

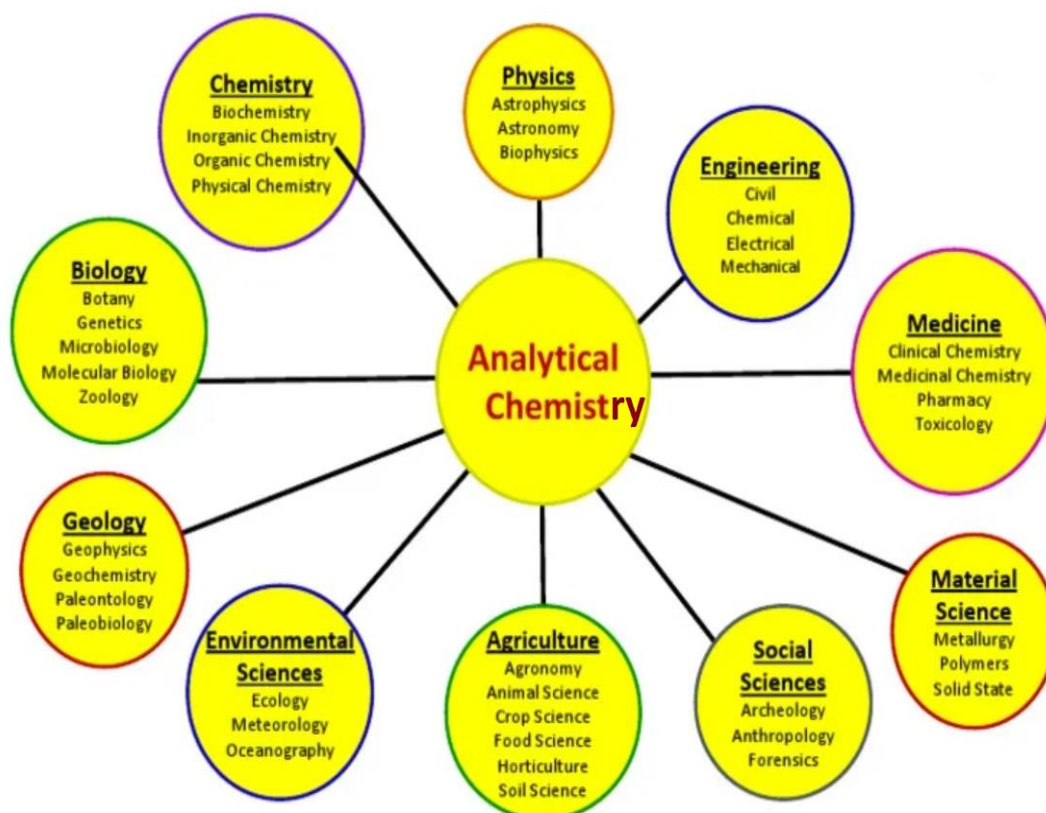


**4th ICABC 2022**

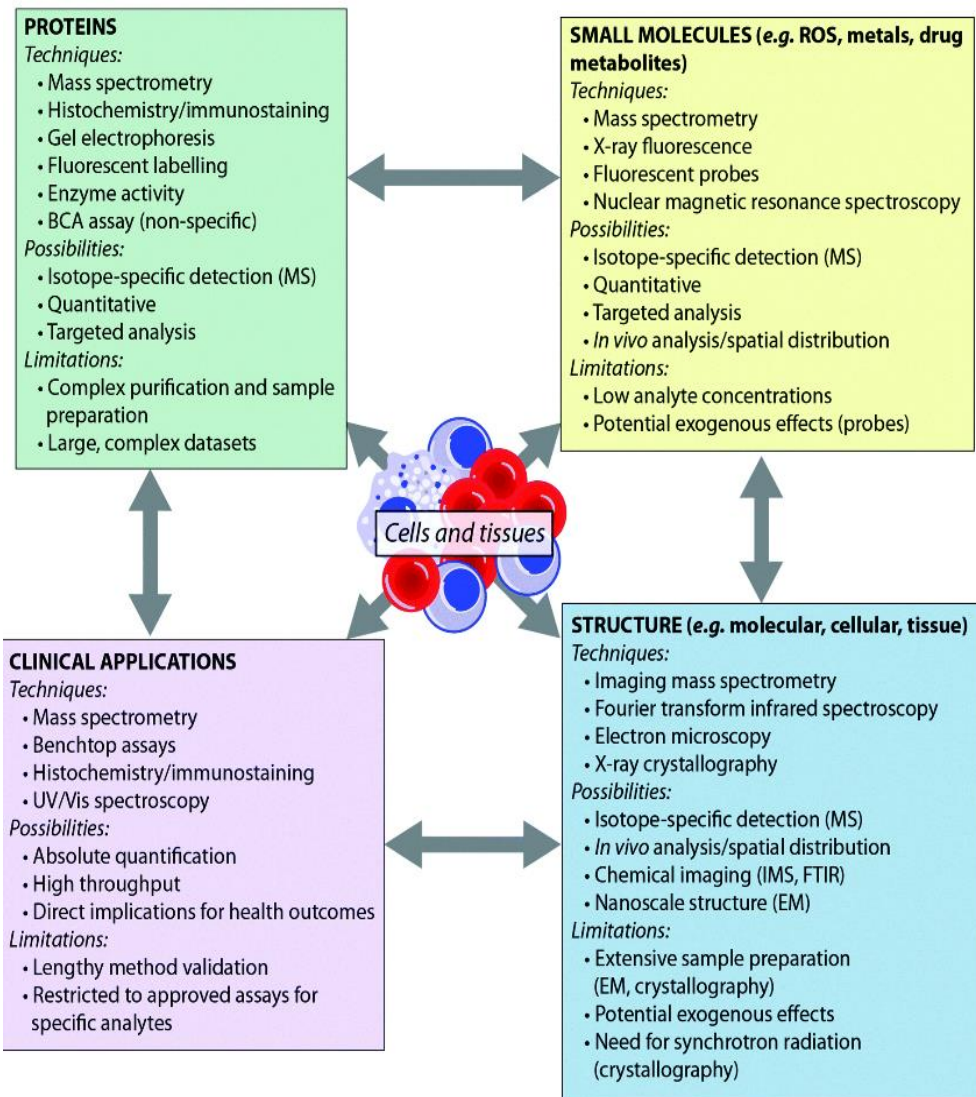
**23-26 March 2022-Online-Turkey**

## **4th International Congress on Analytical and Bioanalytical Chemistry**

# **BOOK of ABSTRACT**



**23-26 March 2022-Online-Turkey**



# 4th ICABC 2022

## **Preface**

The organizing committee of the **4th ICABC 2022** would like to welcome all participants to the "**4th International Congress on Analytical and Bioanalytical Chemistry**", held **as online** between 23-26 March 2022. The **ICABC** meeting was started three years ago and covers all areas of Analytical and Bioanalytical Chemistry as well as applications of Chemical and Biochemical Analysis.

The scientific congress program consists of **9** sessions that include **18 invited and 32 oral** presentations as well as **1 virtual** to be presented in the respective sessions. In addition, researchers of Academia (**32 universities from 11 countries**) and Research Institutes will present up-to-date developments on analytical and bioanalytical chemistry as well as applications to a wide range of environmental, biological and food matrices.

We strongly believe that the discussions and the exchange of ideas among the participants during the 4 days of the meeting will make **4th ICABC** a brilliant platform to initiate new research collaborations, particularly in favor of the young scientists participating in the conference.

We wish you all to enjoy this conference and have a pleasant to joining, hoping to meet you again during the next **ICABCs**.

With our best regards

The Chair (on behalf of Organizing Committee)

Prof. Dr. Mehmet YAMAN

Firat University, Science Faculty, Department of Chemistry, Elazig-Turkey

## **COMMITTEES**

### **INVITED SPEAKERS**

Antony Calokerinos (Athen U/GR)  
Mustafa K. Sezginturk (Canakkale 18 Mart U/TR)  
Mutay Aslan-(Akdeniz U/TR)  
Boguslaw Buszewski (N. Copernicus U/PL)  
Sibel A. Ozkan (Ankara U/TR)  
Trajce Stafilov (SS Cyril Meth U/MC)  
Sezgin Bakirdere (Yildiz Tech. U/TR)  
Arturs Viksna Latvia U.-LV  
Yusuf Dilgin, Canakkale 18 Mart U./TR

Ryszard Lobinski - Pau U-FR  
Mustafa Culha-South Florida U/USA  
Bekir Salih-Hacettepe U/TR  
Wan Jeffrey Basirun- Malaya U/MY  
Mustafa Soylak- Erciyes U/TR  
Durisehvar Unal-Istanbul U/TR  
Slawomira SKRZYPEK- Lodz U/PL  
Utkan DEMIRCI, Stanford U/USA  
Arūnas RAMANAVICIUS Vilnius U/LT

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 Altan Ercan-Abdullah Gul-U/TR  
 Kasim Ocakoglu-Tarsus U/TR  
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 Sibel A. OZKAN Ankara U.-TR  
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 Najma MEMON, Sindh U.-PK  
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 Mehmet Emin DURU- Mugla. U.-TR  
 Elif TUMAY OZER-Uludag U.-TR  
 Ersin KILINC, Dicle U.-TR  
 Emirhan NEMUTLU, Hacettepe U.-TR  
 Sadin OZDEMIR-Mersin U.-TR  
 Serap Saglik ASLAN, Istanbul U.-TR

Nagihan KARAASLAN AYHAN, Munzur U. TR  
Umran SEVEN ERDEMİR-Uludag U. TR  
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Gulberk UCAR, Hacettepe U. TR  
Andrzej LENIART- Lodz U.-PL

Barbara BURNAT- Lodz U.-PL  
Lukasz Poltorak- Lodz U.-PL

## **Organizing Committee Members**

Prof. Dr. Seref GUCER-Uludag U.  
Prof. Dr. Belgin İzgi-Uludag U  
Prof. Dr. F. Nil Ertas - Ege U.  
Assoc. Prof. Dr. Aysu Yarman- Potsdam U/DE  
Prof. Dr. Gokce KAYA-Firat U.  
Prof. Dr. Mehmet YAMAN-Firat U

# 4th ICABC 2022

## **Chair**

Prof. Dr. Mehmet YAMAN-Firat University.

## **Organizing Committee-Secretariat**

**Mehmet Yaman** (Firat U.),

**Gokce Kaya** (Firat U.),

**Hazal Ozyurt** (Near East U),

**Nagihan Karaaslan Ayhan** (Munzur U),

**Sevda Gultekin** (Firat U.),

**Gulsah Ozcan Sinir**-Uludag U

**Emel Akbaba**- Firat U.

**Sevda Akay Sazaklioğlu**- Gazi U/TR

## **GENERAL INFORMATION**

### **Introduction**

The **4th International Congress on Analytical and Bioanalytical Chemistry** will be held on 23-26 March 2022 as **Online**-Turkey is a four-days scientific meeting covering all areas of Analytical and Bioanalytical Chemistry and applications of Chemical and Biochemical Analysis. The international congresses have provided an excellent framework for the presentation of new concepts, instruments, methods, and applications in the area of modern chemical and biochemical analysis. Researchers and scientists from Universities, Research Institutions, State Organizations, and the Industry come together during the meeting to present and discuss the current state of the art in those areas. At the same time, it provides the grounds for the graduate and postgraduate students to present their projects, discuss scientific collaborations with other groups, as well as to explore employment opportunities.

I strongly believe that young researchers will have chance to improve their knowledge in deep of the analytical and bioanalytical chemistry by coming together with experienced scientists including invited speakers and scientific committee members.

## 4th ICABC 2022

### Topics

To promote collaboration among analytical and bioanalytical (including biochemists, food engineering, molecular biology and genetics and similars) scientists from different countries, “4th ICABC 2022” will provide adequate opportunities.

The topics include all areas of analytical and bioanalytic chemistry in applications such as, but not limited to, environmental, biological and food matrices, environmental protection, biochemical studies, drug characterisation, method innovation and validation, instrumental development and applications, sensors and nanobiosensors, chromatography, spectrometry and electrochemistry.

The congress covers determination of inorganic and organic components in environmental, biological and food matrices as well as the following subjects: Food Safety: Omics analysis including GMO, all studies on interactions between metabolic disorders and foodstuffs.

The main aim and theme of the congress is to enlighten the innovations and current trends with analytical and bio analytical chemistry (including organic and food chemistry).

### Location of Conference

4th ICABC 2022 will be held as ONLINE from Turkey

### Papers presentation

Scientific program will include Invited Speakers, which will provide an up-to-date presentation of modern trends of Analytical and Bioanalytical Chemistry as well as of related subjects of chemical and biochemical analysis-interest. Oral Presentations will be presented as online. Contributed papers describing original research work will be also presented as virtual in order to promote efficient discussion on new scientific ideas and results. The presenting authors should record their sound before virtual time. All presentations should be in English. Virtual and oral presentation will be accepted if at least one of the authors is registered and present at the conference for personal communication.



### **OPENING SPEECH**

**Dear reputable Professors, Colleagues and Participants,**

I am very happy to welcome all the participants coming to "4th International Congress on Analytical and Bioanalytical Chemistry "

**Respectable academics and friends;**

Nowadays, role of analytical and bioanalytical chemistry is better understood and increasing day by day. This is valid across a broad spectrum, from global nutrition to health, from advanced technological research to detection of environmental pollution. So, cooperation among analysts for up to date research is gaining importance.

This Congress mainly aims to promulgate knowledge in Life Science Analysis and Industrial Analytical techniques. Both life and Industrial sciences need Analytical and Bioanalytical Techniques in course of research work and therefore, Analytical Meetings would be a perfect venue to share and develop knowledge on key Analysis tools.

Related with Bioanalytical Methodology,

the guideline impact of bio-investigation in the pharmaceutical region is to gain a quantitative proportion of the medication and its metabolites. The purpose behind existing is to play out the pharmaco-kinetics, toxic-energy, bioequivalence and introduction response like pharmacokinetics-pharmaco-dynamics examines.

So, analytical chemistry is indispensable of bio-scientific strategies and hyphenated instruments in assessing the bio-examination of the drugs including,

Hyphenated techniques,

Chromatographic strategies,

Ligand bio-diagnostic procedures.

The first objective of this congress is to provide the opportunity for researchers interested in different disciplines to come together and to exchange ideas in analytical-perspective meetings.

Furthermore, the purpose of congresses and similar scientific meetings is to make presentations of young researchers, to participate the presentations and in an environment of well-known scientists.

This congress was launched for all those purposes.

Hereby, I would like to thank the invited speakers, the members of Science Committee and especially for your participation, and

I would like to express our honor to host the congress.

Statistical Information about the Congress

The scientific conference program consists of 9 sessions that include 18 invited and 32 oral presentations as well as 1 virtual. The participants are of 32 universities from 11 countries.

I believe that the discussions and the exchange of ideas among the participants during the 4 days will make this conference a brilliant platform to initiate new research collaborations.

I wish you all to enjoy this conference.

- I wish the congress will be useful.
- With my best regards.

**Prof. Dr. Mehmet Yaman-  
Chair**



# CONFERENCE PROGRAM

## 4th International Congress on Analytical and Bioanalytical Chemistry (4th ICABC 2022)

23-27 March, 2022, Online

	<b>23 March, 2022</b>
16:00-16:30	<p>➤ Welcome Ceremony</p> <p>➤ Prof. Dr. <b>Mehmet Yaman</b> (Chair)</p> <p>Prof. Dr. <b>Seref GUCER</b> (on behalf of continuation committee)</p> <p><i>Honorable</i></p>
16:35-17:05	<p><b>Inv. 1: Prof. Dr. Boguslaw BUSZEWSKI</b>- N. Copernicus U/PL</p> <p><i>Separation and spectral techniques in determination and characterization of colloids and biocolloids</i></p>
17:05-17:35	<p><b>Inv. 2: Prof. Dr. Mustafa CULHA</b> – South Florida U/USA-Sabancı U/TR</p> <p><i>Surface-enhanced Raman Scattering for Single Living Cell Analysis</i></p>
	<b>24 March, 2022</b>
	<b>Session 1</b> - Chairs: Prof. Dr. F. Nil Ertas- Prof. Dr. Sezgin Bakirdere
10:00-10:30	<p><b>Inv. 3: Prof. Dr. Utkan DEMIRCI</b>, Stanford U/USA</p> <p><i>Microfluidic label free technologies for applications in biofabrication and medicine</i></p>
10:40-11:10	<p><b>Inv. 4: Prof. Dr. Sibel A. OZKAN</b>- Ankara U/TR</p> <p><i>An Overview of Electroanalytical Carbon Based Nanosensors: The Current and Future Prospect</i></p>
11:10-12:00	<p><b>OP1-Hilal Torul</b>-Gazi U/TR</p> <p>Gold Nanoparticle Decorated Paper Electrode Assemblies for Sensor Applications</p>
	<p><b>OP2-Ceren Kaçar Selvi</b>-Ankara U/TR</p> <p>Amperometric Bisphenol A Biosensor Based on Carbon Quantum Dots, Ionic Liquid and Gold Nanoparticles</p>
	<p><b>VP1-Ceren Kaçar Selvi</b>-Ankara U/TR</p> <p>Development of Hydrogen Peroxide Biosensor Based on poly-L-Histidine Modified Glassy Carbon Electrode</p>
	<p><b>OP3- Sevda Akay Sazaklıoğlu</b>- Gazi U/TR</p> <p>Label-Free Determination of Extracellular Vesicles onto Gold Screen Printed Electrodes by Electrochemical Impedance Spectroscopy</p>
	<p><b>OP4- Andrzej Leniart</b>-Lodz U/PL</p> <p>Preparing, characterization and application of glassy carbon electrode modified with carbon nanotubes and palladium for electrooxidation of formaldehyde</p>
12:00-14:00	Lunch
	<b>Session 2</b> - Chairs: Prof. Dr. Hüseyin Çelikkan; - Prof. Dr. Almira RAMANAVICIENE
14:00-14:30	<p><b>Inv. 5: Prof. Dr. Mustafa Kemal SEZGINTURK</b>-Canakkale 18 Mart U/TR</p> <p><i>New Developments in Lateral Flow Assays for SARS-CoV-2 Diagnosis</i></p>
14:30-15:00	<p><b>Inv. 6: Prof. Dr. Slawomira SKRZYPEK</b>- Lodz U/PL</p> <p><i>Fenhexamid Sensing On Planar Boron-Doped Diamond Electrode</i></p>
15:00-15:40	<p><b>OP5- Aušra Valiūnienė</b>-Vilnius U/LT</p> <p>Fast Fourier transform electrochemical impedance spectroscopy for monitoring surface modification in the process of biosensor formation</p>
	<p><b>OP6- Inga Gabriunaite</b>-Vilnius U/LT</p> <p>Tethered Bilayer Lipid Membrane Formation on Metal Oxides</p>
	<p><b>OP7- Laura Sakalauskiene</b> -Vilnius U/LT</p> <p>Gold nanostructure and Prussian blue nanocomposite for the development of amperometric glucose biosensor</p>
	<b>OP8- Mariola Brycht</b> - Lodz U/PL

	<i>Dopamine Sensing on Novel Porous Boron-doped Diamond Electrodes With Various Thickness</i>
15:40-15:50	<i>Tea/Coffee break</i>
	<b>Session 3: Chairs: Assoc. Prof. Dr. Emine A. Torumtay –Assoc. Prof. Dr. Mustafa Celebier</b>
15:50-16:20	<b>Inv. 7: Prof. Dr. Arturs VIKSNA- Latvia U/LV</b> <i>Investigation of floral origin markers of honey by different instrumental methods</i>
16:20-17:00	<b>OP9- Agnese Osīte- Latvia U/LV</b> Metallic elements of residential dust as a measure of indoor air quality
	<b>OP10- Ayşegül Erdoğan -Ege U/TR</b> Investigation of document forgery by XPS via chemical imaging: Analysis of blue commercial ballpoint pen inks
	<b>OP11- Didem Peren Aykas Cinkilic</b> Aydın Adnan Menderes U/TR <i>The Application of Vibrational Spectroscopy and Chemometrics in the Discrimination of Pig Derivatives in Halal Food</i>
	<b>OP12-Zeynep Atasayar- Istanbul U-Mimar Sinan G.S. U/TR</b> Strontium Isotopes and Chemical Analysis In The Investigation of Iron Age Glass Beads Production
	<b>25 March, 2022</b>
	<b>Session 4 - Chairs: Prof. Dr. Belgin İzgi- Prof. Dr. Aysu Yarman</b>
10:00-10:30	<b>Inv. 8: Prof. Dr. Wan Jeffrey BASIRUN- Malaya U/MY</b> <i>Beyond mechanochemical transformation– latest advancements of recycling of sodium borate into sodium borohydride</i>
10:40-11:10	<b>Inv. 9: Prof. Dr. Mustafa SOYLAK- Erciyes U/TR</b> <i>From Activated Carbon to MOF: Solid Phase Extraction</i>
11:10-12:00	<b>OP13- Aliyu B. Abdullahi –Near East U/TRNC</b> Deep eutectic solvent-liquid-liquid microextraction prior to smartphone digital image colorimetry for the determination of cobalt in milk and dairy products
	<b>OP14- Salihu Ismail– Near East U/TRNC- Yusuf Maitama Sule U/NG</b> Switchable-hydrophilicity solvent liquid-liquid microextraction combined with smartphone digital image colorimetry for the determination of palladium in catalytic converters
	<b>OP15- Cansu Demir- Yildiz T. U/TR</b> Development of a novel and effective solid phase extraction method based on reduced graphene oxide-zinc oxide nanoflowers for the sensitive determination of cadmium by flame atomic absorption spectrophotometry
	<b>OP16- Meltem Saylan-Yildiz Techn. U/TR</b> <i>A novel cadmium sulfide nanoparticles based dispersive solid phase extraction for accurate determination of copper ions in artichoke leaves extract by flame atomic absorption spectrophotometry</i>
	<b>OP17- Miray Oner- Yildiz T. U/TR</b> Development of a rapid and efficient dispersive solid phase extraction for the determination of lead at trace levels: Manganese oxide nanoflower based dispersive solid phase extraction prior to flame atomic absorption spectrometry measurement
12:00-14:00	<i>Lunch</i>
	<b>Session 5 -: Chairs: Assoc. Prof. Dr. Onder Metin– Prof. Dr. Agnese Osite</b>
14:00-14:30	<b>Inv. 10: Prof. Dr. Bekir SALIH- Hacettepe U/TR</b> <i>The Role of Hyphenated Mass Spectrometric Techniques in The Characterization of Biosimilar Therapeutic Protein Drug Products</i>
14:30-15:00	<b>Inv. 11: Prof. Dr. Durisehvar UNAL-Istanbul U/TR</b> <i>Cleaning Validation of Manufacturing Equipment in the Pharmaceutical Industry</i>
15:00-15:40	<b>OP18- Cemile Yucel-Dokuz Eylul U/TR</b> Validation of QuEChERS-GC MS/MS Method for determination of UV Filters in Sludges
	<b>OP19- Mehmet Atakay- Hacettepe U/TR</b> Conformational Analysis of Monoclonal Antibody Subunits Using Trapped Ion Mobility-Mass Spectrometry Technique
	<b>OP20- Daria Janiszewska- Nicolaus Copernicus U/PL</b> New approach for determination and identification of selected antibiotics and microorganisms for biomedical purposes
	<b>OP21- A. Krakowska-Sieprawska-Nicolaus Copernicus U/PL</b> Enzymatic pretreatment of plant material for enhanced release of biologically active compounds by supercritical CO <sub>2</sub>
15:40-15:50	<i>Tea/Coffee break</i>
15:50-	<b>Session 6 -: Chairs: Prof. Dr. Elif Tumay Ozer- Prof. Dr. İlhan Yaylim</b>

16:20	<b>Inv. 12: Prof. Dr. Ryszard LOBINSKI</b> -CNRS Pau/FR <i>MS<sup>2</sup>/MS3 data-dependent HPLC for untargeted analysis for mycosporine-like amino-acids and flavonoids in marine resources</i>
16:20-16:50	<b>Inv. 13: Prof. Dr. Sezgin BAKIRDERE</b> - Yildiz Techn. U/TR <i>COVID-19 and Analytical Chemistry</i>
16:50-17:10	<b>OP22- Sude Oflu</b> - Yildiz Techn. U/TR Quadrupole Isotope Dilution Method for the Determination of Methamphetamine in Human Urine and Serum Samples by Gas Chromatography Mass Spectrometry After Simultaneous Derivatization and Spray Assisted Droplet Formation-Liquid Phase Microextraction
	<b>OP23- Rabia Demirel</b> - Yildiz Techn. U/TR Determination of Trace Levels of Paracetamol in Human Urine and Human Serum with High Accuracy and Sensitivity Using Dispersive Liquid Liquid Microextraction Coupled to Quadrupole Isotope Dilution GC-MS
	<b>OP24- Sylwia Studzińska</b> -Nicolaus Copernicus U/PL In vitro and in vivo metabolism studies of antisense oligonucleotides with the use of ion-pair liquid chromatography coupled with mass spectrometry
	<b>26 March, 2022</b>
	<b>Session 7- Chairs: Assoc. Prof. Dr. Murat Yavuz- Prof. Dr. Hulya Cicek</b>
10:00-10:30	<b>Inv. 14: Prof. Dr. Mutay ASLAN</b> -Akdeniz U/TR <i>The Interaction of Inflammation and Expression of Adhesion Molecules in Colorectal Cancer</i>
10:30-11:20	<b>OP25- Hasan Karadag</b> - Adiyaman U/TR Interactions of Titanium Dioxide Nanoparticles and Zinc Oxide Nanoparticles with Glutathione Reductase
	<b>OP26- Marlena Martyna</b> - Maria Curie-Skłodowska U/PL Electroreduction of Bi(III) ions on the innovative cyclically renewable liquid silver amalgam film electrode (R-AgLAFe) in the presence of cysteine
	<b>OP27- Barbara Burnat</b> - Lodz U/PL Carbon black-modified carbon ceramic electrode – fabrication, characterization, and its electroanalytical performance
	<b>OP28- Didem Aydin</b> -Selcuk U/TR Transport of Methylene Blue Across a Bulk Liquid Membrane using Pillar[5]arene as a Carrier Phase
11:20-11:50	<b>Inv. 15: Prof. Dr. Trajce STAFILOV</b> -Ss Cyril and Methodius U/MK <i>Spatial Distribution and Pollution Assessment of Major and Trace Elements in Soil from the Republic of North Macedonia</i>
12:00-14:00	<b>Lunch</b>
14:00-14:30	<b>Session 8- Chairs: Assoc. Prof. Dr. Ümran Seven Erdemir - Dr. Barbara Burnat</b>
	<b>Inv. 16: Prof. Dr. Antony CALOKERINOS</b> -Athen U/GR <i>Analytical Profiles and Antioxidant Properties of Natural Products after Infusion with Herbs or Spices</i>
14:30-15:00	<b>OP29- Hussam AL Saoud</b> -Bialystok U/PL Diatom biosilica for the chromatographic purposes
	<b>OP30- Justyna Walczak-Skierska</b> - Nicolaus Copernicus U/PL Quantitative structure-retention relationship for identification and quantification in lipidomics using chromatographic techniques
	<b>OP31- Rim Elseblani</b> -CNRS-Pau U/FR Analytical approaches to investigate contaminants transported by microplastics on the Lebanese Mediterranean coast
15:00-15:10	<b>Tea/Coffee</b>
	<b>Session 9- Chairs: Prof. Dr. Mustafa Ersoz- Prof. Dr. Serife Tokalioglu</b>
15:10-15:40	<b>Inv. 17: Prof. Dr. Yusuf DILGIN</b> -Canakkale 18 Mart U/TR <i>Electrochemical sensors based on bis-neocuproine Cu(II) complex modified electrodes</i>
15:40-16:10	<b>Inv. 18: Prof. Dr. Arūnas Ramanavičius</b> - Vilnius U/LT <i>Conducting Polymers in Sensor Design</i>
	<b>OP32-Aryan F. Qader</b> -Koya U/IQ-Firat U/TR Phytochemical Screening, Antioxidative Activity, Chromatographic Fractionation, and HPLC Analysis of Phenolic Components in Blackberry (Rubus fruticosus L.) Extract
16:20	<b>Closing</b>

<b>INVITED SPEAKERS (IS)</b> .....	18
<b>IS1- Spatial Distribution and Pollution Assessment of Major and Trace Elements in Soil from the Republic of North Macedonia</b> .....	18
Trajče Stafilev <sup>1</sup> , Robert Šajn <sup>2</sup> .....	18
<sup>1</sup> Institute of Chemistry, Faculty of Natural Sciences and Mathematics, Ss Cyril and Methodius University, Arhimedova 5, 1000 Skopje, North Macedonia; .....	18
<b>IS2- Cleaning Validation of Manufacturing Equipment in the Pharmaceutical Industry</b> .....	19
Durişehvar Özer Ünal .....	19
Istanbul University, Faculty of Pharmacy, Dept. Of Analytical Chemistry, .....	19
Istanbul University, Drug Research and Application Center .....	19
<b>IS3- An Overview of Electroanalytical Carbon Based Nanosensors: The Current and Future Prospect</b> .....	20
Sibel A. OZKAN .....	20
Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, 06560, Ankara, Turkey .....	20
<b>IS4- Investigation of floral origin markers of honey by different instrumental methods</b> .....	21
Arturs Viksna <sup>1*</sup> , Kriss Davids Labsvards <sup>1,2</sup> , Lauma Busa <sup>1</sup> , Janis Rusko <sup>2</sup> , .....	21
Rihards Kluga <sup>1</sup> , Maris Bertins <sup>1</sup> , Agnese Osite <sup>1</sup> .....	21
<sup>1</sup> Faculty of Chemistry, University of Latvia, Jelgavas street 1, LV-1004, Riga Latvia .....	21
<b>IS5- MS<sup>2</sup>/MS<sup>3</sup> data-dependent HPLC for untargeted analysis for mycosporine-like amino-acids and flavonoids in marine resources</b> .....	22
Maroussia Parailoux, Simon Godin and Ryszard Lobinski .....	22
CNRS, Institute for Analytical and Physical Chemistry (IPREM), UMR 5254, Hélioparc, 64053 Pau, France. ....	22
<b>IS6- Microfluidic label free technologies for applications in biofabrication and medicine</b> ....	23
Utkan Demirci, PhD .....	23
Professor, Department of Radiology at Stanford University School of Medicine .....	23
Interim Chief, Director of the Canary Center at Stanford for Cancer Early Detection, USA.....	23
<b>IS7- Fenhexamid Sensing On Planar Boron-Doped Diamond Electrode</b> .....	24
Mariola Brycht <sup>1</sup> , Sławomira Skrzypek <sup>1*</sup> .....	24
University of Lodz, Faculty of Chemistry, Tamka 12, 91-403 Lodz, Poland .....	24
<b>IS8- Separation and spectral techniques in determination and characterization of colloids and biocolloids</b> .....	25
Bogusław Buszewski .....	25
Department of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, .....	25
Interdisciplinary Centre for Modern Technologies, Nicolaus Copernicus University ,Toruń, Poland, .....	25
<b>IS9- Analytical Profiles and Antioxidant Properties of Natural Products</b> .....	26
after Infusion with Herbs or Spices .....	26
Antony C. Calokerinos .....	26
National and Kapodistrian University of Athens, School of Science, Department of Chemistry, Laboratory of Analytical Chemistry, Panepistimiopolis, 15771 Athens, Greece .....	26
<b>IS10- Surface-enhanced Raman Scattering for Single Living Cell Analysis</b> .....	27
Mustafa Culha <sup>1,2</sup> .....	27
<sup>2</sup> The University of South Florida, Department of Internal Medicine, Morsani College of Medicine, Tampa, FL .....	27
<b>IS11- From Activated Carbon to MOF: Solid Phase Extraction</b> .....	27

<b>Mustafa Soylak<sup>a,b</sup></b> .....	27
<sup>a</sup> Erciyes University, Faculty of Sciences, Department of Chemistry, 38039 Kayseri-TURKEY ..	27
<b>IS12- COVID-19 AND ANALYTICAL CHEMISTRY</b> .....	28
<b>Sezgin BAKIRDERE<sup>1,2*</sup></b> .....	28
<sup>1*</sup> Yıldız Technical University, Faculty of Art and Science, Department of Chemistry, 34210, Davutpasa, Esenler, Istanbul, Turkey .....	28
<b>IS13- Electrochemical sensors based on bis-neocuproine Cu(II) complex modified electrodes.</b> .....	29
<b>Yusuf Dilgin<sup>1*</sup>, Selen Ayaz<sup>1</sup>, Gamze Emir<sup>1</sup>, Ayşem Arda<sup>2</sup>, Reşat Apak<sup>2</sup></b> .....	29
<sup>1</sup> Çanakkale Onsekiz Mart University, Science and Art Faculty, Department of Chemistry, 17100-Çanakkale .....	29
<b>IS14- The Interaction of Inflammation and Expression of Adhesion Molecules in Colorectal Cancer</b> .....	31
<b>Mutay Aslan<sup>1,2</sup></b> .....	31
Departments of 1Medical Biochemistry, 2Gene and Cell Therapy, Akdeniz University, Faculty of Medicine, 07070, Antalya, Turkey .....	31
<b>IS15- The Role of Hyphenated Mass Spectrometric Techniques in The Characterization of Biosimilar Therapeutic Protein Drug Products</b> .....	32
<b>Bekir Salih<sup>1*</sup>, Mehmet Atakay<sup>1</sup>, Ülkü Güler<sup>1</sup>, Sema Baltacı<sup>1</sup>, Buse Kardelen Varlıoğlu Özdemir<sup>1</sup>, Emine Yılmaz<sup>2</sup></b> .....	32
1. Hacettepe University, Department of Chemistry, 06800 Ankara-TURKEY .....	32
<b>IS16- Beyond mechanochemical transformation– latest advancements of recycling of sodium borate into sodium borohydride</b> .....	33
<b>Wan Jefrey Basirun</b> .....	33
Department of Chemistry, University Malaya, Kuala Lumpur 50603, Malaysia .....	33
<b>IS17- New Developments in Lateral Flow Assays for SARS-CoV-2 Diagnosis</b> .....	34
<b>Mustafa Kemal Sezgintürk</b> .....	34
Çanakkale Onsekiz Mart University, Faculty of Engineering, Bioengineering Department, Çanakkale, Turkey .....	34
<b>IS18- Conducting Polymers in Sensor Design</b> .....	35
<b>Arūnas Ramanavičius<sup>1,2</sup>, Juratė Petronienė<sup>1</sup>, Urtė Bubnienė<sup>1,2</sup>, Eivydė Andriukonis<sup>1,2</sup>, Asta Kaušaitė<sup>3</sup>, Almira Ramanavičienė<sup>3</sup></b> .....	35
<sup>1</sup> Department of Physical Chemistry, Faculty of Chemistry and Geosciences, Vilnius University, Naugarduko st. 24, LT – 03225, Vilnius, Lithuania .....	35
<b>ORAL PRESENTATIONS (OP)</b> .....	36
<b>OP1- Gold Nanoparticle Decorated Paper Electrode Assemblies for Sensor Applications</b> .....	36
<b>Hilal Torul<sup>1*</sup>, İ. Hakkı Boyacı<sup>2</sup>, Uğur Tamer<sup>1</sup></b> .....	36
<sup>1</sup> Department of Analytical Chemistry, Gazi University, Ankara, Turkey .....	36
<b>OP2- Amperometric Bisphenol A Biosensor Based on Carbon Quantum Dots, Ionic Liquid and Gold Nanoparticles</b> .....	37
<b>Demet Örenli<sup>1</sup>, Ceren Kaçar Selvi<sup>1*</sup>, Funda Öztürk<sup>2</sup>, Pınar Esra Erden<sup>3</sup>, Esmā Kılıç<sup>1</sup></b> .....	37
<sup>1</sup> Department of Chemistry, Faculty of Science, Ankara University, Ankara/Turkey .....	37
<b>OP3 Label-Free Determination of Extracellular Vesicles onto Gold Screen Printed Electrodes by Electrochemical Impedance Spectroscopy</b> .....	38
<b>Sevda Akay Sazaklıoğlu<sup>1,2*</sup>, Hilal Torul<sup>3</sup>, Hafize Seda Vatansever<sup>4,5</sup>, Uğur Tamer<sup>3</sup>, Hüseyin Çelikkan<sup>6</sup></b> .....	38
<sup>1</sup> Department of Medical Services and Techniques, Ankara Medipol University, 06050, Ankara, Turkey .....	38

<b>OP4- Preparing, characterization and application of glassy carbon electrode modified with carbon nanotubes and palladium for electrooxidation of formaldehyde</b> .....	39
Andrzej Leniart*, Mariola Brycht, Barbara Burnat, Sławomira Skrzypek.....	39
University of Lodz, Faculty of Chemistry, Department of Inorganic and Analytical Chemistry 39 Tamka 12, 91-403 Lodz, Poland).....	39
<b>OP5- Fast Fourier transform electrochemical impedance spectroscopy for monitoring surface modification in the process of biosensor formation</b> .....	40
Aušra Valiūnienė <sup>1*</sup> , Povilas Virbickas <sup>1</sup> , Gabija Kavaliauskaitė <sup>1</sup> , Margarita Poderyte <sup>1</sup> , Inga Gabriunaite <sup>1</sup> .....	40
<sup>1</sup> Faculty of Chemistry and Geosciences, Vilnius University, Naugarduko 24, Vilnius LT-03225, Lithuania .....	40
<b>OP6- Tethered Bilayer Lipid Membrane Formation on Metal Oxides</b> .....	41
Inga Gabriunaite <sup>1*</sup> , Aušra Valiūnienė <sup>1</sup> .....	41
Faculty of Chemistry and Geosciences, Vilnius University, Naugarduko str. 24, Vilnius, Lithuania .....	41
<b>OP7- Gold nanostructure and Prussian blue nanocomposite for the development of amperometric glucose biosensor</b> .....	42
Laura Sakalauskiene, B. Brasiunas, A. Ramanaviciene.....	42
NanoTechnas– Center of Nanotechnology and Materials Science, Faculty of Chemistry and Geosciences, Vilnius University Vilnius, Lithuania.....	42
<b>OP8- Dopamine Sensing on Novel Porous Boron-doped Diamond Electrodes With Various Thickness</b> .....	43
Mariola Brycht <sup>1</sup> , Simona Baluchová <sup>2</sup> , Andrew Taylor <sup>3</sup> , Silvia Sedláková <sup>3</sup> , Vincent Mortet <sup>3</sup> , Karolina Schwarzová-Pecková <sup>2</sup> .....	43
<sup>1</sup> University of Lodz, Faculty of Chemistry, Tamka 12, 91-403 Lodz, Poland .....	43
<b>OP9- Metallic elements of residential dust as a measure of indoor air quality</b> .....	44
Agnese Osīte*, Māris Bērtiņš, Arturs Vīksna, Gunita Celma and Oto Poišs .....	44
University of Latvia, Faculty of Chemistry, Jelgavas Str. 1, Riga, Latvia.....	44
<b>OP10- Investigation of document forgery by XPS via chemical imaging:</b> .....	45
<b>Analysis of blue commercial ballpoint pen inks</b> .....	45
Ayşegül Erdoğan* .....	45
Ege University Application and Research Center For Testing and Analysis, (EGE MATAL) Turkey .....	45
<b>OP11- The Application of Vibrational Spectroscopy and Chemometrics in the Discrimination of Pig Derivatives in Halal Food</b> .....	46
Didem Peren Aykas Cinkilic <sup>a*</sup> , Gulsah Ozcan-Sinir <sup>b</sup> .....	46
<sup>a</sup> Department of Food Engineering, Faculty of Engineering, Aydın Adnan Menderes .....	46
University, Aydın, Turkey, .....	46
<b>OP12- Strontium Isotopes and Chemical Analysis In The Investigation of Iron Age Glass Beads Production</b> .....	47
Özden Ormancı <sup>1*</sup> , Erol Uslu <sup>2</sup> , Zeynep Atasayar <sup>1,3</sup> .....	47
<sup>3</sup> Istanbul University-Cerrahpaşa, Graduate Education Institute, Department of Analytical Chemistry, İstanbul, Turkey .....	47
<b>OP13- Deep eutectic solvent-liquid-liquid microextraction prior to smartphone digital image colorimetry for the determination of cobalt in milk and dairy products</b> .....	48
Aliyu Bawa Abdullahi <sup>1,2</sup> , Salihu Ismail <sup>1,3</sup> , Usama Alshana <sup>1,4,*</sup> .....	48
<sup>1</sup> Department of Analytical Chemistry, Faculty of Pharmacy, Near East University, 99138 Nicosia, TRNC, Mersin 10, Turkey .....	48



<sup>2</sup> Department of Chemistry, Kano State College of Education and Preliminary Studies, PMB 3145, Kano, Nigeria.....	48
<b>OP14- Switchable-hydrophilicity solvent liquid-liquid microextraction combined with smartphone digital image colorimetry for the determination of palladium in catalytic converters .....</b>	49
Salihu Ismail <sup>1, 2</sup> , Aliyu B. Abdullahi <sup>1,3</sup> , Usama Alshana <sup>1, 4,*</sup> .....	49
<sup>1</sup> Department of Analytical Chemistry, Faculty of Pharmacy, Near East University, 99138, Nicosia, TRNC, Mersin 10, Turkey .....	49
<sup>2</sup> Department of Chemistry, Faculty of Science, Yusuf Maitama Sule University, PMB 3220, Kano, Nigeria.....	49
<b>OP15- Development of a novel and effective solid phase extraction method based on reduced graphene oxide-zinc oxide nanoflowers for the sensitive determination of cadmium by flame atomic absorption spectrophotometry .....</b>	50
Cansu Demir <sup>1</sup> , Miray Öner <sup>1</sup> , Gülten Çetin <sup>1</sup> , Sezgin Bakirdere <sup>1,2</sup> .....	50
<sup>1</sup> Yıldız Technical University, Department of Chemistry, 34349 İstanbul, TURKEY .....	50
<b>OP16- A novel cadmium sulfide nanoparticles based dispersive solid phase extraction for accurate determination of copper ions in artichoke leaves extract by flame atomic absorption spectrophotometry.....</b>	51
Meltem Şaylan <sup>1,2</sup> , Berfin Metin <sup>1</sup> , Hilal Akbıyık <sup>3</sup> , Fatma Turak <sup>1</sup> , Gülten Çetin <sup>1</sup> , Sezgin Bakirdere <sup>1,4</sup> .....	51
<sup>1</sup> Yıldız Technical University, Department of Chemistry, 34349 İstanbul, TURKEY .....	51
<b>OP17- Development of a rapid and efficient dispersive solid phase extraction for the determination of lead at trace levels: Manganese oxide nanoflower based dispersive solid phase extraction prior to flame atomic absorption spectrometry measurement .....</b>	52
Miray Öner <sup>1</sup> , Cansu Demir <sup>1</sup> , Gülten Çetin <sup>1</sup> , Sezgin Bakirdere <sup>1,2</sup> .....	52
<sup>1</sup> Yıldız Technical University, Department of Chemistry, 34349 İstanbul, TURKEY .....	52
<b>OP18- Validation of QuEChERS-GC MS/MS Method for determination of UV Filters in Sludges .....</b>	53
Cemile Yücel <sup>1*</sup> , Serenay Ceren Tüzün <sup>1</sup> , Hasan Ertaş <sup>2</sup> , F. Nil Ertaş <sup>2</sup> , İlgi Karapınar <sup>1</sup> .....	53
<sup>1</sup> Dokuz Eylül University, Department of Environmental Engineering, Tınaztepe Campus, İzmir, Turkey .....	53
<b>OP19- Conformational Analysis of Monoclonal Antibody Subunits Using Trapped Ion Mobility-Mass Spectrometry Technique .....</b>	54
Mehmet Atakay <sup>1*</sup> , Ülkü Güler <sup>1</sup> , Sema Baltacı <sup>1</sup> , Buse Kardelen Varlıoğlu Özdemir <sup>1</sup> , Emine Yılmaz <sup>2</sup> , Bekir Salih <sup>1</sup> .....	54
1. Hacettepe University, Department of Chemistry, Ankara, TÜRKİYE .....	54
<b>OP20- New approach for determination and identification of selected antibiotics and microorganisms for biomedical purposes.....</b>	57
Daria Janiszewska <sup>1</sup> , Małgorzata Szultka-Młyńska <sup>1</sup> , Bogusław Buszewski <sup>1,2</sup> .....	57
<sup>1</sup> Department of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University, Gagarina 7, 87-100 Toruń, Poland .....	57
<b>OP21- Enzymatic pretreatment of plant material for enhanced release of biologically active compounds by supercritical CO<sub>2</sub> .....</b>	58
A. Krakowska-Sieprawska <sup>2</sup> , K. Rafińska <sup>1</sup> , J. Walczak-Skierska <sup>1</sup> , B. Buszewski <sup>1,2</sup> .....	58
<sup>2</sup> Interdisciplinary Centre of Modern Technologies, Nicolaus Copernicus University, ..... Wileńska 4, 87-100 Toruń, Poland.....	58
<b>OP22- Quadrupole Isotope Dilution Method for the Determination of Methamphetamine in Human Urine and Serum Samples by Gas Chromatography Mass Spectrometry After</b>	



<b>Simultaneous Derivatization and Spray Assisted Droplet Formation-Liquid Phase Microextraction</b> .....	59
Sude Oflu <sup>a</sup> , Sezin Erarpat <sup>a</sup> , Buse Tuğba Zaman <sup>a</sup> , Ömer Tahir Günkara <sup>a</sup> , Sezgin Bakirdere <sup>a,b</sup> , Fatma Turak <sup>a</sup> .....	59
<sup>a</sup> Yıldız Technical University, Faculty of Art and Science, Department of Chemistry, 34210, Davutpasa, Esenler, Istanbul, Turkey .....	59
<b>OP23- Determination of Trace Levels of Paracetamol in Human Urine and Human Serum with High Accuracy and Sensitivity Using Dispersive Liquid Liquid Microextraction Coupled to Quadruple Isotope Dilution GC-MS</b> .....	60
Rabia Demirel <sup>1*</sup> , Sezin Erarpat <sup>1</sup> , Süleyman Bodur <sup>1</sup> , Ömer Tahir Günkara <sup>1</sup> , Sezgin Bakirdere <sup>1,2</sup> , Fatma Turak <sup>1</sup> .....	60
<sup>1</sup> Yıldız Technical University, Faculty of Art and Science, Department of Chemistry, 34220, İstanbul, Turkey .....	60
<b>OP24- In vitro and in vivo metabolism studies of antisense oligonucleotides with the use of ion-pair liquid chromatography coupled with mass spectrometry</b> .....	61
Sylvia Studzińska <sup>*</sup> , Bogusław Buszewski .....	61
Chair of Environmental Chemistry & Bioanalytics, Faculty of Chemistry, .....	61
Nicolaus Copernicus University in Toruń, 7 Gagarin St. PL-87 100 Toruń, Poland. ....	61
<b>OP25- Interactions of Titanium Dioxide Nanoparticles and Zinc Oxide Nanoparticles with Glutathione Reductase</b> .....	62
Hasan Karadag <sup>*</sup> , Beytullah Atkan .....	62
Adiyaman University, Science and Letters Faculty, Chemistry Department, 02040, Adiyaman, Türkiye .....	62
<b>OP26- Electroreduction of Bi(III) ions on the innovative cyclically renewable liquid silver amalgam film electrode (R-AgLAFe) in the presence of cysteine</b> .....	63
Marlena Martyna <sup>1</sup> , Aleksandra Bazan-Woźniak <sup>2</sup> , Robert Pietrzak <sup>2</sup> , Agnieszka Nosal-Wiercińska <sup>1*</sup> .....	63
<sup>1</sup> Faculty of Chemistry, Maria Curie-Skłodowska University, Maria Curie-Skłodowska Sq. 3, 20-031 Lublin, Poland .....	63
<b>OP27- Carbon black-modified carbon ceramic electrode – fabrication, characterization, and its electroanalytical performance</b> .....	66
Barbara Burnat <sup>*</sup> , Mariola Brycht, Andrzej Leniart, Sławomira Skrzypek .....	66
University of Lodz, Faculty of Chemistry, Department of Inorganic and Analytical Chemistry, 12 Tamka St., 91-403 Lodz, Poland .....	66
<b>OP28- Transport of Methylene Blue Across a Bulk Liquid Membrane using Pillar[5]arene as a Carrier Phase</b> .....	67
Didem AYDIN <sup>*</sup> , Mustafa ERSOZ, Ahmet Nuri KURSUNLU .....	67
Department of Chemistry, Selçuk University, Campus, 42031 Konya, Turkey .....	67
<b>OP29- Diatom biosilica for the chromatographic purposes</b> .....	68
Hussam AL Saoud <sup>a,c</sup> , Michał Szumski <sup>b</sup> , Myroslav Sprynskyy <sup>c</sup> , Michał Kawalec <sup>a</sup> , Bogusław Buszewski <sup>b,c</sup> .....	68
<sup>a</sup> Białystok University of Technology, Faculty of mechanical engineering, Department of Materials Engineering and Production, Wiejska 45C, 15-351, Białystok, Poland .....	68
<b>OP30- Quantitative structure-retention relationship for identification and quantification in lipidomics using chromatographic techniques</b> .....	69
Justyna Walczak-Skierska, Bogusław Buszewski .....	69
Department of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, .....	69

Interdisciplinary Centre for Modern Technologies, Nicolaus Copernicus University, Toruń, Poland .....	69
<b>OP31- Analytical approaches to investigate contaminants transported by microplastics on the Lebanese Mediterranean coast .....</b>	<b>70</b>
Rim ELSEBLANI <sup>1,2*</sup> , Milad FAKHRI <sup>2</sup> , Simon GODIN <sup>1</sup> , Javier JIMENEZ-LAMANA <sup>1</sup> , Ryszard LOBINSKI, Joanna SZPUNAR <sup>1</sup> .....	70
<sup>1</sup> Institute of Analytical and Physical Chemistry for the Environment and Materials (IPREM), UMR 5254, CNRS, University of Pau, Hélioparc, 2, av. Pr. Angot, 64053 Pau, France.....	70
<b>OP32- Phytochemical Screening, Antioxidative Activity, Chromatographic Fractionation, and HPLC Analysis of Phenolic Components in Blackberry (<i>Rubus fruticosus</i> L.) Extract .....</b>	<b>71</b>
Aryan Qader <sup>1,2*</sup> , Mehmet Yaman <sup>2</sup> .....	71
<sup>1</sup> Koya University, Faculty of Science and Health, Department of Chemistry, Erbil, Irak .....	71
<sup>2</sup> Firat University, Faculty of Science, Department of Chemistry, Elazig, Turkey .....	71
<b>Virtual Presentations .....</b>	<b>72</b>
<b>VP1- Development of Hydrogen Peroxide Biosensor Based on poly-L-Histidine Modified Glassy Carbon Electrode .....</b>	<b>72</b>
Funda Öztürk <sup>1</sup> , Elif Ulukan <sup>1</sup> , Ceren Kaçar Selvi <sup>2*</sup> .....	72
<sup>2</sup> Department of Chemistry, Faculty of Science, Ankara University, Ankara, Turkey .....	72

**INVITED SPEAKERS (IS)**

**IS1- Spatial Distribution and Pollution Assessment of Major and Trace Elements in Soil from the Republic of North Macedonia**

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Beside the research activities on heavy metal pollution in specific areas in the Republic of North Macedonia, information about soil quality on a national level is limited. Therefore, a geochemical investigation of soil across the whole country were performed, and this information deficit is being addressed with this project which aims to prepare the first Geochemical Atlas of the Republic of North Macedonia. In this atlas, the basic geochemical properties of soils are described, as revealed by a detailed large-scale survey across the country and analyses of the findings. It will provide a sound, well-structured baseline of soil geochemical properties relevant to sustainable land use and soil management to decision makers in the Republic of North Macedonia in order to reduce the environmental, agronomic and health-related pressures. The project includes soil sampling and analysis from 1,024 locations with a grid of 5×5 km distance between the sampling locations. Each sample represents a mixture of five subsamples to the depth of 0–30 cm. Areas which are known as polluted areas (surroundings of mines, metallurgical factories or larger towns) are investigated taking additional samples on a much denser sampling grid (1×1 km or 0.5×0.5 km). All samples are analysed for contents of about 60 elements. For this purpose, several analytical techniques are applied: inductively coupled plasma – atomic emission spectrometry (ICP-AES), atomic absorption spectrometry (AAS), inductively coupled plasma – mass spectrometry (ICP-MS) and neutron activation analysis (NAA). All data are statistically processed, and appropriate maps of distribution are prepared for 39 chemical elements. Based on a comparison of statistical parameters, spatial distribution of particular elements and results of cluster and factor analysis, four main geochemical associations were identified: 1. The association connected with the Neogene and Quaternary volcanism (Ba, Be, Ce, Hf, K, La, Rb, Th, Tl, U, and Zr); 2. Association of siderophile elements (Co, Cu, Fe, Mn, Sc, Ti, and V); 3. Association connected with ophiolites and Mesozoic ultrabasic magmatic rocks of Vardar zone (Cr and Ni) and 4. Chalcophile (sulphide) elements (As, Bi, Cd, Pb, Sb, Sn, and Zn). The regional distribution was prepared according to the eight statistical regions in Macedonia, distribution according to 15 most common geological formation and distribution according to 13 pedological units.

**Key words:** geochemistry, soil, major elements, trace elements, North Macedonia

## **IS2- Cleaning Validation of Manufacturing Equipment in the Pharmaceutical Industry**

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Cleaning validation is a process that demonstrates effectiveness and consistency in cleaning pharmaceutical manufacturing equipment. Validations of equipment cleaning procedures are often used in the pharmaceutical industries to prevent cross-contamination and mixing of drug products, therefore, is critical. Cleaning validation has been a topic of increasing interest in the pharmaceutical industry recently. The main purpose of validating a cleaning process is to ensure compliance with national and international standard regulations. The primary benefit of conducting such a validation study is the identification and correction of potential previously unsuspected problems that could compromise the safety, efficacy or quality of subsequent batches of drug product produced in the equipment. Contamination of a product line with significant residual active ingredient from a previous batch cannot be tolerated.

There are many analytical techniques that can be used in cleaning validation. The most important factor of the analytical method is the determination of the properties or parameters to be measured. If at least three cleaning verification runs have been completed and results meet acceptance criteria, cleaning procedures will be adequately and consistently demonstrated to remove chemical and detergent residues from equipment surfaces during operation.

The developed analytical method should be performed to characterize the active pharmaceutical ingredient, impurities, and unknown compounds that affect the continued series. Quantification and detection limits should be of appropriate value.

The rinse or wash solvent sample can be used to determine the carryover of residues over a large surface area. Direct surface sampling is designed to test all major equipment items.

In this study, validation analytical methods of vaccine, immunosuppressive and antineoplastic drug clearance will be discussed.

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### **IS3- An Overview of Electroanalytical Carbon Based Nanosensors: The Current and Future Prospect**

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A sensor is a device that detects and responds to some type of input from the physical environment. The sensor can be convert the measurement into a readable signal. For electroanalytical sensor technologies, nanomaterials are mostly used for creating a biosensor, biomarker or nanosensor. In recent years, sensor technology with its wide applications has become very popular in the biomedical and pharmaceutical area. Sensor studies provide an overview of some of the important and recent developments brought about by the application of carbon-based nanostructures to nanotechnology for both chemical and biological sensor development and their application in pharmaceutical and biomedical area.

Nanoscience is simply science and engineering carried out on the nanometer scale, that is, 10<sup>-9</sup> meters. Electrochemical nanosensors have recently found extensive applications in pharmaceutical and biomedical industries with some advantages such as lower detection limits, wider linear response range, sensitivity, good stability and reproducibility when compared with other sensors and techniques.

As the demand for smaller, faster, cheaper, and ultrasensitive qualification and quantification of samples rapidly increases, these methods provide a viable path toward the next generation of electrochemical sensors. They have recently found extensive applications in pharmaceutical and biomedical industries with some advantages such as lower detection limits, sensitivity, good stability and reproducibility when compared with other sensors and techniques. As the demand for smaller, faster, cheaper, and ultrasensitive quantification of samples rapidly increases, these methods provide a viable path toward the next generation of electrochemical sensors. Nowadays, different analytical methods are used in environmental, pharmaceutical, or clinical laboratories and a number of the commercial point-of-care devices work using nanosensors.

**IS4- Investigation of floral origin markers of honey by different instrumental methods**

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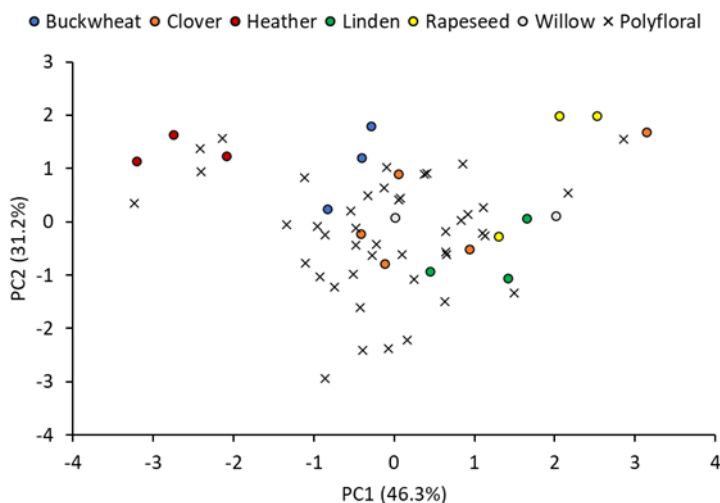
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Significance of honey as healthy food product is crucial and modern and efficient methods of honey authentication are needed. Various data processing methods and a combination of several instrumental methods have been increasingly used in food analysis. In this study, the chemical composition of monofloral buckwheat (*Fagopyrum esculentum*), clover (*Trifolium repens*), heather (*Calluna vulgaris*), linden (*Tilia cordata*), rapeseed (*Brassica napus*), willow (*Salix cinerea*) and polyfloral honey samples of Latvian origin have been investigated using several instrumental analysis methods [1]. Honey is relatively high-priced sweetener that floral origins can be from one or several plants. Demand of honey in EU is larger than EU can produce so large part is imported and consumers have rights to be informed about their product. In order to inform consumers different instrumental and chemometric methods are performed to do that.

78 honey samples were collected directly from the beekeepers in the territory of Latvia, declared as of natural origin and of specific monofloral varieties. The true botanical origin of the samples was further examined by melissopalynology analysis. The data from light stable isotope ratio mass spectrometry (IRMS), ultra-high performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS) and nuclear magnetic resonance (NMR) analysis methods were used in combination with multivariate analysis to characterize honey samples (Fig. 1).



**Fig. 1.** PCA of monofloral buckwheat, clover, heather, linden, rapeseed, willow and polyfloral honey samples obtained by IRMS.

This study proves the validity of the combination of multiple analytical methods, statistical data treatment and PCA to differentiate various natural monofloral honey classes, thus guaranteeing botanical authentication and the honey quality and origin.

**Keywords:** Honey; Isotope ratio mass spectrometry; <sup>1</sup>H-NMR; UHPLC-HRMS; Principal component analysis

**Reference:**

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**IS5- MS<sup>2</sup>/MS<sup>3</sup> data-dependent HPLC for untargeted analysis for mycosporine-like amino-acids and flavonoids in marine resources**

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The potential of marine biota as a source of active ingredients for cosmetics, pharmaceuticals, and nutraceuticals remains largely unexplored. During evolution, algae and marine plants have developed several physiological mechanisms for the synthesis of metabolites to cope with environmental pressure. The biological functions of these compounds often converge with active properties requested by industry. The characterization of the chemodiversity of these metabolites depends critically on the development of exploratory analytical methods for their high-throughput screening and characterization.

High-resolution multistage mass spectrometry and dedicated annotation strategies involving the fragment ion search and neutral loss monitoring will be discussed as a tool for untargeted screening for mycosporine-like amino-acids (MAAs) and flavonoids. The generic methodology developed has been based on the coupling of zwitterionic hydrophilic-interaction liquid chromatography (HILIC) with multistage electrospray mass spectrometry MS<sup>2</sup>/MS<sup>3</sup> using an Orbitrap analyzer and fragment ion search (FISH). Structural elucidation for unknown mycosporines has been implemented by the use of nano-electrospray ionization coupled with MS<sup>n</sup> after the on-line fraction collection and multi-step fragmentation.

The method was applied to study the MAA contents of several algae species extracted with 50% methanol. Candidate-MAAs were detected by mining eight characteristic fragment ions in their HILIC data-dependent MS<sup>2</sup> mass spectrum. Their exact masses were measured with 3 ppm mass accuracy and their structures were elucidated on the basis of the MS<sup>3</sup>/MS<sup>4</sup> mass spectra. The method developed was validated with a targeted analysis using an extract of *Gymnogongrus devoniensis* which confirmed the detection of 14 MAAs reported in literature. In addition, 23 previously unreported MAAs were detected and the structures could be assigned for seven of them. The developed method was applied to the analysis of four algae: *Gelidium sesquipedale*, *Halopytis incurva*, *Porphyra rosengurrtii* and *Cystoseira tamariscifolia* allowing the detection of MAAs, including some reported here for the first time.

Another separation mechanism, a reversed phase HPLC - electrospray Orbitrap ddMS<sup>2</sup>/MS<sup>3</sup> was developed for the non-targeted screening of flavonoids and their derivatives extracted from *Salicornia* plants. The optimization focused on the maximizing of the extraction yields of flavonoids in terms of quantity and structural diversity. An approach was developed that allowed the prediction of the structure of unknown compounds and the discrimination of their isomers by matching a database of all structural modifications known amongst the flavonoids.





## **IS6- Microfluidic label free technologies for applications in biofabrication and medicine**

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### **Abstract**

Micro- and nano-scale technologies can have a significant impact on medicine and biology in the areas of cell manipulation, biofabrication, diagnostics and monitoring. At the convergence of these new technologies and biology, we research for enabling solutions to real-world problems at the clinic. Emerging nano-scale and microfluidic technologies integrated with biology offer innovative possibilities for creating intelligent, mobile medical lab-chip devices that could transform biofabrication, diagnostics and monitoring, tissue engineering and regenerative medicine. We will present so interesting applications of microfluidic and 3-D assembly technologies in biofabrication and microrobotics. Some of our innovative microfluidic devices have been translated into FDA approved and CE marked products, *i.e.*, Zymōt, where they have been widely used by fertility clinics around the world to serve patients, leading to an estimated 10,000+ live births globally.

### **Bio**

**Dr. Utkan Demirci** is a professor with tenure at Stanford University School of Medicine, and serves as the interim director and division chief at the Canary Center for Cancer Early Detection in the Department of Radiology. His group is focused on the development of point-of-care technologies and creating microfluidic platforms for sorting rare cells and exosomes for infectious diseases and cancer. Dr. Demirci is a fellow-elect of the American Institute of Medical and Biological Engineering elected in 2017. He received his PhD from Stanford University in Electrical Engineering in 2005 as well as M.S. degrees in 2001 in Electrical Engineering, and in Management Science and Engineering in 2005. He has published over 200 peer-reviewed articles, 24 book chapters and editorials, four edited books, and over 25 patents pending or granted and serves as an editorial board member for various peer-reviewed journals. His group focuses on developing innovative point-of-care technologies and creating microfluidic platforms for cancer with broad applications to multiple diseases. Dr. Demirci's seminal work in microfluidics has led to the development of innovative platform technologies in medicine. His inventions have been licensed to numerous companies. He has co-founded several startups and serves as advisor to multiple companies including DxNow, LevitasBio, and Koek Biotech. He has translated several FDA approved and CE marked technologies.

**IS7- Fenhexamid Sensing On Planar Boron-Doped Diamond Electrode****Mariola Brycht<sup>1</sup>, Sławomira Skrzypek<sup>1\*</sup>**

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Fenhexamid (FH) is a hydroxyanilide fungicide used to prevent fungal diseases in strawberries, blueberries, and grapes. FH determination was carried out by square-wave voltammetry (SWV) on various carbon-based electrodes (glassy carbon electrode, graphite-and glassy carbon-based paste electrodes, graphite paste electrode modified with multi-walled carbon nanotubes) <sup>1</sup>. However, FH oxidation on these electrodes led to the formation of undesirable oxidation (by)products. Thus, labor- and time-consuming surface cleaning between each scan had to be performed.

Boron-doped diamond (BDD) electrode can be used as an alternative to the above-mentioned electrodes as its use for sensing purposes is widely recognized due to its unique properties including wide electrochemical potential window in aqueous solutions, low and stable background current, long-term stability, resistance to electrode fouling, and low sensitivity to dissolved oxygen <sup>2</sup>. BDD surface pretreatment has been identified as a crucial factor which determines performance of BDD electrodes. Hydrogen-termination (H-BDD) leads to non-polar, hydrophobic BDD surfaces with considerable surface conductivity and negative electron affinity, whereas oxygen-terminated BDD (O-BDD) electrodes possess hydrophilic, polar surfaces with decreased conductivity and positive electron affinity. It is also worth mentioning that untreated (as-received) BDD surface (both commercially available and lab-made) is mainly H-terminated (deposition of BDD films is carried out in hydrogen atmosphere), and it is initially free of oxygen-containing functionalities. However, H-BDD electrode surface ages, *i.e.* becomes gradually oxidized. One of the most common way how to perform re-hydrogenation of progressively oxidized H-BDD electrode surface or conversion from H- to O-termination is *in situ* electrochemical activation <sup>2</sup>.

In this work, manual mechanical polishing of a commercially available planar BDD electrode surface as well as different electrochemical activation programs, *i.e.* the application of highly positive (+2.4 V) and highly negative (−2.4 V) potentials for 300 s in a 0.1 mol L<sup>−1</sup> H<sub>2</sub>SO<sub>4</sub> were tested towards FH sensing in Britton-Robinson buffer of pH 2.0 by SWV. Anodic pretreatment of the BDD electrode surface resulted in a better electrochemical response of FH in comparison with cathodic pretreatment. In addition, anodic pretreatment was found to be more effective at re-activating a fouled electrode by FH oxidation (by)products when compared to tedious mechanical polishing.

Keywords: boron-doped diamond electrode, surface pretreatment; fenhexamid, square-wave voltammetry.

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## **IS8- Separation and spectral techniques in determination and characterization of colloids and biocolloids**

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A number of analytical and instrumental techniques are available to provide information on particle size distributions, including microscopic approaches, chromatography, centrifugation, laser scattering, filtration, spectroscopy and flow fractionation. Compared to the methods enumerated, FFF is a rapidly developing technique with increased resolution, sensitivity and selectivity. It is carried out in a specially designed channel. Generated laminar transfer of the mobile phase (Newtonian parabolic profile) taking into account the gradient of the external field. The difference in flow rate and the control of the field strength (gravitational, magnetic, electric, etc) allows the fractionation and separation of small and large molecules. The FFF is designed to separate a wide variety of analytes including nanoparticles, colloids, biocolloids and macromolecules based on diffusion coefficient and mass transfer. The mean size and size distribution of the analytes can vary considerably depending on the technique. For this reason, to obtain very detailed information, the FFF is combined with various detectors (inductively coupled plasma mass spectrometry (ICP-MS), ultraviolet and visible (UV-VIS), multi-angle laser light scattering (MALLS), dynamic light scattering (DLS). They can be connected to the FFF *on-line* or *off-line*.

In this study, an attempt was made to separate different sizes of bioactive silver composites using the FFF-UV-MALLS-DLS method. The particle size distribution and shapes were performed by transmission electron microscopy (TEM). Moreover, in order to illustrate the presence of silver isotopes, organic silver combinations and silver aggregates in the separated fraction, spectrometric ionization with laser desorption matrix assisted - time of flight (MALDI TOF MS) was performed.

**Key words:** pathogens, silver nanoparticles, colloids, nano-colloids, separation techniques, FFF, MALDI TOF MS.

### **Acknowledgement**

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**IS9- Analytical Profiles and Antioxidant Properties of Natural Products  
after Infusion with Herbs or Spices**

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During the last decades, the beneficial properties of herbs and spices have been re-discovered and consumption is increasing dramatically mainly because they are considered safe for consumption. It is now well-established that herbs and spices are excellent sources of antioxidants due to the high content of phenolic compounds and a plethora of scientific papers have been published and are published in Special Issues [1-3].

Recently, herbs and spices are added into many natural products such as wine, distillates, and olive oil in order to improve the taste and organoleptic properties in general. Analytical methods have proven that infusion of natural products with herbs or spices also improve the chemical properties such as stability and storage time of the final product which will be presented and discussed in this lecture.

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## IS10- Surface-enhanced Raman Scattering for Single Living Cell Analysis

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Surface-enhanced Raman spectroscopy (SERS), a very sensitive mode of Raman Spectroscopy, can provide label-free fingerprint molecular information from a molecular mixture. Thus, in theory, RS can be used to extract molecular level information from complex mixtures. As different from RS, a nanostructured noble metal surface, gold or silver, is used to enhance Raman scattering up to  $10^{11}$  in SERS<sup>3</sup>. Since its discovery during early 1970s, it has been applied for the detection of numerous analytes with biological and nonbiological origins. In recent years, it has also been investigated for its potential for living cell analysis. In this presentation, I covered our effort to utilize SERS for living cells with the hope that the technique as nanospectroscopic one can provide molecular information taking place in living cells.

## IS11- From Activated Carbon to MOF: Solid Phase Extraction

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Solid phase extraction is an important separation-preconcentration technique for the inorganic and organic species at traces levels from the environmental samples prior to their instrumental detection. Our study on this way was start 35 years ago by using activated carbon for these purposes and nowadays it will continue by using metal organic frameworks and its nanocomposites. In this presentation, this long story will be summarized. The application of these works for the environmental samples including natural waters and food samples were explained. Some clues for the future works will also discussed.

**Keywords:** Activated carbon, Amberlite XAD resins, MOF, nanocomposite, Preconcentration, Separation, Environment

**IS12- COVID-19 AND ANALYTICAL CHEMISTRY****Sezgin BAKIRDERE<sup>1,2\*</sup>**<sup>1</sup>Yıldız Technical University, Faculty of Art and Science, Department of Chemistry, 34210, Davutpasa, Esenler, Istanbul, Turkey<sup>2</sup>Turkish Academy of Sciences (TÜBA), Vedat Dalokay Street, No: 112, Çankaya, 06670, Ankara, Turkey  
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During the last two years, the world has encountered one of the worst worldwide crises known as COVID-19 pandemic [1]. COVID-19 has greatly spread and devastated the healthcare system in developing countries [2]. Different antiviral drugs including chloroquine, hydroxychloroquine, favipiravir have been proposed and used for the treatment of COVID-19 [3]. However, these drugs have also various negative impacts on human health. For example, cardiovascular diseases, diarrhea, retinopathy and gastrointestinal diseases can be observed as a result of the usage of these drugs [4]. In addition, drug accumulation in vital organs like liver, lungs and kidneys is another impact on human bodies [5]. Despite the usage of these drugs, there is no specific drug for the treatment of COVID 19. Scientific studies have been continued to test these drugs in terms of their safety and efficacy. For this reason, analytical methods for the accurate detection of drugs used in COVID 19 gain importance among scientific communities [6]. Sample preparation methods such as extraction, clean-up, preconcentration are used before chromatographic methods to minimize matrix effects and improve analyte recoveries from biological samples [7]. Microextraction methods are popular and simple way to preconcentrate and purify target analyte(s) presented in complex matrices [8]. In recent years, hyphenated methods generally liquid chromatography and gas chromatography coupled to mass spectrometry have been used for the separation and detection of antiviral drugs [9]. Further, isotope dilution method is another analytical strategy to achieve high accurate and precise analytical methods for the determination of drugs [10]. The combination of microextraction and isotope dilution methods gives point of view for scientists to detect drugs in biological samples.

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### IS13 Electrochemical sensors based on bis-neocuproine Cu(II) complex modified electrodes.

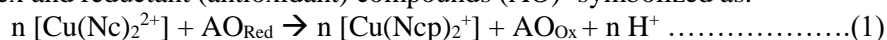
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The copper(II) neocuproine complex  $[\text{Cu}(\text{Nc})_2]^{2+}$  was first developed by the Apak research group in 2004 as the chromogenic oxidizing agent for the determination of total antioxidant capacity (TAC) of phenolic compounds.<sup>1</sup> They named this method as CUPRAC (cupric reducing antioxidant capacity) because the measurement of Cu(II) ion reducing ability of polyphenols was used. In the CUPRAC method, the reaction proceeds *via* electron transfer between  $[\text{Cu}(\text{Nc})_2]^{2+}$  complex and reductant (antioxidant) compounds (AO)<sup>1</sup> symbolized as:



After this reaction, the slightly blue colored  $[\text{Cu}(\text{Nc})_2]^{2+}$  is rapidly reduced to yellow-orange colored cuprous chelate:  $[\text{Cu}(\text{Nc})_2^+]$  absorbing light at 450-455 nm, while the reduced form of AO is oxidized (to  $\text{AO}_{\text{Ox}}$ ), and the liberated protons are neutralized in an ammonium acetate buffer<sup>1</sup>. Thus, CUPRAC reagent has been extensively used in optical sensor studies based on this mechanism. However, optical methods have some limitations such as the challenges in the application to colored and turbid media, involving time-consuming sample preparation steps, low spectral resolution, dependence of sensitivity on optical path length, inconvenience to miniaturization and portability. A robust method that may overcome the limitations of spectrophotometry is the electrochemical technique having high sensitivity and selectivity, rapidity, simplicity, ease of miniaturization, portability and applicability to colored or turbid samples, low cost, and flexible analysis in varying environments. However, few reports have been published on the electrochemical behavior of CUPRAC reagent and voltammetric determination of antioxidant capacity of phenolic compounds<sup>2</sup>. In our recent studies, chemically modified electrodes have been prepared using CUPRAC reagent for the electrochemical determination of various analytes. We have found that CUPRAC reagent-modified pencil graphite electrodes (PGE) and multi-walled carbon nanotube (MWCNT)-screen-printed carbon electrode (SPCE) had a well-formed reversible redox couple and exhibited excellent electrocatalytic activity towards the oxidation of some oxidizable compounds such as  $\text{H}_2\text{O}_2$ , hydrazine and hydroxylamine. In addition, these electrodes were integrated with flow injection analysis (FIA) and rapid, highly sensitive, selective, and low-cost FI amperometric methods were proposed for the determination of these compounds. In our last study, a FI amperometric TAC determination method was developed for the first time by using the CUPRAC reagent-modified electrode exploiting the anodic oxidation current of antioxidant-reduced cuprous-neocuproine. Although voltammetric TAC determination studies based on both the use of CUPRAC reagent in the supporting electrolyte media at bare electrodes and the use of chemically modified electrodes (CMEs) prepared with CUPRAC reagent were performed before, no study has yet been reported in which FIA was integrated with a CUPRAC reagent-modified electrode. Our results show that the analytical figures of merit of the FI amperometric sensor designed using  $[\text{Cu}(\text{Nc})_2]^{2+}/\text{Nf}@f\text{-MWCNT}/\text{GCE}$  were found to be better than those of voltammetric methods. In addition, the developed FI amperometric method using the CUPRAC reagent-modified  $f\text{-MWCNT}/\text{GCE}$  was responsive to a wide variety of polyphenolic compounds with high sensitivity, *i.e.* the micromolar-level LOD of conventional CUPRAC method was brought down to one-tenth micromolar levels.

**Keywords:** Chemically modified electrode; Copper(II)-neocuproine complex; Voltammetry; Amperometry.

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## IS14- The Interaction of Inflammation and Expression of Adhesion Molecules in Colorectal Cancer

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**Background:** Cyclooxygenase-2 (COX-2) is expressed in a variety of human colorectal cancer cells and can contribute to carcinogenesis. This study aimed to investigate the effect of diclofenac (DCF), a COX-2 inhibitor, on cell adhesion molecules and apoptosis in human colon adenocarcinoma cells.

**Materials and methods:** Levels of homing cell adhesion molecule (H-CAM, CD44), intercellular adhesion molecule-1 (ICAM-1, CD54), vascular cell adhesion molecule-1 (VCAM-1, CD106) and epithelial cell adhesion molecule (EpCAM, CD326) were evaluated in cancer cells overexpressing (HT29) or not expressing (HCT116) COX-2. Cell viability was determined by MTT assay, COX-2 protein levels and activity were assessed by immunofluorescence and fluorometric analysis, respectively. Endogenous levels of polyunsaturated fatty acids (PUFA) were measured by liquid chromatography tandem mass spectrometry (LC-MS/MS) while expression of cell adhesion molecules were analyzed by flow cytometry. Annexin V-FITC/propidium iodide-labelling and fluorometric caspase-3 activity measurements were carried out to determine apoptosis.

**Results:** Flow cytometry analysis revealed that the percentage of CD44 and ICAM-1 staining in HCT116 cells were significantly lower compared to HT29 cells. Phorbol 12-myristate 13-acetate (PMA)-induced COX-2 expression, increased CD44 and ICAM-1 levels in HT29 cells which were down regulated by diclofenac. Stimulation of COX-2 activity in HT29 cells via PMA significantly decreased diclofenac associated increase in PUFA levels. Treatment of both diclofenac and PMA significantly increased the number of apoptotic cells and caspase-3 activity in colon adenocarcinoma cells compared to control groups.

**Conclusions:** Diclofenac's effect to retard colorectal tumor growth and metastasis occurs in COX-2 over expressing colon cancer cells by increased apoptosis and decreased expression of CD44 and ICAM-1. Funding: This work was supported by a grant by Akdeniz University Research Foundation TYL-2020-5435. Keywords: cancer, COX-2, diclofenac.

**Funding:** This work was supported by a grant by Akdeniz University Research Foundation TYL-2020-5435.

**Keywords:** Cancer, COX-2, diclofenac.

## IS15- The Role of Hyphenated Mass Spectrometric Techniques in The Characterization of Biosimilar Therapeutic Protein Drug Products

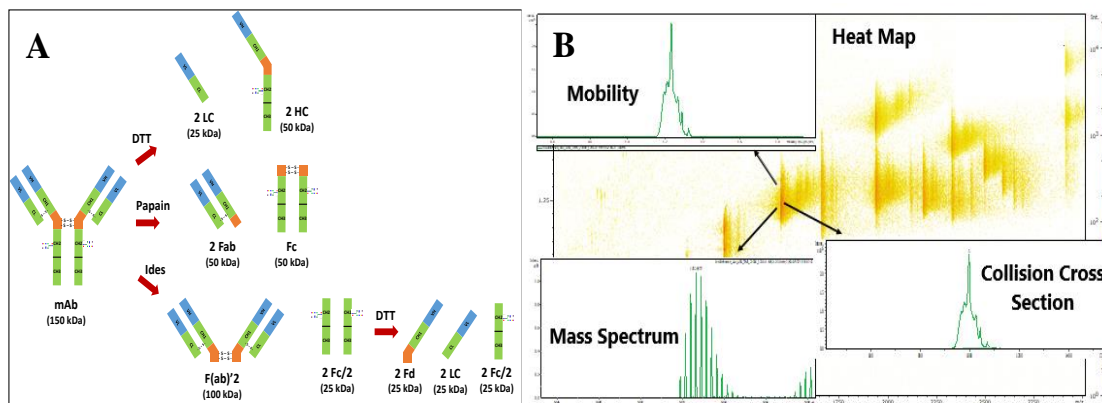
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Therapeutic monoclonal antibodies (mAb) is the largest class of biomolecule-based drugs in the pharmaceutical industry. The production and analysis of mAbs are quite difficult due to their complex chemical structures. They contain more than one variant of the same protein due to variable post-translational modifications. Therefore, the total evaluation of the structural features of mAbs is important for the development of mAb-based drugs. Various separation techniques, namely liquid chromatography and capillary electrophoresis coupled mass spectrometry and without separation technique, direct infusion mass spectrometric techniques were used for the detailed mAbs analysis in their intact form and after reduction process using various enzymes and reducing agents. In addition, conformational analyzes of mAbs are carried out using ion mobility technique combined mass spectrometry (IM-MS) based on the separation of ions in the gas phase according to their collision cross-section (CCS) values as a size parameter<sup>1</sup>. In this way, fast and straightforward configured systems were applied to perform full mAb analyses. Dithiothreitol (DTT) denaturing agent, papain and IdeS enzymes were used to define the monoclonal antibody (mAb) fragments using various hyphenated mass spectrometric techniques. All mAb fragments obtained by the treatment of DTT, papain, and Ides are given in Figure 1. Bevacizumab therapeutic drug was used as model drug and hyphenated mass spectrometric techniques were used for analysis. It was examined which technique was more practical and give more detailed structural information for therapeutic proteins. It could be noted that IM-MS and direct infusion-MS techniques were yielded detailed and fast information for the analysis of biosimilar tests of mAbs.



**Figure 1.** Formation of the monoclonal Antibody fragments after the treatment by reducing agent and the digestion of papain and IdeS enzymes (A). IM-MS outputs of differently charged ions of Intact Monoclonal Antibody (B).

**Keywords:** Monoclonal Antibody, Trapped Ion Mobility, Mass Spectrometry, Biotechnological Drug, Conformational Analysis

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### Acknowledgment

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**IS16- Beyond mechanochemical transformation– latest advancements of recycling of sodium borate into sodium borohydride**

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**Abstract**

Ball milling or mechanochemical transformation of reactants into products has dominated the recycling process of sodium borate ( $\text{NaBO}_2 \cdot x\text{H}_2\text{O}$ ) into sodium borohydride ( $\text{NaBH}_4$ ). There is a need for the recycling of sodium borate ( $\text{NaBO}_2 \cdot x\text{H}_2\text{O}$ ) into sodium borohydride ( $\text{NaBH}_4$ ), as sodium borohydride is the solid state material for the storage and transport of hydrogen (in the form of the borohydride  $\text{BH}_4^-$  anion) which precludes the requirement of heavy high pressurized metal cylinders or pipelines for the storage and transportation of hydrogen gas. Several works have reported the mechanochemical transformation of borate into borohydride using Mg metal, hydrogen gas, magnesium hydride with high efficiency conversion. However there are several drawbacks of mechanochemical methods for this type of recycling, and newer methods such as electrochemical reduction should be explored as electrosynthesis is a proven method of synthesis of specialized chemicals since more than 150 years ago. Thus this talk focuses on the challenges associated with the electrochemical reduction of sodium borate into sodium borohydride.

## **IS17- New Developments in Lateral Flow Assays for SARS-CoV-2 Diagnosis**

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A lateral flow assay (LFA) is composed of a chromatographic system (separation of components of a mixture on the basis of differences in their movement through reaction membrane) and immunochemical reaction (between antibody-antigen, nucleic acid-target analyte). It is based on the movement of sample across the membrane via capillary force. The standard lateral flow assay has four parts: a sample pad, the area on which sample is dropped; conjugate pad, on which labelled tags combined with biorecognition elements; reaction membrane (usually nitrocellulose membrane), on which location containing test line and control line for target DNA-probe DNA hybridization or antigen-antibody interaction; and absorbent pad, which reserves waste [1].

A new severe acute respiratory syndrome (SARS)-like coronavirus (SARS-CoV-2) caused a global coronavirus disease 2019 (COVID-19) which became a pandemic. This is very important to know how SARS-CoV-2 enters human cells in terms of the lighting its mystery and curbing its spread. In this frame, it was understood that the surface spike protein of the virus was playing leading role in entry into the cells. Consequently, most of LFA is targeted to antibodies for the surface spike protein of the virus. Beside the other important target for this sense was the antibodies for N-capsid protein of the viruses. Moreover these antigens were also targeted themselves in many antigen based LFA systems. In this study, LFA systems for SARS-CoV-2 developed for both commercially and scientifically since the beginning of the global pandemic were discussed.

**Keywords:** Lateral Flow Assay, LFA, SARS-CoV-2, COVID-19

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## IS18- Conducting Polymers in Sensor Design

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During this presentation electrochemical [1], chemical [2] and biochemical [3-6] synthesis of conducting polymers (CPs). CPs-based structures in the design of various types of electrochemical biosensors will be outlined [6]. Applicability of CPs development of glucose biosensors based on glucose oxidase (GOx) will be discussed. Enzymatic reaction catalysed by GOx can be applied for the initiation of polymerization of some conducting polymers. We have shown that this method is suitable for the synthesis of polypyrrole [3], polyaniline [4], polythiophene [5] and some other conducting polymer based layers and nanoparticles. We also have demonstrated that formed nanostructures and nanoparticles shows good biocompatibility with living cells and when they were injected in mice. We have demonstrated that during such kind of synthesis of nanoparticles and/or nanostructured layers the enzymes becomes entrapped within CP-layer. We have shown that redox processes that are part of metabolism of living cells can be applied for the synthesis of conducting polymer – polypyrrole (Ppy), and formed Ppy nanoparticles could be entrapped within cell wall of yeast cells [6]. CPs-based nanoparticles and nanostructured layers are suitable for the design of bioelectrochemical devices. Applicability of CPs in the design of molecularly imprinted polymer based electrochemical sensors will be discussed.

**Acknowledgement:** Support by Ukrainian-Lithuanian project No P-LU-18-53 is acknowledged.

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## ORAL PRESENTATIONS (OP)

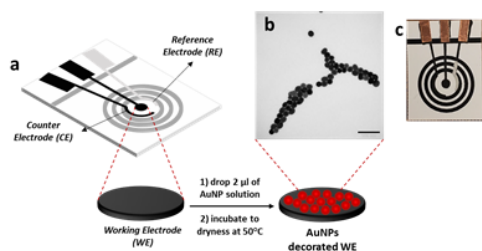
## OP1- Gold Nanoparticle Decorated Paper Electrode Assemblies for Sensor Applications

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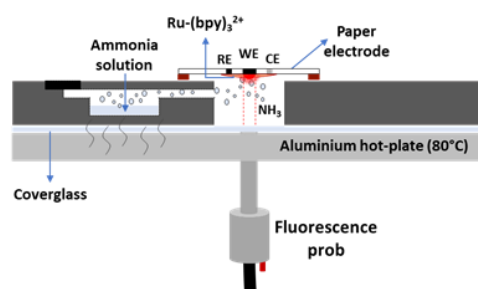
In recent years, paper-based sensor systems have been started over again to be used because of their unique properties such as low cost, biocompatible, disposable, flexible, easily prepared, and simple to use. In the literature, several applications of paper-based electrochemical sensors were reported for chemical industry fields, food safety, environmental, and clinical analysis<sup>1</sup>. Furthermore, researchers focus on working with paper-based sensors as point-of-care (POC) diagnostics because of their eco-friendly behavior. Basically, a paper-based electrochemical sensor consists of a paper membrane as a substrate material, an electrode area, and two or three electrodes. In general, the electrode area is created by forming hydrophobic barriers on the paper substrate surface. Then, the two/three electrodes assembly can be fabricated by using various techniques.

Recently, different types of nanomaterials were reported in the literature to increase the surface area and enhance the conductivity of electrochemical sensors. Gold nanoparticles (AuNPs) are one of the most widely used nanomaterials with unique characteristics such as high surface energy and high surface area. Therefore, AuNPs can be used to serve an efficient conducting interface and unique catalytic activity for the construction of robust and sensitive electrochemical sensors<sup>2</sup>. We have fabricated the paper electrodes and modified them with nanomaterials. In our works, we have utilized the gold nanoparticles which were formed by using both chemical and/or electrochemical synthesis mechanisms. Subsequently, the modified paper electrodes have been used for various applications. Here, we focused on using the gold nanoparticle modified paper electrodes for the determination of ammonia via an electrochemiluminescence technique.

**Keywords:** paper electrode, gold nanoparticle, sensor



**Figure 1.** a) The fabrication procedure of the AuNP decorated PE, b) TEM image of AuNPs, c) the photograph of PE



**Figure 2.** The schematic illustration of the detection of ammonia using the paper-based ECL gas sensor

## Acknowledgment

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## OP2- Amperometric Bisphenol A Biosensor Based on Carbon Quantum Dots, Ionic Liquid and Gold Nanoparticles

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Bisphenol A (BPA), an organic monomer, is widely used for the production of plastic food packaging and containers. Development of simple and accurate analytical techniques for BPA determination is highly desirable in environmental analysis, health care and food safety. Electrochemical techniques have been widely used for the determination of BPA due to their good sensitivity, simplicity and high selectivity <sup>1,2</sup>.

In this study, an amperometric biosensor was developed for BPA determination. For the construction of the BPA biosensor, screen printed carbon electrode (SPCE) was modified with carbon quantum dots (CQDs), ionic liquid (IL) and gold nanoparticles (AuNPs). The AuNP/CQD-IL/SPCE was further modified with tyrosinase (TyOx) enzyme for the construction of the biosensor. Central composite design approach was used to optimize the electrode surface composition for three critical variables: amounts of CQDs and IL and deposition time of AuNPs. The results of the central composite design study showed that the highest amperometric response was obtained with the electrode fabricated with 3.0  $\mu$ L IL and 5.1  $\mu$ L CQDs. Optimum deposition time of the AuNPs was determined as 284 s. After the optimization of electrode surface composition and other experimental parameters, analytical performance characteristics of the TyOx/AuNPs/CQDs-IL/SPCE biosensor was investigated by chronoamperometry measurements and the biosensor exhibited a linear response to BPA in the range of 0.02–4.0  $\mu$ M. Other performance parameters such as response time, sensitivity, detection limit and stability were also addressed. The combination of CQDs, IL and AuNPs improved the analytical performance and the presented biosensor showed short response time, good sensitivity, low detection limit, high sensitivity and good stability towards BPA. Thus, our future study will be focused on the analytical application of the TyOx/AuNPs/CQDs-IL/SPCE biosensor for BPA determination in real samples.

**Keywords:** Bisphenol A, Biosensor, Carbon Quantum Dot, Ionic liquid, Gold nanoparticle

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### OP3 Label-Free Determination of Extracellular Vesicles onto Gold Screen Printed Electrodes by Electrochemical Impedance Spectroscopy

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#### Abstract

Extracellular vesicles (EVs) are heterogeneous particles released by healthy or cancerous mammalian cells. EVs in the size range of 40 nm to 200 nm are called exosomes. Exosomes originated due to the inward budding of endosomal membranes and were released in the plasma membrane and extracellular environment<sup>1</sup>. Recognition and rapid analysis of exosomes are pioneering stages in clinical research. Nucleic acid, lipid recognition, and specific protein information of exosomes are used to improve the efficiency, sensitivity, and reliability of exosome assays<sup>2</sup>. We indicate a biosensor fabrication for exosome detection in lower concentrations regarding their biological recognition strategies. Implementation of the biosensor consists of two steps: i) Functionalization of screen-printed electrodes (SPEs) with tetraspanin anti-CD63 antibodies, ii) Capturing exosomes by the electrode surface. Label-free detection of exosomes was succeeded with Bode and Nyquist diagrams to be different representations of electrochemical impedance spectroscopy (EIS). The proposed biosensor as a sensitive and reliable impedimetric method for detecting exosomes was established in a working range of  $3.7 \times 10^3$  to  $3.7 \times 10^8$  particles mL<sup>-1</sup> with a detection limit of  $2.0 \times 10^3$  particles mL<sup>-1</sup> which have the potential to be a suitable surface for clinical analysis.

**Keywords:** Exosome; Label-free detection; Electrochemical impedance spectroscopy

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#### Acknowledgment

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**OP4- Preparing, characterization and application of glassy carbon electrode modified with carbon nanotubes and palladium for electrooxidation of formaldehyde**

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Palladium, due to its catalytic properties and ability to absorb hydrogen, is used to build fuel cells operating on the basis of oxidation of simple organic compounds such as: methanol or formaldehyde. The type of electrode and the surface development, microstructure and size and dispersion of the catalyst on the electrode strongly influence the performance of such an electrode and its efficiency. The glassy carbon electrode, which has unique properties such as high electrical and thermal conductivity, hardness, resistance to high temperatures and chemicals, stability over a wide range of polarization potentials, and an easily renewable surface, can be a carrier for catalysts. In order to improve the reactivity of the electrode, the surface of the support can be further modified with other materials, for example graphene, carbon nanotubes or metallic nanoparticles.

The aim of this work was to prepare modified glassy carbon (GC) electrodes with multi-walled carbon nanotubes (MWCNTs) and electrochemically generated palladium nanoparticles (Pd), to characterize them in terms of surface morphology and to study the electrocatalytic properties of these electrodes in the electrooxidation reaction of formaldehyde.

The preparation of GC/MWCNTs-Pd electrodes consisted of depositing multi-walled carbon nanotubes on the glassy carbon surface and electrochemically generating palladium nanoparticles at a potential of  $E = 0.1$  V vs. NEK from a solution of 0.1 M HCl + 0.01 M  $\text{PdCl}_4(\text{NH}_4)_2$  with programmed charges (-0.005 C, -0.020 C, -0.050 C, and -0.100 C). GC/MWCNTs-Pd electrodes with different contents of palladium nanoparticles were characterized for morphology by atomic force microscopy (AFM) and scanning electron microscopy with elemental microanalysis (SEM-EDS). Whereas, the evaluation of electrocatalytic activity in formaldehyde oxidation reaction was carried out in 0.1 M  $\text{HClO}_4$  solution containing 0.1 M formaldehyde.

Electrochemical generation of Pd catalyst yielded palladium nanoparticles in four size ranges: 10-30 nm, 20-40 nm, 50-60 nm and 70-90 nm. From the measured results, it was observed that the amount and size of the obtained nanoparticles of deposited palladium on the electrode depends on the charge applied during the electrodeposition. It was found that as the amount of palladium increased, the electrocatalytic activity of the formaldehyde oxidation reaction increased

**Keywords:** palladium, multi-walled carbon nanotubes, glassy carbon, electrodeposition, cyclic voltammetry, atomic force microscope, scanning electron microscopy, formaldehyde oxidation

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**OP5- Fast Fourier transform electrochemical impedance spectroscopy for monitoring surface modification in the process of biosensor formation**

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Electrochemical impedance spectroscopy (EIS) can provide a set of useful information enabling physical modelling of an electrochemical interface in order to extract relevant physico-chemical parameters, which determine the properties of the electrode-electrolyte boundary. However, conventional EIS technique is relatively slow and, therefore, it is hardly applicable for investigation of very dynamic biological samples. Advances in the development of electrochemistry enabled the development of fast Fourier transform electrochemical impedance spectroscopy (FFT-EIS).

FFT-EIS technique is one of the fastest and the most informative electrochemical techniques, which can provide EIS spectra in very short period of time. Therefore, FFT-EIS is very useful for the investigation of very dynamic electrochemical systems and for monitoring formation of various layers on different surfaces in real time.

FFT impedance spectrometer was constructed by Professor Georgi Popkirov who developed and advanced FFT EIS based technique together with prof. R. N. Schindler<sup>1</sup> in 1990. In this technique, system is perturbed not by the consequently applied sine waves of different frequencies, but by the superposition of 30-50 sine waves with properly chosen frequencies. If frequency range from 1.5 Hz to 50 kHz is applied, then the measurement time is just 0.67 s. It is a big advantage of this technique to acquire EIS spectra many times faster in comparison with conventional EIS based techniques.

A significant advantage of FFT-EIS spectrometer is that it is easily controlled. All “bad” data points can be controlled/avoided. All information we need is presented on the screen and everyone who measures impedance spectra can see “good” or “bad” measurement is performed.

The advantages of FFT-EIS equipment enable monitoring various dynamic processes in real time, such as (i) evolution of surface modification during the process of phospholipid membrane formation; (ii) degradation of bioselective layer of biosensor. Recently it was demonstrated that application of FFT-EIS in scanning electrochemical microscopy (SECM) opens a new avenue in fast localized electrochemical impedance measurements <sup>2</sup>.

**Keywords:** Fast Fourier transform electrochemical impedance; phospholipid membrane; biosensor.

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## OP6- Tethered Bilayer Lipid Membrane Formation on Metal Oxides

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Tethered bilayer lipid membranes (tBLMs) formed on gold substrates have been applied as biosensors of bacterial toxins<sup>1</sup>. However, the opacity, availability and expensive preparation of thin film gold substrates limit the application as biosensors. Therefore Au can be substituted for transparent and cheaper metal oxide surfaces, such as tin oxides.

In this study, fluorine doped tin oxide was modified with phosphatidylcholine and cholesterol tBLM, that was achieved by formation of synthesised molecular anchor self-assembled monolayer (SAM). Electrochemical impedance spectroscopy (EIS) measurements showed the existence of water reservoir between solid support and tBLM. It ensures reconstitution of transmembrane proteins inside the tBLM. Bacterial toxin  $\alpha$ -hemolysin (from *Staphylococcus aureus*) and toxin melittin (from bee venom) disrupted the tBLM in concentration dependant manner. Therefore, the described tBLM can be used as a biosensor for the detection of bacterial or venom toxins.

**Keywords:** tethered bilayer lipid membrane, self-assembled monolayer, impedance spectroscopy, toxin detection

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**OP7- Gold nanostructure and Prussian blue nanocomposite for the development of amperometric glucose biosensor****Laura Sakalauskiene, B. Brasiunas, A. Ramanaviciene**

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With the growing number of people with diabetes, a lot of attention has been brought to monitoring blood glucose levels. It is the most effective way to prevent and diagnose diabetes. Therefore, sensitive, selective, stable, and easy-to-use biosensors with miniaturization capability are needed. Electrochemical enzymatic biosensors have highlighted outstanding advantages, including low limit of detection (LOD), high sensitivity, and good selectivity [1]. Precious metal nanoparticles have been widely used in recent years due to their unique and special properties. The electrochemically active  $H_2O_2$  produced during enzymatic reaction of glucose oxidase (GOx) with glucose might be registered using different electrochemical methods. Prussian blue (PB) has been extensively explored for glucose detection due to its electrocatalytic property for  $H_2O_2$  reduction. Electrochemical glucose biosensors manufactured using PB have short response times, high electrocatalytic activity, and sensitivity to glucose. The nanocomposite of PB and gold nanostructures (PB/AuNS) present on the graphite rod (GR) electrode provides a few merits for design of glucose biosensor. Firstly, the stability of PB on the GR electrode was improved by forming PB/AuNS nanocomposite. Secondly, the rapid transfer of electrons due to satisfactory AuNS conductivity was achieved. Finally, a favorable microenvironment for retaining biological activity of immobilized enzymes was established [2].

In this study a glucose biosensor based on the GR electrode modified with AuNS and PB nanocomposite was developed (PB/AuNS/GR). PB, as an electrocatalyst for  $H_2O_2$ , was electrochemically synthesized on a GR electrode which in turn was electrochemically premodified with AuNS. In the next step, GOx was adsorbed on PB/AuNS/GR surface and cross-linked with glutare aldehyde vapour. All electrochemical measurements were performed with a computerized potentiostat/galvanostat PGSTAT 30/Autolab (EcoChemie, The Netherlands) with GPES 4.9 using a three-electrode system. The evaluation of analytical parameters of the developed electrochemical glucose biosensor was performed.

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## OP8- Dopamine Sensing on Novel Porous Boron-doped Diamond Electrodes With Various Thickness

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Novel porous boron-doped diamond (BDD<sub>porous</sub>)-based materials have attracted lots of research interest due to their enhanced detection ability, compared to conventional planar BDD films <sup>1</sup>. However, one of the most crucial factors determining final characteristics of BDD<sub>porous</sub> electrodes is the number of deposited porous layers <sup>2</sup>.

This contribution reports on comprehensive characterization and electroanalytical performance of three BDD<sub>porous</sub> electrodes of different thickness given by a number of deposited layers (2, 3 and 5) prepared using microwave plasma-enhanced chemical vapor deposition on SiO<sub>2</sub> nanofiber-based scaffolds (B/C of 4000 ppm). Scanning electron micrographs showed homogenous coverage of the 3D template by polycrystalline diamond for all studied samples. Raman spectroscopy was used to give a qualitative indication of the purity, and increased content of sp<sup>2</sup> carbon in electrodes with more layers was evaluated. Cyclic voltammetric experiments with various redox probes were performed to assess electrochemical behavior of prepared BDD<sub>porous</sub> electrodes, and it was found that with increasing number of porous layers, enhanced rates of electron transfer kinetics along with the larger effective surface (from 5.04 mm<sup>2</sup> to 7.72 mm<sup>2</sup>) areas were observed. The effect of number of layers on sensing properties towards neurotransmitter dopamine in phosphate buffer medium (pH 7.4) was investigated. A significant enhancement in analytical performance was recognized with increasing layer number using square-wave voltammetry (SWV): the highest sensitivity of 574.1  $\mu\text{A } \mu\text{mol}^{-1} \text{ L}$  was achieved on a BDD<sub>porous</sub> electrode with five layers and dropped to 35.9  $\mu\text{A } \mu\text{mol}^{-1} \text{ L}$  when the number of layers decreased to two. Consequently, the lowest detection limit of 200.0 nmol L<sup>-1</sup> was obtained on a BDD<sub>porous</sub> electrode with five layers. Moreover, the applicability of the developed SWV procedure was verified in complex environment, namely in the multicomponent Neurobasal medium (containing 37 compounds, including 17 amino acids, 8 vitamins, 8 inorganic salts, inositol, D-glucose, sodium pyruvate and HEPES; at least 8 of these components were identified as electroactive), and the results confirmed that the proposed SWV procedure do not suffer from any substantial matrix effects, and hence dopamine can be accurately quantified on BDD<sub>porous</sub> electrode even in a very complex Neurobasal medium.

Evidently, the different number of deposited porous layers influences structural, electrochemical, and sensing properties of fabricated BDD<sub>porous</sub> electrodes.

**Keywords:** Porous boron-doped diamond; number of layers; dopamine sensing; electroanalysis.

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**OP9- Metallic elements of residential dust as a measure of indoor air quality****Agnese Osīte\*, Māris Bērtiņš, Arturs Vīksna, Gunita Celma and Oto Poišs**

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In addition to the research and the monitoring of ambient air, already for decades, scientists have been paying attention to indoor air quality in schools and offices and demonstrating that human exposure to indoor contaminants is no less important and an emerging area of health concern, especially because people spend up to 90% of their time indoors<sup>1</sup>. The growing evidence on hazardousness of contaminated air and the respective health outcomes then led the World Health Organization (WHO) to the formulation of various priority indoor air pollutants, including particulate matter (PM). Since the Covid-19 pandemic, people around the world have been forced to stay in their living dwellings. That is why it would be extremely important to find out residential indoor air quality, identifying the total concentrations of metallic elements, considering the toxicity of many metals, their persistence, and lack of degradability, and their potential to accumulate in tissues and internal organs of the human body<sup>2</sup>.

During current research, 30 residential dust samples were collected from the apartments in Riga city and rural areas. Residential indoor dust is compounded of particulate matter derived from a range of indoor and outdoor sources, which can act as both a sink and transport medium for persistent contaminants such as toxic metals. A sampling of residential dust was conducted mostly in living rooms as these were the most representative of the space where people spent their time while at home; in 25% of the inspected homes sampling of the dust was conducted in bedrooms. For the dust sampling, the household vacuum cleaner was used, an unused dust container was applied for each sample. After manual removal of the pet and human hair and larger refuse, an amount of 0.5g of each dust sample were subjected to conventional microwave-assisted acid digestion performed with the Milestone Start E, microwave power of 1500W. Obtained sample extracts were measured by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 8900 Triple Quadrupole). Sample batches consisted of 10 field samples and a reagent blank after which stability check of the ICP-MS system was performed using two quality control samples (ICP-MS multi-element standard solutions). For the calibration graph, six different standard solutions in concentrations ranging from 0.1 to 50.0 µg/L were prepared from stock standard solutions. The total concentration of more than 30 metallic elements including Cr, Cu, Pb, Ba, Sn, Sb, V, Ni, etc. was determined.

Obtained results might be useful in different types of further investigations. Concentrations of measured chemicals in residential dust could be useful surrogates for indoor chemical exposures, which can also be compared and linked to ambient air pollutants. Since chemicals in dust can enter the body via inhalation, or inadvertent ingestion after hand-to-mouth contact, notably infants and children typically engage in more hand-to-mouth activity than other age groups, or via direct absorption through the skin, such measures should allow epidemiologists to observe true associations between exposure and disease more effectively.

**Keywords:** Indoor air, residential dust, metallic elements

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**OP10- Investigation of document forgery by XPS via chemical imaging:  
Analysis of blue commercial ballpoint pen inks**

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Ink analysis is an important tool in forensic examination of forged documents. It provides significant information which helps in determination of ink age, backdating, fraud, and forgery. Ink mismatch is a key indicator of forgery in questioned documents. A document containing text written with more than one inks can be a forged document. Forensic specialists explore the ink type in an addressed report, if it consists of more than one kind of inks; it depicts that some manipulations have been made in that document. Ink discrimination techniques can broadly be destructive or non-destructive techniques. whereas the former is more promising. X-Ray Photoelectron Spectroscopy (XPS) is also a very attractive and novel technique for ink analysis since it can discriminate the chemical differences easily and rapidly. In addition, it is non-destructive to the evidence and does not require any sample preparation [3]. The modern non-destructive ink examination methods use multispectral image analysis-that is chemical imaging which has proved its effectiveness in many areas.

This study aims to demonstrate the great potential of the XPS by providing an overview of its application to forensic examinations of ink evidence. Moreover, it presents the chemistry of problematic intersecting lines with the same and different pens. In the present study, 10 blue ballpoint pens were analyzed by XPS at 5 different points. Then, chemical mapping was realized on an area of  $\sim 10 \text{ mm}^2$  for crossed inks. Herein, monochromated X-ray ( $\text{Al K}\alpha$ ) spot size was fixed at  $100 \text{ }\mu\text{m}$ . XPS mapping resulted in the acquisition of spectra at each pixel, in an array of  $30 \times 35$  pixels with a step size of  $100 \text{ }\mu\text{m}$ . Their chemistry revealed that each blue pen differed from each other. Moreover, chemical imaging feature of XPS using Principal Component Analysis (PCA) showed that which line was drawn first even if the type and color of the pens were alike. Consequently, developing more informative and effective tools for questioned document examinations become more necessary. It was proven that XPS was a very novel technique and there will be great demand on this research area in future.

**Keywords:** Document forgery, ink analysis, XPS, chemical imaging, PCA

**OP11- The Application of Vibrational Spectroscopy and Chemometrics in the Discrimination of Pig Derivatives in Halal Food**

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Issues related to halal products, including adulteration and authentication, are emerging in Muslim communities. It is necessary to assure being halal of the products such as foods, pharmaceuticals, and cosmetics for the Muslim communities. Pig meat and byproducts of pig (lard and porcine gelatin) are usually cheaper than those of sheep and cattle. Therefore, it has been reported that pig meat and pig derivatives have been mixed in many foods and goods and it is important to detect those products. Traditional methods, including UV-vis spectroscopy, nuclear magnetic resonance spectroscopy (NMR), chromatography, histological tests, electrophoretic separation of proteins, immunological procedures, DNA based techniques, are the main ones to authenticate halal food. Nonetheless, requiring expensive equipment, well-trained analyst, tedious sample preparation steps and extended sample preparation time make them less appealing for routine analysis. On the other hand, vibrational spectroscopy sensors combined with chemometrics are good alternatives to authenticate halal products by being rapid, easy-to-use, sensitive, robust, relatively cheap, and analyzing various sample structures simultaneously. Additionally, the advances in micro-electro-mechanical systems, semi-conductor detectors, optical fibers, solid-state lasers, and microprocessors led to the production of miniaturized sensors that can be used in slaughterhouses, customs, and markets.

Key words: Vibrational spectroscopy; Chemometrics; Halal food; Pig meat and derivatives; Miniaturization

## OP12- Strontium Isotopes and Chemical Analysis In The Investigation of Iron Age Glass Beads Production

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The glass beads, reflecting the Iron Age, from Urartian Kingdom founded in the capital Tushpa (Van) on the eastern shore of Lake Van have great importance for the determination of the glassmaking technologies and glass trading of the period. During the excavation works at the Necropolis of Karagündüz located at 34 km away from Tushpa, some iron age glass beads have been unearthed. The aim of this study is to identify the recipe used to make these glass beads, based on their chemical and isotopic composition by using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Scanning Electron Microscopy/Energy Dispersive X-Ray Spectroscopy (SEM-EDS). The chemical fingerprint of the raw materials can be related to geographical locations and some of the chemical characteristics of the raw materials are present in the final product unchanged. In this study, we, therefore, investigated the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of the possible sand raw material from the Lake Van, and the Sr isotopic signature of the glass beads. The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios obtained are higher than the previous studies have been made with using Iron Age glass beads. This results support the fact that the use of igneous and metamorphic rocks around the Lake Van as a sand raw material increase the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios. According to the elemental and isotopic analysis results, it can be said that there was a local glass production and even recycled glass have made.

**Keywords:** Glass Provenance, isotopic ratio, ICP-MS, SEM-EDS

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**OP13- Deep eutectic solvent-liquid-liquid microextraction prior to smartphone digital image colorimetry for the determination of cobalt in milk and dairy products**

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**Abstract**

Deep eutectic solvent-liquid-liquid microextraction (DES-LLME)<sup>1</sup> was combined with smartphone digital image colorimetry (SDIC)<sup>2</sup> for the determination of cobalt as its chelate with 1-(2-pyridylazo)-2-naphthol. The sample extract was placed in a quartz cuvette inside a laboratory-designed colorimetric box from which images of the extract were captured and then split into their red-green-blue channels. The intensity of the red channel, found to give the highest intensity, was used to calculate the concentration of cobalt. Optimum SDIC performance were obtained at a distance of 8.0 cm between the cuvette and the detection camera with a 60.0% brightness of the light source emitting light at a wavelength of 560 nm and using 1600 px<sup>2</sup> as the region of interest. Optimum DES-LLME conditions were found as follows: complexation pH of 5.0, volume of DES (Choline chloride/ phenol, 1:4) of 300 µL containing 900 µL of tetrahydrofuran, 3.0 min complexation and 2.0 min extraction time. The limits of detection and quantitation were found as 22.0 and 73.3 ng g<sup>-1</sup>, respectively, while the coefficient of determination (R<sup>2</sup>) was higher than 0.9965 and relative standard deviation below 6.5%. The proposed method was applied for the determination of cobalt in milk and dairy samples with percentage relative recoveries between 84.4 and 106.4%. The results pointed out that DES-LLME-SDIC can serve as a good alternative to conventional sophisticated instruments for the determination of cobalt in milk and dairy products due to its simplicity, low cost, greenness and potential of continuous improvement with the rapid advancement in smartphone technology and image processing programs.

**Keywords:** Cobalt, deep eutectic solvent, dairy samples, liquid-liquid microextraction, smartphone

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## OP14- Switchable-hydrophilicity solvent liquid-liquid microextraction combined with smartphone digital image colorimetry for the determination of palladium in catalytic converters

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### Abstract

Switchable-hydrophilicity solvent liquid-liquid microextraction (SHS-LLME)<sup>1</sup> was coupled with smartphone digital image colorimetry (SDIC)<sup>2</sup> for the determination of palladium in catalytic converters as its metal chelate with *N,N*-diethyl-*N'*-benzoylthiourea<sup>3</sup>. Images of the colored extract were captured in a homemade colorimetric box, which were then split into their red-green-blue channels. The blue channel, giving the highest intensity, was used to calculate the concentration of palladium. Optimum SDIC conditions were achieved with a distance of 7.0 cm between the sample solution and the detection camera, a region of interest of 145 px<sup>2</sup> at a maximum absorption wavelength of 480 nm and 30.0% brightness of the monochromatic light source. Optimum SHS-LLME extraction efficiency was achieved using 600 µL of triethylamine as the extraction solvent and 4.0 mL of 10 M sodium hydroxide as the hydrophilicity-switching trigger. Optimum complexation conditions were obtained with sample pH at 4.5 and metal/ligand mole ratio of 1:2 within 3.0 min complexation time. Under optimum SHS-LLME-SDIC, the method's limits of detection and quantitation were found as 0.30 and 1.0 µg g<sup>-1</sup> respectively. A good linearity with R<sup>2</sup> above 0.9972 was obtained over the linear dynamic range of 1.0-50.0 µg g<sup>-1</sup>. The proposed SHS-LLME-SDIC method was applied for the determination of palladium in catalytic converters with recoveries between 95.7 to 103.7% and relative standard deviation (RSD) lower than 2.0%. The results showed that this method offers a viable, cost-effective and green alternative to sophisticated instruments in developing countries and small laboratories for the determination of palladium in catalytic converters.

**Keywords:** Catalytic converter, microextraction, palladium, smartphone camera, switchable hydrophilicity solvent.

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**OP15- Development of a novel and effective solid phase extraction method based on reduced graphene oxide-zinc oxide nanoflowers for the sensitive determination of cadmium by flame atomic absorption spectrophotometry**

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Cadmium, one of the elements found in the earth's crust, is a nonessential and toxic heavy metal for human body. It was reported that this element can be found in trace levels in nutrients, and humans can be exposed to this element through diet. Moreover, some industrial processes, tobacco consumption and the presence of cadmium in air and water are among the main causes of exposure to this element [1,2]. Even though cadmium shows genotoxic effects in vital organs such as kidney and liver as well as bones and cardiovascular system, it can accumulate in all organs and tissues in human body [2]. This element has a quite long half-life up to 30 years and some of the substantial effects on human health are bone demineralization and kidney dysfunction [1,3]. Considering all these toxic properties, accurate and precise determination of cadmium at trace levels is very significant. In this study, reduced graphene oxide-zinc oxide nanoflowers (RGO-ZnO NFs) based dispersive solid phase extraction combined with slotted quartz tube-flame atomic absorption spectrometry (SQT-FAAS) system was applied for sensitive determination of cadmium. RGO-ZnO NFs was synthesized using a novel and rapid microwave-assisted procedure. NFs was characterized by using SEM (Scanning Electron Microscope) images and XRD (X-ray Diffraction) pattern. Significant extraction parameters of the developed method were optimized by univariate optimization approach to enhance the signal to noise ratio and improve extraction efficiency of the analytes. Under the optimum experimental conditions, limit of detection (LOD) and quantification (LOQ) values and dynamic range were indicated and enhancement in detection power was calculated.

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**OP16- A novel cadmium sulfide nanoparticles based dispersive solid phase extraction for accurate determination of copper ions in artichoke leaves extract by flame atomic absorption spectrophotometry**

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Copper (Cu), an essential trace metal in human being and animals, is involved in diverse biological processes including iron metabolism, antioxidant defense, neuropeptide synthesis, and immune function. Cu deficiency may result in cardiovascular system disorders, bone malformation, and neurologic and immunologic problems<sup>1</sup>. It has been reported that chronic exposure to high levels of trace metals leads to potential health risks and toxicity<sup>2</sup>. In this study, the cadmium sulfide nanoparticles based dispersive solid phase extraction (CdS NPs-DSPE) strategy was improved for the extraction/preconcentration of Cu ions. Analyte was measured using slotted quartz tube-flame atomic absorption spectrometry system (SQT-FAAS). A new, rapid, and simple synthesis procedure for the CdS NPs was developed with the aid of a microwave system. In this context, the CdS NPs were synthesized by the microwave-assisted reaction of cadmium sulfate, thiourea, and sulfur powder in ethylene glycol. Various experimental parameters affecting the extraction and desorption of Cu ions were examined. Under the optimum extraction conditions, the calibration graph was linear in the range of 1.0-30 µg/L. The detection and quantification limits were obtained as 0.71 and 2.38 µg/L with high repeatability, respectively. The enhancement of detection power was found as 162.9-folds compared to the FAAS system. The practicability and reliability of the extraction strategy were validated using spiked artichoke leaves extract. The percent recoveries were calculated using the matrix matching calibration strategy as 87.0±12.6 % and 116.8±3.2 % for the spiked samples at 50 and 150 µg/L, respectively. These results have demonstrated the suitability and capability of the developed method for the accurate determination of trace levels of Cu in the food matrices.

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**OP17- Development of a rapid and efficient dispersive solid phase extraction for the determination of lead at trace levels: Manganese oxide nanoflower based dispersive solid phase extraction prior to flame atomic absorption spectrometry measurement****Miray Öner<sup>1</sup>, Cansu Demir<sup>1</sup>, Gülten Çetin<sup>1</sup>, Sezgin Bakırdere<sup>1,2</sup>**<sup>1</sup>Yıldız Technical University, Department of Chemistry, 34349 İstanbul, TURKEY<sup>2</sup>Turkish Academy of Sciences (TÜBA), Vedat Dalokay Street, No: 112, 06670, Çankaya, 06690, Ankara, TurkeyEmail address: [miiray.oneer@gmail.com](mailto:miiray.oneer@gmail.com)

The exposure of animals and humans to lead (Pb) has increased dramatically as a result of industrial development. Pb is among the most toxic heavy metals, and it is one of the significant environmental contaminants [1]. Its hazardous effects on the environment and human health can lead to serious problems even at small doses. Several disorders in the nervous, immune, reproductive, and gastrointestinal systems can be caused by this element. The major sources of Pb are air, water and food, and human beings are mainly exposed to this metal from these sources [2]. It is also known that this metal has a tendency to accumulate in living organisms [3]. In this study, an accurate and precise analytical strategy was developed for the determination of Pb at trace levels. For this purpose, the manganese oxide nanoflowers-based dispersive solid phase extraction (MnO<sub>2</sub> NFs-DSPE) method was used to separate and preconcentrate the target analyte prior to the measurement in flame atomic absorption spectrometry system. The morphologies of MnO<sub>2</sub> NFs were characterized by scanning electron microscopy (SEM) and X-ray diffraction (XRD). All variable parameters of the presented method were optimized step by step with a univariate optimization strategy. Under the optimum experimental conditions, the limit of detection (LOD), the limit of quantification (LOQ), and linear range were determined. In order to test the reliability and applicability of the developed method, recovery studies were performed in selected samples. The recovery results demonstrated that the development of a new extraction strategy provides an excellent alternative for the detection method of lead in various samples.

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**OP18- Validation of QuEChERS-GC MS/MS Method for determination of UV Filters in Sludges****Cemile Yücel<sup>1\*</sup>, Serenay Ceren Tüzün<sup>1</sup>, Hasan Ertaş<sup>2</sup>, F. Nil Ertaş<sup>2</sup>, İlgi Karapınar<sup>1</sup>**<sup>1</sup>Dokuz Eylül University, Department of Environmental Engineering, Tinaztepe Campus, Izmir, Turkey<sup>2</sup>Ege University, Science Faculty, Department of Chemistry, Bornova, Izmir, Turkey\*Corresponding Author: [cemile.yucel@deu.edu.tr](mailto:cemile.yucel@deu.edu.tr) , [fatma.nil.ersoyertas@ege.edu.tr](mailto:fatma.nil.ersoyertas@ege.edu.tr)

Ultraviolet filters (UVFs) are the general name of the chemical group that absorbs ultraviolet light and prevents the transition of light. Organic UVFs are widely used in cosmetic sector, these compounds are also employed in plastics, auto paints and rubber industries. Their residue levels in the environment are gradually increasing. In particular, the use of sewage sludge as fertilizer in agricultural areas raises concerns due to the potential of UV filters to penetrate soil and crops<sup>2</sup>. UVFs have been included in the Emerging pollutants and Watch List of the EU in recent years since they behave like an endocrine disruptor, altering the normal functioning of organisms<sup>1</sup>. Therefore, reliable, and sensitive methods of analysis for their trace level residues in environmental samples is of great importance. Generally, chromatographic methods coupled with a mass detector are preferred for their analysis in surface waters where the success of the method is based on the efficiency of the sample preparation technique. For complex samples such as sludges, this step becomes even more crucial for minimizing the interferences.

Present study describes the use of QuEChERS technique prior to the detection of some selected UVFs namely Benzophenone-3 (BP-3), 3- Benzylidene camphor (3BC), 2-ethyl hexyl-4-(dimethyl amino) benzoate (EDP), 2-ethyl-hexyl-4- trimethoxy cinnamate (EHMC), Ethylhexyl salicylate (EHS), Homosalate (HMS), Isoamyl p-methoxycinnamate (IAMC) and 4-Methylbenzylidene camphor (4-MBC) in sludge samples. Sludge samples spiked with standard mixture of UV filters for matrix-match calibration run through the extraction procedure and the UVFs content was determined by injector port silylation coupled with GC-MS/MS. Linearity for targeted UV filters was in the range of 40–1200 µg/kg in sludge. High recovery (63-123%) low RSD values indicated the high performance of this method. The method provides a reliable and fast analysis methods for UVFs in real sludge samples.

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## OP19- Conformational Analysis of Monoclonal Antibody Subunits Using Trapped Ion Mobility-Mass Spectrometry Technique

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**Abstract:** Analyzing the structural properties of monoclonal antibody (mAb) based therapeutic proteins is very important in developing these drugs. To meet this analytical requirement, various chromatographic, spectroscopic, and spectrometric techniques are being developed to characterize mAbs. In this study, a hyphenated method including trapped ion mobility spectrometry (TIMS) technology and liquid chromatography technique was developed for the conformational analysis of mAbs. This way, detailed data with high accuracy could be obtained for such samples. Conventional analytical techniques could not determine the conformational features of these biomacromolecules. TIMS technology provides an advantage in the conformational analysis of these species. In this study, mAb subunits could be obtained due to the reduction of mAb samples using appropriate chemical reagents and enzymes. The effects of using different enzyme and analytical method strategies on the conformational properties of mAb fragments could be monitored using the ion mobility-mass spectrometry technique.

### Keywords

Monoclonal Antibody, Trapped Ion Mobility, Mass Spectrometry, Conformational Analysis

### Introduction

Protein-based biopharmaceuticals generally have much more complex structural features than small molecule drugs. Therapeutic mAbs stands out as the most effective drug used among these structures.<sup>1</sup> mAbs are tetrameric glycoproteins with a molecular weight of about 150 kDa produced by recombinant DNA technology. These proteins have a Y-shaped structure. Four polypeptide chains in its structure are connected by disulfide bonds from the hinge regions, inside and outside the chain.<sup>2-3</sup> In addition to its advantages such as high sensitivity, accuracy, analysis speed, and low sample consumption compared to other analytical methods, mass spectrometry can provide detailed data on the characteristics of mAbs such as structural characterization, detailed post-translational modification analysis, and precise molecular mass determination during their production steps.<sup>4</sup> The ion mobility-mass spectrometry (IM-MS) technique provides detailed data on ions' size, shape, and conformational properties, besides conventional mass spectrometric analysis data. In this study, conformational LC-IM-MS analyses of mAb-based biopharmaceutical drugs were performed using trapped ion mobility – time-of-flight mass spectrometry (TIMS-TOF-MS) technique.

### Materials and Method

Dithiothreitol (DTT), papain, and IdeS enzymes were used to obtain subunits of mAb samples. Bevacizumab (Altuzan Vial 25 µg/µL stock) sample produced in the same production batch was used in all reduction processes and analyses performed in the study. TIMS-TOF-MS (Bruker Daltonics) instrument with electrospray ionization source was used online with high pressure liquid chromatography (Agilent 1200 HPLC) system. A reverse phase separation based column (Agilent Poroshell 300SB-C8, 2.1 × 75 mm, 5 µm) was used in the LC-IM-MS analyses. During the separation, the column was kept constant at 60 °C. LC mobile phase flow rate was 0.3 mL/min. Mobile phase A: Water (0.1% formic acid, v/v), mobile phase B: acetonitrile (0.1% formic acid, v/v) were used in the analyses. Samples were analyzed in positive ion mode, and their mass spectra were recorded in the m/z range of 300-5000. IM-MS analyses were performed using the following ESI source settings: 4500 V capillary voltage, 0.5 bar nebulizer gas pressure, 4.0 L/min drying gas flow, 200 °C drying gas temperature. TIMS parameter settings: IMS ramp start: 0.53 V.s/cm<sup>2</sup>, ramp end 1.95 V.s/cm<sup>2</sup>; ramp time 350 ms; ΔV deflection transfer and capillary exit: -

20 V, funnel 1 and deflection transfer: 135 V, ramp start and accumulation exit: 120 V. Nitrogen was used as a bath gas at ca. 300 K.

## Results and Discussion

CCS-z graphs were created using data obtained from LC-IM-MS analyzes of reduced bevacizumab samples. Light chain obtained from DTT-reduced bevacizumab have two conformer transition at 21+, 20+, 19+, 14+, 13+, and 12+ charge states (Figure 1A). More than one conformer was also observed for heavy chain at 33+, 32+, 31+, 30+ charge states (Figure 1B). For this sample, it was determined that the amount of compact conformer with low CCS value increased at lower charge states. Conformer transitions of ions with 35+, 34+, 33+, 32+ and 31+ charges for Fc fragment (Figure 1C) and 33+, 32+, 31+ and 30+ charges for Fab fragment (Figure 1D) could be observed in CCS-z plots of papain-reduced bevacizumab. In the CCS-z plot of the Fc/2 fragment of Ides-reduced bevacizumab, 3 conformer transitions were determined in 11+, 10+ in the lower CCS region, 15+, 14+, 13+ in the middle CCS region, and 21+ in the upper CCS region (Figure 1E). No conformer transition could be observed in the CCS-z graph of the F(ab)'2 fragment of Ides-reduced bevacizumab (Figure 1F). These data show that this large fragment with a molecular mass of about 100 kDa cannot form more compact conformers the high charge states because they cannot overcome intramolecular charge repulsion. For the Fc/2 fragment of bevacizumab treated with DTT after reduction with Ides, 2 conformer transition regions are obtained for ions with charges of 11+, 10+ in the lower CCS region and 15+, 14+, 13+ in the middle CCS region (Figure 1G). For the light chain, there are 2 conformer transitions in 14+, 13+, 12+ charge states in the middle CCS region and 19+ in the upper CCS region (Figure 1H). The Fd fragment has only one conformer in the 14+ and 13+ charge states in the middle CCS region (Figure 1).

## Conclusion

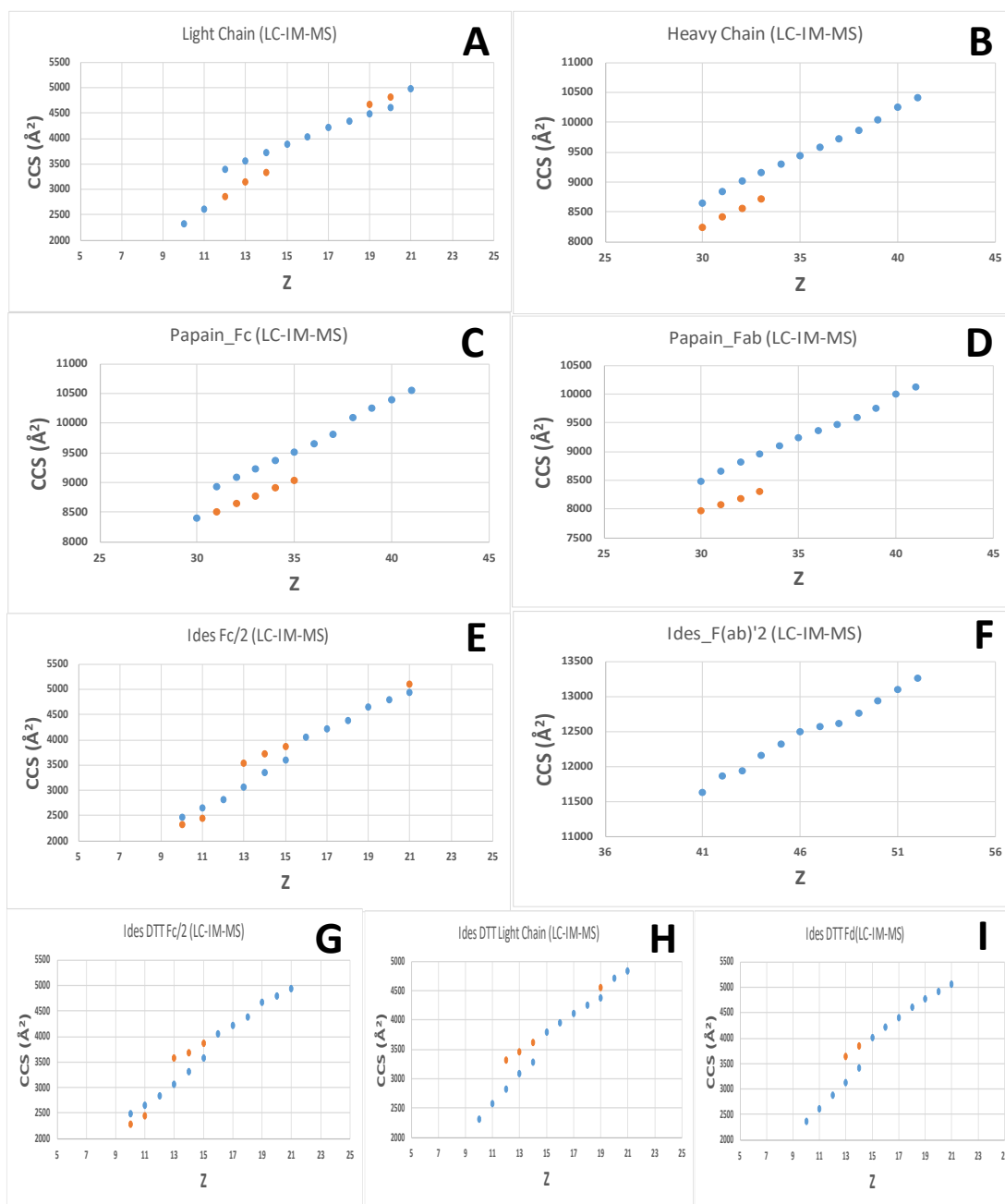
The conformational properties of bevacizumab subunits were unique depending on the data distribution profiles in the CCS-z plots. In this way, it is possible to determine the conformational differences of bevacizumab subunits obtained from different samples by evaluating the graphics obtained from such analyzes performed in the study.

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**Figure 1.** CCS-z plots A) DTT-reduced, light chain B) DTT-reduced, heavy chain C) Papain-reduced, Fc D) Papain-reduced, Fab E) Ides-reduced, Fc/2 F) Ides-reduced F(ab)'2, G) Ides-DTT-reduced, Fc/2 H) Ides-DTT-reduced, light chain I) Ides-DTT-reduced, Fd.

**OP20- New approach for determination and identification of selected antibiotics and microorganisms for biomedical purposes**

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Antibiotics are widely prescribed biologically active compounds for therapeutic and prophylactic use against bacterial infections. They can be classified into many different groups of drugs and vary a lot in structure, mode of action and spectrum of activity.

Physicians determine the dosage of antibiotic based on the ability of the patient's body to metabolize and excrete of target compounds. Therefore, new analytical methods are created to facilitate the monitoring of body fluids taking into account content of antibiotics and their metabolites. Developing of analytical methods for clinical use is facing occurrence more than one antibiotics as well as their metabolites in the tested samples. For this reason, it is necessary to develop methods that enable simultaneous determination of antibiotics from different therapeutic classes and their metabolites. Nowadays, the most frequently used methods in the analysis of antibiotics are chromatographic techniques coupled with different detection systems. On the other hand, for microorganisms identification gene sequencing and the matrix-assisted laser desorption ionization-time of flight mass spectrometry have been evaluated in terms of its analytical accuracy to identify clinical isolates by many diagnostic laboratories.

This study aimed at developing an efficient analytical protocol for the determination of in vitro electrochemical products of selected antibiotic drugs. Combination of electrochemistry (EC) and mass spectrometry (MS) was applied for the in vitro determination of the studied antibiotics and their electrochemical products. To identify the structure of the detected electrochemical products, MS/MS experiments were performed. This was one of the first applications of the EC system for generation of electrochemical products produced from antibiotic drugs. The electrochemical results were compared to in vivo experiments by analyzing urine samples from patients after antibiotic drugs have been administered. Overall, the comparison of electrochemistry to in vivo experiments shows the high potential of EC-MS as a fast analytical tool in the prediction of electrochemical conversion that could be applied to therapeutic drug monitoring and pharmacokinetic studies as well.

In addition, the identification of microorganisms was also an essential element of the research. For this purpose, the identification of bacteria was performed based on protein and lipid profiles using the Matrix-Assisted Laser Desorption/Ionization-Time Of Flight (MALDI-TOF) mass spectrometry (MS) technique. Sequencing of the 16S rDNA gene was used as a reference method. Quick and accurate determination of pathogenic bacteria is associated with the appropriate selection and individual antibiotic therapy.

#### **Acknowledgements**

Daria Janiszewska, Małgorzata Szultka-Młyńska, and Bogusław Buszewski are members of Toruń Center of Excellence "Towards Personalized Medicine" operating under Excellence Initiative-Research University.



**OP21- Enzymatic pretreatment of plant material for enhanced release of biologically active compounds by supercritical CO<sub>2</sub>**

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Biologically active compounds, naturally occurring in plants, are an extremely interesting subject of research due to the health-promoting properties assigned to them. Development of the so-called Green Chemistry techniques enabling the isolation of these compounds with a low investment of time and reagents have resulted in increased interest in this subject. It is especially noticeable in the cosmetics and pharmaceutical industries, where the possibility of using raw materials of natural origin is constantly being sought.

The aim of the research was to develop a methodology for isolation of biologically active substances from selected plant materials, characterized by a high content of compounds with antioxidant properties, in particular polyphenolic compounds. To improve the extraction efficiency, enzymatic hydrolysis of plant material was used, based on the use of a preparation that degrades cell walls. Thanks to this process, the isolated compounds were released more easily. Supercritical fluid extraction (SFE) with the addition of a polar co-solvent was used for the research, due to the low toxicity of carbon dioxide and the high selectivity of the process. A statistical response surface methodology was used to optimize the enzymatic hydrolysis and extraction conditions. The obtained extracts were characterized by the use of spectrophotometric and chromatographic techniques, namely high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS / MS). The total content of polyphenolic compounds in the extract obtained after two-step optimization was much higher ( $545.84 \pm 21.40 \mu\text{g/g}$ ) than in the extract obtained from non-hydrolyzed material ( $274.68 \pm 22.50 \mu\text{g/g}$ ) and in the extract obtained by maceration ( $161.86 \pm 19.80 \mu\text{g/g}$ ).

It has been proven that the extracts produced by EA-SFE with the highest content of flavonoids can support the cellular antioxidant system by direct neutralizing free radicals or activating enzymatic antioxidant mechanisms, which indicates the practical application in various industries.

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Aneta Krakowska-Sieprawska, Katarzyna Rafińska and Bogusław Buszewski are a members of Toruń Center of Excellence "Towards Personalized Medicine" operating under Excellence Initiative-Research University.

**OP22- Quadrupole Isotope Dilution Method for the Determination of Methamphetamine in Human Urine and Serum Samples by Gas Chromatography Mass Spectrometry After Simultaneous Derivatization and Spray Assisted Droplet Formation-Liquid Phase Microextraction**

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The psychostimulant methamphetamine has a highly addictive feature [1]. Overuse and abuse of methamphetamine can lead to adverse health problems such as aggression, paranoia, memory impairment, hypertension, depression, anxiety, Parkinson's disease and stroke [2][3]. For these reasons, detection of methamphetamine in biological samples is important. In this study, an analytical method with high sensitivity/accuracy was developed for the determination of trace level of methamphetamine in human serum and urine samples using gas chromatography-mass spectrometry (GC-MS). A simultaneous derivatization and spray assisted droplet formation-liquid phase microextraction (SADF-LPME) method used in the study was used for preconcentration of the analyte. Quadrupole isotope dilution (ID<sup>4</sup>) was used to provide high accuracy and precision in the selected matrices. By optimizing the appropriate parameters of the SADF-LPME method, the limit of detection (LOD) was found as 48.0 µg/kg. Synthesis and purification of methamphetamine acetamide-d<sub>3</sub>, which was used as a methamphetamine isotope analog in the ID<sup>4</sup> method, was carried out in our research laboratory. Recovery studies were carried out to verify the accuracy and applicability of the developed method for selected matrices. When the SADF-LPME method was combined with ID<sup>4</sup>, the percent recovery values for urine and serum samples were calculated as 99.7-100.0% and 99.4-100.2%, respectively. This result demonstrated the applicability of the proposed method in selected matrices for trace level determination of methamphetamine.

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**OP23- Determination of Trace Levels of Paracetamol in Human Urine and Human Serum with High Accuracy and Sensitivity Using Dispersive Liquid Liquid Microextraction Coupled to Quadruple Isotope Dilution GC-MS**

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Paracetamol is known to be one of analgesic drugs used by children and adults and it has antipyretic and analgesic effects. Although it is generally considered as a safe drug, its overdose usage can cause serious and fatal hepatotoxicity.<sup>1,2</sup> For these reasons, it is crucial to determine paracetamol at trace levels in biofluids. In this study, an analytical method was developed for the determination of trace levels of paracetamol in human urine and serum samples by gas chromatography-mass spectrometry (GC-MS). Paracetamol was derivatized and then preconcentrated by dispersive liquid-liquid microextraction (DLLME) method. Benzoyl chloride was dissolved in dichloromethane and used as the derivatization agent. The limit of detection (LOD) and limit of quantification (LOQ) of the DLLME-GC-MS system under the optimum DLLME conditions were determined as 0.13 and 0.43 mg/kg, respectively. The microextraction method used was combined with quadrupole isotope dilution (ID<sup>4</sup>) strategy to increase accuracy/precision of the DLLME-GC-MS method. When the DLLME-GC-MS method was combined with the ID<sup>4</sup> method, high recovery results were obtained for human urine (98.8-99.9%) and serum (99.3-101.0%). The combined DLLME-QID-GC-MS method offers highly accurate and precise results for analyte determination in biological matrices. Paracetamol concentration in human urine and serum samples can be monitored in clinical studies by the developed method.

**Acknowledgement**

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**OP24- In vitro and in vivo metabolism studies of antisense oligonucleotides with the use of ion-pair liquid chromatography coupled with mass spectrometry**

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Antisense oligonucleotides (ASO) are synthetic, modified compounds tested as potential therapeutic agents against e.g. myotonic dystrophy type 1, spinal muscular atrophy. On the other hand some of them are already used in antisense therapy. During last seven years, as many as 14 ASO have now found use in the treatment of various diseases, mostly genetic, such as e.g., Duchenne Muscular Dystrophy, Spinal Muscular Atrophy, Familial Amyloid Neuropathies, Acute Hepatic Porphyria. ASO act by binding to complementary sequences of the targeted ribonucleic acids in cells, since they are synthetic, single-stranded, short (up to 20 nucleotides) fragments of nucleic acids. The use of oligonucleotides as therapeutic agents has enforced the need for their isolation, identification and determination in serum, blood, tissues or urine samples. This is especially important when studying metabolism and therapeutic monitoring of ASO. The higher the degree of metabolism, the lower the therapeutic effect of a given drug in which the active substance is ASO. For these reasons, it is very important to develop sensitive methods for the determination of these compounds with their effective separation. One of the most commonly used method in oligonucleotides analysis is ion pair reversed-phase liquid chromatography.

The aim of the study was the analysis and identification of ASO metabolites after their incubation with human liver microsomes regarding. Four different modification types of ASO were studied. The method development included the optimization of incubation conditions, selection of proper chromatographic conditions (in order to obtain separation selectivity in short time), as well as optimization of mass spectrometry parameters (in order to obtain low limits of quantification). Obtained results indicated that 3'-exonucleases and 5'-exonucleases contributed to the biotransformation of oligonucleotides. The number of metabolites depends on oligonucleotide modification and consequently its resistance to enzymatic attack. The method developed in the study was next successfully applied to the analysis of serum extracts obtained from children treated with an antisense therapy drug.

## OP25- Interactions of Titanium Dioxide Nanoparticles and Zinc Oxide Nanoparticles with Glutathione Reductase

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Titanium dioxide nanoparticles (TiO<sub>2</sub> NPs) uses in sunscreens, cosmetics and ointments to protect the human skin against UV radiation, and also uses in toothpastes and foods as a whitening agent<sup>1</sup>. Zinc oxide nanoparticles (ZnO NPs) are commonly used in sunscreens. They are used because they absorb ultraviolet light effectively but have a large enough bandgap to be completely transparent to visible light<sup>2</sup>.

Glutathione reductase (GR) (EC 1.8.1.7) is an antioxidant enzyme. Oxidized glutathione (GSSG) is converted to reduced glutathione (GSH) in the presence of NADPH (β-nicotinamide adenine dinucleotide 2'-phosphate reduced) by GR enzyme<sup>3</sup>.

In this study, GR from baker's yeast (*Saccharomyces cerevisiae*) exposed to 0, 25, 50, 100, 250 and 500 mg/L TiO<sub>2</sub> NPs and ZnO NPs. The results of the present study showed that when the concentrations of TiO<sub>2</sub> NPs and ZnO NPs increased, there was no statistically significant changes in GR enzyme activities compared to the control groups (p> 0.05, n=3).

At conclusion, we did not observe any statistically significant changes related to effects of TiO<sub>2</sub> NPs and ZnO NPs on GR activities.

**Key Words:** Glutathione Reductase, Nanoparticles, Titanium Dioxide Nanoparticle, Zinc Oxide Nanoparticle.

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**OP26- Electroreduction of Bi(III) ions on the innovative cyclically renewable liquid silver amalgam film electrode (R-AgLAFE) in the presence of cysteine**

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**Abstract**

The cyclically refreshable liquid silver amalgam film silver based electrode (R-AgLAFE) to study of electrode processes under the “cap-pair” conditions was used. The catalytic effect of cysteine (CE) on the Bi(III) ions electroreduction processes has been demonstrated. The magnitude of the catalytic effect is related to the formation of the Bi(III) – Hg(SR)<sub>2</sub> active complexes mediating electron transfer equilibrium.

**Keywords:** Cysteine, R-AgLAFE electrode, catalytic effect

**Introduction**

Determination of the active substance content is a routine activity in the process of pharmaceutical preparation production. Due to various structures and properties, a proper analytical method should be chosen for each analysed substance. The most commonly used analytical techniques include UV-VIS spectrophotometry, liquid chromatography, gas chromatography, mass spectrometry and electrochemical methods. Voltammetry is an example of an electrochemical method which, in addition to chromatography, is becoming increasingly common due to its very large sensitivity at the level of pM, selectivity, or the possibility of conducting speciation analysis in real samples without their pre-treatment.

The “cap-pair” rule<sup>1</sup> determines the conditions that indicate the catalytic activity of a substance<sup>2-3</sup> developed and used to electrode processes.

In the present study the kinetics and mechanism of Bi(III) reduction in the presence of cysteine have been studied using the innovative R–AgLAFE electrode<sup>4</sup>.

The electrode used has the advantages of a commercial mercury electrode, allowing for equally accurate determination of analytical parameters and offering a significant reduction in the toxic mercury needed to form the amalgam film and the waste generated.

**Materials and Method**

The following reagents were used to prepare the solutions: NaClO<sub>4</sub>, HClO<sub>4</sub> (Sigma) and cysteine (Fluka) as well as redistilled water. The basic electrolyte was a mixture of 0.5 mol·dm<sup>-3</sup> NaClO<sub>4</sub> and 0.5 mol·dm<sup>-3</sup> HClO<sub>4</sub>. The solutions of 1 mol·dm<sup>-3</sup> chlorate(VII) were subsidized with the Bi(III) ions at a concentration of 1·10<sup>-3</sup> mol·dm<sup>-3</sup>. The measurements were performed with the cysteine concentration between 1·10<sup>-5</sup> and 1·10<sup>-3</sup> mol·dm<sup>-3</sup>. All electrochemical measurements were performed with Autolab Fra 2/ GPES (Version 4.9) frequency response analyser (Eco Chemie, Utrecht, Netherlands). A three-electrode system composed of Ag/AgCl/3M KCl electrode as a reference and a platinum wire as an auxiliary electrode, and R–AgLAFE as a working electrode.

Additionally, the working electrode surface morphology was examined using an optical microscope Nikon Eclipse MA200 with the lens "Nikon Lu Plan Fluor 10x/ 0.30A" and the polarization filter "MA2-PA".

Figure 1 shows that the liquid supersaturated (1% mas.) silver amalgam forms a thin film on the surface of silver base (wire) without destruction of its texture. The slight discontinuity of the film is caused by its small mechanical strength during electrode preparation. However, it does not affect the reproducibility of the voltammetric curves.

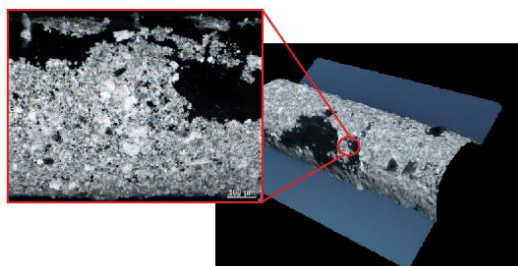
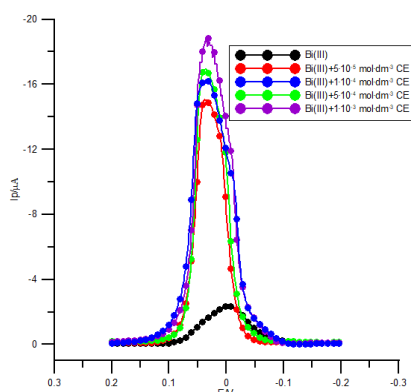


Fig. 1. R-AgLAFe electrode surface.

The applied electrochemical techniques that is: direct current (DC) voltammetry, cyclic voltammetry (CV), square wave voltammetry (SWV) and electrochemical impedance spectroscopy (EIS) were used to determine the kinetic parameters of the process of Bi(III) ions electroreduction as well as in the presence of cysteine.

## Results and Discussion

Introduction of cysteine to the solutions of Bi(III) ions in  $1 \text{ mol} \cdot \text{dm}^{-3}$  chlorates(VII) causes the increase of SWV peak current of Bi(III) ions electroreduction (Fig.2) and a simultaneous decrease in the peak width in the middle of its height. This indicates the increase of reversibility of the Bi(III) ions electroreduction process<sup>2</sup>. The magnitude of these effects depends on the CE concentration.

Fig. 2. SWV peaks of electroreduction of Bi(III) ions in  $1 \text{ mol} \cdot \text{dm}^{-3}$  chlorate(VII) with the increasing cysteine concentration included in the legend.

Such qualitative estimation of the effect of the studied substances on the electroreduction process of Bi(III) ions in  $1 \text{ mol} \cdot \text{dm}^{-3}$  chlorates(VII) is confirmed by the results obtained from the CV curves (Fig.3). Slight changes with a change in the rate of electrode polarisation (particularly at low polarisation rates) indicate that the stage controlling the rate of Bi(III) ions electroreduction in the presence of CE is a chemical reaction<sup>2</sup>. According to the assumptions of the “cap-pair” rule and the literature data<sup>2-3, 5</sup>, it will probably be the reaction of active complexes Bi(III) – Hg(SR)<sub>2</sub> formation on the surface of the electrode which can mediate the electron transfer.

Based on the parameters of the cyclic voltammetry CV curves, the values of the transfer coefficients  $\alpha$  and the standard rate constants  $k_s$  of the Bi(III) ions electroreduction and in the presence of cysteine (Table 1) were determined.

The increase in the values of the transition coefficients  $\alpha$  after the addition of cysteine into the basic electrolyte solution indicates an increase in the reversibility of the Bi(III) ions electroreduction process<sup>2</sup>. This also translates into an increase in the standard rate constants, confirming the catalytic action of the amino acid.



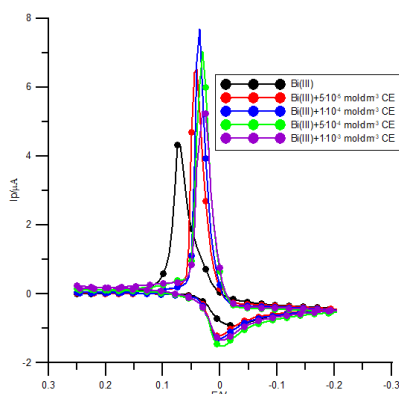


Fig. 3. Voltammetric CV curves of the electroreduction of Bi(III) ions in 1 mol·dm<sup>-3</sup> chlorate(VII) with the increasing cysteine concentration shown in the legend.

Table 1. Values of cathodic transition coefficients ( $\alpha$ ) and standard rate constants ( $k_s$ ) of  $1 \cdot 10^{-3}$  mol·dm<sup>-3</sup> Bi(III) electroreduction in 1 mol·dm<sup>-3</sup> chlorate(VII) and in the presence of cysteine.

$10^3 c_{\text{Bi(III)}} + 10^4 c_{\text{CCE}} / \text{mol} \cdot \text{dm}^{-3}$	$\alpha$	$10^4 k_s / \text{cm} \cdot \text{s}^{-1}$
<b>0</b>	<b>0.25</b>	<b>0.36</b>
0.5	0.53	5.37
1.0	0.57	8.11
5.0	0.59	10.05
10.0	0.61	12.78

## Conclusion

The studies using an innovative electrode of cyclically renewable liquid silver amalgam film R-AgLAFE pointed out to the catalytic effect of cysteine on the multistep electroreduction process of Bi(III) ions. The magnitude of the catalytic effect is related to the formation of active complexes mediating electron transfer equilibrium.

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**OP27- Carbon black-modified carbon ceramic electrode – fabrication, characterization, and its electroanalytical performance****Barbara Burnat\*, Mariola Brycht, Andrzej Leniart, Sławomira Skrzypek**University of Lodz, Faculty of Chemistry, Department of Inorganic and Analytical Chemistry,  
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Improvements in the quality of the analytical signal, as well as the sensitivity and selectivity of the carbon-based electrodes, are mainly obtained by modification of their surface with various materials. An alternative to surface modification is the modification of the electrode in its entire volume, however, this strategy is useful mainly for those electrodes where the user prepares the electrode material himself. An interesting but underestimated type of carbon-based electrodes is carbon ceramic electrode (CCE), the development of which began with the work of Lev and co-workers [1]. In general, ceramic carbon electrodes are produced using sol-gel technology based on hydrolysis and condensation processes of silica precursors. The resulting silica matrix acts as a binder for the conductive carbon powder, typically graphite powder, but not limited to. Our previous studies on the partial and total replacement of graphite by carbon-based and non-carbon materials showed that reduced graphene oxide, carbon nanotubes, and bismuth oxide improve the electroanalytical performance of CCEs.

Carbon black (CB) is a low-cost nanomaterial characterized by excellent electrical conductivity, high surface area, dispersibility in solvents, and possibility of facile functionalization. CB shows  $I_D/I_G$  ratio greater than 0.9, and valuable heterogeneous rate constants. As shown in review paper of Silva et al [2] there are many reports on electrochemical biosensors based on nanostructured carbon black applied to the determination of pharmaceuticals, environmental contaminants, food additives, and biomolecules. CB has been widely exploited in the design of carbon paste electrodes, glassy carbon electrodes, and screen-printed electrodes. However only a few papers treat about application of CB in the modification procedure of ceramic carbon electrodes dedicated for electroanalysis.

The aim of this work was to verify the effect of CB incorporated in a silica matrix on the topographical and electrochemical characteristics of ceramic carbon electrodes. For that purpose, both unmodified (GP-CCE) and carbon black-modified (CB-CCE) electrodes were characterized by atomic force microscopy (AFM), cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS). On the basis of the AFM images, the effect of CB on the topography and roughness of the electrodes was evaluated. The electrochemical properties of the investigated electrodes were compared in terms of the reversibility of the  $\text{Fe}(\text{CN})_6^{4-}/\text{Fe}(\text{CN})_6^{3-}$  redox model system. Both voltammetric and impedance analyzes confirmed that the modification of the ceramic carbon electrode with the use of carbon black improves its electrochemical properties. The developed CB-CCE was successfully applied for the voltammetric determination of syringic acid (SA) as a phenolic model compound. Due to the incorporation of CB, increased sensitivity of the SWV procedure at CB-CCE was achieved. Furthermore, the linear dynamic range was improved and it was 0.5 - 16.0 and 3.0 - 18.0  $\mu\text{mol L}^{-1}$  for CB-CCE and GP-CCE, respectively. The calculated detection limit (LOD) for CB-CCE (0.15  $\mu\text{mol L}^{-1}$ ) was found to be 3 times lower than for GP-CCE. Additionally, the CB-modified electrode was used to quantify syringic acid in a red wine sample. In summary, all of the analyzes performed confirmed the positive effect of CB on the electroanalytical performance of the ceramic carbon electrode. Therefore, CB-CCE was found to be a promising material for the electroanalytical applications.

Keywords: carbon ceramic electrode, sol-gel process, electroanalysis, voltammetric analysis, syringic acid,

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## **OP28- Transport of Methylene Blue Across a Bulk Liquid Membrane using Pillar[5]arene as a Carrier Phase**

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Pillar[n]arenes, a new class of supramolecular chemistry, have a negatively charged electron-rich domain due to the attachment of electron donor groups to the main pillararene skeleton, and thus Pillar[n]arenes can cause intermolecular interactions and form binding affinities of host-guest complexes. In general, many type of macrocyclic derivatives structures have also been frequently preferred in membrane studies, since they are based on host-guest interaction, however, pillararenes has not been used as carriers in membrane transport systems when compared to other conventional macrocyclic host compounds [1] [2].

Within the scope of this study, Pillar[5]arene was used and they are attributed as one of the newest members of macrocyclic structures, as a carrier, considering that Pillararene can interact with positively charged electroactive molecules (for example, methylene blue) via electrostatic interaction, methylene blue is produced from a donor phase through an aqueous membrane system. Detection of transport to the acceptor phase was achieved by using an H type tube according to the Bulk Liquid Membrane (BLM) system. Accordingly, as the stirring speed increased, the rate of transport of methylene blue by Pillararene increased. Likewise, as the concentration of Pillararene increases, the transmission of methylene blue to the opposite side also increases. (Calculated methylene blue concentrations confirm this). Therefore, we have seen the success of Pillararene in carrying methylene blue and that it can be used as a new macrocyclic structure in liquid membrane systems.

*Keywords: Bulk Liquid Membrane, Methylene Blue, Pillar[5]arene*

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### *Acknowledgment*

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**OP29- Diatom biosilica for the chromatographic purposes**

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**Abstract**

Diatoms are widespread unicellular photosynthetic algae that produce unique highly ordered siliceous cell wall, called frustule. Biosilica is an inorganic polymer formed by organisms such as diatoms or siliceous sponges of orthosilicate units in which two silanol groups are joined together to one bond or siloxane. Biosilica has been used in various applications, such as water treatment systems, pharmaceutical applications, and as a tool for drug delivery systems. The surface of the amorphous silica exhibits high levels of free reactive hydroxyl groups (-OH) which can be used to modify the frustule with chemical groups (-NH<sub>2</sub>, -COOH, -SH, and -CHO). As the given population of biosilica particles is characterized by a narrow size distribution and practically the same shape and morphology it would be, in our opinion, interesting to use it in liquid chromatography. Hence, the aim of this study is to prepare a stationary phase based on biosilica obtained from diatom(microalgae), then use it to fill the chromatographic column and test it in capillary high-performance liquid chromatography.

**Key words:** Diatom, Biosilica, chromatographic column, high-performance liquid chromatography.

**Acknowledgement:** This work was financially supported in the frame of the project “Advanced Bio composites for Tomorrow's Economy BIOG-NET”, FNP POIR.04.04.00-00-1792/18-00, project is carried out within the TEAM-NET program of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund.

**OP30- Quantitative structure-retention relationship for identification and quantification in lipidomics using chromatographic techniques**

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Lipids are a group of organic small molecule compounds, occurring in all living organisms as active components of biological membranes. Lipids play the role of condensed cellular energy, in the form of triacylglycerols, they are a precursor for many signalling molecules, they play a significant role in intercellular communication as primary and secondary signal transducers, play an insulating and protective function, and building blocks of biological membranes surrounding cells and also inside them. Phospholipids are the most abundant components found in the biological membrane. In addition, phospholipids lower the level of triglycerides and cholesterol in the blood. Due to their structure, they belong to the molecules of an amphiphilic character, because they consist of a polar, hydrophilic "head" and one or two chains of fatty acids, which show a non-polar character.

Quantitative structure-retention relationship (QSRR) can be used as an optimization tool to determine and understand chromatographic separation on a molecular basis. QSRR is a statistically determined relationships between chromatographic parameters and values (descriptors), which characterize the structure of the analyzed analytes. QSRR methodologies are used to describe the retention mechanism, for classification of chromatographic columns, to predict the elution order of analytes and to predict the retention of new compounds. The QSRR methodology is based on the knowledge of two types of data. One of them is the chromatographic parameters obtained for a sufficiently large series of analytes. The second type of data is a set of parameters describing the molecular structure of the studied compounds. In the case of retention data, the most frequently used parameter is the n-octanol-water partition coefficient. Multiple Regression Analysis is the basic statistical method for determining the relationships between variables, which is usually used in the QSRR procedure. The QSRR methodology was used for the first time in the study of phospholipids with the chromatographic techniques.

**OP31- Analytical approaches to investigate contaminants transported by microplastics on the Lebanese Mediterranean coast**

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Anthropogenic activities are increasingly putting pressure on the environment of the Lebanese Mediterranean coast. One of the main emerging sources of pollution is plastic ending up in the marine environment and persisting as small (so called microplastics, 1  $\mu$ m - 5 mm) particles which become a vector for the transport of a variety of contaminants. The assessment of microplastics related environmental risks requires the development of multi-technique analytical approaches targeting different classes of organic and inorganic pollutants.

A comprehensive study of microplastic pollution of the Lebanese Mediterranean coast has been performed. The samples were collected from the shoreline in 2020-2021, sieved and sorted into different sizes. The nature of the microplastics was investigated by FTIR revealing the predominant presence of polyethylene and polypropylene. For the study of adsorbed organic pollutants, solid-liquid (solvent) extraction procedures targeting polar and non-polar compounds have been optimized. For the analysis of metals, an acid microwave digestion was carried out.

Prior to quantitative analysis, the organic pollutants were screened on the basis of their accurate mass, chromatographic behavior and fragmentation and the lists of compounds for subsequent targeted analysis was established. Compounds that are used in plastic manufacturing, plastics additives and plastics pigments, as well as metals naturally present in the marine environment, were excluded.

The first ever LC-MS/MS data on microplastics-adsorbed organic pollutants present on Lebanese Mediterranean coast demonstrate the persistency of several pesticides and pharmaceuticals, such as phenacetin and minoxidil pesticides. The quantification of metals was carried out by ICP-MS and the obtained data discussed in the context of their possible environmental and anthropogenic sources.

The performed study clearly shows that microplastics are not only a source of pollution by themselves, but also transport organic and inorganic contaminant in the marine environment. Moreover, as microplastics are becoming important targets of environmental studies, the perspectives for future green methodologies reducing the use of organic solvents in sample preparation step, *e.g.*, by employing liquid-extraction surface analysis, will be discussed.

**Keywords:** microplastics, organic pollutants, metals

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**OP32- Phytochemical Screening, Antioxidative Activity, Chromatographic Fractionation, and HPLC Analysis of Phenolic Components in Blackberry (*Rubus fruticosus* L.) Extract**

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Plants contain natural substances known as phytonutrients. Various fruits and vegetables assist in receiving more phytonutrients and enhancing health. The blackberry belongs to the genus *Rubus* (Rosaceae family, *Rubus* genus). It is a widely planted fruit around the globe. The current work investigates blackberry extract's phytochemistry and antioxidant potential and its chromatographic separation by utilizing a glass column on silica gel and analyzing each fraction using high-performance liquid chromatography (HPLC). The phenolic (38.08 mg GE/g), flavonoid (14.58 mg QE/g), flavonol (6.95 mg R/g), and anthocyanin content (7.73 mg cyanidin-3-glucoside/kg) were all elevated. Additionally, the extract exhibited ferric reduction and chelating metal properties, containing 20.53 mg FeSO<sub>4</sub>/g and 182.12 mg Fe<sup>2+</sup>/g, respectively. Blackberry has a high antioxidant capacity and was very effective in inhibiting amylase activity (76.01%). Column chromatography was run using silica gel and toluene-ethyl acetate-formic acid (65:45:25) as the solvent system, with each fraction being tested using thin-layer chromatography (TLC). HPLC analysis of the crude extract and fractions revealed that blackberry extract and fractions are rich sources of natural antioxidants.

**Keywords:** *Rubus fruticosus*, blackberry, phenolics, antioxidant activity, chromatographic fractionation



## Virtual Presentations

### VP1- Development of Hydrogen Peroxide Biosensor Based on poly-L-Histidine Modified Glassy Carbon Electrode

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Determination of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) by rapid and reliable methods has an important role in chemistry, food industry, clinical control and environmental monitoring. Amperometric biosensors have the characteristics of simplicity, high selectivity and sensitivity<sup>1</sup>. Thus, these biosensors are good alternatives for the detection of hydrogen peroxide.

In this study an amperometric  $\text{H}_2\text{O}_2$  biosensor was constructed using horseradish peroxidase enzyme (HRP) immobilized in the poly-L-histidine (PLH), ionic liquid (IL) and multiwalled carbon nanotubes (MWCNTs) modified glassy carbon electrode (GCE) and the analytical performance of this biosensor was investigated. For this purpose, the GCE was first modified with a mixture of MWCNT, IL and gelatin. The PLH modified GCE was prepared using the following procedure: IL and MWCNT modified GCE was placed in a 0.05 M phosphate buffer solution (pH 5.0) containing 0.005 M L-histidine (monomer). The IL-MWCNT/GCE was submitted to 30 cycles between 0.0 and  $-1.5$  V (vs. Ag/AgCl) at a scan rate of  $50 \text{ mV s}^{-1}$  to obtain the PLH-IL-MWCNT/GCE<sup>2</sup>. HRP was dropped onto the PLH-IL-MWCNT/GCE for the immobilization of the enzymes. Parameters such as the amount of IL and MWCNTs were optimized in order to achieve high amperometric response. The best analytical response was obtained with  $2.5 \mu\text{L}$  IL and  $1.5 \text{ mg mL}^{-1}$  MWCNTs. Optimum experimental conditions including pH and potential were also determined. Analytical performance of the fabricated biosensor was determined under optimum experimental conditions. Linear operating range of HRP/PLH-IL-MWCNT/GCE was determined. Other parameters such as detection limit, sensitivity, response time and reproducibility were also explored. The use of the PLH, IL and MWCNT improved the performance of the presented biosensor and the biosensor showed good analytical performance towards  $\text{H}_2\text{O}_2$  determination. It can be concluded that the presented biosensor is a good alternative for practical analysis of  $\text{H}_2\text{O}_2$ .

**Keywords:** Hydrogen peroxide, ionic liquids, multiwall carbon nanotubes, poly-L-Histidine.

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## Index

### A

A.Krakovska-Sieprawska, 58  
 Agnese Osite, 21  
 Agnese Osite, 44  
 Agnieszka Nosal-Wiercińska, 63  
 Aliyu B. Abdullahi, 49  
Aliyu Bawa Abdullahi, 48  
 Almira Ramanavičienė, 35  
 Andrzej Leniart, 39, 66  
 Antony C. Calokerinos, 26  
Arturs Viksna, 21  
 Arturs Viksna, 44  
 Arūnas Ramanavičius, 35  
 Aryan Qader, 71  
Aušra Valiūnienė, 40, 41  
 Ayşegül Erdoğan, 45

### B

B. Buszewski, 58  
 Barbara Burnat, 39, 66  
Bekir Salih, 32, 54  
 Bogusław Buszewski, 68  
 Bogusław Buszewski, 25, 57, 61, 69

### C

Cansu Demir, 50, 52  
Cemile Yücel, 53  
Ceren Kaçar Selvi, 37, 72

### D

Daria Janiszewska, 57  
 Didem AYDIN, 67  
 Didem Peren Aykas Cinkilic, 46  
 Durişehvar Özer Ünal, 19

### F

F. Nil Ertaş, 53

### G

Gulsah Ozcan-Sinir, 46

### H

Hasan Karadag, 62  
Hilal Torul, 36, 38  
 Hussam AL Saoud, 68

### I

Inga Gabriunaite, 40, 41

### J

Justyna Walczak-Skierska, 69

### L

Laura Sakalauskiene, 42

### M

Mariola Brycht, 24, 39, 43, 66  
Marlena Martyna, 63  
 Mehmet Atakay, 32, 54  
 Mehmet Yaman, 71  
Meltem Şaylan, 51  
 Miray Öner, 50, 52  
 Mustafa Culha, 27  
 Mustafa Kemal Sezgintürk, 34  
 Mustafa Soylak, 27  
 Mutay Aslan, 31

### R

Rabia Demirel, 60  
Rim ELSEBLANI, 70  
 Ryszard LOBINSKI, 70  
 Ryszard Lobinski, 22

### S

Salihu Ismail, 48, 49  
 Sevda Akay Sazaklıoğlu, 38  
 Sezgin Bakirdere, 50, 51, 52, 59, 60  
 Sezgin BAKIRDERE, 28  
 Sibel A. OZKAN, 20  
 Sławomira Skrzypek, 24, 39, 66  
Sude Ofli, 59  
Sylvia Studzińska, 61

### T

Trajče Stafilov, 18

### U

Utkan Demirci, 23

### W

Wan Jeffrey Basirun, 33

### Y

Yusuf Dilgin, 29

### Z

Zeynep Atasayar, 47