its consistency between batches, as it directly impacts the therapeutic outcomes.

Having this in mind, the aim of this work is the development of an analytical method for the accurate determination of active ingredients' encapsulation efficiency within nanoparticles. Briefly, methotrexate (MTX)-loaded lipid and polymeric nanoparticles were separated from free MTX present in solution through Amicon® ultracentrifugal filters. The resulting portions were collected and analyzed by HPLC with UV detection set at 302 nm. Chromatographic separation was accomplished using a reversed-phase C18 monolithic column and a mixture of acetonitrile (ACN):phosphate buffer (100 mM, pH 7.0) (9:91, v/v) as mobile phase. Elution was performed in isocratic mode at a flow rate of 1.5 mL min^{-1}, resulting in a total run time of 3.5 min. Nanoparticles were submitted to ultrafiltration after dilution in water, phosphate buffer (100 mM, pH 7.0), or ACN:phosphate buffer (100 mM, pH 7.0) (10:90, v/v). Buffered media was the most suitable for ultrafiltration, providing total MTX recoveries > 92%. In fact, different ultrafiltration media provided different MTX % recovery in top (retained sample) and bottom (filtrate) compartments. From an initial mass loading of 27.6 ± 0.3 µg, 12.5 ± 0.1 µg and 4.56 ± 0.01 µg were retained at the top part of the filter when buffer and ACN:buffer were used as dilution media, respectively. DLS results have shown an increase in nanoparticles' size when organic solvent was added (223 ± 2.9 nm to 297 ± 4.5 nm). These results indicate the need for adequate solvents for accurate determination of entrapment efficiency in nanoparticles.

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