

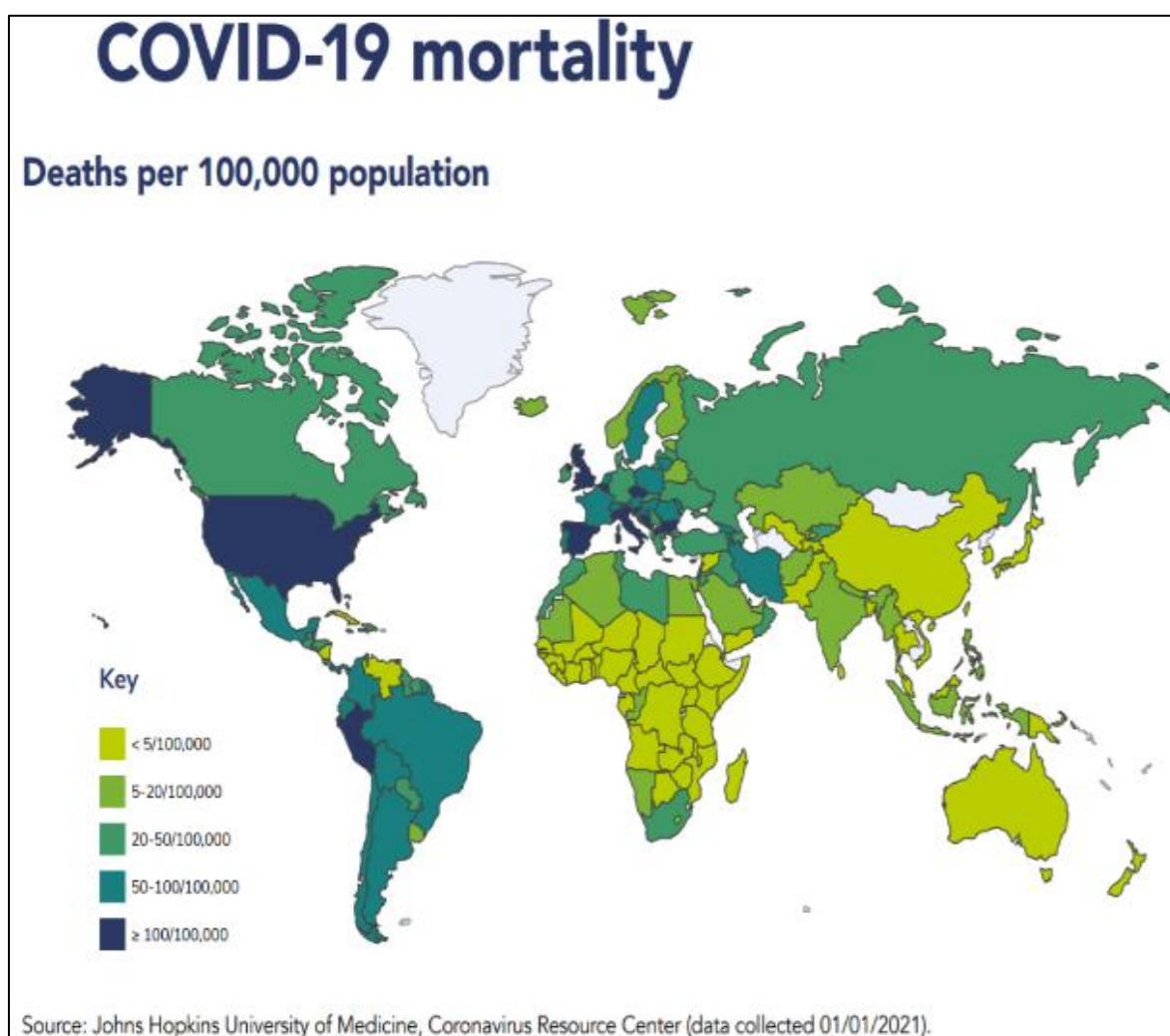


**3<sup>rd</sup> ICABC 2021**

**3rd International Congress on Analytical  
and Bioanalytical Chemistry**

**PROCEEDING BOOK**

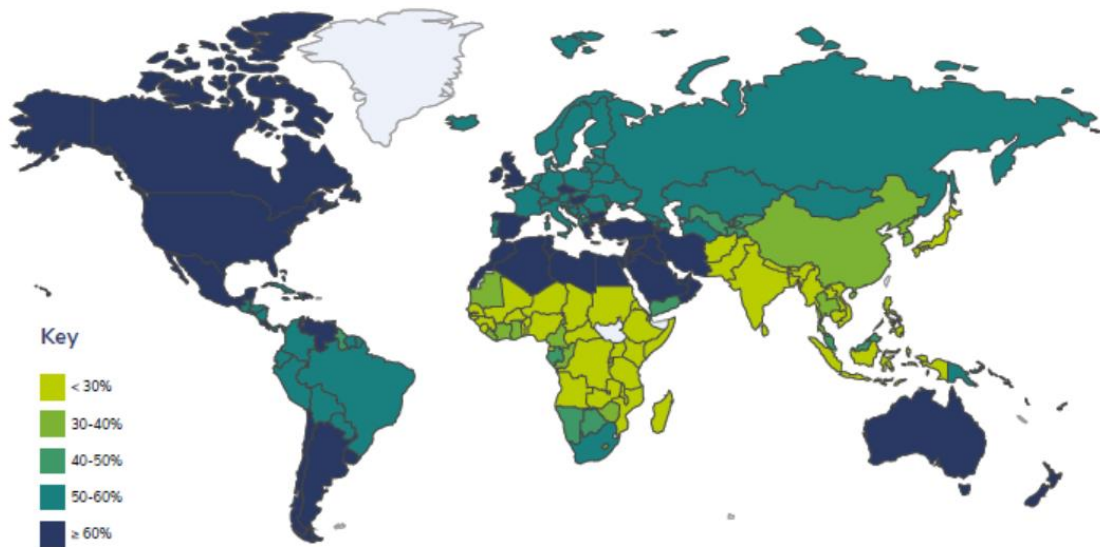
**Editor: Mehmet Yaman**



**22-26 March 2021-Online-Turkey**

# Prevalence of overweight in adults

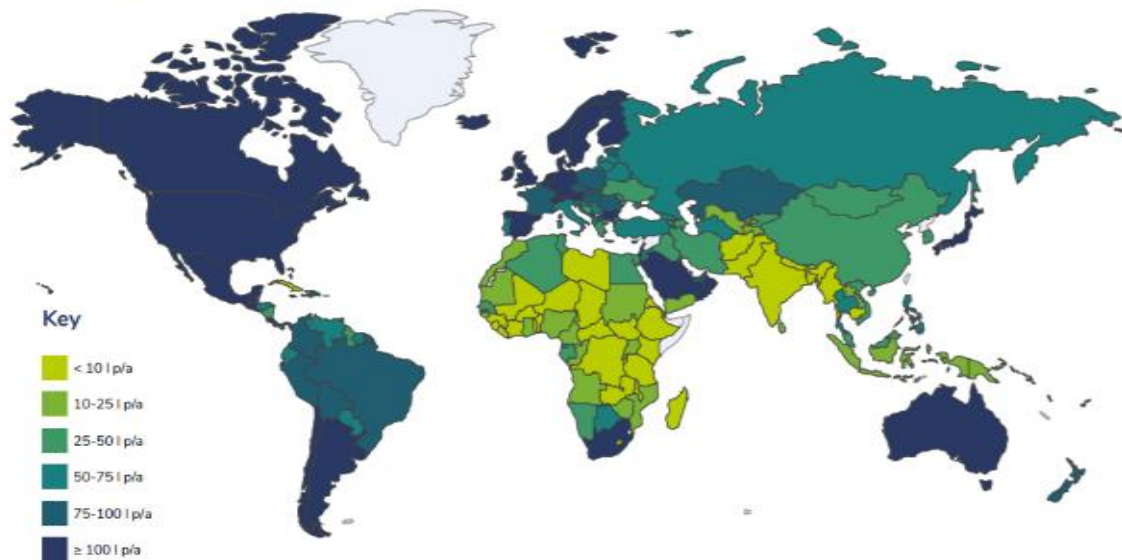
Adult overweight BMI > 25kg/m<sup>2</sup>



Source: World Health Organization, Global Health Observatory.

# Consumption of sugar-sweetened beverages

Litres per person per year

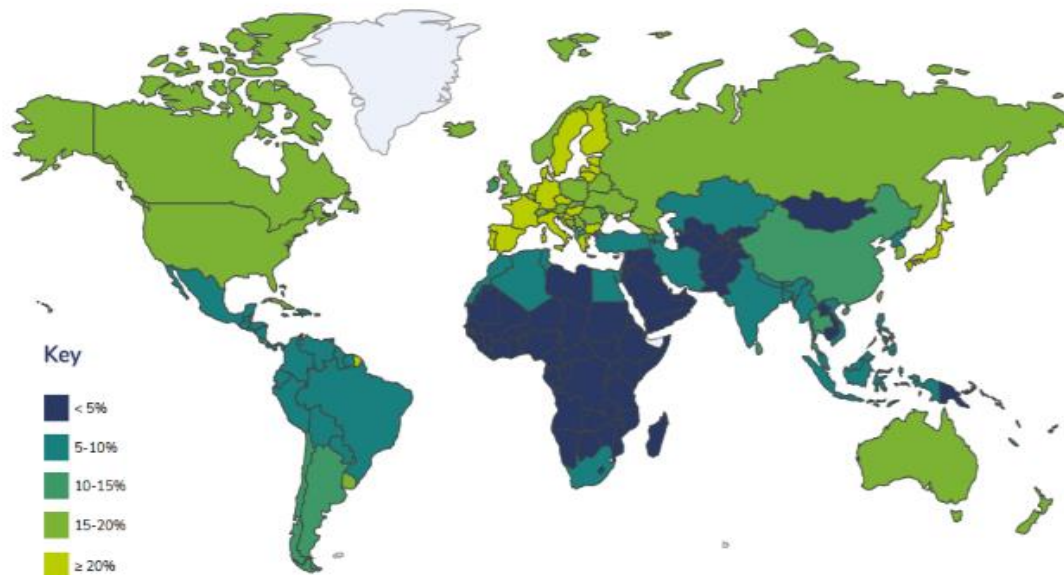


Source: Ferretti and Mariani, 2019.

22-26 March 2021-Online-Turkey

# Percentage of adults aged over 65

Percentage of Adults over 65



Source: World Health Organization, Global Health Observatory.

# 3<sup>rd</sup> ICABC 2021

## **Preface**

The organizing committee of the **3rd ICABC 2021** would like to welcome all participants to the "**3rd International Congress on Analytical and Bioanalytical Chemistry** ", held **as online** between 22-26 March 2021. The 3rd ICABC 2021 was started two 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 **10** sessions that include **18 invited and 52 oral** presentations as well as **5 virtuals** to be presented in the respective sessions. In addition, researchers of Academia (**48 universities from 10 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 5 days of the meeting will make **3rd 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**

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Antony Calokerinos (Athen U/GR)

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Mutay Aslan-(Akdeniz U/TR)

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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

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Ersin KILINC, Dicle U/TR

Łukasz Półtorak-Lodz U/Poland

Barbara Burnat -Lodz U/PL

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Tugba Boyunegmez Tumer- Canakkale 18 Mart U/TR

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Ibrahim Kivrak-Mugla U/TR

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Iryna Kravchenko-Odessa N. P. U/UA

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Aysu Yarman-Potsdam U/DE

Nabil A. Fakhre- Salahaddin U/IQ

Bengi Uslu-Ankara U/TR

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Mustafa SOYLAK Erciyes U.-TR

F. Nil ERTAS-Ege U.-TR

Arzum ERDEM GURSAN-Ege U.-TR

Mustafa ERSOZ-Selcuk U.-TR

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Elif TUMAY OZER-Uludag U.-TR

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Sadin OZDEMIR-Mersin U.-TR  
Serap Saglik ASLAN, Istanbul U.-TR  
Nagihan KARAASLAN AYHAN, Munzur U. TR  
Umran SEVEN ERDEMİR-Uludag U. TR  
Ugur TAMER, Gazi U. TR

Gulberk UCAR, Hacettepe U. TR  
Andrzej LENIART- Lodz U.-PL  
Barbara BURNAT- Lodz U.-PL  
Lukasz Poltorak- Lodz U.-PL

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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

# 3<sup>rd</sup> ICABC 2021

## **Chair**

Prof. Dr. Mehmet YAMAN-Firat University.

## **Organizing Committee-Secretariat**

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

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

**Umrhan Seven Erdemir** (Uludag U),

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

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

**Emine Akyuz Turumtay**-Recep Tayyip Erdogan U

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

**Gulsah Ozcan Sinir**-Uludag U

**Emel Akbaba**- Firat U.

## **GENERAL INFORMATION**

### **Introduction**

The **3rd International Congress on Analytical and Bioanalytical Chemistry** will be held on 22-26 March 2021 as **Online**-Turkey is a five-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.



## 3<sup>rd</sup> ICABC 2021

### Topics

To promote collaboration among analytical and bioanalytical (including biochemists, food engineering, molecular biology and genetics and similars) scientists from different countries, “3rd ICABC 2021” 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

3rd ICABC 2021 was held as ONLINE

### 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 or Turkish. 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 Honorable Professors, Colleagues and Participants,**

I am very happy to welcome all the participants coming to "3rd 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 to more and the update in analysis methods is gaining importance.

Particularly, the global issue (Covid19) and the requirements such as testing and immune system enhancing to prevent from similar infectious, increase the cooperation among analysts.

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.

On the other hand, the purpose of congresses and similar scientific meetings is to make presentations of young researchers, to listen to presentations and to up date themselves in an environment of well-known scientists.

This congress was launched for such purposes.

Hereby, I would like to give some statistical information related with the current global issue (COVID19) <https://ourworldindata.org/mortality-risk-covid>

Taking into consideration the deaths per 100 thousand population in the World, the highest mortality ratio of COVID19 was reported in America and European countries (more than 50 per 100 thousand). To determine the causes of this situation, we should look to the following information.

The distribution of the mortality rates across more than 160 countries appears to be strongly associated with overweight, with a dramatic increase in risk at higher levels of overweight prevalence. I would like to emphasise that Turkey have also more than 60% in prevalence of overweight in adults together with North America not European countries.

Again, many research reported that there is a correlation between obesity and covid19. Comparing countries around the globe, we find a close association between deaths from COVID-19 and the prevalence of overweight in the adult population. No country where the average adult BMI is below 25 kg/m<sup>2</sup> has a high COVID-19 mortality rate. In other words, no country where less than half the adults are experiencing overweight has a high COVID-19 mortality rate.

Similarly, the obesity rates significantly differ depending on countries. It is clear that the obesity rate in a developed country is significantly higher than a developing country.

To support this topic, I would like to more information.

There is a correlation between the consumption of sugar-sweetened beverages and obesity, and this correlation agree with the above information.

It is clearly seen that America and West European that have high mortality rate in Covid19 and overweight rates have also highest consumption of sugar sweetened beverages.

Finally, the consumption of sugar (HFCS) in the World is highly correlated with obesity and covid19 mortality.

In conclusion, researchers estimated that more than 130 000,000 COVID-19 cases, about 3 million deaths in the World, 30% of them could be attributed to obesity; more 25% to high blood pressure; more than 20% to diabetes; and more than 10% to heart failure. The results are all quite consistent that diabetes, obesity, and cardiovascular disease are the top three [COVID-19] risk factors, in addition to age. While age is a risk factor for severe COVID-19, it's not one we can

change. But conditions like obesity, diabetes, and cardiovascular diseases are potentially modifiable, either through lifestyle changes or medications.

Further, the experimental medicine scientists use the HFCS as comparison group to obtain obesity and diabete in animals. Briefly, HFCS is the mother of the more significant dieases. Therefore, managers of countries should ban the use of HFCS, as well as encaurage by going to increase the production of sugar beet that is alternative to HFCS.

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 10 sessions that include 18 invited and 52 oral presentations as well as 5 virtuals. The participants are of 48 universities from 10 countries. I believe that the discussions and the exchange of ideas among the participants during the 5 days will make this conference a brilliant platform to initiate new research collaborations.

I wish you all to enjoy this conference and have a pleasant to joining.

- I wish the conference will be useful.
- My best regards.

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

# CONFERENCE PROGRAM

## 3rd International Congress on Analytical and Bioanalytical Chemistry (3rd ICABC 2021)

22-26 March, 2021, Online

	<b>22 March, 2021</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>➤ Bioanalytics from Micro- to Nano- Dimension</p>
17:05-17:30	<p><b>Inv 2: Prof. Dr. Ersin KILINC</b>, Dicle U/TR</p> <p>siRNA Delivery by Formulated Non-viral Vectors</p>
	<b>23 March, 2021</b>
	<b>Session 1-</b> Chairs: <b>Prof. Dr. Durisehvar Unal- Prof. Dr. Dilsad Ariksoysal</b>
10:00-10:30	<p><b>Inv. 3: Prof. Dr. Sibel A. OZKAN</b>-Ankara U/TR</p> <p>Nanostructured Materials-Based Nanosensors for Sensitive Monitoring of Pharmaceuticals</p>
10:30-10:40	Tea/Coffee break
10:40-11:10	<p><b>Inv 4: Prof. Dr. Yusuf DILGIN</b>-Canakkale 18 Mart U/TR</p> <p>Chemically modified disposable electrodes as amperometric sensors and biosensors in Flow Injection Analysis</p>
11:15-12:05	<p><b>OP1-Andrzej Leniart</b>-Lodz U/PL</p> <p>Preparing, characterization and application of glassy carbon electrode to the determination of Fluometuron</p> <p><b>OP2- Melike BİLGİ KAMAÇ</b>-Çankiri Karatekin U/TR</p> <p>A dual immunosensor for the simultaneous electrochemical detection of CA 125 and HE4</p> <p><b>OP3-Hasret Subak</b>-100. Yıl U/TR</p> <p>Electrochemical Detection of Interaction Between Helichrysum pallasii (Spreng.) Ledeb. and DNA by Using Disposable Biosensors</p> <p><b>OP4- Tugba Oren Varol</b>-Muğla Sıtkı Koçman U/TR</p> <p>A Nanomaterial Enriched Plant Tissue Based Biosensor for Phenol Detection</p> <p><b>OP5- İrem Aydın Kırılancı</b>-Ege U/TR</p> <p>A Label-Free Aptamer Based Impedimetric Biosensor for Ochratoxin A Detection</p>
12:00-14:00	Lunch
	<b>Session 2 -</b> Chairs: <b>Prof. Dr. Belgin Izgi- Assoc. Prof. Dr. Mustafa Celebier</b>
14:00-14:30	<p><b>Inv 5: Prof. Dr. Mutay ASLAN</b>-Akdeniz U/TR</p> <p>Liver-Kidney Functions, Sphingolipid Levels and Inflammation in Experimental ER Stress Model</p>
14:35-15:05	<p><b>Inv 6: Prof. Dr. Perihan UNAK</b>-Ege U/TR</p> <p>Theranostics</p>
15:05-15:30	<p><b>OP6-Mahmure Ayşe Tayman</b>-Ankara Yıldırım B U/TR</p> <p>Investigation of the Interaction between Oral Antiseptic Hormone Reagent</p> <p><b>OP7-Elif Tutun</b>-Ege U/TR</p> <p>Extraction of Hypericin and Hyperforin from St. John's Wort (Hypericum perforatum L.) and Investigation of Wound Healing Potential</p> <p><b>OP8- Songul Ulusoy</b>-Cumhuriyet U/TR</p> <p>Development new solid phase materials for determination of anticancer drugs at trace levels</p>

15:35-15:40	Tea/Coffee break
	<b>Session 3:</b> Chairs: <b>Prof. Dr. Ozlem Sogut- Prof. Dr. Slawomira SKRZYPEK</b>
15:40-16:10	<b>Inv 7: Prof. Dr. Trajce Stafilov-Ss Cyril and Methodius U/MK</b> Studies on Environmental Pollution with Potentially Toxic Elements in the Republic of North Macedonia
16:15-16:45	<b>Inv 8: Prof. Dr. –Mustafa Tuzen-Gaziosmanpasa U/TR</b> Selenium speciation in water and food samples by using green extraction techniques
	<b>OP9-Mais Al-Nidavi-Near East U/TRNC</b> Smartphone digital image colorimetry combined with reversed-phase switchable-hydrophilicity solvent liquid-liquid microextraction for the determination of copper in edible oils
	<b>OP10-Gonul Serdar-Karadeniz Techn. U/TR</b> Microwave Assisted Production of Gold Nanoparticles From Catechin Extract
	<b>OP11- Bahire Filiz Senkal-Istanbul Techn. U/TR</b> <i>Preparation of urethane functional poly (styrene) based sorbent for removing of mercury from water</i>
	<b>OP12- S. Yigit Sain-Kirklareli U/TR</b> Development and validation of an AAS method for quantitation of Class 1 and Class 2A elemental impurities in tablets
16:50-17:40	<b>OP13-Zeynep Atasayar- Mimar Sinan U/TR</b> The Provenance of Iron Age Glass Beads: A Chemical and Isotopic Approach
	<b>24 March, 2021</b>
	<b>Session 4 -</b> Chairs: <b>Prof. Dr. Yusuf Dilgin- Assoc Prof. Dr. Umran Seven Erdemir</b>
10:00-10:30	<b>Inv 9: Prof. Dr. Antony CALOKERINOS-Athen U/GR</b> Recent Developments in the Quality Control of Natural Products
10:30-10:40	Tea/Coffee break
10:40-11:10	<b>Inv 10: Prof. Dr. Arturs VIKSNA-Latvia U/LV</b> Application of chemometric methods for assesment of monofloral and polyfloral honey
	<b>OP14-Etil Guzelmeric-Yeditepe U/TR</b> Chromatographic studies on identification marker of honey originated from Pinus spp.
	<b>OP15- Nur Cebi-Yildiz Techn. U/TR</b> <i>Fish Oil Supplements in Turkey: Discrimination of Natural and Synthetic Forms of Fish Oil by FTIR, Raman and GC-MS Techniques Combined with Chemometrics</i>
	<b>OP16- Tuğba BAYRAKTUTAN- Iğdır U/TR</b> Determination of Photophysical Properties of Coumarin 30 in Biomimic Igepal CO-520 Reverse Micelles
11:10-12:00	<b>OP17- Cemile Yucel- Dokuz Eylul U/TR</b> Development of DLLME-SFOD/GC-MS Method for UV Filter Determination
	<b>OP18- Gülşah ÖZCAN-SİNİR-Uludag U/TR</b> Recent Applications of Analytical Techniques in the Determination of Olive Oil Adulteration
12:00-14:00	Lunch
	<b>Session 5 -:</b> Chairs: <b>Prof. Dr. Mutay Aslan – Dr. Nagihan Karaaslan Ayhan</b>
14:00-14:30	<b>Inv 11: Prof. Dr. Ryszard LOBINSKI-CNRS Pau/FR</b> Mass spectrometry for trace level biological metals speciation analysis
	<b>OP19- Biljana Balabanova-Goce Delčev U/MK</b> Tracking moss bio-indication capacity for anthropogenic elements depositions using SEM analysis
	<b>OP20- Burak Yaman-Gaziantep U/TR</b> Assessment of health risks due to potentially toxic elements intake by infants and toddlers via consumption of Weaning Foods
14:35-15:05	<b>OP21- Emel Akbaba-Firat U/TR</b> Shortening the time of extraction procedure for the determination of health-protective parameters of <i>Rosmarinus officinalis</i> L.
15:05-15:15	Tea/Coffee break
	<b>Session 6 -:</b> Chairs: <b>Prof. Dr. Perihan Unak- Assoc Prof. Dr. Usama Alshana</b>
15:15-15:45	<b>Inv 12: Prof. Dr. Resat APAK- Istanbul U/TR</b> Chemical Reaction Components of Colorimetric Sensor Design, Focusing on Antioxidants and Energetics Detection
15:50-	<b>OP22-Jude Caleb - Near East U/TRNC</b>

16:30	Smartphone digital image colorimetry combined with supramolecular solvent-liquid-liquid microextraction for the determination of curcumin in food samples
	<b>OP23- Gonul Serdar</b> -Karadeniz Techn. U/TR Green Synthesis of Gold Nanoparticles Using Mulberry Leaf Extract
	<b>OP24- Dicle Cevik</b> - Trakya U/TR Search for the Parthenolide Sources in Several Asteraceae Plants and Quality Evaluation of the Marketed Feverfew Supplements by HPTLC
	<b>OP25- Deniz Karatas</b> -Manisa Celal Bayar U/TR Investigation of Paclitaxel Drug Interaction with Molecular Docking
16:30-17:35	<b>OP26- Agnese Osite</b> -Latvia U/LV Impact of wood ash on nitrogen circulation in pine needles, blueberry, and lingonberry plants
	<b>OP27- A. Nosal-Wiercińska</b> - Maria Curie-Skłodowska U/PL Application of cyclically refreshable liquid amalgam film silver based electrode to study the kinetics and mechanism of Bi(III) ion electroreduction in the presence of 2-thiocytosine as regards of the "cap - pair" effect
	<b>OP28- Volkan Yasakci</b> -Ege U/TR A Pilot Study into the Effects of Fluorodeoxyglucose Conjugated Iron Oxide Magnetic Nanoparticles on Adenocarcinoma in Mice, and Lung Cancer Cells in 3D Cell Culture Spheroids
	<b>OP29- Cigdem Ichedef</b> -Ege U/TR Stability Constants of Actinide Metal Ions with Selected Polyphenols
	<b>OP30- Ikbal Gozde Kaptanoglu</b> -Ege U/TR Eco-friendly Synthesis of Graphene for Removal of Pollutants from Aquatic Environments
	<b>OP31 Hasan Karadag</b> -Adiyaman U/TR The Decline of Glutathione Reductase Activity by Copper (II) Oxide Nanoparticles and Copper (II) Chloride
	<b>VP1- Alpaslan Enes Oral</b> -Ege U/TR Preparation of Murexide -Alginate Hydrogel Beads For Strontium Detection Using RGB Coordinates
	<b>VP2- Umitcan Sedir</b> -Ege U/TR Permeable Reactive Barrier Design for The Immobilization of Strontium Radionuclide
	<b>OP32- I. Emir Akyildiz</b> - Marmara U/TR Analysis of Isobaric Advanced Glycation End Products Instead of Furosine as an Early Stage Maillard Reaction Product for Quality Assessment of Food Samples
	<b>OP33- I. Emir Akyildiz</b> - Marmara U/TR Targeted Lipidomic Investigation of Royal Jelly Samples as a Rich Source of Sterols and Fatty Acids
	<b>VP3- Merve Bat OZMATARA</b> - Gebze Techn. U/TR Green Synthesis of Iron Nanoparticles Using Galium aparine Extract, Their Characterization and Antioxidant Activity
	<b>25 March, 2021</b>
	<b>Session 7- Chairs: Prof. Dr. Sibel A. Ozkan- Assoc Prof. Dr. Aysu Yarman</b>
	<b>Inv 13: Prof. Dr. Sezgin BAKIRDERE- Yildiz Techn. U/TR</b> Novel Microextraction Strategies for Sensitive and Accurate Determination of Pesticides
	<b>Inv 14: Prof. Dr. Bezhan CHANKVETADZE- Tbilisi U-GE</b> Recent developments in enantioselective analysis of chiral drugs in capillary electromigration techniques
11:15-12:05	<b>OP34-Hakan Erdogan</b> -Health Sci. U/TR Preparation of Peptide Based Low Molecular Weight Hydrogel Drug Carriers
	<b>OP35-A. Emin Atik</b> -Acibadem U-Turgut Pharm/TR Analytical Characterization for the Comparability Evaluation of TUR03 Drug Substance and Innovator Lots by Mass Spectr.
	<b>OP36-Nagihan Karaaslan Ayhan</b> -Munzur U/TR Evaluation of antioxidant properties of yarrow depending on extraction mixing techniques
	<b>OP37- Ozlem Oz</b> -Harran U/TR Experimental Approach to Drug Interference in Biotinidase Assay with LC-MS / MS
	<b>OP38- Murat Caglayan</b> - Health Sci. U/TR The Effect of Contrast Media on Biotinidase Analysis Performed with Dry Spot Test
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	<b>Session 8- Chairs: Prof. Dr. Nil Ertas- Assoc Prof. Dr. Emine Akyuz Turumtay</b>
14:00-14:30	<b>Inv 15: Prof. Dr. Mustafa CULHA – South Florida U/TR</b> Surface-enhanced Raman Scattering for Early Cancer Detection and Cancer Diagnosis
14:35-15:05	<b>Inv 16: Prof. Dr. Mustafa Kemal SEZGINTURK–Canakkale 18 Mart U/TR</b> Lateral Flow Assays towards point-of-care COVID-19 detection
15:10-15:30	<b>OP40-H. Mehmet Kayili- Karabuk U/TR</b> In-depth Profiling of Milk Whey N-glycans by MALDI-MS using Linkage-Specific Sialic Acid Esterification Approach
15:30-15:40	<b>OP41- Tayyibe Beyza YÜCEL-Giresun U/TR</b> PAH analysis of mussel samples collected from Giresun coastline-Black Sea
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	<b>Session 9- Chairs: Prof. Dr. B. Filiz Senkal- Dr. Emel Akbaba</b>
15:40-16:10	<b>Inv 17: Dr. Lukasz Poltorak-Lodz U/PL</b> Electroanalytical Applications of Miniaturized and Electrified Liquid-Liquid Interfaces
16:15-16:45	<b>OP42- Marina Serin -Ege U/TR</b> Aptamer-based Nanobiosensing Strategies Using Graphene Oxide Devoted for Clinical Analysis
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	<b>VP5-Ozlem Faiz- Recep Tayyip Erdogan U/TR</b> Investigation Of Biological Activities Of Water And Methanol Extracts Of Hazelnut Leaf-Blueberry (Ericaceae), Buckwheat - Sorrel (Polygonaceae) Species The Role Of These Species In Our Nutrition
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	<b>Session 10- Chairs: Prof. Dr. Sezgin Bakirdere- Prof. Dr. Şule AYTAŞ</b>
10:00-10:30	<b>Inv 18: Dr. Barbara BURNAT-Lodz U/PL</b> Carbon ceramic electrode as a powerful tool for electroanalysis
10:35-12:05	<b>OP45- Nur Cebi-Yildiz Techn. U/TR</b> The Famous Turkish Rose Essential Oil: Characterization and Authenticity Monitoring by FTIR, Raman and GC-MS Techniques Combined with Chemometrics
	<b>OP46- Fatma Kursun Baysak-Kirklareli U/TR</b> Pervaporation Separation of Ethyl Alcohol–Water Liquid Mixtures Using Poly(vinyl alcohol)/(Chitosan-g-Poly(N,N-Dimethylacrylamide)) Membranes
	<b>OP47-Hakan Serbest- Yildiz Techn. U/TR</b> Development of a Sensitive Analytical Method for the Determination of Rhodium in Metal Coating Wastewater Samples using Microextraction Strategy-Slotted Quartz Tube-Flame Atomic Absorption Spectroscopy
	<b>OP48- Nurdan Kurnaz Yetim- Kirklareli U/TR</b> Color removal of dyes using immobilized enzyme
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## INVITED SPEAKERS (IS)

### IS1- Bioanalytics from Micro- to Nano- Dimension

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The biologically active compounds are present in biological samples in a relatively low concentration levels. Hence, an essential step is the isolation from complex matrices (whole blood, tissue) and enrich them before the final determination. From a medical point of view, the routine analysis of xenoestrogens, which are one of the causes of cancer, generates a lot of problems. Nowadays, interest of the analytics of this group of compounds need to be developed effective analytical methodologies and procedures for biological monitoring of xenoestrogens and their derivatives and determination of their biotransformation pathways in the bloodstream.

Solid phase extraction (SPE) with a new generation sorbents (e.g. imprinted polymers, nanoparticles with ferromagnetic properties and *shell* structure) and its miniaturized form solid phase microextraction (SPME) with a new generation fibers are a good alternative for implementation of multidimensional separation techniques (LC×LC-MALDI-TOF/MS<sup>n</sup>). To enlarge specificity/selectivity, sorbents will be modified with appropriate chosen molecules (MIPs – Molecularly Imprinted Polymers), which will create a molecular recognition system (host – guest) or monolithic columns for SPE coupled *on-line* with micro- and/or nanocolumns for micro-liquid [micro-LC], electro-chromatography (CEC) or capillary electrophoresis (CZE). Fundamental research are focused on two main areas: (i) macromolecular and supramolecular chemistry and research of the relationship between the structure and properties of polymers and (ii) biotechnology combined with enzymatic applied microbiology. It gives the possibility to track the impact of the enzyme-substrate or antigen-antibody complexes in biological systems. Such approach allows to increase the selectivity and reduce the limit of detection of analyzed compounds as well as automation of determination process.

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#### Professor Bogusław Buszewski

Professor Dr. Bogusław Buszewski graduated at the Maria Curie Skłodowska University, Lublin. In 1986 he has received PhD degree and in 1992 the Doctor of Sciences (habilitation) degree. In 1994 he has received professor position in Nicolaus Copernicus University, Toruń. He has been Humboldt Fellow at Tübingen University (Germany) and visiting professor at several universities in Europe, Asia and the USA. His main scientific interests are concerned with chromatography and related techniques, spectroscopy (HPLC, GC, CZE, FFF, MS), adsorption, sample preparation, environmental and bioanalysis (metabolomics, proteomics, biomarkers), nanotechnology and chemometry. He is author or co-author of 15 books, patents and more than 650 scientific papers (over 15.000 citations, h=56) and member of the editorial boards of 26 national and international journals. Prof. Buszewski is the president of the *Central European Group for Separation Sciences* and the chairman of the *Committee of Analytical Chemistry of Polish Academy of Sciences*. He is a member of Polish Academy of Sciences and European Academy of Sciences and Arts. He was awarded by numerous national and international organizations (including *Doctor Honoris Causa* multiple).



## IS2- siRNA Delivery by Formulated Non-viral Vectors

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The RNAi process is occurred by the incorporation of specific siRNA to the specific target messenger RNA (mRNA) as a means of inhibiting the synthesis of the encoded protein. After its discovery, RNAi has attained special interest due to its spesificity. Thus, it become a powerful tool to study gene functions in biological processes. The ability to induce RNAi in mammalian cells using synthetic small interfering RNA (siRNA) have recently emerged as a promising nucleic acid agent for treating various diseases such as cancer, because of their superior ability to silence target genes in a specific manner<sup>1</sup>.

It was shown that, siRNAs have shown promise for treating a variety of diseases, including influenza and HIV infection, cancer and genetic defects. Although there are many siRNA-based products close to commercialization, efficient intracellular siRNA delivery strategies are urgently required for its potential therapeutic use in targeted cell that is not still accomplished fully because of internalization and intracellular trafficking problems such as low transfection efficiency, low efficiency of carrier agent, low efficiency due to systematic distrubution, enzyme degradation, inappropriate subcellular localization, and endosomal trapping of siRNAs in cells. siRNA cannot cross the cytoplasmic membrane by free diffusion. In the circulation and interstitial space, siRNA is vulnerable to degradation by RNase. Although siRNA can be delivered directly and locally to the target sites in limited applications, a carrier vector is needed to protect siRNA from degradation and efficient targeting <sup>2</sup>.

As carrier system usually contains a key cationic component as a transfection reagent, such as a cationic lipid, a cationic polymer or a cationic peptide, in order to bind siRNA effectively due to its negative surface charge. Other supplementary components help to improve the stability, solubility or pharmacological profiles of siRNA-carrier complexes.

**Keywords:** Non-viral vector, siRNA delivery, targeted cancer therapy

### *Acknowledgment*

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### **IS3- Nanostructured Materials-Based Nanosensors for Sensitive Monitoring of Pharmaceuticals**

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Nanomaterials exhibit properties that are quite different from those of materials at large scales. The objects under study in nanotechnology are the nanomaterials, also called as nanostructured materials. All materials are composed of grains, which in turn are made of molecules and atoms. Nanomaterials are those having grain sizes in the range of nanometers. Nanotechnology has become very popular in the sensor fields. It is thought that the utilization of such technologies, as well as the use of nanosized materials, could well have beneficial effects for the performance of sensors.

Nanotechnology has become very popular in the sensor fields in recent times. It is thought that the utilization of such technologies, as well as the use of nanosized materials, could well have beneficial effects for the performance of sensors. Nano-sized materials have been shown to have a number of novel and interesting physical and chemical properties. There exist various materials of different types for fabricating nanosensors. Especially, functional carbon-based nanomaterials have become important due to their unique combinations of chemical and physical properties, extensive research efforts are being made to utilize these materials for various industrial applications, such as high-strength materials and electronics. These advantageous properties of carbon-based nanomaterials are also actively investigated in several areas of biomedical and drug assay.

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. Nowadays, a lot of different analytical methods are used in environmental, pharmaceutical, or clinical laboratories and also a number of the commercial point-of-care devices work using nanosensors.

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. In recent years, carbon based nanosensors have great use of in pharmaceutical application, further for real sample applications like dosage forms, human body fluids etc.

## IS4- Chemically modified disposable electrodes as amperometric sensors and biosensors in flow injection analysis

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Modification of electrodes is a very useful procedure used in electroanalytical chemistry because modified electrodes exhibit a remarkable improvement in the sensitivity, selectivity, stability, precision, and accuracy of sensors or biosensors compared to unmodified electrodes<sup>1</sup>. Therefore, chemically modified electrodes (CMEs) have gained great attention in electrochemical studies in the last two decades. CMEs can be prepared from various materials such as conducting polymers, metal nanoparticles, carbon nanotubes, bio-recognition elements, quantum dots, graphene oxide using different modification strategies. The choice of the electrode material can also affect the cost, selectivity, sensitivity, and stability of an electrochemical sensor/biosensor. Recently, chemically modified disposable electrodes such as pencil graphite electrode (PGE), indium tin oxide (ITO) electrode and screen printed electrodes (SPEs) have found great attention in electrochemical studies due to their advantages such as their high electrochemical reactivity, commercial availability, good mechanical rigidity, lower cost and the ease of modification<sup>2</sup>. Another useful approach in the electrochemical sensors and biosensors is the use of the Flow Injection Analysis (FIA) procedure with modified electrodes in electrochemical techniques<sup>1</sup>. FIA has some advantages in routine analytical determinations, such as very limited sample consumption and short analysis time. In this context, this study describes the modification of disposable electrodes (especially PGE) with various materials such as quantum dots, organic redox mediators, metal nanoparticles, conductive polymers, and characterization of these modified electrodes with SEM, EDX, XRD, AFM, etc. For example, Fig 1 shows that Cu(II) bis neocuproine complex (CUPRAC reagent) was successfully modified on Nafion (Nf) adsorbed PGE. Moreover, FI amperometric sensors and biosensors for the detection of various analytes such as glucose, H<sub>2</sub>O<sub>2</sub>, hydrazine, NADH have been performed by using modified disposable electrodes, home-made flow cells for PGE, and commercially purchased wall jet type flow cell for SPEs. As a result, highly sensitive, selective, rapid, low-cost, and disposable electrochemical sensors and biosensors have been constructed by combining three useful components (disposable electrode, FIA, and modification materials).

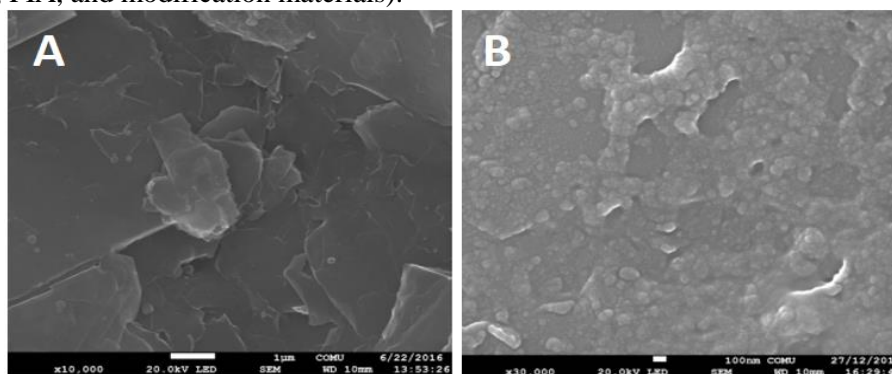


Figure 1: The SEM images of disposable PGE (A) and CUPRAC reagent modified Nf-PGE (B)<sup>2</sup>

**Keywords:** Disposable electrodes; modification; flow injection analysis; electrochemical sensors/biosensors

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## ISS- Liver-Kidney Functions, Sphingolipid Levels and Inflammation in Experimental ER Stress Model

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### Abstract

**Objectives:** Disorders of the endoplasmic reticulum (ER) lead to cellular damage but can cause cell death if ER dysfunction is prolonged. We aimed to examine liver/kidney functions, neutral sphingomyelinase (N-SMase) activity, sphingolipid levels, cytosolic phospholipase A2 (cPLA2) and cyclooxygenase-2 (COX-2) protein expression in rats under ER stress.

**Materials and Methods:** ER stress was induced by tunicamycin (TM) and the ER stress inhibitor taurodeoxycholic acid (TUDCA) was injected before induction of ER stress. ER stress was confirmed by increased tissue levels of GRP78. Hematological and biochemical profiles were measured by autoanalyzers while hepatic and renal injury was evaluated via microscopy and histopathological scoring. Tissue levels of C16-C24 sphingomyelins (SM), C16-C24 ceramides (CERs) and sphingosine-1-phosphate (S1P) were determined by LC-MS/MS. Tissue cPLA2 and COX-2 were measured by western blot and activity assays.

**Results:** Tunicamycin treatment caused kidney and liver function test abnormalities, increased hematocrit and hemoglobin levels but decreased white blood cell counts. Histopathological findings showed hepatic necroinflammation and renal tubular damage in rats treated with TM. TUDCA administration attenuated WBC abnormalities and TM- induced hepatic/renal functional impairment in ER stress, as evident by significantly restored serum ALT, AST, creatinine, and total bilirubin levels. A significant increase was observed in N-SMase activity, tissue levels of C16-C24 CERs, cPLA2 and COX-2 expression in liver and kidney tissue under ER stress. TUDCA administration decreased tissue CER levels, cPLA2 and COX-2 expression as well as prostaglandin E2 (PGE2) formation.

**Conclusion:** These results signify that ER stress causes hepatic and renal toxicity as well as CER-induced PGE2 formation in liver and kidney.

**Funding:** This study was supported by a grant [No: TSA-2019-4280] from Akdeniz University Research Foundation.

**Key words:** Endoplasmic reticulum stress, ceramide, cytosolic phospholipase A2, cyclooxygenase-2

### INTRODUCTION

ER stress is reported to contribute to liver diseases such as nonalcoholic/alcoholic fatty liver and hepatic fibrosis. Recent studies have also shown that ER stress is implicated in the initiation of numerous kidney diseases such as renal fibrosis, diabetic nephropathy, inflammation-induced kidney injury and renal ischemia-reperfusion. The complexity of how ER stress impacts on hepatocyte and renal injury needs to be further clarified (1).

Tunicamycin (TM) is a nucleotide sugar analog. It inhibits the enzyme that catalyzes the first step of protein glycosylation. As a result of inhibition, N-linked glycosylation is blocked, resulting in the accumulation of misfolded proteins in the ER. This situation induces ER stress. Tunicamycin not only induces ER stress but also provides metabolic changes associated with ER stress. Taurodeoxycholic acid (TUDCA), A hydrophilic bile acid and is given as a treatment in cholestatic liver diseases. TUDCA, a chemical molecular chaperone, can prevent unfolded protein response and suppress ER stress (2).

Lipotoxicity in endoplasmic reticulum stress can occur. Lipotoxicity causes the accumulation of lipids and their intermediate metabolites in the cell is termed lipotoxicity and can occur under ER stress. Sphingolipid intermediate metabolites such as ceramide are toxic when they accumulate in cell membranes. They change the structure and organization of the membrane. This condition initiates metabolic dysfunction in peripheral organs such as liver and kidney. The aim of this study was to investigate the link between lipotoxicity and inflammation due to endoplasmic reticulum stress. For this purpose; A rat model of ER stress was created. Histopathological examination of

liver and kidney tissues were performed. Liver / kidney functions were evaluated. Hematological profile was viewed. Liver and kidney neutral sphingomyelinase (N-SMase) enzyme activity was measured. Lipidomic sphingolipid analysis was performed in liver and kidney tissues. Cytosolic phospholipase A2 (cPLA2), COX-2 protein expression and PGE<sub>2</sub> formation were evaluated in liver and kidney tissues (3).

## MATERIAL AND METHODS

Four main groups were formed in the study. Control group (n = 10), tunicamycin (TM) treated group (n = 10), TUDCA treated group (n = 10), TM + TUDCA (n = 10) treated group. The dose of TM selected in the experimental model used was based on the doses used to study ER stress in animal models. Liver and kidney tissues were removed 24 hours after TM administration. Rats were anesthetized by giving a mixture of ketamine and xylazine hydrochloride before blood and tissues were collected. Blood samples were taken before liver and kidney dissections. TM was i.p. injected and given 1 mg/kg body weight. TM was dissolved in 1ml DMSO. TUDCA was i.p. injected and given 250 mg/kg body weight. TUDCA was dissolved in 1 ml of saline. In the TM + TUDCA group (n=10) TUDCA was injected (i.p.) at a dose of 250 mg/kg body weight 30 minutes before induction of ER stress. Control group animals received an equal volume of i.p. DMSO and saline. Demonstration of liver and kidney damage: hematoxylin-eosin (H&E) staining/necroinflammation was evaluated according to the Modified Ishak Hepatic Activity Index. ER stress markers were evaluated by glucose-regulated protein 78 (GRP78) enzyme immunoassay. Hematological and biochemical measurements were done by autoanalyzer (Fujifilm DRI-CHEM NX500 / Mindray BC-2800 Auto Hematology Analyzer). Sphingolipid measurements were done by LC / MSMS analysis (LCMS-8040 Shimadzu). Tissue cPLA2 and COX-2 quantification was measured by activity assays and western blot analysis. Prostaglandin E2 measurement was performed via enzyme immunoassay. Protein Measurements were done by. Modified Bradford method. Statistical Analysis was done by Sigma Stat 2.03 Windows XP program.

## RESULTS AND DISCUSSION

**Liver Damage:** In our study, portal inflammation, confluent and lytic necrosis were observed in rats given TM. Tunicamycin also caused significant hypoalbuminemia, significant increases in TBIL, ALT, and AST, revealing liver irregularities. The mechanism of tunicamycin induced liver damage is still unknown in all aspects. Inflammasome activation is blamed. Hepatic ROS generation has also been reported. Our results show that TUDCA provides partial protection against liver damage caused by TM toxicity. TUDCA lowered ALT and AST levels and normalized TBIL levels.

**Kidney Damage:** The presence of ER stress markers in renal biopsies has been shown to be associated with the severity of acute kidney injury (4). Decreased kidney function was manifested by increased BUN and CRE levels. TUDCA caused partial restoration of kidney damage by reversing the observed increase in both BUN and CRE levels.

**Hematological Parameters in Experimental Groups:** Tunicamycin and/or TUDCA treatment increased RBC, HGB and HCT levels. Tunicamycin has previously been reported to cause a 2.5 to 4-fold increase in erythropoietin (EPO) binding (5). This increase in EPO binding is probably due to decreased EPO receptor-N-glycosylation due to TM therapy. As observed, this can be expected to cause an increase in RBC, HGB and HCT levels. TUDCA has been reported to increase the number of immature erythroid cells, which can lead to increased levels of RBC, HGB and HCT as observed in rats treated with TUDCA (6). TM therapy reduced WBC numbers. Decreased WBC numbers in TM treated rats may be associated with impaired protein N-

glycosylation. N-glycosylation is known to play an important role in lymphocyte differentiation and the development of immune-mediated inflammatory diseases (7). TUDCA is known to maintain lymphocyte homeostasis by significantly reducing lymphocyte apoptosis, and this may be the mechanism that results in relatively stable WBC counts as observed here (8).

**Ceramide Accumulation During ER Stress:** This is the first study reporting increased ceramide levels in a rat model of ER stress induced by TM treatment.

## CONCLUSION:

ER stress in liver and kidney, increased N-SMase activity. Increased CER levels. Caused cPLA2 and COX activation. TUDCA reduced ER stress in liver and kidney tissue. Decreased N-SMase activity in ER stressed liver and kidney. Decreased CERs, cPLA2, COX and PGE2 levels. Our study reveals for the first time that there is a significant increase in N-SMase activity, C16-C24 CER tissue levels, cPLA2 and COX-2 expression in liver and kidney tissues under ER stress.

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## IS6- Theranostics

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**Abstract**-The term theranostic describes technology with concurrent and complementary diagnostic and therapeutic capabilities. In the nuclear medicine the theranostic approach can be realized by using the same targeting platform labelled by gamma or positron emitter for diagnosis and labelled by corpuscular radiation emitters ( $\alpha$  or  $\beta$ -) for therapy. Application of the theranostic nanoparticles hold the potential to revolutionize future disease management. Since the last decade, there has been a growing interest in the engineering of various kinds of theranostic nanoparticles for simultaneous cancer imaging and therapy. Radiolabeling and efficient targeting of theranostic nanoparticles to the tumor site is critical for both diagnostic and therapeutic purposes. However, difficulties still exist in the engineering of biocompatible theranostic nanoparticles with highly specific *in vivo* tumor targeting capabilities.

In terms of radiochemistry and radiopharmacy, the development of radionuclide pairs, which have the same chemical structure but can be used in both diagnosis and treatment with different decomposition pathways, has a special importance in the creation of new generation theranostics. In this way, it is possible to monitor both diagnosis and treatment with the same probe. For example, the application of diagnostic and therapeutic radionuclides of the same element (radioisotopes) would utilize chemically identical radiopharmaceuticals for imaging and subsequent treatment, thereby enabling the radiotheranostic concept. Examples of current situation on radionuclide-labeled nanoparticles with theranostic potential will be given in this presentation.

**Keywords:** *Theranostic, Radiopharmaceutical, PET, SPECT, radionuclide therapy*

## Introduction

Theranostics contain multiple components for the benefit of the patient for personalized therapy. Nowadays, various nanoparticles (NPs) have been developed to diagnose and treat cancer with the contribution of nanomedicine. These nanoparticles can provide support for the creation of theranostics that combine multiple properties needed by personal medicine. However, very few of these nanoparticles have Food and Drug Administration (FDA) approval and have been used in humans. Recently, efforts have been made to develop nanoparticles for theranostic purposes with emerging approaches. As a new generation nano-carrier system, theranostic NPs not only offer patients a variety of treatment options, but also monitor therapeutic efficacy following treatments. Therefore, it can accelerate clinicians' personalized treatment decisions. Today, many NPs have been used as medical agents for cancer diagnosis and treatment, including quantum dots, magnetic NPs, carbon nanotubes, gold nanostructures, polymeric NPs, and silica NPs<sup>1-4</sup>.

This extended summary outlines the theranostic potential of  $\text{Fe}_3\text{O}_4$ ,  $\text{Pt@TiO}_2$ ,  $\text{MnO}$  nanoparticles labelled with several theranostic radionuclides such as Sc-46, Tc-99m, Zr-89.

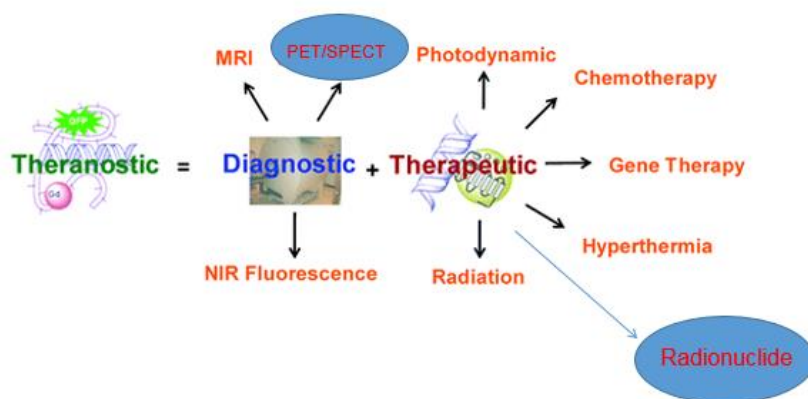


Figure-1 Multifunctional Theranostics



## Material and Methods

All nanoparticles and nanoconjugates were robustly synthesized according to literature methods.<sup>1-4</sup>

For characterization, size and morphologies were determined using HPLC, DLS, TEM. It is aimed to have a potential for SPECT (Single Photon Emission Tomography) by radiolabeling with nanoconjugate <sup>99m</sup>Tc and <sup>46</sup>Sc and for PET (Positron Emission Tomography) imaging by radiolabeling with <sup>89</sup>Zr. Stability and lipophilicity studies were carried out with radiolabeled samples, which were quality controlled by HPLC and TLRC, and cell studies were started with PC3, LNCaP and RWPE-1 cells. Cytotoxicity, cell involvement, photodynamic therapy / radiotherapy simulation and ELISA studies were carried out within the scope of cell studies. Based on the data obtained from cell studies, immunocompromised animals and PC3 and LNCaP xenograft tumor models were developed and PET images were performed with male mice injected with <sup>89</sup>Zr radiolabeled nanoconjugate.

## Results and Discussion

The application of diagnostic and therapeutic radionuclides of the same element (radioisotopes) would utilize chemically-identical radiopharmaceuticals for imaging and subsequent treatment, thereby enabling the radiotheranostic concept. Scandium presents theranostic potential with three radioisotopes. <sup>43</sup>Sc ( $T_{1/2}$  = 3.9 h) and <sup>44</sup>Sc ( $T_{1/2}$  = 4.0 h) can both be used for PET, while <sup>47</sup>Sc ( $T_{1/2}$  = 3.35 d) is the therapeutic match—also suitable for SPECT. Currently, <sup>44</sup>Sc is most advanced in terms of production, as well as with pre-clinical investigations, and has already been employed in proof-of-concept studies in patients. The development of <sup>47</sup>Sc is still in its infancy, however, its therapeutic potential has been demonstrated preclinically. Due to relatively short of the half-life (<sup>47</sup>Sc), the study of chemical nature and its stability of scandium are mostly common using <sup>46</sup>Sc instead of <sup>47</sup>Sc even both had similar chemical behavior, but only had different half-life ( $T_{1/2}$  = 83 days).

For that, we aim to synthesis new multifunctional theranostic probes labeled with <sup>44/47/46</sup>Sc and study interaction of these probes with prostate cancer cells. This probe based on Super Paramagnetic Iron Oxide (SPIO) nanoparticles (with HPG and pSMA modification) are intended to target PSMA(+) prostate cancer cells. Thus while prostate tumours can be imaged with PET/MRI systems with <sup>44</sup>Sc labeled PSMA conjugated Fe<sub>3</sub>O<sub>4</sub>/SiO<sub>2</sub>-HPG-NH<sub>2</sub> probe, its <sup>47</sup>Sc derivative with the same chemistry can be used for therapy. In addition to designed probe has a potential for magnetic hyperthermia for local therapy of tumors with magnetic field.

Tekin et al reported that the Pt-TiO<sub>2</sub>-Spx nanoconjugates, it was observed that the synthesized Pt-TiO<sub>2</sub>-Spx nanoconjugate decreased the survival rate in PC3 and LNCaP cells<sup>3</sup>. The fact that nanoconjugate shows toxicity in prostate cancer cells but does not affect healthy cells is explained by the knowledge that Spx molecule works through the SRPK inhibition mechanism in cancer cells. This result was visually supported by AFM images. Since this mechanism works on cancer cells, the nanoconjugate has been selective to cancer cells. The cell uptake results are also in line with the toxicity results, with radioiodine and <sup>89</sup>Zr, the radiolabeled nanoconjugate is highly retained by PC3 and LNCaP cells, while not showing affinity for RWPE-1 cells. According to the result of photodynamic therapy / radiotherapy simulation; It has been observed that nanoconjugate increases radiation damage when stimulated with light in prostate cancer cells and cell death increases because of increased damage. In RWPE-1, which is a healthy prostate cell, it has been observed that the survival rate is higher than prostate cancer cells. This situation is very important in terms of target / non-target tissue ratio *in vivo* applications. According to the results of ELISA studies, VEGF165b ratio, which is the anti-angiogenic form of VEGF165 in the medium containing metabolic wastes of cells, increased after the application of nanoconjugate, and this situation did not lose its anti-angiogenesis feature after the nanoconjugation. As a result of animal studies, it was observed that <sup>89</sup>Zr-Pt-TiO<sub>2</sub>-Spx had a greater effect on PC3 xenograft tumor model compared to LNCaP and no involvement was observed in vital organs. The *in vitro* and *in vivo* data obtained revealed that the designed Pt-TiO<sub>2</sub>-Spx nanoconjugate radiolabeled with radioiodine and <sup>89</sup>Zr has a strong potential for multimodal diagnosis and treatment of prostate cancer, especially in terms of target / non-target tissue ratio.

Diethylenetriaminepentaacetic acid (DTPA) conjugated manganese oxide nanoparticles (MnO NPs) were synthesized and encapsulated with protoporphyrin-IX (PP(IX)) encapsulated with niosomes<sup>4</sup>. Niosome-MnO-DTPA-PP(IX)) nanoconjugates present fluorescence properties and MR imaging and photodynamic and radiotherapy potential efficiency<sup>4</sup>.

### Conclusion

Theranostics contain multiple components for the benefit of the patient for personalized therapy. Nowadays, various nanoparticles (NPs) have been developed to diagnose and treat cancer with the contribution of nanomedicine. These nanoparticles can provide support for the creation of theranostics that combine multiple properties needed by personal medicine.

**Acknowledgement:** The work titled 'Radiobioconjugates of magnetic nanoparticles labelled with <sup>44</sup>Sc and <sup>47</sup>Sc for theranostic application' was supported financially by TUBITAK Project No. 218S749. Authors thank to TUBITAK for the support. The Scientific and Technological Research Council of Turkey for their support within the scope of the Bilateral Cooperation project no. 218S749. Volkan Yasakçı was also supported with 100/2000 CoHE Doctorate Scholarship and Turkish Higher Education Council (YÖK) 100/2000 Scholarship. The authors thank to the Turkish Higher Education Council (YÖK) and 100/2000 CoHE PhD for their support.

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## **IS7- Studies on Environmental Pollution with Potentially Toxic Elements in the Republic of North Macedonia**

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Anthropogenic environmental changes, associated mainly with chemical pollution, lead to a degradation of the natural human environment. Among all chemical pollutants, potentially toxic elements (PTEs) are of a special ecological, biological and health significance. Soil, as a part of the ecosystem, is vital for the survival of mankind which is closely connected to its productivity. Therefore, the surveys of the pollution with PTEs of soil, waters, sediments, air and food on the whole territory of North Macedonia were performed. For that purpose, various spectroscopic techniques such as (atomic absorption spectrometry (AAS), inductively coupled plasma - atomic emission spectrometry (ICP-AES), inductively coupled plasma-mass spectrometry (ICP-MS), and neutron activation analysis (NAA) were applied. Air pollution was investigated by the application of moss biomonitoring and dust samples (attic dust and house-hold dust). The pollution with heavy metals in the particular regions was additionally studied. It has been established that the highest pollution is present in the areas with mining (Pb, Zn, Cu, As, Sb, Ni, Cr) and metallurgical activities (Pb, Zn, Cd, Fe-Ni, Fe-Cr, Fe-Si, Fe, steel), as well as in the area of thermoelectric power plants. High contents of some heavy metals were also found in the areas where their contents usually vary gradually across the geochemical landscape and depend on the geochemistry of the underlying lithology. The obtained data are statistically processed and spatial distribution maps for each specific element are prepared to give a proper interpretation of the obtained results.

**Keywords:** Potentially toxic elements, environmental pollution, air, water, soil, food, North Macedonia

## **IS8- Selenium speciation in water and food samples by using green extraction techniques**

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Selenium is known essential trace element for human and animal health. High concentration of selenium can be toxic effect. Selenium is found two different oxidation states as Se(IV) and Se(VI). The toxicity of Se(IV) is higher than Se(VI). So, speciation of selenium in water samples is very important rather than determination of its total concentration. Direct determination of traces analyte ions in atomic absorption spectrometry (AAS) is difficult because of low detection limit and matrix ions. In order to solve these problems, several separations and preconcentration methods such as solid phase microextraction and dispersive liquid liquid microextraction have been used [1].

In this study, selenium speciation studies will be discussed by using green extraction techniques. Ultrasound-assisted liquid phase microextraction (UALPME) based on environmentally friendly extractants, deep eutectic solvent (DES) was used for speciation of selenium [2]. Solidified floating organic drop microextraction was used for speciation of Se (IV) and Se (VI) in water samples prior to electrothermal atomic absorption spectrometry [3]. Ultrasound-assisted ionic liquid dispersive liquid-liquid microextraction combined with graphite furnace atomic absorption spectrometry was used for selenium speciation in foods and beverages [4]. Switchable liquid-liquid microextraction method was developed for assessment of selenium in food and soft drink samples by using hydride generation atomic absorption spectrometry [5]. Synthesized polystyrene-g-polyoleic acid-g-polyethylene glycol graft copolymer was used as adsorbent in the solid phase microextraction of selenium ions by using electrothermal atomic absorption spectrometry [6].

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## IS9- Recent Developments in the Quality Control of Natural Products

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One of the oldest remedies for humans are natural products which can be used either as medicine products or nutrient supplements. During the last decades, the beneficial properties of natural products have been re-discovered and consumption is increasing dramatically mainly because they are considered safe for consumption. The interest of researchers in studying the properties of natural products can be shown by the increasing number of scientific contributions to special issues for natural products [1]. Nevertheless, since natural products are plant-driven and properties may vary according to weather conditions, soil composition, etc, their chemical composition should be carefully monitored. It is important to establish their beneficial properties such as antioxidant activity as well as the nature and content of important compounds such as polyphenols, sugars, etc.

The evolution of Analytical Chemistry during the last few decades is noteworthy and now we have techniques and methods which can be used for the selective and sensitive determination of all parameters required for the establishment of the nutritive value of every natural product. Wines [2], honey [3], date-pits [4], chestnuts [5] and many others have been investigated in detail and will be presented and discussed during the lecture.

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Samir Scandar, Spyros Grigorakis, Sofia Loupassaki, and Antony Calokerinos (submitted)

## IS10- Application of chemometric methods for assesment of monofloral and polyfloral honey

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Elemental composition of plants is not constant. Among others, temporal variations of concentrations of different elements are of special interest. Till now, however, little is known about short-term, for example, over a period of hours, variations of element concentrations in plants and in the rhizosphere soil.

Honey is relatively high-priced sweetener that floral origins can be from one plant or several. 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 [1]. In order to inform consumers different instrumental and chemometric methods are performed to do that.

60 honey samples from origins of Latvia were analysed. Isotope ratio mass spectrometry (IRMS) was performed to determine carbon and nitrogen isotopes ratio. Liquid chromatography–high performance mass spectrometry was performed to quantify 18 different polyphenols and flavonoids. NMR working frequency of 300 MHz for <sup>1</sup>H was performed to acquire <sup>1</sup>H-NMR spectra from 0 to 10 ppm with bin width of 0,005 ppm. FT-IR spectrum was acquired using attenuated total reflectance (ATR) mode.

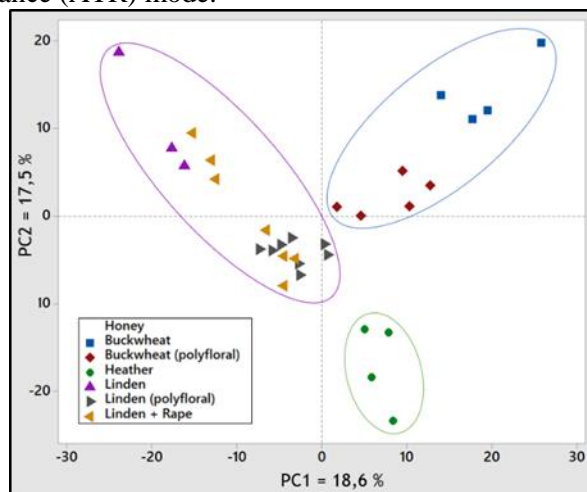


Fig. 1. 2D scatterplot construed from principal component values of <sup>1</sup>H-NMR spectra of monofloral buckwheat, heather, linden honey and polyfloral honey containing linden, rape or buckwheat honey.

The results show that the  $\delta^{15}\text{N}$  values might be useful indicator for heather honey due values were statistically different after ANOVA one way test. Construed PCA scatterplots from <sup>1</sup>H-NMR data shows best results to distinguish monofloral from polyfloral honey and do assesment of botanical origin of polyfloral honey.

**Keywords:** Trace elements; Macronutrients; Natural grasses; Rhizosphere soil; Temporal variability

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## **IS11- Mass spectrometry for trace level biological metals speciation analysis**

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Metal species play an essential role in maintaining the structure and the enzymatic activity of biological cells. However, an excess of certain metals can lead to severe toxicity because of metal substitutions in enzymes and oxidative damage. As a consequence, it is crucial to determine the nature of metal species to better comprehend their fate and their role in organisms.

Generally associated with organic molecules, metals are usually present in low concentrations (nM to  $\mu$ M ranges) and occur distributed in a plethora of chemical forms with various properties (covalent or noncovalent species and a wide range of molecular weights). These species are often unstable (labile and prone to oxidation), which makes the analysis cumbersome.

Whereas covalent species can be approached by canonical metabolomic procedures, the identification of noncovalent metal complexes at basal concentrations remains a challenge. X-ray absorption spectroscopy (XAS) and micro-X-ray fluorescence ( $\mu$ XRF) techniques are of particular interest to obtain information on the valence, coordination environment and spatial localization of elements within intact tissues but they have a number of limitations. These include low sensitivity rendering the analysis of biological fluids at basal concentrations virtually impossible, and the critical dependence on a priori knowledge of the nature of the metal complexes present because their signals have to be compared with appropriate standards. *De novo* identification of previously unreported species and detection of minor species are therefore difficult to achieve.

An alternative is liquid chromatography with mass spectrometric (MS) detection which has been developed largely for metabolomics during the last decade. However, successful applications to metal-speciation in biological samples in-vivo have been rare and limited mostly to single elements with few ligands at relatively high concentrations. The reasons are the difficult-to-control dissociation of metal complexes during sample preparation and during their exposure to the column stationary phase and electrospray source.

The lecture discusses the recent advances in the development of coupling of chromatographic techniques with dual: elemental (ICP MS) et molecular (electrospray) mass spectrometric detection,<sup>1</sup> highlighting their advantages and limitations about history of the subject.

**Keywords:** mass spectrometry, metals, speciation

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## **IS12- Chemical Reaction Components of Colorimetric Sensor Design, Focusing on Antioxidants and Energetics Detection**

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**Abstract-** Although antioxidants are known as health-beneficial compounds fighting against oxidative stress initiator reactive species, their use cannot be restricted to biological media because they can be used for preserving food and protecting almost every kind of organic substance from oxidation. Thus, it becomes increasingly important to measure antioxidant activity/capacity in different media and complex matrices other than conventional food and biological extracts. Energetic substances include explosives, pyrotechnics and propellants, and their prompt detection is important for the safety and well-being of modern societies exposed to various security threats. Frequently, in field/on site detection of these important substances may require the use of colorimetric sensors and probes enabling naked-eye detection, or low-cost and easy-to-use fluorometric sensors working either in turn-on or turn-off mode. Thus, this presentation will focus on the design strategies and main themes of chemical reaction components of colorimetric sensors and nanoprobe in characterizing antioxidant and energetic substances.

**Keywords:** *Colorimetric Sensor, Nanoprobe, Antioxidant, Energetic Material*

### **Introduction**

For total antioxidant capacity (TAC) determination, chromogenic reagents of conventional electron transfer (ET)-based TAC assays may be impregnated, covalently bound, or electrostatically retained on natural or synthetic membranes for subsequent color development<sup>1-2</sup>. The incorporation of nanotechnology has greatly contributed to colorimetric sensor design due to the superb physico-chemical properties of nanomaterials, including the effects of size, geometry, specific surface area, surface plasmon resonance (SPR) absorption and nanozyme catalysis. As for possible mechanisms of analyte detection, a wide spectrum of physico-chemical interactions between analytes and nanoprobe may be significant, depending on the purpose. Optical nanosensors and nanoprobe will be treated in terms of their different mechanisms of operation<sup>3,4</sup>. Especially aggregation/disaggregation sensors using nanoparticles may have high sensitivity at the expense of selectivity.

### **Materials**

In the developed sensing methods in our laboratories based on nanoparticles, H<sub>2</sub>AuCl<sub>4</sub>, AgNO<sub>3</sub>, FeCl<sub>2</sub>, and FeCl<sub>3</sub> were used for nanoparticle synthesis. Also, sodium citrate, 4-Aminothiophenol (4-ATP) were used as reducing (stabilizing) and modifying agents. In the design of colorimetric sensors, CuCl<sub>2</sub>, neocuproine (Nc), Nafion membrane, N,N-dimethyl-*p*-phenylenediamine (DMPD) were used. NED: (1-naphthyl)ethylenediamine was used for azo-coupling. Nitramine and peroxide-based energetic substances and different classes of antioxidant substances were analyzed.

### **Methods**

#### **Cupric Reducing Antioxidant Capacity (CUPRAC) Membrane Sensor**

A Nafion membrane, cut in dimensions of 4.5×0.5 cm, was immersed in a solution containing 2 mL of 1.0×10<sup>-2</sup> M Cu(II), 2 mL of 7.5×10<sup>-3</sup> M Nc, and 2 mL of 1 M NH<sub>4</sub>Ac and left for 30 minutes. Then, these reagent-impregnated membranes were immersed in the 8.2 mL standard antioxidant or real sample solutions to be analyzed (x mL of sample + (8.2-x) mL of EtOH). Phenolic antioxidants and L-ascorbic acid were prepared at 1 mM concentration in EtOH and water, respectively. After 30 min, absorbance measurements were made against the reference membrane at 450 nm.

#### Silver Nanoparticles Antioxidant Capacity (SNPAC) Method

*Preparation of initial SNP solution:* Fifty milliliters of 1.0 mM AgNO<sub>3</sub> was boiled and then 5 mL of %1 trisodium citrate was added. The solution was continued to boil until its color change to pale yellow.

All antioxidants and L-ascorbic acid were prepared at 1.0 mM concentration in EtOH and water, respectively. The developed SNPAC method is summarized as:

2 mL of initial SNP solution + x mL of antioxidant solution + (0.8-x) mL of H<sub>2</sub>O

total volume = 2.8 mL, measure A<sub>423</sub> against a reagent blank after 30 minutes.

Depending on the concentration of antioxidants, the yellow color of the initial SNP solution was intensified with the reduction of Ag<sup>+</sup>.

#### Modified Gold Nanoparticles (AuNPs) for Analyzing Nitramine Energetic Materials

##### *Hydrolysis of Nitramine Explosives*

A volume of 2 mL of hydrolysis solution (1 M Na<sub>2</sub>CO<sub>3</sub> + 0.4 M NaOH) was added to RDX or HMX solution. For the analysis, RDX solutions at 10–50 mg L<sup>-1</sup> (in water bath at 2.5–25 mg L<sup>-1</sup>) or HMX solutions at 2.5–25 mg L<sup>-1</sup> were used. This mixture was allowed to stand for 30 min for NO<sub>2</sub><sup>-</sup> formation. While RDX was hydrolyzed at room temperature, a 60°C water bath was required for HMX.

##### *Modified Griess (4-ATP–AuNP+NED) Method*

RDX/HMX hydrolyzate + 0.5 mL 1 M HCl + 0.6 mL (in 0.1 mL portions) 5 M HCl + 1 mL 4-ATP–AuNP + 1 mL 1 M H<sub>3</sub>PO<sub>4</sub> (pH ≈ 2) + 0.5 mL 20 mM NED.

The absorbance measurements were made at 565 nm against a reagent blank after 30 min of NED addition.

#### DMPD Membrane Sensor for Determining Triacetone triperoxide (TATP)

The scheme for the method is summarized as follows:

1 mL of ( solution (10-100 mg L<sup>-1</sup> concentrations) + 2 mL of 4 M HCl (allow to stand for 5 min for hydrolysis into H<sub>2</sub>O<sub>2</sub>) + 2 mL of 4 M NaOH + 2 mL of pH 3.6 buffer + 2 mL of 100 mg L<sup>-1</sup> Fe<sub>3</sub>O<sub>4</sub> MNPs + 1 mL of 5×10<sup>-3</sup> M DMPD in this order.

After the addition of DMPD, the Nafion membrane cut in appropriate size was placed in the solution. After 30 min, measurements were made against a blank membrane at 550 nm.

## Results and Discussion

**CUPRAC Sensor:** The developed sensor gave a linear absorbance (A) response to antioxidant solutions over a wide concentration (c) range (Table 1). Most common food and biological antioxidants (three exemplified) could be successfully determined with the CUPRAC sensor.

**Table 1:** The analytical figures of merit and trolox-equivalent antioxidant capacity (TEAC) values with the CUPRAC sensor for selected antioxidants.

Antioxidant	Linear equation and linear range (μM)	TEAC (sensor based CUPRAC)	TEAC (solution based CUPRAC)
Quercetin	$A = 9.88 \times 10^4 c + 0.015$ (0.35–13.00)	4.11	4.38
Ascorbic acid	$A = 1.71 \times 10^4 c - 0.059$ (6.36–79.30)	0.71	0.96
Catechin	$A = 4.61 \times 10^4 c + 0.054$ (0.13–27.00)	1.92	3.09

The TEAC coefficients of the developed CUPRAC sensor gave a good correlation ( $r=0.957$ ) with those of the solution based CUPRAC method. The linearity, precision, and recovery parameters were tested for the CUPRAC sensor and reliable and robust results were obtained. The sensor gave additive absorbance responses in mixtures, complying with Beer's law. As common food ingredients did not interfere with this method, commercial fruit juices could be analyzed successfully without pretreatment.

**SNPAC Method:** Antioxidants were analyzed with the aid of localized surface plasmon resonance (LSPR) absorbance *via* enhancing the LSPR peak of citrate-stabilized silver nanoparticles. The linear equation, linear range ( $\mu\text{M}$ ) and TEAC values with the developed method for three selected antioxidants are tabulated in Table 2:

**Table 2:** The analytical figures of merit and TEAC values for proposed SNPAC method

Antioxidant	Linear equation and linear range ( $\mu\text{M}$ )	TEAC <sub>SNPAC</sub>	TEAC <sub>CUPRAC</sub>
Quercetin	$A = 2.99 \times 10^4 c + 0.01$ (1.33–39.80)	3.83	4.38
Ascorbic acid	$A = 1.13 \times 10^4 c + 0.04$ (0.88–103.00)	1.44	0.96
Catechin	$A = 2.82 \times 10^4 c + 0.04$ (0.35–41.13)	3.61	3.09

Although most literature methods exploiting the formation of silver nanoparticles gave nonlinear responses with concentration due to variable kinetics of antioxidants, the SNPAC method based on the growth of nanoparticles yielded concentration-dependent linear responses. The method was validated for many parameters and was not affected by common food additives. Some fruit juices and herbal teas were analyzed successfully for total antioxidant capacity with the developed method.

**Analysis of Nitramine Energetic Materials with the aid of modified AuNPs:** The linear calibration equations and LOD values of RDX and HMX are depicted in Table 3:

**Table 3:** The linear calibration curve and LOD values of nitramine energetic materials.

Energetic Material (conditions)	Linear Equation	LOD ( $\text{mg L}^{-1}$ )
RDX (at room temperature)	$A_{565\text{nm}} = (7.94 \pm 0.57) \times 10^{-2} C_{\text{RDX}} - (7.22 \pm 5.1) \times 10^{-2}$	0.55
RDX (water bath at $60^\circ\text{C}$ )	$A_{565} = (1.48 \pm 0.03) \times 10^{-1} C_{\text{RDX}} - (5.05 \pm 1.3) \times 10^{-2}$	0.20
HMX (water bath at $60^\circ\text{C}$ )	$A_{565} = (1.25 \pm 0.1) \times 10^{-1} C_{\text{HMX}} + (4.95 \pm 4.0) \times 10^{-2}$	0.24

Contrary to the literature, the developed method was studied with an increase in absorbance over a wide range of nitrite concentrations (derived from alkaline hydrolysis of tested nitramines). Finally, the developed method was validated against HPLC for (RDX+HMX) mixtures.

**TATP Sensor:** When the developed method was performed on a Nafion membrane, the calibration curve was obtained as below:

$$A_{550\text{ nm}} = (6.82 \pm 0.42) \times 10^{-2} C_{\text{TATP}} + (1.08 \pm 2.56) \times 10^{-2} \quad (r=0.9990)$$

The LOD value was found  $0.1 \text{ mg L}^{-1}$ . The retention of DMPD radical cation on the Nafion membrane caused an increase in the sensitivity of the developed method by a preconcentration factor. The method was not affected by most handheld luggage items, common soil and groundwater ions, and nitro energetic materials. Finally, the method was validated against GC-MS.

## Conclusions

The developed colorimetric sensors using membranes and nanoparticles proved to be selective and sensitive in the determination of antioxidants and energetic materials. The CUPRAC and SNPAC sensors could precisely measure the antioxidant capacity of plant and food extracts without pretreatment. Both CUPRAC and SNPAC reagents had suitable redox potentials to

selectively oxidize antioxidants but not other food components (*e.g.*, sugars). The internationally patented CUPRAC sensor provided a simple and efficient solution to TAC measurement like a pH-paper. Colorimetric analysis of nitramine type energetic materials using modified AuNPs yielded a low-cost method, successfully applicable to the field and contaminated land remediation, discriminating between RDX and HMX. The TATP sensor showed important prospects for the fight against terrorism, especially with its increased sensitivity.

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**IS13- Novel Microextraction Strategies for Sensitive and Accurate Determination of Pesticides****Sezgin BAKIRDERE<sup>1,2\*</sup>**<sup>1</sup>Yıldız Technical University, Faculty of Art and Science, Department of Chemistry, 34210, Davutpasa, Esenler, İstanbul, Turkey<sup>2</sup>Turkish Academy of Sciences (TÜBA), Piyade Street No: 27, Çankaya, 06690, Ankara, Turkey\*Email: [bsezgin23@yahoo.com](mailto:bsezgin23@yahoo.com)

Pesticide determination in variety of matrices is one of the main research areas among scientists due to their serious health effects and common usage in many countries. Pesticides are known as their adverse impacts on human health such as neurological impairments, cancer and reproductive disorders [1]. Their mobility in different environmental bodies leads to pesticide-contaminated food, drinking water, soil, air and creates public health issues. For these reasons, it is very crucial and urgent to perform pesticide determination by sensitive and accurate analytical methods [2,3]. Different chromatographic methods such as gas chromatography (GC) and liquid chromatography (LC) allow to reach the detection limits at ppm levels for several pesticides [4]. Although instrumental methods have become more advanced and sophisticated, sample preparation methods have a great deal of importance for analytical procedures to simplify matrix, clean sample solution and preconcentrate target analyte(s) [5]. Microextraction methods have become indispensable sample preparation methods in pesticide determination area. Dispersive liquid-liquid microextraction (DLLME) [6], switchable solvent liquid phase microextraction (SS-LPME) [7] and dispersive solid phase extraction (DSPE) [8] are some common methods applied in literature. The main purpose of these methods is to enrich target analyte(s) to lower detection limits and eliminate the possible interference effects. In these methods.

**Keywords:** Pesticides, microextraction methods, low detection limits, preconcentration.

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## **IS14- Recent developments in enantioselective analysis of chiral drugs in capillary electromigration techniques**

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Capillary electrophoresis (CE) is very useful technique for analytical scale separation of enantiomers. It is clear that even only at the expense of higher peak efficiency CE may allow to observe enantioseparation for certain chiral analyte-selector pairs where the separation power of HPLC is insufficient for achieving this goal. In addition, chiral CE offers almost unlimited possibility from the viewpoint of adjustment of separation factor. Together with aforementioned conceptual advantages CE offers some favorable technical characteristics for achieving high separation selectivity. Thus, chiral stationary phases (CSPs) in HPLC contain commonly limited and predefined amounts of a chiral selector, whereas the concentration of a chiral selector is easily variable and just limited by the solubility (for charged selectors also with Joule heating) of a chiral selector in a CE buffer. In addition, the combination of two or more (chiral) selectors is technically much easier and not associated with instrumental difficulties in CE compared to column-coupling in HPLC. Again, two columns are coupled with given amounts of the chiral selectors in HPLC whereas the ratio of chiral selectors in a combination can be easily optimized in CE. Thus, chiral CE offers really enormous flexibility from the viewpoint of the adjustment of the separation selectivity. This in combination with the inherently high separation efficiency makes chiral CE a very powerful technique for enantioseparations. Chiral CE is a powerful technique not only for separation of enantiomers but also for understanding fine mechanisms of enantioselective selector-selectand interactions. This presentation discusses most recent examples from our research on separation of chiral drug enantiomers and on understanding of chiral recognition mechanisms of cyclodextrins by combined use of CE, nuclear magnetic resonance spectroscopy and molecular modeling calculations<sup>1</sup>. Capillary electrochromatography (CEC) is another miniaturized separation technique combining some advantages of CE and HPLC. The final part of the presentation describes our recent studies on application of chiral CEC to separation of enantiomers of chiral drugs<sup>2</sup>.

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## **IS15- Lateral Flow Assays towards point-of-care COVID-19 detection**

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A lateral flow assay (LFA) is a paper-based device for the sensitive and on-site detection and quantification of related targets in a complex sample. In a LFA device, the sample is placed on test device and the results can visually be monitored within 5-15 minutes. In the past two decades, relatively low development and fabrication costs of LFA based technologies has made them the most important tools for any fields in which rapid tests are required. LFAs are applied to the variety of clinical samples such as whole blood, sweat, urine, saliva, and other physiological fluids. As well as further fields in which LFA based technologies are frequently used include food control, veterinary medicine, environmental risk assessment, and bioprocess monitoring. This is possible to make a wide variety of analysis for any biomarker/pollutants/metabolites in these fields by the help of LFA based systems.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral infection causes the respiratory illness Coronavirus disease-19 (COVID-19). At the first weeks of the pandemic, there is no doubt that, the global diagnostic healthcare industry has a great difficulty in supply accurate, sensitive, and cost-effective tests for the diagnosis of SARS-CoV-2 viral infection. Consequently, since last year there has been an increasing demand for point-of-care LFA diagnostic tests allowing the accurate, rapid, and economical detection of SARS-COV-2 in samples.

In this study, the fundamentals of lateral flow assay based systems are summarized. Beside, LFA based tools for COVID-19 detection are discussed as the most important candidates which could overcome the problems with the other bioanalytical methods because of their important advantages.

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

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## **IS16- Surface-enhanced Raman Scattering for Early Cancer Detection and Cancer Diagnosis**

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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 for the differentiation of healthy, and diseased tissues and body fluids. RS and almost all of its modes are long investigated for diagnosis, and their potential is well-demonstrated in the literature. 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 label-free cancer diagnosis. In this presentation, I covered our effort to utilize SERS for label-free cancer diagnosis from “liquid-biopsy”.

## IS17- Electroanalytical Applications of Miniaturized and Electrified Liquid-Liquid Interfaces.

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In electrochemistry, the electrified liquid-liquid interfaces are known as the interface between two immiscible electrolyte solutions (ITIES). Corresponding platforms can be applied in electrochemical sensing. Here, the detection is not limited to the redox reactions (oxidation and reduction) and usually arise from the interfacial ion transfer reaction which can be followed with all available methods offered by the electrochemical toolbox.

In this work, significant attention was given to the development of simple protocols allowing for the ITIES miniaturization (increasing the stability of the soft junction, decreasing the values of LODs, and increasing the detection sensitivity). The protocols we have studied or developed are based on the (i) metal micro-wire embedded in and then dissolved from a glass tubing,<sup>1</sup> (ii) fused silica capillaries,<sup>2</sup> and (iii) self-adhesive polyamide films micro punched with an array of ordered needles. All these platforms were characterized by ion transfer voltammetry and scanning electron microscopy.

These platforms are then employed to study analytes holding high societal relevance. Our examples will cover the detection of cocaine from real street samples, electroanalytical screening of a family of fluoroquinolone antibiotics, and detection of ephedrine from urine samples or solutions containing caffeine. In all cases, obtained values of LODs were found in the range from 1 to 5  $\mu\text{M}$ .

Conclusions. All examples that will be covered during this presentation describe simple, fast, and very cheap sensing platforms fabrication protocols. Developed devices gave fully functional platforms allowing for the extraction of a number of analytical and physicochemical parameters pertaining to studied analytes.

**Keywords:** illicit drugs, antibiotics, miniaturization, soft junctions, voltammetry.

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### Acknowledgment

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## **IS18- Carbon ceramic electrode as a powerful tool for electroanalysis**

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Carbon electrodes are widely known and commonly used by many researchers around the world. Apart from carbon paste electrodes, glassy carbon electrodes, boron-doped diamond electrodes, also carbon ceramic electrodes (CCEs) deserve attention due to their unique properties. For the first time, CCEs were introduced in 1994 and have been of interest to many scientists since then.

In general, ceramic carbon electrodes are produced using the 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 the graphite powder, but not limited to). Electrodes made in this way have unique features when compared to other carbon-based electrodes. CCEs are characterized by a large surface area, porosity, surface renewability, mechanical rigidity, chemical stability, and low production costs. In addition, the sol-gel procedure is particularly suitable for the preparation of an electrode material modified with admixtures of other compounds, giving the electrode certain pre-determined properties. There is a wide range of factors determining the final properties of the CCEs, including parameters of the sol-gel process (silica precursor, ratio of reactants, pH, deposition technique) as well as the type of carbon powder (graphite, carbon nanotubes, reduced graphene oxide, carbon black, etc.) used for electrode preparation. The application field of the CCEs is very wide. There is a lot of examples of their application in voltammetric procedures for the determination of bioactive compounds.

During this lecture, the factors determining the functional properties of ceramic carbon electrodes will be discussed. Moreover, an overview of possible applications of CCEs in electroanalytical procedures will be presented, as well as their advantages and disadvantages will be pointed out.

**Keywords:** carbon ceramic electrode, sol-gel process, electroanalysis, voltammetric analysis, biologically active compounds,

## ORAL PRESENTATIONS (OP)

### OP1- Preparing, characterization and application of glassy carbon electrode to the determination of Fluometuron

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Due to the fact that carbon has unique electrochemical properties, it has become the basic electrode material used in the production of electrodes used, inter alia, in electroanalysis. The presence of carbon in various forms, including graphite, carbon nanotubes, graphene or glassy carbon, has contributed to the production of various types of carbon electrodes. Powdered carbon is used to produce carbon paste electrodes (CPE), which are successfully used in the study of biologically active compounds.

The main purpose of the presented work was the preparing, characterization and application of a carbon paste electrode based on glassy carbon powder for the determination of Fluometuron, which is used to control weeds in the cultivation of cotton and sugar cane. It works by inhibiting plant photosynthesis and carotenoid biosynthesis. It was found that this compound and its metabolites are very stable in soil and resistant to water hydrolysis and photolysis. This means that Fluometuron and its metabolites may pollute soil and groundwater.

The first stage of the research included the preparing of a glassy carbon paste electrode and its electrochemical characterization using the cyclic voltammetry (CVC) technique and topographic characteristics performed with the use of an atomic force microscope (AFM). The next, second step involved the use of a carbon paste electrode for quantitative determination of Fluometuron using square wave voltammetry (SWV). The research included the selection of the reaction environment and optimization of the measurement parameters typical for the SWV technique, i.e. frequency ( $f$ ), amplitude ( $\Delta E_{\text{step}}$ ) and potential step ( $E_{\text{step}}$ ). The last step was to determine the linear range (LR), limit of detection (LOD) and quantification (LOQ).

It was found that Fluometuron is oxidized in an acid medium, and the best analytical signal was obtained in sulfuric acid with a concentration of  $0.5 \text{ mol L}^{-1}$ . After optimization of the SWV parameters, the linearity was determined, which is  $(3.7 - 86.6) \times 10^{-6} \text{ mol L}^{-1}$ , and the limit of quantification (LOQ) and the limit of detection (LOD), which are respectively  $2.2 \times 10^{-6} \text{ mol L}^{-1}$  and  $0.7 \times 10^{-6} \text{ mol L}^{-1}$ .

The production of a paste carbon electrode on the basis of glassy carbon powder is not complicated by refreshing the electrode surface in a simple and easy way. The effect of this is obtaining repeatable and reproducible results.

**Keywords:** square wave voltammetry, cyclic voltammetry, glassy carbon paste electrode, atomic force microscope, Fluometuron

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## OP2- A dual immunosensor for the simultaneous electrochemical detection of CA 125 and HE4

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One of the most dangerous types of cancer found in female patients is ovarian cancer<sup>1</sup>. It is important to determine the levels of CA 125 and HE4 in body fluids for the early diagnosis of ovarian cancer. In this study, label-free dual CA125-HE4 immunosensors are prepared by using dual screen-printed electrodes as working electrodes. For this purpose, firstly, the dual screen-printed electrodes (SPCDE) were modified with reduced graphene oxide (RGO), polythionine (PTH) and gold nanoparticle (AuNP). Then, the surfaces of the working electrodes (SPCDE/RGO/PTH/AuNP) were modified by 3-mercaptopropionic acid (3-MPA) which introduced –COOH ends for the covalent attachment of the antibodies Anti-CA125 and Anti-HE4 onto the electrode surfaces. Next, the remaining active –COOH ends were blocked by bovine serum albumin (BSA) to prevent unspecific interactions. The preparation stages of the immunosensors and optimization studies were characterized by electrochemical methods (CV, DPV and SWV). Analytical characterizations (limit of detection, linear range, repeatability, operation stability, and storage stability) of dual immunosensors were also performed. Simultaneous electrochemical detections of CA 125 and HE4 were carried out in human blood serum samples with developed dual immunosensors.

**Keywords:** CA125, HE4, dual biosensor, ovarian cancer.

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**OP3 Electrochemical Detection of Interaction Between *Helichrysum pallasii* (Spreng.) Ledeb. and DNA by Using Disposable Biosensors**

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Small molecules, nucleic acids and proteins can be analysed with label-free or label-based electrochemical methodologies that contain nanotubes, semiconductors, nanowires, nanofilaments and nanoparticles in their detection scheme. In particular, carbon nanotubes are used to detect sensitive interactions with high electron transfer potentials<sup>1,2</sup>. A still popular trend is to identify the binding of small molecules in plant extract to deoxyribonucleic acid structure. The aim of this study is to investigate the effects of extracts that obtained recently growing *Helichrysum pallasii* (Spreng.) Ledeb. plants on DNA with the support of nanomaterials used in the study. Plant extract contents are still being investigated worldwide.

On the other hand, the positive or negative effects of many of plants used for therapeutic purposes on human health have not been experimentally proven yet. In this study, the effects of some other herbs used for traditional treatment on DNA will also be examined with the developed electrochemical biosensor.

**Keywords:** Biosensor, DNA hybridization, *Helichrysum*, plant extract-DNA interaction

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## OP4- A Nanomaterial Enriched Plant Tissue Based Biosensor for Phenol Detection

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Crude tissues and tissue homogenates of various plants (e.g. mushroom, apple, banana, potato) have been utilized as natural enzyme sources for the fabrication of efficient electroanalytical platforms through a practical and low cost procedure. Within this framework, a special emphasis has been given to the development of plant tissue based biosensors for the accurate and precise detection of environmentally important phenol and phenolic compounds in the literature.<sup>1,2</sup>

Based on the pointed idea above, a nanomaterial enriched plant tissue based biosensor has been fabricated in order to improve the biosensor response. Within this purpose, phenol oxidation catalyzed by polyphenol oxidase was examined by monitoring the consumption of dissolved oxygen in the measurement media via chronoamperometry. The effects of operating parameters such as applied potential, tissue amount in biosensor composition and pH on biosensor response were also evaluated. Analytical characteristics of the plant tissue based biosensor were investigated in the range of 10-200  $\mu$ M phenol at pH 6.5 in terms of linearity, sensitivity and repeatability. Additionally, storage stability and selectivity of the developed system have revealed its potential for analytical applications. The plant tissue based biosensor was tested for phenol detection in tap water samples with satisfactory recovery values ranging between 101.03%-105.75%.

**Keywords:** Plant tissue, biosensor, phenol, nanomaterial

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### Acknowledgment

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## **OP5- A Label-Free Aptamer Based Impedimetric Biosensor for Ochratoxin A Detection**

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Transition metal oxides are abundant and cost-effective materials which display unique catalytic performance and excellent selectivity when coupled to biorecognition components with simple design<sup>1</sup>. Present study describes pulsed deposited transition metal oxides films at a PGE as a novel platform of for the aptamer based electrochemical sensor for a mycotoxin, OTA. Initial studies were devoted to the screening of the transition metal oxides namely, manganese, molybdenum and tungsten oxide film deposited onto a PGE to reveal their performances as a platform for aptasensor studies and covalent bonding of the amino terminal aptamer was tested upon EIS studies. In addition, the deposition mode was also searched in pursue of sensitive and yet selective method development.

Among the metal oxides studied, pulsed deposited manganese oxide modified PGEs have shown superior performance as an aptasensor for label-free impedimetric detection of OTA and under optimal conditions, more specific and reproducible results obtained with the electrode against other mycotoxins. The calibration graphs obtained with developed aptasensors has given a linear range in nanomolar levels and the LOD values were found well below of the lowest limit for OTA in grape juice set by European Community being 2 nM (EC no. 1881/2006).

**Acknowledgement:** Authors would like to thank Ege University for financial support (project no: FDK-2020-21648).

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## OP6- Investigation of the Interaction between Oral Antiseptic Hormone Reagent

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**Objective:** Oral antiseptics are solutions commonly used in daily life to control dental plaque biofilm. It contains many organic and inorganic molecules. Different side effects may occur depending on the passage of these molecules into the bloodstream. The presence of oral antiseptics in the bloodstream may cause false low or high results, especially in the analysis of large hormone molecules. The aim of this study is to show the effect of frequently used oral antiseptic on the analysis of different hormones in an in vitro environment.

**Methods:** The quaternary ammonium containing mouthwash, which does not contain ethyl alcohol, was used as an oral antiseptic. The ingredients of the antiseptic are aqua, glycerin, propylene glycol, sorbitol, poloxamer 407, cetylpyridinium chloride, potassium sorbate, sodium fluoride, sodium saccharine. The mixture was prepared by taking 20µL of solution and 180µL of biochemical control solution with the same content as blood. CKMB mass, troponin I, estradiol (E2), FSH, LH, TSH, FT3, FT4, progesterone, prolactin, Testosterone, ferritin, folate, vitamin B12 tests were performed on the hormone autoanalyzer from the prepared mixture. The same experiment was repeated by adding 20µL of distilled water to the control solution. Deviation amounts were calculated with bias%.

**Results:** The detected deviation range was calculated between -10.63% and 3.54%. The greatest deviation was in folate with -10.63%. CKMB mass deviated from the target value by -7.12% and FSH by -6.01%. Deviation rates in other tests were below 5%.

**Conclusion:** Depending on the content of the antiseptic, the folate level was found to be false low by -10.63%. The deviation rates in other tests are not clinically important in terms of diagnosis and follow-up of the diseases. Since the contents of oral antiseptics are different, the deviation rates caused by hormone tests may vary.

**Keywords:** Antiseptic, CKMB mass, folate, hormone, analytical error

## **OP7- Extraction of Hypericin and Hyperforin from St. John's Wort (*Hypericum perforatum* L.) and Investigation of Wound Healing Potential**

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**Abstract**-Several plant species are used to treat different skin diseases such as melanoma, dermatitis, and skin wounds. For wound healing, alternative medicines to improve wound healing are a continuing area of research. St. John's Wort, known as healing wounds for a long time, is a medicinal plant whose antidepressant activity has been proven as a result of clinical trials and has become widespread in the world. Within the scope of this research, St. John's Wort collected from the Aegean Region was extracted separately in ethanol, glycerine / water and olive oil. The obtained extracts were analyzed by LC-QTOF-MS (a Quadrupole Time-of-Flight LC/MS) and Hypericin and Hyperforin were analysed from active ingredients. In addition, a wound healing model was conducted with BJ (normal human diploid foreskin fibroblasts) and HaCaT (Normal human keratinocyte cell) cell lines and it was concluded that they could be used in wound healing.

**Keywords:** *St. John's Wort, wound healing, LC-QTOF-MS, in vitro, normal human diploid foreskin fibroblasts cell line, Normal human keratinocyte cell line*

### **Introduction**

Cutaneous wounds are the result of a breakdown in the integrity of the skin. The healing process depends on local wound factors, systemic mediators, underlying disease and type of injury, including a number of well-organized cellular and molecular events. The basic principle of optimal wound healing is to provide adequate tissue perfusion and oxygenation, proper nutrition and moist wound healing environment to minimize tissue damage and restore the anatomical continuity and function of the affected part<sup>1</sup>. Plants have come to the fore, which play a major role in the use of herbs in the treatment of different disorders. St. John's wort, known as a medicinal plant whose antidepressant activity has been proven as a result of clinical trials and its use has become widespread in the world. *Hypericum perforatum* L. is widely consumed in the world<sup>2</sup>. For this purpose, St. John's Wort plant was extracted with a mixture of Ethanol and Glycerin Water by separate methods and analyzed with LC-QTOF-MS device. Subsequently, wound healing studies were performed with BJ (normal human diploid foreskin fibroblasts) and HaCaT (Normal human keratinocyte cell) cell lines.

### **Materials and Method**

In the St. John's Wort extraction procedure, two different extracting media, ethanol and purified water, were selected. While St. John's Wort-Ethanol Extraction was carried out according to the study of Wölflé et al.<sup>3</sup>, St. John's Wort-Ultra Pure Water Extraction was performed according to the study of Karakashov et al.<sup>4</sup>. The extraction of the dried St. John's Wort plant with olive oil was performed by putting it in olive oil and waiting in the sun. St. John's wort was analyzed with liquid chromatography quadrupole time-of-flight mass spectrometry (LC/QTOF/MS). LC/QTOF/MS analysis of St. John's wort was performed at the Ege University Central Research Test and Analysis Laboratory Application and Research Center (EGE MATA). Human epidermal keratinocyte (HaCaT) and human foreskin fibroblast (BJ) cell lines were used in in vitro cell culture studies. HaCaT cells were maintained in continuous culture in DMEM (Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002) and BJ cells were maintained in continuous culture in MEM (Eagle's Minimum Essential Medium, Catalog No. 30-2003.) supplemented with 10% fetal bovine serum (FBS) (Thermo Fisher) in a low oxygen atmosphere

(5% CO<sub>2</sub>) at 37 °C. Only centaury-ethanol extract dissolved in 10 mg / 1 mL ethanol was used on the study groups. Single concentration was studied in 3 replicates (n=3). Cells were incubated in 37 °C, 5% CO<sub>2</sub> environment until 0, 3, 48 hours. At the end of each hour, images of the cells were taken and the effect of the extracts on the cells was examined.

### Results and Discussion

Table1 shows the results from LC/QTOF/MS analysis performed to determine the amount of all Hyperforin and Hypericine contained in the St. John's Wort extract. The following molecules were identified in St. John's Wort extract: Hyperforin (537,39m/z), Hypericine (505,44m/z) on positive mode.

Compound Label	Mass	Name	DB Formula	m/z
Hyperforin	536,38	Hyperforin	C <sub>35</sub> H <sub>52</sub> O <sub>4</sub>	537,39
Hypericin	504,44	Hypericin	C <sub>30</sub> H <sub>16</sub> O <sub>8</sub>	505,44

Table1. LC-QTOF-MS Results Chart of St. John's Wort extract.

In the LC-QTOF-MS analysis of Glycerin/Water extracts, no results were obtained for St. John's Wort. Therefore, cell culture studies were continued over ethanol and olive oil extracts.

Results of wound healing studies are shown in Figures1 and Figure2. Figure1 shows the wound healing of a treated BJ cell, while Figure2 presents the wound healing of a treated HaCaT cell in the same period. Time-dependent wound healing effects of St. John's Wort extract on BJ and HaCaT cells are shown in Figure3.

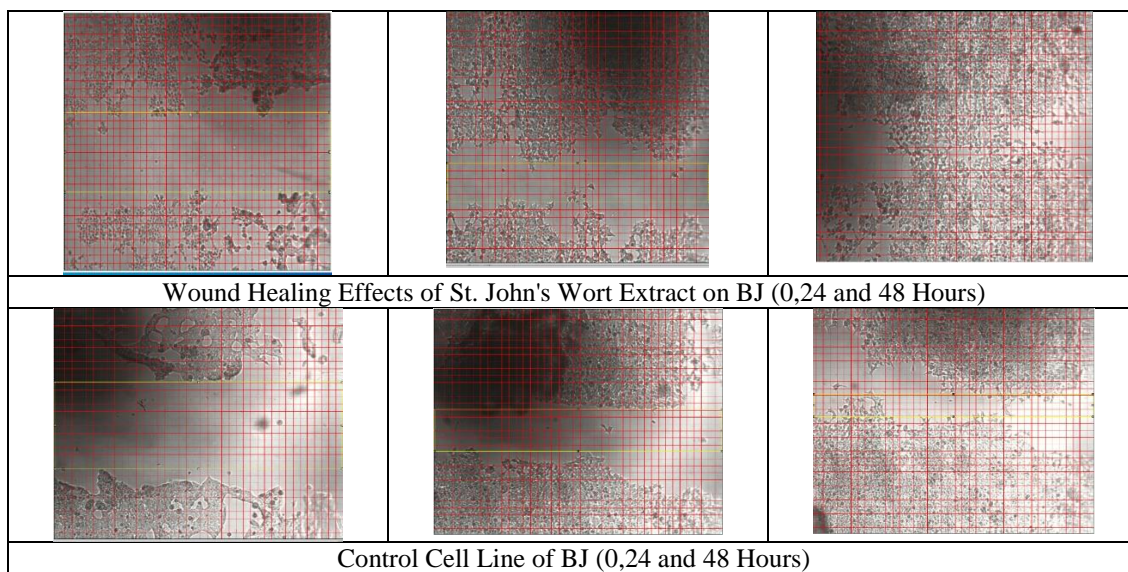
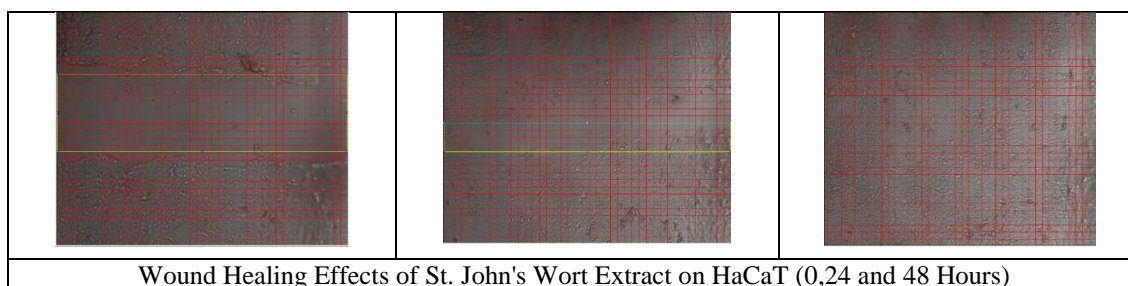


Figure1. Wound Healing Effects on BJ Cell Line.



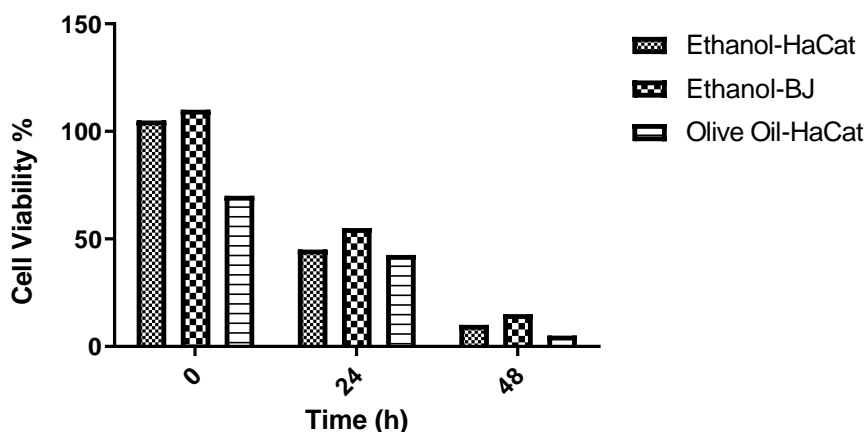
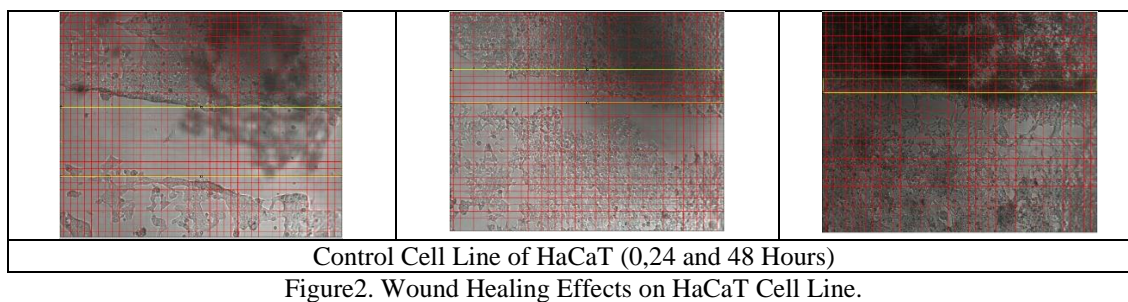


Figure3. Wound closure on BJ, HaCaT cell lines.

As a result, in the study conducted, centaury extracts were applied to BJ and HaCaT dermal fibroblast cells and it was observed that the extracts increased the antioxidant enzyme activity in fibroblast cells and thus positively affected the proliferation mechanisms in the cells under the wound model and stress conditions. Wound healing of treated HaCaT cells reached 60% after 24 h and 100% after 48 hours whereas wound healing of untreated BJ cells reached 100% after 48 hours ( $P < 0.05$ ).

### Conclusion

In conclusion, all experimental data showed that St. John's Wort extract based wound healing treatment was capable of accelerating wound healing. St. John's Wort extract is promising for future medicinal applications for wound healing.

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## **OP8- Development new solid phase materials for determination of anticancer drugs at trace levels**

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Cancer is one of the most dangerous disease and a serious cause of mortality in humans for last decades. Scientist are trying to find new ways to treat it every day by using various synthetic or natural drugs. Today, chemotherapy is main way of treatment with chemical drugs. A lot of type of active molecules are known for treatment of different kind of cancer[1]. Anastrozole and Letrozole are anti-cancer drugs used in mainly breast cancer. Therapeutic effects of drugs are expected in the first step for treatment but they have so negative side effects, unfortunately. The pain, nausea or vomiting, low blood counts, hair loss and mouth sores are the main side effect of drugs in human body. The analysis of anticancer drugs is very important due to quality control of drug molecules and getting the ideal therapeutic drug concentration in biological samples. It is a big challenge to perform these analyses due to complex structure of samples and the trace concentration of drug molecules. Any bio-analytical method includes several steps, all of them being important in order to achieve reliable results. The first step is collection of samples for the analysis, followed by the extraction procedure and sample clean-up, chromatographic analysis and detection[2].

Determination of these anticancer drugs in complex matrix is very important task for analytical chemists. Generally, this type analysis needs hybrid instrumental systems such as LC-MS which is so expensive for a lot of laboratory. For this reason, generally a sample pre-treatment approaches are used in this trace analysis. Solid phase micro extraction (SPME) techniques are preferred due to simplicity and cost effect sides. In this study, a new solid phase material was developed for anticancer drugs including Anastrozole and Letrozole. Experimental variables, and determination conditions were studied and optimized step by step. Finally, the developed method was tested by model solutions in order to validate analytical merits.

**Keywords:** Anticancer drugs, Anastrozole, Letrozole, HPLC, Magnetic Solid Phase Extraction

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**OP9- Smartphone digital image colorimetry combined with reversed-phase switchable-hydrophilicity solvent liquid-liquid microextraction for the determination of copper in edible oils**

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Smartphone digital image colorimetry (SDIC) was combined with reversed-phase switchable-hydrophilicity solvent liquid-liquid microextraction (RP-SHS-LLME)<sup>1</sup> for the determination of total copper concentration. A colorimetric box was designed to capture images for sample solutions, which were split into their red-green-blue channels. A linear correlation was obtained between the intensity of the blue channel and the concentration of copper ions in the solution. Optimum SDIC conditions were achieved with a distance of 7.0 cm between the cuvette and the detection camera, a region of interest of 2500 px<sup>2</sup> at a maximum absorption wavelength of 400 nm. Optimum RP-SHS-LLME extraction efficiency was attained using 80 µL of triethylamine as the extraction solvent, 140 µL of 7.5 M nitric acid as a hydrophilicity-switching trigger within 1.0 min extraction time. Optimum complexation conditions were obtained with sample pH at 5.5, 200 µL of N,N-diethyl-N'-benzoylthiourea as the chelating agent within 4.0 min complexation time. The limit of detection (LOD) was found to be 0.5 µg mL<sup>-1</sup>. The calibration graphs showed good linearity with coefficients of determination greater than 0.9966 and relative standard deviations less than 5.0%. The proposed method RP-SHS-LLME-SDIC was applied to determine the total copper concentration in edible oil samples (i.e., sunflower, olive, black seed and corn), from which percentage relative recoveries ranged between 94.5 and 109.2%.

**Keywords:** Digital image colorimetry, Copper, Edible oil, Smartphone, Switchable-hydrophilicity solvent.

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## OP10- Microwave Assisted Production of Gold Nanoparticles From Catechin Extract

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In this study, the synthesis and characterization of Au nanoparticles from *green tea leaves* collected the Eastern Black Sea region (Of-TRABZON) were investigated. We isolated catechin extract using Supercritical Fluid Extraction (SFE) equipped with a modifier pump was used. 10 g of dried sample was shaken in 200 mL distilled water containing citric acid (0.1 M) for 90 min at room temperature and extracted in a laboratory microwave device (Milestone, Start S Microwave, USA) at 4 minutes, 600 W and left cooling. The extracts obtained by applying microwave extraction were completely dried in the lyophilizer and transferred to the SFE system. Caffeine was separated by applying at 250 bar, 60 ° C and for 3 hours and then the catechin extract was separated by applying 0.5 mL / min ethanol, modified CO<sub>2</sub>-SFE extraction under the same conditions<sup>1</sup>. The catechin mixture was dissolved in water (2% w/v) and used for AuNP production. Different portions of extract solution was added to HAuCl<sub>4</sub> solution (0.5 mM-1 mM) and the mixture was exposed to a household microwave at 90 W for 1–30 min. The color of the mixture started in pale yellow and become purple-red and most of the nanoparticle resonance absorbance were between 500 and 600 nm range <sup>2</sup>. Absorption spectra were measured on a Shimadzu UVP-1240 spectrophotometer. All experimental work was repeated three times.

**Key Words:** Green synthesis, Catechin, AuNP, MAE, SFE

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## OP11- Preparation of urethane functional poly (styrene) based sorbent for removing of mercury from water

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**Abstract**-Crosslinked Polyvinyl benzyl chloride (PVBC) as a beads form was prepared copolymerization of vinyl benzyl chloride (0.90 mol) and Ethylene glycole (EGDMA) (0.10 mol) using suspension polymerization method. PVBC beads was aminated with diethanol amine. Urethane modified beads (UR-resin) were obtained starting from reaction of aminated beads with butyl isocyanate in the presence of dibutyl tin laurate in DMF at 60 °C for 24 h. The UR-resin was used to remove mercury ions from water. The UR-resin has 2.50 mmol/g of mercury loading capacity in nonbuffered conditions.

**Keywords:** Mercury removal; polymeric sorbent; urethane modified polymer

### Introduction

Chelating polymeric adsorbents have attracted attention as the metal selective adsorbents in the field of wastewater treatment <sup>1</sup>. They can bind metal ions with coordination, yielding chelate complexes that are efficient even for trace quantities of as little as a few parts per million (ppm). Mercury is used in many industries such as electrical paints, fungicides, chlor-alkali, paper and pulp, pharmaceutical, etc.

Generally, conventional methods for treatment of mercury ions consist of chemical precipitation, ion exchange, solvent extraction and adsorption. Adsorption is an effective and economical method to remove mercury ions.

The use of polymer-bonded ligands in selective mercury removal has been the subject of many research articles<sup>2,3</sup>. Polymeric sorbents modified with sulfur and amide are used for binding mercuric ions. Compounds with amide groups easily react with mercury ion and give mono-mercury or diamido-mercury compounds <sup>2</sup>.

In this study, urethane modified poly (styrene) based polymeric sorbent was synthesized. Mercury sorption properties of the polymeric sorbent were investigated with respect to different initial mercury concentration, depending on pH and sorption other toxic cations. Also, kinetic models were also studied.

### Experimental

#### Materials

Vinyl benzyl chloride, ethyleneglycole dimethacrylate (EGDMA), AIBN (2,2'-Azobis(2-methylpropionitrile), diethanolamine, butyl isocyanate, dibutyltin dilaurate, diphenylcarbazide, mercury(II) chloride were supplied from Sigma-Aldrich company.

#### Preparation of The UR-resin

Cross-linked PVBC beads were prepared by suspension polymerization according to the literature<sup>1</sup>. 4 g of PVBC resin was aminated using 8 mL of diethanolamine in 1-methyl-2-pyrrolidone (20 mL) solvent at room temperature for 48 h and then at 60 °C for 3 h. The yield was 4.30 g.

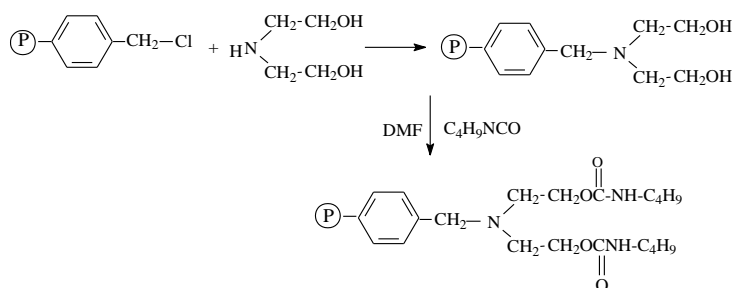
The aminated resin was reacted with butyl isocyanate to produce urethane functions via two hydroxyl function on the sorbent. For this purpose, 2 g of aminated resin and 2.50 mL of butyl isocyanate (22.20 mmol) were added to DMF (30 mL). Three drops of dibutyltin dilaurate was added into the reaction mixture as a catalyst. The mixture was stirred at 60 °C for 48 h. The yield of The UR-resin was 2.88 g.

#### Mercury sorption experiments

0.075 g of the samples were mixed with 10 mL of Hg(II) solutions (0.005 M - 0.10 M ) to determine effect of different Hg(II) concentrations on sorption capacity of the resin. The mixtures were shaken with a continuous shaker for 24 h at room temperature. The sorption capacities of the resin were examined colorimetrically according to the literature<sup>4</sup>. Also, pH depending sorption capacities of the UR-resin was examined . To examine resin characteristics against other toxic metal ions, 10 mL of  $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ,  $(\text{CH}_3\text{COO})_2\text{Pb} \cdot 3\text{H}_2\text{O}$  and  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  metal salts solutions (0.10 M) were used in the sorption experiments.

## Results and Discussion

Polymers or reagents which have urethane, urea, amide etc. functional group capture mercury ions from water via covalent binding. For this purpose, poly (styrene) based urethane modified polymeric sorbent was prepared in this study (Scheme 1).



**Scheme 1.** Preparation of urethane modified resin (PS-UR -resin)

## Mercury Adsorption Experiments of Polymeric Sorbent

All of experiments were performed at room temperature.  $\text{HgCl}_2$  salt was used in the sorption experiments. Mercury sorption experiments of the PS-UR-resin were studied depending on different initial concentration of mercury and pH. Also, other toxic metal sorption properties of the resin was investigated. The sorption experiments give a mercury loading capacity of about 2.50 mmol/g, sorption capacity of the resin increases depending on initial mercury concentrations. According to the experimental results, the maximum sorption capacity of the resin was found at pH 7. The sorption amount of the resin increases at high pH values. Because high amount of  $\text{H}^+$  present at low pH and protonation of the amino groups on the surface of the resin (Table 1).

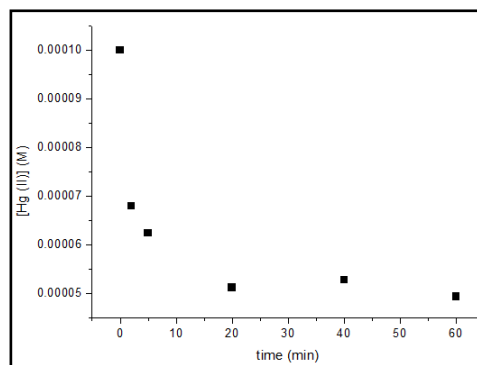
**Table 1:** Mercury sorption capacities depending on different Hg(II) concentrations and buffered conditions.

<i>Initial concentrations, M</i>	<i>Hg(II)</i>	<i>Capacity (mmol.g<sup>-1</sup>)</i>	<i>Medium condition</i>	<i>Recovered metal (mmol.g<sup>-1</sup>)</i>
0.100		2.50	non-buffered	2.32
0.075		1.95	non-buffered	-
0.050		1.61	non-buffered	-
0.100		2.06	buffered (pH =4)	-
0.100		2.86	buffered (pH =5)	-
0.100		3.13	buffered (pH =7)	-

Small sorptions ( $0.01\text{--}0.67\text{ mmol g}^{-1}$ ) were detected for these metal ions according to mercury ions.

### Mercury sorption kinetics of resin

Batch kinetic sorption of the PS-UR-resin experiments were carried out highly diluted mercury solutions ( $1.10^{-4}\text{ M}$ ). According to the concentration–time plot in Fig. 1 shows that within about 60 min of contact time, mercury concentration falls to zero.



**Figure 1 .** Hg(II) concentration in solution depending on time

### Conclusion

A new urethane functional polymeric resin can be used an alternative sorbent for removal of mercury from water. Mercury sorption capacity was found as  $2.50\text{ mmol/g}$  sorbent. The results indicated that the polymeric sorbent has high sorption capacity for mercury ions.

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## **OP12- Development and validation of an AAS method for quantitation of Class 1 and Class 2A elemental impurities in tablets**

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According to the international harmonization conference guide ICH (the international council for harmonisation of technical requirements for pharmaceuticals for human use) Q3D (R1) published on March 22, 2019, the methods by which the limits for elemental impurities can be determined by applying inductively coupled plasma (ICP)-Mass spectrometry (MS) analysis in drugs were announced [1]. Due to the difficult availability of ICP devices and the cost of procurement from scratch, method adaptation was achieved using the same sample preparation techniques as atomic absorption spectroscopy (AAS), which is more common in laboratories and the AAS device. The ICH guide complies with the lowest detection and quantitative detection limits, detection limits of graphite furnace atomic absorption spectroscopy equipment (GFAAS) [2]. Method development and validation studies were carried out for the most critical and the worstcase limit Class 1 and Class 2A metals specified in the relevant guideline, and the drugs in tablet form samples which are used most frequently [2].

In this study, sample preparation was performed by microwave-assisted acid digestion using a mixture of 65% HNO<sub>3</sub> and 37% HCl (5:1, v/v). In house validation results showed good performance in low limits (LOQ: %25 of limits for each metals) and the low percent relative standart deviation obtained for six replicate measurements of LOQ (%RSD<8.0 %). The linearity was studied for three standard concentrations (50%, 100% and 150%). The linearity correlation coefficient results obtained at least 0.99. Two real samples were spiked with at 50% and 100% metals. The recovery results obtained between 80% and 120%.

**Keywords:** Elemental impurities, ICH, GFAAS, drug. heavy metals.

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### OP13- The Provenance of Iron Age Glass Beads: A Chemical and Isotopic Approach

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The study of production technology and trade of ancient glass became a popular topic of archaeological science research and little is known about the glass produced during the Iron Age. Urartu, founded in the capital Tushpa (Van) on the eastern shore of Lake Van in mid 9<sup>th</sup> century BCE, was one of the powerful states in the Near East in the Middle Iron Age. During the excavation works at the Mound of Van Fortress, 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). 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 <sup>87</sup>Sr/<sup>86</sup>Sr ratio of the possible sand raw material from the Lake Van, and the Sr isotopic signature of the glass beads.

The sample preparation procedure was as follows: after 2.4 mL of 12 M HCl, 2.4 mL of 22 M HF, and 1.2 mL of 14 M HNO<sub>3</sub> acid solutions were added into the PTFE vessel containing 50 mg of the glass bead sample, it was digested in microwave system under the optimized parameters. When the temperature of the vessel reached to room temperature, the homogenous solution was diluted to 50 mL with ultra pure water (18 MΩ cm<sup>-1</sup>). The major and trace elements and isotopic ratios of the glass beads were measured using an Agilent 7700e ICP-MS system equipped with Cetac ASX-520 autosampler.

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

#### **Acknowledgment**

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**OP14- Chromatographic studies on identification marker of honey originated from *Pinus* spp.**

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Honey is a sweet product of honey bees (*Apis mellifera*) formed by the collection of flowers or nectars and secretion of insects living on the plants. According to the source the bees collect, honey is classified into two main groups as blossom honey and honeydew honey. The source of blossom honey is the nectar of plants whereas the source of honeydew honey is the secretion of plants or insects living on the plants. Typical examples of this group are pine and oak honey<sup>1</sup>. Nowadays, melissopalynology has been accepted as the "gold standard" in ascertaining the botanical origin of honey. However, adulteration with pollen grains may result in falsifying its botanical origin to increase its market value. Therefore, chromatographic approach is crucial for authentication of bee products<sup>2</sup>.

In this study, high-performance thin-layer chromatography (HPTLC) was used to determine floral markers of honeydew honey originated from *Pinus* spp. Quercetin, caffeic acid and an unknown component were determined as identification markers of honeydew honey. Then, we aimed to identify the unknown compound through chromatographic separations and nuclear magnetic resonance (NMR) spectroscopy. Based on the studies, this unknown marker component was determined as protocatechuic acid. Moreover, its quantification was performed by a validated high-performance liquid chromatographic (HPLC) method.

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**OP15- Fish Oil Supplements in Turkey: Discrimination of Natural and Synthetic Forms of Fish Oil by FTIR, Raman and GC-MS Techniques Combined with Chemometrics**

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People are eager to consume fish oil supplements in their daily diet due to their beneficial Omega-3 fatty acids (EPA and DHA). Fish oils are generally sold as natural triglycerides and ethyl ester forms in the market. The synthetic ethyl ester form of fish oil has lower bioavailability with resistance to enzymatic digestion, but it has the advantage of low-cost compared to triglycerides. In other words, human digestion is designed to absorb natural triglyceride form. This study was built for evaluation of FTIR, Raman, and GC-MS techniques combined with chemometrics for rapid quality classification of natural triglyceride and ethyl ester forms of fish oils which are sold in the Turkey market.

Fish oil samples (n=20) were obtained from different brands (Turkey), respectively. Analyses were performed by FTIR (Bruker Tensor 27, Germany) and Raman (Rigaku, USA) spectroscopy techniques. Fatty acid methyl ester (FAME) compositions of fish oil samples were determined by GC-MS (Shimadzu, Italy) technique. HCA (hierarchical cluster analysis) and PCA (principal component analysis) were applied to the FTIR, Raman and GC-MS data of all samples (OPUS program Version 7.2, SIMCA)

As results, characterizations of fish oils were performed by FTIR, Raman, and GC-MS techniques. Natural triglyceride and ethyl ester formed fish oils were clearly discriminated by using FTIR, Raman, and GC-MS techniques. Three of the twenty commercial samples were determined as synthetic ethyl ester formed fish oils.

Findings show that FTIR and Raman techniques could be used rapidly for the determination of the quality of fish oils in the Turkey market.

**Key Words:** *fish oil, FTIR, Raman, GC-MS, quality, HCA, PCA*

## OP16- Determination of Photophysical Properties of Coumarin 30 in Biomimic Igepal CO-520 Reverse Micelles

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**Subject:** Reverse micelles are biomimic systems in which nanosized water pools are formed.

The photophysical properties of coumarin 30 (C30) were investigated using UV-vis absorbance, steady-state fluorescence and time resolved fluorescence spectroscopy techniques in the nanocavities of these biomimic systems.

**Experimental:** It is known that Igepal CO-520 concentration should be greater than  $3.5 \times 10^{-3}$  M when creating reverse micelle<sup>1</sup>. Therefore, when creating reverse micellar environments, solutions were prepared in n-heptane using  $4.0 \times 10^{-3}$  M Igepal CO-520. Deionized water was added to the medium depending on the parameter  $W_0$  ( $W_0 = [\text{H}_2\text{O}]/[\text{surfactant}]$ ). Different reverse micelle solutions were prepared containing  $1 \times 10^{-6}$  M C30. Quinine Sulphate was used as a reference for quantum yield calculations. Absorbance and fluorescence spectra were taken in different reverse micelles and water medium.

**Results:** Reverse micellar environments are regarded as model enzyme catalysis and a way of determining the mechanical direction of some reactions. Because it is accepted that micellar centers have the ability to bind and direct, just like enzymes<sup>2</sup>. Absorbance and fluorescence band maximum of C30 was observed red-shift and increase in absorbance and fluorescence intensity in reverse micelles media. Fluorescence quantum yields and fluorescence lifetime values also differed in reverse micellar environments. Steady-state fluorescence anisotropy measurements of the dye showed that the probe moved differently in different reverse micellar environments. Anisotropy values of C30 were higher than pure water.

**Conclusion:** This study showed that the photophysical properties of C30 dye change as the size of the water pools changes in different reverse micellar environments. This suggests that C30 will be a good probe in determining the microenvironment and its possible location in the reverse micellar environment.

**Keywords:** Reverse micelles, Steady-state fluorescence anisotropy, coumarin 30, Igepal CO-520

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## OP17- Development of DLLME-SFOD/GC-MS Method for UV Filter Determination

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The term of UV filters refers to a group of chemicals those absorb the ultraviolet light and since they are widely used in plastic, paint, and rubber industry as well as the cosmetic sector, their levels in surface waters are increasing in last decades<sup>1</sup>. These compounds tend to accumulate in water, sewage sludge and biota and behave like an endocrine disruptor, altering the normal functioning of organisms<sup>2</sup>. Due to their harmful effects, UV filters have been included in the European Union monitoring list (2015/495/EU) and therefore, their accurate and sensitive analysis in environmental samples is of great importance.

Present study described a method development for selected group of UV filters including 2,4-dihydroxybenzophenone or benzophenone-1 (**BP-1**), Benzophenone-3 (**BP-3**), 3- Benzylidene camphor (**3BC**), 2-ethyl hexyl-4-(dimethyl amino) benzoate (**EDP**), 2-ethyl-hexyl-4-trimethoxycinnamate(**EHMC**), Ethylhexyl salicylate (**EHS**), Homosalate (**HMS**), Isoamyl p-methoxycinnamate (**IAMC**), 4-Methylbenzylidene camphor (**4-MBC**) and Octocrylene (**OC**) in surface waters. The standard mixture was subjected to solidification of floating organic droplet (DLLME-SFOD) followed by injector port silylation coupled with gas chromatography – tandem mass spectrometry for determining organic UV filters in ultra pure water. A series of parameters, including injection port temperature, purge off time, pH of water, extraction solvent type and dispersive solvent type enhancing the response of the UV filters were systematically investigated by using different statistical experimental design methods to determine the significant factors.

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## **OP18- Recent Applications of Analytical Techniques in the Determination of Olive Oil Adulteration**

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Olive oil is one of the unique foods, which is a top target for food adulteration. Adulteration may not necessarily result in a food security problem, but it does result in the substitution of a more valuable product with a cheaper and lower quality option. When this substitution occurs, the food industry might face decreased market acceptance, increased costs of a recall, liability, and withdrawals, damaged brand, and even a bankruptcy amounting to billions of dollars per year.

The International Olive Council (2019) categorizes olive oils according to their quality and the production methodology. Different grades of olive oils, including extra virgin olive oil (EVOO), virgin olive oil, refined olive oil, olive oil, olive-pomace oil, and lampante oil. EVOO is the first pressed oil and the difference is between EVOO and virgin olive oil based on their free fatty acid level. Refined olive oil is a lower quality olive oil produced from the virgin olive, which goes through a refining process to lower the acidity of the oil. Olive oil is a mixture of refined olive oil and virgin olive oil.

Some of the oldest information sources date back to the ancient Romans, who reported food fraud incidents related to olive oil due to its limited production and superior quality. The most used adulterants of olive oil are cheaper seed oils such as soybean, corn, canola, cotton and sunflower or mixing higher grade olive oils (ex. EVOO, virgin olive oil) with other lower-grade olive oils (ex. Refined olive oil, lampante, pomace).

Current analytical methods to authenticate food products and to determine their quality rely mainly on chromatographic, elemental, and isotopic methods. Some of these techniques are time-consuming, costly, require complicated sample preparation steps, and a skilled person.

New and alternative techniques proposed by several studies are thermal techniques (differential scanning calorimetry, spectroscopic techniques, including FT-IR, NIR and Raman spectroscopy, selected ion mass spectroscopy, fluorescence spectroscopy, electronic nose, electronic tongue, and digital imaging, voltammetric analysis, and DNA analysis. They are fast, simple, reliable, cost-effective, and green methods.

This review has summarized the advantages and disadvantages of recent applications of alternative techniques.

**Key Words:** Olive oil, authenticity, adulteration, cost-effective, green methods

**OP19- Tracking moss bio-indication capacity for anthropogenic elements depositions using SEM analysis**

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In the last decade there has been a research expansion of methodologies that establish fast and effective methodological approaches for monitoring atmospheric deposits. Air pollution has been alarming in recent decades globally. The distribution of microparticles originating from different emission sources poses a huge threat to the health of the human population. The number of researches done so far is huge, but the most common problem is to find a fast, effective and transparent research model. In this research we proposed a model, which can serve as an initial research for subsequent screening models. This approach, unlike the typical analysis with existing chemical instrumentation techniques, does not require chemical destruction of the sample and additional costs for chemical agents. But on the other hand this model is suitable for screening of large areas in order to identify critically affected areas with anthropogenic born elements. Bryophytes are a very useful tool for monitoring atmospheric deposition. Moss samples of *Hypnum cupressiforme* (Hedw.) and *Homalothecium lutescens* (Hedw.) were collected at the critical mining areas in the Eastern part of the Republic of North Macedonia. The unique structure and physiology of mosses allows dust particles to remain trapped on their surface. With the help of electron microscopy, the composition of anthropogenic elements in the dust particles trapped on the surface of these structures was monitored. Chemical characterization of the moss surface in comparison with the deposited dust. Analyzes have shown that both moss species have similar surface adsorption to dust particles, as well as insignificant variability in the chemical composition. *Hypnum cupressiforme* dominate in the background content for the biogenic elements carbon and oxygen, macroelements Mg, Al, Si, K, Ca, and microelements Fe, Cu and Zn.

**Keywords:** moss species, SEM, dust deposition, pollution

## OP20- Assessment of health risks due to potentially toxic elements intake by infants and toddlers via consumption of Weaning Foods

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**Abstract:** In this study, toxic trace elements were determined in applesauce and apple juice because those are consumed by babies and toddlers at relatively high ratio. The samples were digested using microwave oven, and analyzed by ICPMS. It was found that, there are high risk of metals depending on commercial brand of producer. Particularly, applesauce was found seriously hazard due to higher toxic element concentrations. So, it is need to following the concentrations of toxic metals intensively.

**Keywords:** toxic elements, health risk, fruit juices, nutrition

### Introduction

The harmful effects of potentially toxic elements (PTEs) are well-documented (1). Depending on how long children are exposed to these toxins and how much they are exposed to, they may be at risk for lowered IQ, behavioral problems (such as attention deficit hyperactivity disorder), type 2 diabetes, and cancer, among other health issues (1). The recorded per capita consumption of fruit products including fruit juices has increased by about 95.5% of the children of 2–4 years and 92% of the children of 5–12 years consume the minimum recommended intake of fruit juice in each day. Fruit juices are known to be high in sugars, but they could also pose another health risk: potentially harmful levels of heavy metals, according to new testing conducted by Consumer Reports (CR) in USA, the nonprofit advocacy organization (2). In 2011, Consumer Reports found elevated levels of inorganic arsenic and lead in apple and grape juices. Recently, in its analysis of 45 popular fruit juices sold across the USA country, CR found that nearly half contained concerning levels of inorganic arsenic, lead, or cadmium, which are elements commonly known as toxic heavy metals at the ppb levels. Previous tests from consumer reports (CR) and others have found elevated levels of heavy metals not just in juices but also in infant and toddler foods, rice and rice products, protein powder, some types of fish, and sweet potatoes (2).

As a results of the results obtained, some authors say that “The risk comes from chronic exposure”, “Minimizing consumption of juices and other foods that have heavy metals can reduce the chance of negative outcomes in the future.” (2).

According to the findings from CR report,

- Every product had measurable levels of at least one of these heavy metals: cadmium, inorganic arsenic, lead, or mercury.
- Juice brands marketed for children did not fare better or worse than other juices.
- Organic juices did not have lower levels of heavy metals than conventional ones.

One way to reduce exposure to heavy metals would be through government-established limits-but few are in place. As for lead, the FDA has set a guideline for juice-50 ppb-but CR thinks it should be much lower (3-4). “Babies and toddlers are particularly vulnerable due to their smaller size and developing brains and organ systems”.

By comparison, the F.D.A. has said that lead should not exceed 5 ppb in bottled water, 50 ppb in juices and 100 ppb in candy. Cadmium should not exceed 5 ppb in bottled water. The European Union limits cadmium to 15 ppb. in infant formula.

Purees, fruit juices, and other processed fruit ingredients are commonly used across product sub-categories and in products marketed at babies older than 6 months although Sweet drinks such as fruit juice, fruit drinks, cordials and soft drinks are not recommended for infants and toddlers. In this study, potentially toxic elements including, As, Cd, Pb, Ni, Cr and similar were determined in both apple juices and applesauce that consumed commonly by babies and toddlers.

### Materials and Methods

The food samples including applesauce and apple juices that are consumed commonly by infants and toddler, were obtained from the supermarkets.

To digestion of the samples, microwave oven was used. Trace elements in the digested samples were determined by using ICP-MS (PerkinElmer ELAN 9000 ICP-MS).

### Results and Discussion

The concentration of arsenic, cadmium, lead, chromium, nickel, selenium, cobalt, copper and manganese were measured in two commercially available brands of applesauce and one organical (Fig. 1) brand. Further, two commercially available brands of apple juice were also analyzed for those elements. The concentrations of As, Cd and Pb in some apple juice brands sampled were higher than U.S. Food and Drug Administration (FDA) maximum contaminant level for bottled water. FDA is setting an action level for arsenic in single-strength apple juice of 10 µg/kg or 10 ppb (3-4). The main risk was found in applesauce because all studied elements were found at very high concentrations in these samples.

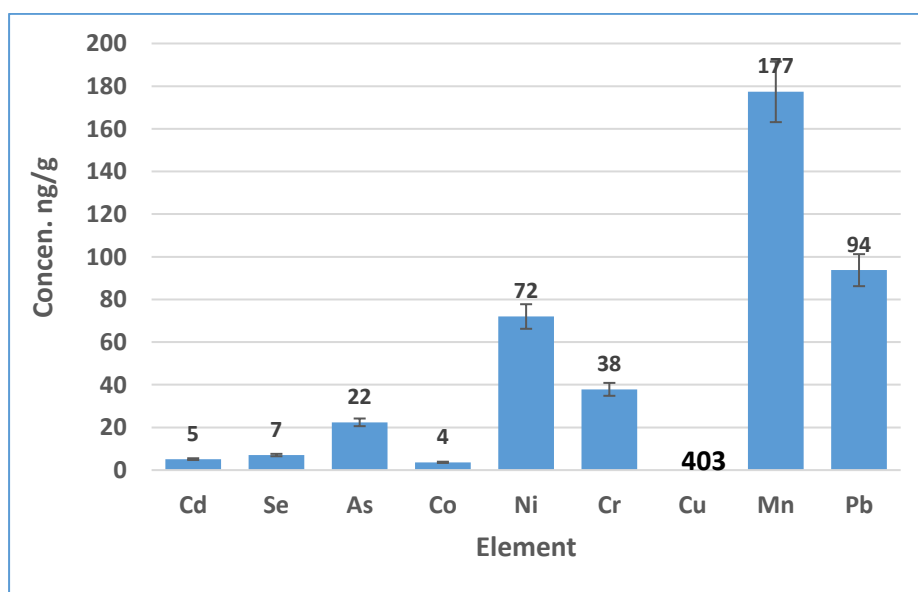


Figure 1. Metal concentrations in brand3 (named organical) applesauce

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## **OP21- Shortening the time of extraction procedure for the determination of health-protective parameters of *Rosmarinus officinalis* L.**

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**Abstract**-Numerous studies have shown the high correlation between the consumption of medicinal plants in prevention and treatment of various diseases. Phenolic compounds may significantly decrease the adverse effects of chemically reactive species on normal physiological functions in humans. A mechanism for antioxidant activity of natural compounds is the inhibition and suppression of the formation of reactive species either by inhibiting enzymes or by chelation of the trace elements involved in the formation of free radicals.

In this study, conventional extraction and microwave-assisted extraction methods were compared in terms of effectiveness and extraction time, as well as solvent quantity for rosemary. The parameters of total phenolic content, total flavonoids, total antioxidant capacity and metal chelating analyzes were examined in the extracts obtained. While the classical extraction took 3 days with requiring much more solvent, MW-assisted extraction took 20-30 minutes, also required less solvent.

In conclusion, microwave-assisted extraction method was found to be superior to the classical method in *Rosmarinus officinalis*

**Keywords:** total phenolic content, total flavonoid, antioxidant capacity, Rosemary, MW

### **Introduction**

The physiological roles of polyphenols as antioxidants include preventing various conditions associated with oxidative stress, such as cancer, atherosclerosis, cardiovascular and neurodegenerative diseases. Polyphenolic compounds also participate as free radical scavengers by regulating or protecting the endogenous antioxidant defence. Antioxidants are either natural or synthetic. Synthetic antioxidants have phenolic structures of various degrees of alkyl substitution, whereas natural antioxidants can be phenolic compounds including 1- tocopherols, flavonoids, and phenolic acids, 2- nitrogen compounds such as alkaloids, chlorophyll derivatives, amino acids, and amines, and 3- carotenoids as well as ascorbic acid. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) among synthetic antioxidants have been used since the beginning of the 20th century, as antioxidants (1-3). However, restrictions on the use of these compounds are being imposed due to their toxicity and carcinogenicity (3-5). As a result, natural antioxidants have attracted much attention in recent years because synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butylhydroquinone (TBHQ), have been used as potent compounds in food preservation for decades due to their low cost and bland flavour.

Although measurements of particular species (such as MDA) of oxidation products based on lipid oxidation were used in the past, various chemical assays coupled with highly sensitive and automated detection technologies are employed, nowadays, to measuring antioxidants activity. On the other hand, recent methods are based on scavenging activity against certain types of free radicals or ROS, reducing power, and metal chelation among others (4-6).

In this study, total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP) assay, and metal chelating capacity were applied to evaluate protective effect of *Rosmarinus officinalis* L. methanolic extracts against many health problems such as cancer, cardiovascular and neurodegenerative diseases

### **Materials and Methods**

The samples of *Rosmarinus officinalis* L. were collected from Elazig area. Firstly, conventional extraction was applied by adding methanol of 70 mL to 10 g of sample during 24 h. After filtration

and separation, this process was repeated three times, in total 72 h. The filtrates were combined and evaporated using rotary evaporatory with vacuum. Alternatively, microwave oven (MW) (MarsExpress) was used at different conditions as described in Table 1.

The commonly used health-protective parameters including total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP) assay, and metal chelating capacity were examined for the extracts obtained by both classical and MW methods.

Table 1. Applied conditions in MW assisted extraction method.

Temperature	Time		
60	8	14	20
70	8	14	20
85	8	14	20

*Total phenolic content:* The TPC was measured by UV spectrophotometer.

Method is based on a colorimetric oxidation / reduction reaction using the Folin-Ciocalteu reagent. After experimental studies, absorbances were measured at 760 nm.

*Total Flavonoid content:* The total flavonoid content was determined according to the aluminium chloride colorimetric method. After complexing products, absorbances were measured at 415 nm.

*Ferric Reducing Antioxidant Power (FRAP) assay:* Reducing power of the methanolic Rosemary extracts was determined on the basis of their antioxidant principles to form colored complex with potassium ferricyanide. At the end of method, the absorbance was measured at 700 nm.

*Metal chelating capacity* was performed by applying phenanthroline method.

## Results and Discussions

Flavonoids are potent antioxidants and have stimulated considerable interest recently because of their potential beneficial effects on human health in fighting degenerative diseases. To determine the assays considered as health-protective parameters, medicinal plants are commonly examined by using conventional extraction technique to extract phenolic compounds from solid phase. This technique is high time consumed method.

From the results, the concentration of phenolic compounds obtained via MW-assisted extraction in rosemary extract were found to be higher than conventional method. On the other hand, in the MW-assisted extraction, the phenolic compounds were determined to be significantly low at 85°C for 20 minutes, suggesting the total phenolic components to be degraded at high temperature. On the contrary, this outcome was not observed at the same temperature for 8 and 14 minutes procedures, suggesting that the degradation of phenolics is also related to the time of the procedure. As a result, 20 minutes and 85°C were selected as the optimum conditions for the TPC. Related with TFC, the concentration of flavonoids was found to be significantly higher (1.5 times) than the other extraction conditions, at 85°C for the 8-minutes of extraction, suggesting that the molecules contributing to the total flavonoids in this assay are extremely sensitive to the temperature and extraction time. This evaluation was found to be in similar to the results of total phenolic content.

Related with the antioxidant power of rosemary, the extract obtained via MW-assisted extraction have significantly higher values than that of the extract obtained via conventional method. In MW-assisted extraction procedure, on the other hand, for the 20-minutes of extraction time, 60°C was not found to be efficient, while at 85°C the antioxidant molecules contributing the FRAP assay were possibly degraded. Therefore, the maximum concentration was obtained at 70°C for 20-minutes of extraction. For the 14-minutes of extraction, relatively higher antioxidant profiles

of rosemary extract was obtained at 60°C and 85°C of extraction temperature. Furthermore, at the 8-minutes of extraction the highest degree of antioxidant power was found at 85°C, suggesting that the antioxidant molecules contributing FRAP assay are highly susceptible to the temperature and time of extraction.

Related with metal chelating capacity, the molecules contributed to the metal chelating capacity in rosemary extract was not interconnected to the time and temperature conditions of MW-assisted extraction method.

In conclusion, 85 °C and 8 minutes of extraction time was found to be the optimum conditions for rosemary, considering the four assays studied in this research. In other words, microwave-assisted extraction method was found to be superior to the classical method in *Rosmarinus officinalis*.

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**OP22- Smartphone digital image colorimetry combined with supramolecular solvent-liquid-liquid microextraction for the determination of curcumin in food samples**

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Smartphone digital image colorimetry (SDIC)<sup>1</sup> in combination with supramolecular solvent-based liquid-liquid microextraction (SMS-LLME)<sup>2</sup> was used for the determination of curcumin. A homemade colorimetric box was designed and used to capture reproducible images of the sample solutions with a smartphone. The images were split into their red-green-blue channels. The most intense response was obtained with the blue channel which was correlated to the concentration of curcumin. Optimum SDIC conditions include a distance of 9.0 cm between the cuvette and the detection camera, a region of interest of 1600 px<sup>2</sup> and a light source at 15% brightness using a second smartphone backlight illuminating a color corresponding to 428 nm. Optimum SMS-LLME efficiency was achieved with 1000 µL of tetrahydrofuran/1-undecanol (4:1, v/v) as the supramolecular extraction solvent, pH of sample solution at 7.0, 2.0% (w/v) sodium chloride in 60 s extraction time. Limits of detection (LOD) were within the range of 0.2-0.9 µg mL<sup>-1</sup> (0.04-0.18%, w/w). Coefficients of determination ( $R^2$ ) of the calibration graphs were higher than 0.9965 and relative standard deviations below 8.5%. The proposed SMS-LLME-SDIC method was applied for the determination of curcumin in turmeric and tea samples with percentage relative recoveries between 94.0 and 104.0%.

**Keywords:** Curcumin, Digital image colorimetry, Food, Smartphone, Supramolecular solvent

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## OP23- Green Synthesis of Gold Nanoparticles Using Mulberry Leaf Extract

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Gold nanoparticles (AuNPs) were synthesized using mulberry leaf extract and  $\text{HAuCl}_4$  solution at room temperature and household microwave by applying two different methods in this study. Freshly leaves of Mulberry were collected from the Eastern Black Sea region (TRABZON) in Turkey. Firstly, 10 g of dried sample was shaken in 100 mL of distilled water- citric acid (0.1 M) mixture (1:1) for 90 min at room temperature and extracted in a laboratory microwave device at 4 minutes, 600 W and left cooling. For the biosynthesis of Au-NPs, the different volume of leaf extract (0.5 and 1 mL) was added  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  solution (0.5 mM-1 mM) at room temperature (First method). In second method, the same mixture was exposed to a household microwave at 90 W for 1–60 min. Gold nanoparticles were characterized using UV-visible absorption spectroscopy and FT-IR. UV-Vis absorption spectrum of Au-NPs was done in UV-Vis spectrophotometer (Shimadzu UV-1240) in a wave length range from 300 to 800 nm<sup>1</sup>. The characteristic plasmon resonance peak of gold nanoparticles was observed at around 538-550 nm. The color of the mixture was purple-red indicating the formation of Au-NPs<sup>2</sup>. All experimental work was repeated three times.

**Keywords:** Gold nanoparticle, MAE, Green synthesis, UV-Vis spectroscopy

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**OP24- Search for the Parthenolide Sources in Several Asteraceae Plants and Quality Evaluation of the Marketed Feverfew Supplements by HPTLC**

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*Tanacetum parthenium* (L.) Schulz Bip. (Asteraceae) is known as feverfew and its leaf has been traditionally used for the treatment of fever, asthma, stomach ache, menstrual problems, and for the prophylaxis of migraine<sup>1</sup>. The sesquiterpene lactone parthenolide is the major bioactive compound responsible for the antimigraine activity and also is used as a chemical marker for the quality assessment of *T. parthenium* products<sup>2</sup>. This study aimed to assess possible parthenolide sources in Asteraceae species and evaluate the quality of commercially available herbal products containing feverfew extract. For this purpose, qualitative analysis of nineteen extracts obtained from the flower heads of several Asteraceae plants including *Anthemis* sp. (*A. altissima*, *A. austriaca*, *A. coelopoda* var. *coelopoda*, *A. cotula*, *A. cretica*, *A. pseudocotula* and *A. tinctoria*), *Bellis* sp. (*B. perennis* and *B. sylvestris*), *Chrysanthemum coronarium*, *Tripleurospermum* sp., and *Matricaria recutita* as well as four marketed capsule formulations of feverfew supplements were evaluated by High-Performance Thin-Layer Chromatography (HPTLC) by using parthenolide and apigenin as the standards. Moreover, HPTLC fingerprinting profiles of these marketed feverfew samples were compared with that of *T. parthenium* as a reference plant. Separation was performed on the precoated HPTLC silica gel 60 F<sub>254</sub> glass plates using the developing solvent system of *n*-hexane:ethyl acetate:acetic acid (20:10:1, v/v/v). Consequently, parthenolide was not detected in any of the flower extracts except the reference plant, whereas apigenin was only detected in *A. coelopoda* var. *coelopoda* and *M. recutita* extracts. The comparison of the HPTLC fingerprints of reference plant and the marketed feverfew supplements indicated that the specifications of these samples were not consistent. Furthermore, parthenolide and apigenin were not detected in any commercial feverfew products. Additionally, fading zones on the HPTLC chromatogram of feverfew samples indicated the lower quality of these products. In conclusion, none of the tested Asteraceae flowers can be considered as substitution for *T. parthenium* due to absence of parthenolide. On the other hand, the marketed feverfew products were devoid of parthenolide and apigenin, and they had different HPTLC fingerprint profiles than the reference plant extract.

**Keywords:** *Tanacetum parthenium*, Asteraceae, parthenolide, apigenin, High-Performance Thin-Layer Chromatography (HPTLC)

**References:**

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## OP25- Preparation of Peptide Based Low Molecular Weight Hydrogel Drug Carriers

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Low molecular weight dipeptide based hydrogel structures have started to attract attention in drug delivery studies in recent years<sup>1</sup>. In particular, the use of these peptide structures as drug delivery system is often preferred because of their biocompatibility and easy preparation<sup>2</sup>. In this study, Fmoc-Phe-Phe-OH dipeptide hydrogel structures were prepared with epirubicin molecule embedded in Oil/Water system. The morphological and dimensional properties of these bio-inspired low molecular weight hydrogel structures were investigated by the Scanning Electron Microscopy (SEM) and Photon Correlation Spectroscopy (PCS) method respectively. Its structural features are characterized by Fourier Transform Infrared Spectroscopy (FTIR) method. Their thermal behavior was also examined by the DSC method. It was determined that the size of Fmoc-Phe-Phe particles prepared were approximately 1 micrometer in size. This size may be an indication that the prepared particles are arranged in gel form microparticles. The release behavior of epirubicin that encapsulated into the bioinspired dipeptide-based hydrogel, was followed by UV-spectrophotometer method. The results obtained in the conducted studies are of the nature to contribute to the potential use of Fmoc-Phe-Phe dipeptide hydrogels in drug release applications. In addition, the literature shows that Fmoc-Phe-Phe-OH particles can be prepared in smaller sizes with the help of ultrasonic probe in Oil-Water system. These prepared structures show that they can be used in applications such as catalysis and dye removal.

**Keywords:** Fmoc-diphenylalanine, Drug Delivery, Epirubicin

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## OP26- Analytical Characterization for the Comparability Evaluation of TUR03 Drug Substance and Innovator Lots by Mass Spectrometry

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**Abstract-** A biosimilar is defined as a similar version of the licensed biotherapeutic reference product (innovator) in terms of quality, safety, and efficacy. TUR03 has been developed as a biosimilar candidate to one of the best-selling biologicals. With the advent of state-of-the-art analytical techniques, it is now possible to assess the physicochemical and functional attributes of the biosimilar drugs in comparison with the innovator with great sensitivity. In this study, a comprehensive mass spectrometric characterization was performed on TUR03 and innovator lots for intact and deglycosylated-reduced intact mass, peptide mapping, and released *N*-glycan level. The obtained results were compared by side-by-side with the innovator.

**Keywords:** *biosimilar, monoclonal antibody, mass spectrometry*

### Introduction

Therapeutic monoclonal antibodies (mAbs) are the fastest growing class in biopharmaceutical market and extensively used against various oncological, autoimmune and inflammatory diseases. The patents for most of the mAbs are either expired or are about expire, therefore many pharmaceutical companies around the world have started to develop their biosimilar versions.

TUR03 is a recombinant humanized mAb produced by Chinese hamster ovary (CHO) cells. It is composed of two light chain (LC) and two heavy chain (HC) and these chains form a tetramer structure through intra- and inter-chain disulfide bonds. The approximate molecular weight is 148 kDa and each LC and HC consists of 214 and 448 amino acid residues, respectively. There is only one *N*-linked glycosylation site at Asn298 of the heavy chain.

To demonstrate the biosimilarity, high-resolution mass spectrometry systems have emerged the greatest interest in recent years. Here, comprehensive analytical similarity assessments at intact and peptide levels were conducted on TUR03 drug substance and innovator lots by mass spectrometry. Through peptide mapping analysis, post-translational modification (PTM) levels were also calculated. Additionally, *N*-linked glycosylation was analyzed and quantified.

### Materials and Method

All chromatographic and mass spectrometric analysis were performed on a Waters Acquity H-Class Bio UPLC system coupled with Xevo G2-XS QToF MS (Waters Corporation, Milford, USA), equipped with an electrospray ionization (ESI) source. The system was controlled by UNIFI™ software (v1.9.4). The mass spectrometer was run in positive mode and leucine enkephalin was continuously applied as a reference lock mass standard throughout data acquisition.

Intact and deglycosylated-reduced intact mass analysis were performed using a C4 reversed-phase column combined with a short UPLC gradient prior to ESI and ion detection. The processing of acquired data was done by applying maximum entropy deconvolution (MaxEnt1™).

For peptide mapping analysis, samples were denatured, reduced, and alkylated. The samples were then desalted, and buffer exchanged into an ammonium bicarbonate buffer using polyacrylamide desalting column. Each sample was digested using trypsin at a final concentration of 1:25 (w/w). Digestion was proceeded for 30 min at 37°C. The obtained peptides were analyzed using C18 reverse-phase column with a 90 minute UPLC run prior to ESI and ion detection. Identification

of PTMs was achieved through inclusion of potential amino acid mass modifications in the UNIFI peptide search algorithm.

The released *N*-glycan analysis were carried out by using Waters GlycoWorks *Rapi*Fluor-MS *N*-glycan kit. First, the sample was denatured and deglycosylated. The released glycans were then labeled with the *Rapi*Fluor-MS solution. Samples were cleaned using solid-phase extraction on a GlycoWorks HILIC elution plate. The glycans were then separated on an UPLC-FLR system and the results were integrated and labeled based on retention times and mass data.

## Results and Discussion

Intact mass analysis revealed that the molecular masses of 147,664 Da, 147,869 Da, 148,026 Da, and 148,175 Da represent G0F/G0F-GN (-2Lys), G0F/G0F (-2Lys), G0F/G1F (-2Lys) and G0F/G1F (-Lys) glycoforms (glycosylation at the heavy chain Asn298) occupancies, respectively. The masses of all detected species were consistent with N-terminal pyroglutamate (pyroQ) formation from glutamine on both heavy chains.

The major components detected by deglycosylated-reduced intact mass analysis were reduced mAb LC and deglycosylated/reduced mAb HC having N-terminal pyroQ and C-terminal Lys truncation. The results for intact and deglycosylated-reduced intact mass analysis were consistent with the expected structure of mAb and demonstrated comparability between TUR03 and tested innovator lots.

According to the guideline on biosimilar drugs, a biosimilar must have the same amino acid sequence as its innovator. The amino acid sequence of TUR03 and innovator were determined by peptide mapping analysis. Peptide-level sequence coverage was 100% when trypsin and Lys-C enzymes were used sequentially. TUR03 and innovator have the same amino acid sequence. Post-translation modifications (PTMs) detected by peptide mapping included N-terminal pyroQ, C-terminal Lys truncation, Met oxidation, and Asn deamidation. The most oxidized tryptic peptide was HC:T18 with a sequence of DTLMISR. Met253 was more prone to oxidation compared to the other Met residues. The major clip detected by peptide mapping was C-terminal Lys truncation of the heavy chain (~25%). Moreover, peptide mapping analysis showed that glycosylation site was Asn298 on the heavy chain of both products. The calculated PTM levels are comparable between TUR03 and tested innovator lots.

The major detected *N*-glycan was the complex biantennary fucosylated *N*-glycan G0F, which ranged from ~60% to ~70%. The levels of fucosylated complex *N*-glycans were more than 90% and were comparable between TUR03 and innovator lots. The results for *N*-glycan profiling demonstrated comparability between TUR03 and tested innovator lots.

## Conclusion

The overall results have revealed that TUR03 is highly similar to its innovator in terms of critical quality attributes (CQAs) measured by high resolution mass spectrometry. This aspect offers affordable alternative drugs to the patients and provides huge savings to healthcare systems.

## Acknowledgment

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## OP27- Experimental Approach to Drug Interference in Biotidinase Assay with LC-MS / MS

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**Objective:** Biotidinase enzyme activity is studied by fluorometric method from dry blood of whatman paper. Affecting enzyme activity may cause some erroneous clinical interpretations. Drugs may also be among the molecules that cause this condition. The aim of this study is to show the effect of different drugs on biotidinase analysis in in vitro environment.

**Methods:** Materials used in routine control determination of biotidinase activity 20uL medication (Calcium folinate 100 mg / 10 ml, Clarithromycin 500 mg IV, Topotecan HCL 4 mg, Cisplatin 10 mg / 20 mL, Trabektedine 1 mg, Doxorubicin 2 mg / ml, Vancomycin HCL 500 mg) and distilled water was dried at room temperature after dropping. Blood extracted by taking 1 mm punches was studied by fluorometric method. Biotidinase analysis results obtained with 3 repetitions were recorded where each drug affected. Deviation amounts were calculated with bias%.

**Results:** The detected deviation range was calculated between -54.06% and 643.33%. The largest deviation was at 643.33% calcium folinate. The deviation was 74.58% with topotecan HCL, -54.06% with doxorubicin and 79.58% with vancomycin HCL.

**Conclusion:** Calcium solution caused significant biotidinase activity change. Deviations in biotidinase activities in patients treated with topotecan HCL, doxorubicin, vancomycin HCL may cause incorrect clinical interpretation. Confirmation of analytical errors is recommended by supporting this study in in vivo.

**Keywords:** biotidinase, clarithromycin, calcium, cisplatin, vancomycin, analytical error

## OP28- The Effect of Contrast Media on Biotidinase Analysis Performed with Dry Spot Test

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**Objective:** Biotinidase enzyme deficiency is also examined in the Ministry of Health metabolic screenings. Using contrast agents before enzyme activity in these patients may cause a change in activity. Diagnosis delay may cause complications and malpractice. The aim of this study is to show the effect of different contrast agents on biotinidase analysis in an in vitro environment.

**Methods:** 20uL contrast agent (Iohexol 647 mg/100 mL, Ioversol 636mg/mL(100 mL), Iohexol 350mg/50 mL, Gadopentetik dimeglumine 469.01 mg/1 mL, Gadoteric acid 376,9 mg/20 mL, Gadoxetic acid disodium 181.43 mg/mL, Iobitridol 658.1 mg/mL) and distilled water were instilled and dried at room temperature. The extracted blood was studied by fluorometric method by taking 1 mm punches. The biotinidase analysis results obtained from the affected area of each drug in 3 repetitions were recorded. Deviation amounts were calculated with bias%.

**Results:** The detected deviation range was calculated between -35.10% and 13.02%. The greatest deviation, -35.10%, occurred in Iohexol (647 mg) preparation. Deviations of -9.74% with Ioversol (636mg/mL, 100 mL), - 19.79% with Gadoxetic acid disodium (181.43 mg/mL) and - 23.55% with Iobitridol (658.1 mg/mL) were seen.

**Conclusion:** Negative deviation was detected for contrast agents in general. False negative results in biotinidase activity may cause erroneous diagnosis and unnecessary further investigation. It should not be forgotten that the prolonged hospitalization of pediatric patients caused by the wrong diagnosis increases the risk of anxiety in the parents.

**Keywords:** Biotidinase deficiency, Iohexol, Iobitridol, Analytical error

**OP29- Synthesis and molecular docking study of some dibenzazepine-triazole hybrid derivatives as potential drug candidates for the treatment of Epilepsy's disease**

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**Abstract-**Herein, a series of dibenzazepine derivatives **3a-d** were designed by molecular hybridization of two important cores namely dibenzazepine and triazole, respectively, and synthesized via Click reaction in high yields. Then, interaction of the dibenzazepine-triazole derivatives **3a-d** with the protein of epilepsy (7CQF) was investigated through *in silico* approach by application of bioinformatics tools. The current study exhibited that the compound **3a, 3b and 3d** has significant activity and their docking scores compared to standard eslicarbazepine acetate (*Zebinix*), Oxcarbazepine (*Trileptal*), and Phenytoin.

**Keywords:** Epilepsy, dibenzazepine, molecular hybridization, Click reaction, molecular docking.

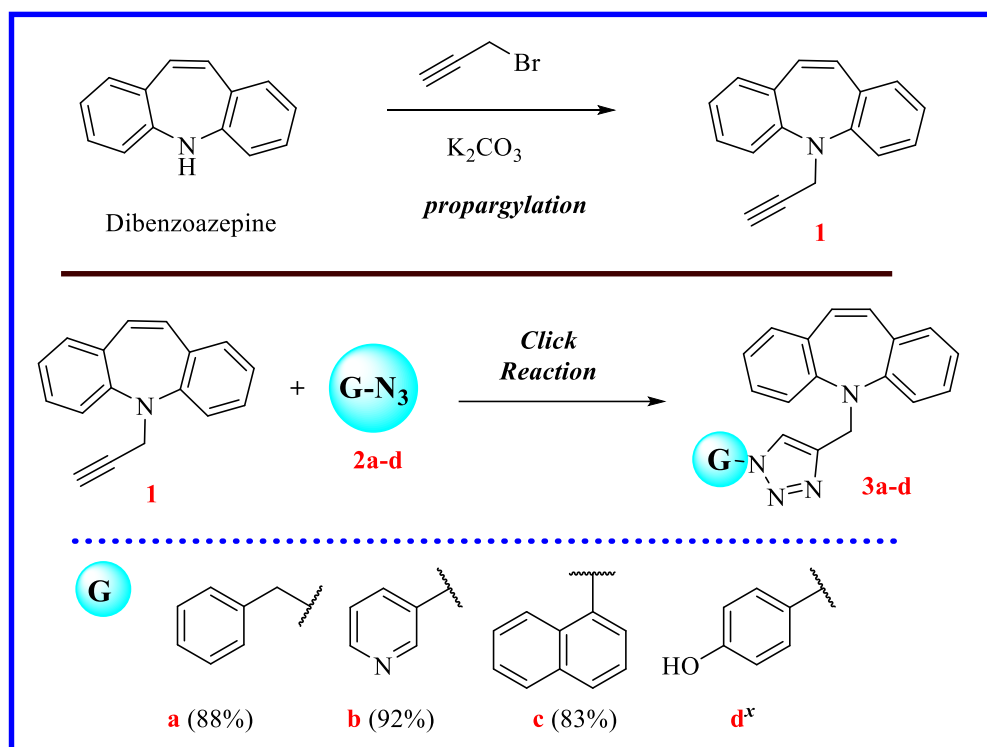
### **Intruduction**

Epilepsy, known as ‘a disease of the brain’, is a common neurological disease characterized by the periodic and unpredictable occurrence of seizures, affecting more than 50 million people worldwide [1,2]. With available marketed antiepileptic drugs complete control of seizures is not achieved still date and also these drugs are associated with a wide range of side effects, and a reduced quality of patient’s life, resulting in a strong medical demand for novel treatment strategies for epilepsy [2].

On the other hand, dibenzazepine (*iminostilbene*) and its derivatives are well recognized heterocyclic compounds in medicinal and pharmacologically important antidepressant drug discovery which are particularly effective in the central nervous system [3]. Carbamazepine (*Tegretol*), oxcarbazepine (OXCZ) and eslicarbazepine acetate (ESCZ) are dibenzazepine derivatives that used in the treatment of epilepsy, schizophrenia, panic attack, etc. one of the common chronic neurological disorders [3]. Molecular docking is a molecular modeling technique that is used to predict how a protein (enzyme) interacts with small molecules (ligands or drugs). The docking is often used in the molecular modeling and computer aided drug design. These *in silico* studies could reduce the costs of research by diminishing the random trial and error, and save the time before testing at *in vivo* and *in vitro* environments [4].

### **Material and Method**

With the aim of obtaining new and effective antiepileptics, herein, a series of dibenzazepine derivatives **3a-d** were designed by molecular hybridization of two important cores namely dibenzazepine and triazole, respectively with the view of structural requirements of pharmacophore for potential antiepileptic agents. The target compounds **3a-d** were synthesized in two steps namely *N*-propargylation of dibenzazepine and Click reaction, respectively in yields (83-92%) (Scheme 1). For this, firstly 5-(prop-2-yn-1-yl)-5*H*-dibenzo[*b,f*]azepine (**1**), which was synthesized according to a previously reported method [3], became the key structure that allowed us to prepare dibenzazepine derivatives **3a-d**. In addition, azide derivatives **2a-d** were synthesized by Sandmeyer reaction starting from their amine derivatives according to methods in the literature [5].

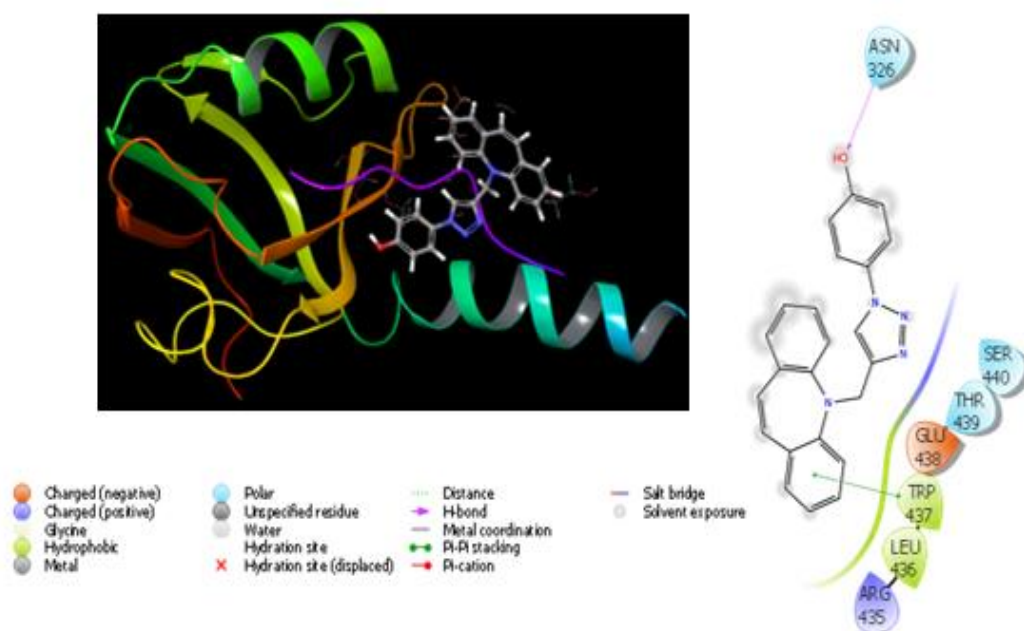


**Scheme 1.** Route of synthesis of the dibenzoazepine-triazole hybrid derivatives **3a-d**. <sup>x</sup>Synthesis of this compound continues (conditions for the Click reaction; azide derivative (1.10 equiv), CuSO<sub>4</sub>·5H<sub>2</sub>O (0.35 equiv), (+)-Sodium *L*-Ascorbate (0.20 equiv), *t*-BuOH/H<sub>2</sub>O (1:1), r.t, 16 h).

**Experimental Results;** NMR data of the obtained compounds confirm molecular structures of the compounds. 5-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-5*H*-dibenzo[*b,f*]azepine (**3a**): 88% Yield, gray crystals, <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.35-7.19 (m, 6H), 7.18-7.05 (m, 4H), 7.01 (t, *J* = 7.4 Hz, 2H), 6.92 (dd, *J* = 7.4, 1.3 Hz, 2H), 6.75 (s, 2H), 5.39 (s, 2H), 5.14 (m, 2H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 149.93, 146.26, 134.99, 133.56, 132.05, 129.14, 129.03, 128.89, 128.28, 127.21, 123.76, 122.94, 120.59, 53.79, 47.46. 5-((1-(pyridin-3-yl)-1*H*-1,2,3-triazol-4-yl)methyl)-5*H*-dibenzo[*b,f*]azepine (**3b**): 92% Yield, white solid, <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.84 (s, 1H), 8.64 (d, *J* = 4.0 Hz, 1H), 8.01 (ddd, *J* = 8.3, 2.5, 1.4 Hz, 1H), 7.74 (s, 1H), 7.43 (dd, *J* = 8.2, 4.8 Hz, 1H), 7.33-7.22 (m, 2H), 7.23-7.08 (m, 4H), 7.03 (t, *J* = 7.4 Hz, 2H), 6.84 (s, 2H), 5.24 (s, 2H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 149.86, 149.55, 147.42, 141.32, 133.70, 133.60, 132.14, 129.26, 129.19, 128.00, 124.15, 123.99, 120.49, 120.39, 47.28. 5-((1-(naphthalen-1-yl)-1*H*-1,2,3-triazol-4-yl)methyl)-5*H*-dibenzo[*b,f*]azepine (**3c**): 83% Yield, <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.94 (dd, *J* = 7.7, 0.9 Hz, 1H), 7.89 (d, *J* = 8.3 Hz, 1H), 7.54-7.44 (m, 4H), 7.38-7.26 (m, 4H), 7.10 (d, *J* = 7.2 Hz, 2H), 7.03 (t, *J* = 7.6 Hz, 3H), 6.83 (d, *J* = 8.5 Hz, 1H), 6.73 (s, 2H), 5.29 (s, 2H).

Then, interaction of the obtained dibenzoazepine-triazole derivatives **3a-d** with the protein of epilepsy (7CQF) was investigated through *in silico* approach (molecular docking) by application of bioinformatics tools. In the calculations made, Maestro Molecular modeling platform (version 12.2) by Schrödinger program. Many modules are used in the calculations. It is made using many modules such as the LigPrep module, the protein preparation module, The Glide ligand docking module, and the Qik-prop module. The X-ray crystal structure of 7CQF (PDB ID) was downloaded from Protein Data Bank.

## Results and Discussion



**Figure 1.** Presentation interactions of the compound **3d** with epilepsy protein *PSD-95 PDZ3 fused with ADAM22 C-terminal peptide* (ID:7CQF in PDB).

**Table 1.** Numerical values of the docking parameters of the compounds **3a-d** and standards against epilepsy protein (7CQF).

AChE	Docking Score	Glide ligand efficiency	Glide Hbond	Glide evdw	Glide ecoul	Glide emodel	Glide energy	Glide einternal	Glide posenum
<b>3d</b>	<b>-3.76</b>	-0.13	-0.32	-3.72	-6.14	-36.58	-29.86	0.60	282
<b>3a</b>	<b>-3.62</b>	-0.11	0.00	-6.42	-4.48	-33.85	-30.90	6.01	184
<b>3b</b>	<b>-3.21</b>	-0.12	0.00	-24.26	-2.52	-30.75	-26.78	3.33	380
<b>ESCBZ</b>	<b>-3.13</b>	-0.14	0.00	-20.66	-1.35	-26.00	-22.00	1.94	358
<b>OXCBS</b>	<b>-3.11</b>	-0.16	0.00	-17.25	0.04	-21.60	-17.20	0.00	217
<b>3c</b>	<b>-2.95</b>	-0.10	0.00	-25.00	-1.93	-30.04	-26.93	4.95	142
<b>Phenytoin</b>	<b>-1.81</b>	-0.10	-0.34	-0.71	-5.98	-21.46	-16.69	0.73	269

As a result, the compounds **3a-d** were obtained in good yields in two steps. Table 1 show numerical values of the docking parameters of the compounds **3a-d** and standards against epilepsy protein (7CQF). The parameter used to compare pharmacological activity between these parameters, is the docking score. If the numerical value of this parameter of a molecule is more negative than other molecules, the pharmacological activity of this molecule is higher than others. Among other parameters, Glide ligand efficiency, Glide ecoul, and Glide emodel parameters are the parameters obtained as a result of chemical interactions of molecules. However, other parameters obtained are Glide energy, Glide einternal, and Glide posenum parameters are parameters that occur from the pose formed between epilepsy protein and molecules.

The compound **3d** was found to have one hydrogen bond and one  $\pi$ - $\pi$  stacking interactions with the most important active site residues (Figure 1). The present study exhibited that the compound **3d**, bearing hydroxyl group at *para* position of aromatic ring in triazole skeleton, has the best activity and their docking scores compared to its other derivatives (**3a-c**), and standards (Phenytoin, ESCBZ and OXCBS) (Table 1).



## Conclusion

A series of dibenzoazepine derivatives **3a-d** were designed, and synthesized by Click reaction in good yields. Interaction of the synthesized compounds **3a-d** with the protein of epilepsy was investigated through molecular docking study. The docking scores of the compounds gave better results compared to standards against epilepsy protein. The synthesized compounds may serve as leads in the discovery and development of new antiepileptic drugs.

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**OP30- In-depth Profiling of Milk Whey N-glycans by MALDI-MS using Linkage-Specific Sialic Acid Esterification Approach**

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Glycosylation is one of the most common important post-translational modifications in cell proteome. Many glycoproteins contain different types of glycans in a glycosylation site (in the polypeptide chain). It is very important to understand the function of the glycan varieties in cellular activities. Post-translational modifications of milk whey proteins expanded the complexity of milk compositions and contributed to their biological functions. It has emphasized that the milk *N*-glycans have an anti-pathogenic effect, and glycans containing sialic acids in milk whey's proteome play a role in newborns' immune system. Although there is considerable information about the types and roles of free oligosaccharides in the milk, very little is known about glycans' biological properties associated with milk glycoproteins. In the study, a MALDI-MS-based methodology using a linkage-specific sialic acid esterification approach was applied to milk whey glycoproteins derived from different species (human, cow, buffalo, goat, and donkey). First, *N*-glycans were released from milk whey glycoproteins, and linkage-specific ethyl-esterification of sialic acid residues was performed. Then, MALDI-MS analysis of the ethyl-esterified *N*-glycans was performed in reflectron and positive ionization mode. Relative abundances of the detected *N*-glycan structures were calculated. Comparisons were also performed concerning to relative abundances of *N*-glycans and *N*-glycosylation traits. In the study, the number of the detected whey *N*-glycans were 67, 75, 71, 99, and 66 for the species of human, cow, buffalo, goat, and donkey, respectively. It was determined that cow and buffalo whey *N*-glycan profiles were mostly overlapped. Goat milk whey *N*-glycan profile showed the most heterogeneity. Acetylated *N*-glycans were only observed in donkey milk *N*-glycan profiles (16.06 %). Human milk *N*-glycans was found to be the most fucosylated among species (62.72 %). *N*-glycosyl neuraminic acid-containing *N*-glycan structures were found in all species except human milk whey glycoproteins. Human milk whey *N*-glycans contained mostly alpha2,6-linked *N*-acetyl neuraminic acid type sialic acids (65.16 %). it was detected goat whey milk *N*-glycans included a high abundance of *N*-glycosyl neuraminic acid-containing *N*-glycans (28.24 %).

**Keywords:** Milk whey proteins, glycosylation, glycomics, mass spectrometry

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**OP31- PAH analysis of mussel samples collected from Giresun coastline-Black Sea**

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PAH compounds formed as a result of the combustion of fuel types containing large amounts of carbon are industrial waste discharge, atmospheric particles, organic pollutants mixed with water, air and soil with the pouring of fossil fuels into the water environment<sup>1</sup>. The United States Environmental Protection Agency (US-EPA) named 16 of these compounds as priority pollutants<sup>2</sup>.

In the spring of 2019, the mussel(*Mytilus galloprovincialis*) samples collected from Giresun center and 7 district seaports were weighed as approximately 15-20 grams, and the saponification reaction was performed to remove the lipid molecules in the wet mussels with the prepared 2N methanolic KOH solution. PAH compounds with cyclohexane were extracted into the organic phase. PAH compounds were separated by column chromatography using the hexane: DCM solvent system deactivated florisil or alumina filling, dissolved with n-hexane. The sample, whose solution was evaporated with the evaporator, was analyzed in the GC/MS device.

It was found in the range of 0.54-406.94 ppb in the spring sampling and the lowest fluorine in Bulancak station; the highest was found at Fenantren Espiye station..Phenanthrene, acenaphthene and benzo [a] anthracene compounds were found to be the highest in the spring sampling. Fenantrene concentration is quite high at 7 stations, and the mussel sampling with the highest concentration of 3 compounds was determined as Giresun center site. The PAH values of the mussel sample collected from Görele locality are seen as the least amount compared to the values in other stations.

**Keywords:** Mussel, PAH, GC/MS, Black Sea

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**OP32- The Famous Turkish Rose Essential Oil: Characterization and Authenticity Monitoring by FTIR, Raman and GC-MS Techniques Combined with Chemometrics**

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Oil rose “*Rosa damascena*” is an emblematic rose specie for both Isparta and Turkey. Monitoring of the authenticity of rose essential oil has great importance for protection of consumer and quality properties of unique natural product. This study was built for evaluation of FTIR, Raman and GC-MS techniques combined with chemometrics for discrimination and classification of authentic rose essential oil from fraudulent commercial rose essential oils.

In the current research, rose essential oil samples (n=12) and commercial samples (n=20) were obtained from producers (Isparta, Turkey) and different brands, respectively. Analyses were performed by FTIR (Bruker Tensor 27, Germany) and Raman (Rigaku, USA) spectroscopy techniques. Volatile composition of rose essential oil samples were determined by GC-MS (Shimadzu, Italy) technique. HCA (hierarchical cluster analysis) and PCA (principal component analysis) were applied to the FTIR and Raman data of all samples by using OPUS program Version 7.2 (Bruker GmbH). HCA dendrogram was obtained from GC-MS data of all samples by using the supervised SIMCA 15 (Umetrics, Umea, Sweden) chemometrics software.

As results, characterization of authentic rose essential oil was performed by FTIR, Raman and GC-MS techniques. Rose essential oil (n=12) were clearly distinguished from fraudulent samples by using FTIR, Raman and GC-MS techniques. A separate methodology was built for each of analytical technique combined with chemometrics. Two of the twenty commercial samples were determined to be authentic Rose essential oil. Identical results were obtained by using the three different analytic techniques (FTIR, Raman and GC-MS). Obtained results proved that each of the FTIR, Raman and GC-MS techniques could be used effectively for determination of the authenticity of Rose essential oil.

**Key Words:** *Rosa damascene essential oil*, rose, FTIR, Raman, GC-MS, authenticity

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**OP33- Pervaporation Separation of Ethyl Alcohol–Water Liquid Mixtures Using Poly(vinyl alcohol)/(Chitosan-g-Poly(N,N-Dimethylacrylamide)) Membranes**

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Pervaporation (PV) is a membrane-based process which is considered one of the most viable, economical, and eco-friendly and energy-efficient alternatives to traditional separation methods [1, 2]. In this work, poly(vinyl alcohol)/(chitosan-g-poly(N,N-dimethylacrylamide)) (PVA/CS-g-PDMAAm) membranes were used for separation of ethyl alcohol/water (EtOH/water) azeotropic mixtures using pervaporation method.

The effect on the pervaporation efficiency of various parameters such as CS-g-PDMAAm yield, membrane thickness, temperature and feed concentration was investigated and the optimum conditions were obtained. It has been found that the selectivity is higher at low temperatures. However, it was found that the selectivity increased by increasing the amount of CS-g-PDMAAm in the blend membrane and feed concentration but increased the membrane thickness decreasing the selectivity. The best separation factor was determined to be 33.5 at a flux of 50 g/m<sup>2</sup>.h at 15 °C temperature.

**Keywords:** Pervaporation, Ethyl Alcohol, Poly(vinyl alcohol), Chitosan, N,N-dimethylacrylamide

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**OP34- Development of a Sensitive Analytical Method for the Determination of Rhodium in Metal Coating Wastewater Samples using Microextraction Strategy- Slotted Quartz Tube-Flame Atomic Absorption Spectroscopy**

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It is possible to improve the accuracy of the system by developing different analytical strategies for the flame atomic absorption spectrometer<sup>1</sup>. In this study, it was aimed to develop a simple, sensitive and cheap analytical method for the determination of rhodium as one of the platinum group metals in metal plating wastewater. The analytical method was developed to lower the detection limit of rhodium with a slotted quartz tube (SQT) cut in accordance with the burner head of the device. A flame atomic absorption spectrometer (FAAS) was used for rhodium determination with a hollow cathode lamp. Solid phase microextraction strategy was applied to obtain high sensitivity for the analyte. All the system parameters were optimized to enhance the detection power. After optimizing the system parameters, enhancement in detection power was calculated using the detection limits of FAAS and developed analytical method. The data obtained as a result of this study showed that the sensitivity of the system can be increased with the combination of a simple and inexpensive microextraction strategy and SQT-FAAS system. The developed method was applied to metal coating wastewater sample for their rhodium content which is harmful to the environment and health.

**Keywords:** Rhodium, slotted quartz tube, spectroscopy

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### OP35- Color removal of dyes using immobilized enzyme

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Reactive dyes are widely used in the textile industry due to their easy handling, cost effectiveness in synthesis, stability, color diversity and better dye processing conditions. On the other hand, many synthetic dyes are resistant to biodegradation due to the large amount of aromatic structure, complex molecules and synthetic origin<sup>1</sup>. It is known that the horseradish peroxidase (HRP) enzyme has the capacity to remove phenolic compounds and aromatic amines from aqueous solutions and also to de-color textile waste<sup>2</sup>.

In this study, enzymatic decolorization of methyl orange (MO) and Rhodamine B dyes were monitored with UV-Vis spectrophotometer. For this purpose, tris (2-aminoethyl) amine polystyrene (2AEPS) was reacted with 2-hydroxy-1-naphthaldehyde and polymeric microspheres containing Schiff base were obtained (2AEPS-napht). HRP enzyme was immobilized on these spheres by adsorption method. Color removal studies were performed against various parameters such as pH, temperature, dye concentration, and H<sub>2</sub>O<sub>2</sub> concentration.

Optimum pH value for dye removal using immobilized enzyme was determined as pH: 7 and pH: 3 for MO and Rhodamine B, respectively. After 24 hours, the color removal for MO dye was about 70%, while the color removal for Rhodamine B dye was about 80%. was obtained.

**Keywords:** Methyl orange, Rhodamine B, horseradish peroxidase, Schiff bases, enzyme immobilization

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**OP36- Recovery of Boron by Using Poly (vinyl alcohol)/(Chitosan-g-Poly (N, N-Dimethylacrylamide)) Membranes by Pervaporation Method**

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As an alternative of distillation, pervaporation is an energy-efficient and environmentally-friendly membrane process for water–organic, organic–water, or organic–organic mixtures, and thermally sensitive compounds. In the pervaporation separation, a membrane acts as the separating barrier for the component of minor affinity [1]. While boron is widely dispersed in natural waters as one of the vital elements for plants, animals and humans, high concentrations of boron in water present a serious problem for domestic and agricultural uses [2].

In this work, poly(vinyl alcohol)/(chitosan-g-poly(N,N-dimethylacrylamide)) (PVA/CS-g-PDMAAm) membranes were used for recovery of boron using pervaporation method. The effects of the CS-g-PDMAAm percentage, membrane thickness, working temperature, feed solution concentration and pH on the rejection and flux were investigated by separating Boron/water mixtures with the pervaporation technique. Also, Boron removal from wastewater was performed using the pervaporation technique under optimum conditions. Also, Boron was not detected in any permeates, and membranes could be used over and over again.

**Keywords:** Pervaporation, Boron, Poly(vinyl alcohol), Chitosan, N,N-dimethylacrylamide

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### **OP37- Evaluation of antioxidant properties of yarrow depending on extraction mixing techniques**

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Medicinal plants are one of the most important resources for the protection of human health due to their nutritional properties and bioactivities. They are preferred in traditional medicine, food and pharmaceutical products because they are safer and more beneficial. Yarrow belonging to genus *Achillea* L. from *Asteraceae* family has high bioactivity due to terpenes, flavonoids, coumarins, phenolic acids, ligands and essential oils [1, 2]. It is reported that yarrow has positive effects in the treatment of hepatobiliary diseases and amenorrhea [3]. Therefore, it has been still investigated the antitumor, anti-microbial, anti-inflammatory and antioxidant properties of this plant. There are different results about the chemical composition and antioxidant properties of yarrow in the literature. There are different results about the chemical composition and antioxidant properties of yarrow in the literature. The difference between results is not only due to genetic and environmental conditions, but also to the sample preparation procedure.

In this study, the antioxidant activity (DPPH and ABTS radical scavenging activity) and total phenolic content of yarrow were investigated using different extraction mixing techniques reported in a study (soaking, stirring, shaking, sonication-assisted stirring) [4]. It was determined that antioxidant activity and total phenolic content results varied depending on the extraction mixing techniques. When the results obtained were evaluated taking into account all extraction mixing techniques, the DPPH radical scavenging activity, ABTS radical scavenging activity, and total phenolic content results in order from highest to lowest were as follows: sonication-assisted stirring, stirring, shaking, soaking. Sonication-assisted stirring was determined as the best extraction mixing technique in determining the antioxidant properties of yarrow.

**Keywords:** Antioxidant property, extraction mixing technique, yarrow

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### OP38- Purification and Characterization of Polyphenol Oxidase Enzyme from Damson Plum (*Prunus insititia*)

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Polyphenol oxidase (PPO), an oxidoreductase enzyme containing copper that catalyzes the hydroxylation of monophenols to o-diphenols and the oxidation reactions of o-diphenols to o-quinones, performs browning reactions in fruits and vegetables<sup>1</sup>. *Prunus plum* is a species having high phenolic content and high antioxidant activity as the enzyme source<sup>2</sup>.

In our study, PPO enzyme was partially purified by 0-80% ammonium sulfate precipitation and dialysis, respectively. By using partially purified Polyphenol oxidase enzyme extract (PPP), the enzyme characterization were studied to determine optimum activity buffer concentration, pH and temperature values and the kinetic constants were determined for each PPO enzyme substrate called as catechol, 4-methyl catechol, pyrogallol and caffeic acid, respectively. In the last step, the enzyme was purified by affinity chromatography using Sepharose 4B-L-Tyrosine-p-amino benzoic acid affinity gel.

According to the characterization studies performed with the *Prunus insititia* PPP enzyme; optimum values were determined for catechol, 4-methyl catechol, pyrogallol and caffeic acid substrates as 0.05M - pH: 7.2 - 25°C; 0.2M - pH: 4.5 - 10°C; 0.01M - pH: 6.8 - 5°C and 0.2M - pH: 8.5 - 10°C, respectively. The  $V_{max}$  and  $K_M$  kinetic constants were calculated for the same substrates of PPO enzyme as 17219.97 U/(mL\*min) and 11.67 mM; 7309.73 U/(mL\*min) and 5 mM; 12580.12 U/(mL\*min) and 3.75 mM; 12100.41 U/(mL\*min) and 6.25 mM, respectively. The enzyme was purified by affinity chromatography, using Sepharose 4B-L-tyrosine-p-amino benzoic acid affinity gel. The activity of pure PPO enzyme obtained from affinity gel was calculated as 3850.5.U/(mL\*min) with catechol substrate at an equilibrium concentration of 13mM.

PPO enzyme activity was measured by using catechol which the enzyme had the highest  $V_{max}$  value. Since the purification of PPO enzyme from *Prunus insititia* plum by affinity chromatography have not been investigated in literature yet, a new enzyme source has been gained to the literature with this study. The enzyme purification studies by affinity chromatography have been continued.

**Keywords** Polyphenol oxidase, *Prunus insititia*, purification, characterization

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#### Acknowledgment

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**OP39- Colorimetric detection of cysteine using magnetic iron oxide – carbon dots as peroxidase mimetics**

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As one of the non-essential amino acids, cysteine plays important role in many biological functions such as the synthesis of glutathione which is an important antioxidant, strengthening of hair, the regulation of immune system activity and the protection of digestive system <sup>1, 2</sup>. This amino acid has been widely used as a part of dietary supplements alone or with other amino acids, vitamins or minerals. Therefore, it is very important to investigate the cysteine content of the supplements from the viewpoints of its application in food and medicine industries

In this study, a facile and simple colorimetric approach was reported for the determination of cysteine in nutritional supplements. In this regard, a highly effective catalyst, magnetic iron oxide-carbon dots was fabricated through a two-step route including the preparation of carbon dots from aloe vera leaf using microwave-assisted hydrothermal green synthesis and the co-precipitation of magnetic iron oxide in the aqueous medium of carbon dots. The strong affinity of prepared nanoparticles to peroxide and 3,3',5,5'-tetramethylbenzidine (TMB) caused a rapid color change which was decreased with the presence of cysteine in the reaction media. Accordingly, cysteine was detected based on the decrease in color intensity. A wide linear range and low detection limit were obtained for the analyte using the developed method. The method was applied to dietary supplements and acceptable recoveries were obtained.

**Keywords:** cysteine, carbon dots, magnetic iron-oxide, catalyst.

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**OP40- Use of ZnO nanoflowers for heavy metal ion adsorption**

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ZnO nanoparticles (NPs) attract a lot of notice for rudimentary studies and potential applications in different research areas. ZnO NPs represent a multidirectional material, and their superior properties find current and potential applications in water treatment, heavy metal removal, catalysts, transducers, semiconductors, textile, cosmetics<sup>1,2</sup>, etc. In this study, ZnO particles was chosen because it is cheap and has good photocatalytic performance in heavy metal ions removal. This study investigated the removal of Ni<sup>2+</sup> and Pb<sup>2+</sup> from aqueous solutions with the novel ZnO nanoparticle adsorbent. Analytical parameters in the optimal adsorption conditions of ZnO-NPs such as model solution pH, adsorbent amount, eluent type and volume, ligand amount, sample volume and matrix effect were determined for Ni<sup>2+</sup> and Pb<sup>2+</sup>. As a result of these studies, quantitative recovery of Ni<sup>2+</sup> and Pb<sup>2+</sup> was obtained at pH 6.0 and 7.0, respectively. Quantitative recovery of analyte ions was observed with 5 mL 3M HNO<sub>3</sub> solution as eluent.

The developed method has been applied to various water and food samples and the accuracy of the developed method has been confirmed by certified reference material analysis and addition recovery studies. The obtained percent recovery values of Ni<sup>2+</sup> and Pb<sup>2+</sup> (88.4 –103.6 and 75.8 – 101.7, respectively) confirmed the applicability of the method with high accuracy and precision.

**Keywords:** ZnO nanoflowers, heavy metals, atomic absorption spectrometry

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## OP41- Investigation of Paclitaxel Drug Interaction with Molecular Docking

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It has been shown in some studies using capillary electrophoresis that taxol binds to the main and small grooves of DNA and to the backbone phosphate group but does not cause any change in the helix<sup>[1,2]</sup>. The affinity of paclitaxel to DNA by molecular docking by excluding thermodynamic interactions of other molecules has not been investigated before. In this study, the interaction of 2 DNA molecules with different chain lengths and paclitaxel was modeled. Since the total negative charge and surface area of the long-chain DNA molecule is higher, the number of negative conformations quite high. Despite the formation of H-bonds in some conformation of both DNA molecules, positive interaction was calculated especially in the short chain DNA model. It can be said that there is a non-spontaneous interaction in this model. On the other hand, while the free binding energy for the most probable model of the short chain DNA molecule was -1.48 kcal / mol, it was calculated as -3.94 kcal / mol in the other model. Besides the H-bond, another effective force is the van der Waals interaction. From this, it can be concluded that paclitaxel interacts partially with the DNA molecule but cannot disrupt the structure. We believe that the genotoxic effect of taxol is not directly related to itself, but because it is associated with the increase in DNA metabolites triggered by apoptosis.

**Keywords:** DNA, Paclitaxel, molecular docking, genotoxicity

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## OP42- Impact of wood ash on nitrogen circulation in pine needles, blueberry, and lingonberry plants

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In order to provide heat supply as well as local electricity supply, many boiler houses use various materials of organic origin as fuel, most often wood. In the heating process, a lot of wood ash is produced, which contains little organic matter, but a lot of minerals and nitrogen in the form of nitrates, and in practice this ash could be used, for example, to improve forest soils.

Samples of pine needles and blueberry and lingonberry plants were collected from forests, where wood ash and ammonium nitrate were used for soil improvement, as well as control plant samples from forests parts, where no additional improvement of the soil was performed. The Isotope – Ratio Mass Spectrometry (IRMS) method was used to assess the changes of introduced mineral nitrogen in the studied plants.

Obtained results showed that the largest changes in  $\delta^{15}\text{N}$  values between samples from the control plot and plots with dispersed soil ash supplements were observed in the case of pine needles, while in blueberry and lingonberry samples the difference was insignificant, indicating more active nitrogen transport to the youngest parts of the plant (pine needles). In the samples of lingonberry leaves the values of  $\delta^{15}\text{N}$  were 1 ‰ higher and the proportion of nitrogen mass was 0.2% higher than in the lingonberry stems, which indicates a more active nitrogen transfer directly towards the plant leaves.

From the obtained results, it can be observed that it is possible to use wood ash as additives for forest soil improvement, there are considerable changes in nitrogen uptake in pine needles, and IRMS method is a powerful tool for the assessment of nitrogen sources in studied plants.

**Keywords:** wood ash, pine needle, nitrogen,  $\delta^{15}\text{N}$ , IRMS

**Acknowledgement:** This work was supported by Short term scientific mission organized in scope of project "Research program on improvement of forest growth conditions 2016-2021"



**OP43- Application of cyclically refreshable liquid amalgam film silver based electrode to study the kinetics and mechanism of Bi(III) ion electroreduction in the presence of 2-thiocytosine as regards of the "cap - pair" effect**

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**Abstract:** The process of Bi(III) ions electroreduction in chlorates(VII) on the cyclically refreshable liquid amalgam film silver based electrode (R-AgLAFE) is catalyzed by 2-thiocytosine according to the „cap-pair” rule. The research results show that the mechanism of the electrode process is affected the active Bi-(RS-Hg) complexes located in the adsorption layer which mediate the electron transfer.

**Keywords:** „cap-pair”, 2-thiocytosine, R-AgLAFE

**Introduction:**

The electrochemical activity of metal ions is determined by the type of base electrolyte, the presence of organic substances and the material of the working electrode. This problem is related to issues such as charge transfer resistance, concentration polarization, electrode position efficiency, surface super saturation or electrode deactivation which translates into the accuracy and sensitivity of voltammetric measurements.

So far, the „cap-pair” effect<sup>1</sup> has been studied only on the mercury drop electrodes, however, due to restrictions on the use of metallic mercury, the usage of the cyclically refreshable liquid amalgam film silver based electrode (R-AgLAFE) seems to be a good alternative because, like the mercury drop electrode, it guarantees an equally accurate determination of kinetic parameters<sup>2,3</sup>.

This approach fits well with the current green chemistry trend due to the diametrically lower consumption of mercury to produce an amalgam film and the reduction of generated waste.

The mentioned advantages of this electrode meant that R-AgLAFE was used for studying the kinetics and mechanism of Bi(III) ion electroreduction in terms of the „cap-pair” effect<sup>2</sup> and the presence of 2-thiocytosine (TC) as a derivative of canonic base cytosine, known as an anti-leukemia drug detected in the RNA molecules of *Escherichia coli*<sup>4</sup>.

**Materials and methods:**

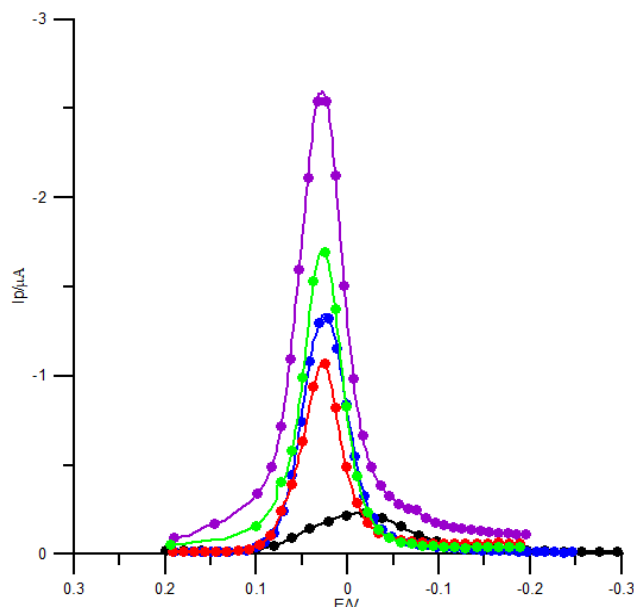
The research has been carried out with the use of an  $\mu$ AUTOLAB electrochemical analyzer controlled by the GPES software (Version 4.9) (Eco Chemie, Utrecht Netherlands). The cell stand included a three-electrode system with a cyclically refreshable liquid amalgam film silver based electrode (R-AgLAFE), used as a working electrode; a silver/silver chloride electrode (Ag/AgCl, 3 MKCl) as a reference electrode; and a Pt wire as a counter electrode.

Research methodology is mostly based on the electrochemical techniques (direct current polarography (DC), cyclic voltammetry (CV) and square wave voltammetry (SWV), electrochemical impedance spectroscopy (EIS)) which allowed to determine the kinetic parameters indicated at the mechanism of Bi(III) ion electroreduction and magnitude of the catalytic effect of 2-thiocytosine.

The supporting electrolyte was  $0.5 \text{ mol} \cdot \text{dm}^{-3} \text{ NaClO}_4 + 0.5 \text{ mol} \cdot \text{dm}^{-3} \text{ HClO}_4$ . The concentration of Bi(III) ions in the solutions was always  $1 \cdot 10^{-3} \text{ mol} \cdot \text{dm}^{-3}$ . Due to the poor solubility of  $\text{Bi}(\text{NO}_3)_3$  in chlorates(VII), the solutions were treated by ultrasonic machining. The concentrations of the 2-thiocytosine were chosen to be  $1 \cdot 10^{-5}$  and  $3 \cdot 10^{-3} \text{ mol} \cdot \text{dm}^{-3}$ .

### Results and discussion:

The addition of  $1 \cdot 10^{-4} \text{ mol} \cdot \text{dm}^{-3}$  of 2-thiocytosine to the solution of the basic electrolyte results in a significant increase of the SWV peak current of Bi(III) ions electroreduction and peak shift towards more positive potentials (Fig.1). This peak becomes better defined. At the same time its width diminishes at half of the height pointing out to the increase of the reversibility of Bi(III) ions electroreduction in the presence of TC. The extent of this effect depends on the concentration of 2-thiocytosine.

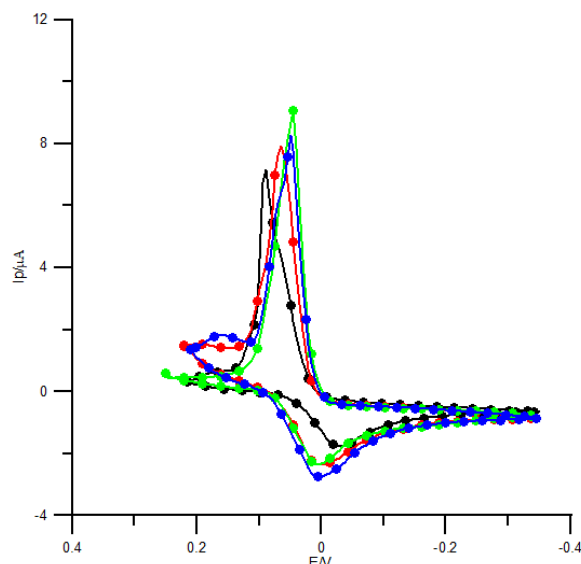


**Fig. 1** SWV peaks showing the electroreduction of  $1 \cdot 10^{-3} \text{ mol} \cdot \text{dm}^{-3}$  Bi(III) ions (●) in the  $1 \text{ mol} \cdot \text{dm}^{-3}$  chlorates(VII) and in the presence of 2-thiocytosine:  $1 \cdot 10^{-5}$  (●),  $5 \cdot 10^{-5}$  (●),  $1 \cdot 10^{-4}$  (●),  $1 \cdot 10^{-3}$  (●).

The effect of 2-thiocytosine on the kinetics of depolariser ions electroreduction also depends on the course of cyclic voltammetry curves (Fig. 2). The presence of TC leads to an increase in the reversibility process. The values  $\Delta E_{a-c}$  decrease significantly compared to those obtained for the basic electrolyte ( $1 \cdot 10^{-3} \text{ mol} \cdot \text{dm}^{-3}$  Bi(III) in  $1 \text{ mol} \cdot \text{dm}^{-3}$  chlorates(VII)).

Moreover,  $\Delta E_{a-c}$  for the Bi(III) ions electroreduction in the presence of 2-thiocytosine to a small extent depends to a small extent on the rate of electrode polarisation (Table 1) which indicates to the chemical reaction controlling the electroreduction rate of Bi(III) ions in  $1 \text{ mol} \cdot \text{dm}^{-3}$  chlorates(VII) in the presence of 2-thiocytosine<sup>2</sup>.

The above mentioned chemical reaction is probably the Bi-(RS-Hg) complex formation on the electrode surface, which is the intermediate during the electron transfer<sup>2,5</sup>. The formation of this complex is favoured by the RS-Hg adsorption on Hg(Ag)FE<sup>2,5</sup>. 2-thiocytosine is a mediator in the formation of these complexes.



**Fig. 2** Cyclic voltammetric curves showing the electroreduction of  $1 \cdot 10^{-3} \text{ mol} \cdot \text{dm}^{-3} \text{ Bi(III)}$  ions (●) in the  $1 \text{ mol} \cdot \text{dm}^{-3}$  chlorates(VII) and in the presence of 2-thiocytochrome:  $1 \cdot 10^{-5}$  (●),  $3 \cdot 10^{-5}$  (●),  $1 \cdot 10^{-4}$  (●); by the electrode polarization speed  $v=50 \text{ mV} \cdot \text{s}^{-1}$ .

**Table 1.** Changes in  $\Delta E_{a-c}$  for the electroreduction of  $1 \cdot 10^{-3} \text{ mol} \cdot \text{dm}^{-3} \text{ Bi(III)}$  ions in  $1 \text{ mol} \cdot \text{dm}^{-3}$  chlorates(VII) as well as with the increasing concentrations of 2-thiocytochrome from the polarization speed  $v$ .

$10^3 C_{TC}$ $/\text{mol} \cdot \text{dm}^{-3}$	$\Delta E / \text{V}$							
	$v / \text{mV} \cdot \text{s}^{-1}$							
	5	10	20	50	100	200	500	1000
<b>0.0</b>	<b>0.098</b>	<b>0.097</b>	<b>0.100</b>	<b>0.102</b>	<b>0.110</b>	<b>0.126</b>	<b>0.148</b>	<b>0.171</b>
<b>0.1</b>	0.0488	0.0498	0.0500	0.0521	0.0632	0.0978	0.1009	0.1162
<b>0.3</b>	0.0436	0.0440	0.4426	0.0503	0.0570	0.0967	0.1000	0.1120
<b>0.5</b>	0.0397	0.0400	0.0400	0.0424	0.0508	0.0817	0.0728	0.0961
<b>1.0</b>	0.0382	0.0389	0.0384	0.0382	0.0482	0.0625	0.0679	0.0907
<b>3.0</b>	0.0344	0.0343	0.0340	0.0342	0.0485	0.0598	0.0601	0.0823
<b>5.0</b>	0.0306	0.0303	0.0302	0.0342	0.0467	0.0588	0.0591	0.0793
<b>10.0</b>	0.0300	0.0302	0.0300	0.0340	0.0464	0.0572	0.0579	0.0669

## Conclusion:

The multistage Bi(III) electroreduction process in chlorates(VII) is affected by the presence of 2-thiocytochrome. The use of the R-Ag/LAFE electrode to study electrode mechanisms has brought the expected results and is definitely in line with the green chemistry trend due to a significant reduction in the toxic mercury consumption.

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## OP44- A Pilot Study into the Effects of FluorodeoxyglucoseConjugated Iron Oxide Magnetic Nanoparticles on Adenocarcinoma in Mice, and Lung Cancer Cells in 3D Cell Culture Spheroids

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**Abstract-** Our study involves the cytotoxic effects of conjugated (fluorodeoxyglucose-magnetic nanoparticles (FDG-mNPs)) and unconjugated magnetic nanoparticles (mNPs) on 2D, 3D-spheroidal H727 *in-vitro* cell cultures, and *in-vivo* MAC-16 tumour models. MTT reduction and trypan blue exclusion techniques are used with 2D and 3D-spheroidal H727 cultures. mNPs, and FDG-mNPs (38.4-714 µg/mL), are used to determine cell viability, optimal dose ranges and cell destruction.

MAC-16 tumour bearing mice received intratumoral FDG-mNPs, mNPs and FDG-mNPs in saline solution (n = 2). The iron concentrations were 83 µg/mL and 95 µg/mL for FDG-mNPs and mNPs, respectively. Two additional MAC-16-tumour mice received no treatment. Tumour volume, (indicating therapeutic response), was measured over 5 days. Our results indicated that: (1) No correlation was found between mNP concentration and spheroid cell viability, (2) FDG-mNP and saline treated tumour growth rates remained low over 5 days. The volume of the tumour decreased (P<0.001) compared to untreated and mNP-treated mouse tumours. We conclude that our results are worthy of further investigation.

**Key words:** Cancer; Iron oxide Nanoparticles; Spheroids; MAC-16 Tumour; Mouse Model

### Introduction

The conjugation of mNPs to the glucose analogue fluorodeoxyglucose (FDG) is thought to improve cellular mNP incorporation and induce cytotoxicity in cancer cell lines. It is well known that cancer cells have a great affinity for the uptake of glucose; The Warburg effect (Atkinson, San-Millán and Brooks, 2017). Since FDG is a glucose analogue, it is believed that it is rapidly taken up by cancer cells. This has been shown by some reports (Subramanian et al., 2016; Atkinson et al., 1984).

Our research involves the destructive effects of FDG-mNP and iron oxide on lung cancer cells in 2D- and 3D-spheroidal H727 *in-vitro* cultures and in *in-vivo* MAC-16 tumour models. The objectives of our study were 1) To assess the percentage of viable cells using the MTT assay. 2) To measure the volume of tumour in MAC-16 bearing mice *in-vivo* after 7-9 days following injection with nanoparticles mNPs, FDG-mNPs and saline.

### Materials and Methods

Synthesis of mNPs was undertaken as described in previous studies (Ozkaya et al., 2013).

H727 cells were grown in RPMI medium with 10% fetal bovine serum (FBS), 1% glutamine and 1% penicillin-streptomycin in 5% CO<sub>2</sub> in an incubator at 37°C. The cell line was maintained in exponential growth by sub-culturing cells at high confluence, using 0.25% trypsin-EDTA for detachment and pelleted following centrifugation at 1000 RPM for 5 minutes. Cells were grown in various 96-well plates; untreated flat-bottomed plates were used to produce cell monolayers, with ultra-low attachment surface plates used for 3D cell culture (Corning Inc.). A Methyl Blue Tetrazolium assay (MTT) was used to assess cell viability.

In order to generate 3D spheroidal cultures, H727 cells were seeded into non-adhesive round-bottomed 96-well microplates at a well density of  $1 \times 10^3$  cells in 200 µL of RPMI-1640 medium and incubated for 48-72 hours in the dark at 5% CO<sub>2</sub> at 37°C for 2, 3, 6, 12, 20 and 28 days.

Thereafter, 3D-spheroids were dosed with FDG-mNPs or mNPs at a concentration of 252 µg/mL, 361 µg/mL, 500 µg/mL, 630 µg/mL or 714 µg/mL for 24 hours or 4 days.

#### **Cell viability assessment**

To determine the live cells viability, spheroids were washed with PBS 3 times. For DAPI staining, cells were suspended in a fresh media (100µL) and DAPI at a concentration of 5mg/mL was added to each well of a 96-well plate (corning plate). DAPI-stained cells were incubated in a dark room temperature for 12 minutes and images were taken at day 12 by a fluorescence microscope (EVOS FL).

#### **In-Vivo testing of mNPs and FDG-mNPs in MAC-16 Tumour Bearing Mice**

All animal research procedures followed were in accordance with the standards of guidance for the Coordinating Committee on Cancer Research Guidelines for the Care and Use of Laboratory Animals according in the U.K. The studies were conducted under Home Office License Number PPL303179.

The in-vivo testing of the effect of mNPs and FDG-mNPs on tumour volume (mm<sup>3</sup>) and percentage of cell death was undertaken in mice. Specifically, eight female NMRI mice (15 - 20 g) were inoculated subcutaneously in the flank region with MAC-16 tumour grafts (~1×10<sup>6</sup>cells/transplant) which were able to grow until their size reached about 2-3 mm in diameter. Six MAC-16-tumour bearing mice were then treated with FDG-mNPs (95 µg/mL iron concentration), mNPs (83 µg/mL iron concentration) or saline solution as control (n = 2 per each treatment group); treatment was administered intratumourally to the outside surface of the tumour. A control group of two MAC-16 tumour bearing mice received no treatment.

### **Results**

Effect on NCI-H727 2D and 3D Spheroidal Cultures:

The MTT results indicated that FDG-mNPs, FDG-mNPs in saline, and mNPs showed no inhibitory effects on the percentage of cell viability of NCI-H727 cells after 24 hours of treatment. Only higher doses of mNPs (800 – 1000 µg/mL) reduced cell viability in a dose-dependent manner. Treating NCI-H727 cells with high doses of mNPs for 28 days at 1000 µg/mL increased the area of cells and caused shape disruption.

Figure 1 shows the growth and structural development in spheroids derived from the 3D cell culture of NCI-H727 non-small cell lung carcinoma cells over 28 days. Following 3D culture, growth stages of the spheroids over 28 days demonstrated growth in shape that were accompanied by changes in the area that comprised the necrotic core and viable outer edge: the size of the necrotic or hypoxic core increased proportionally with the size of the spheroid.

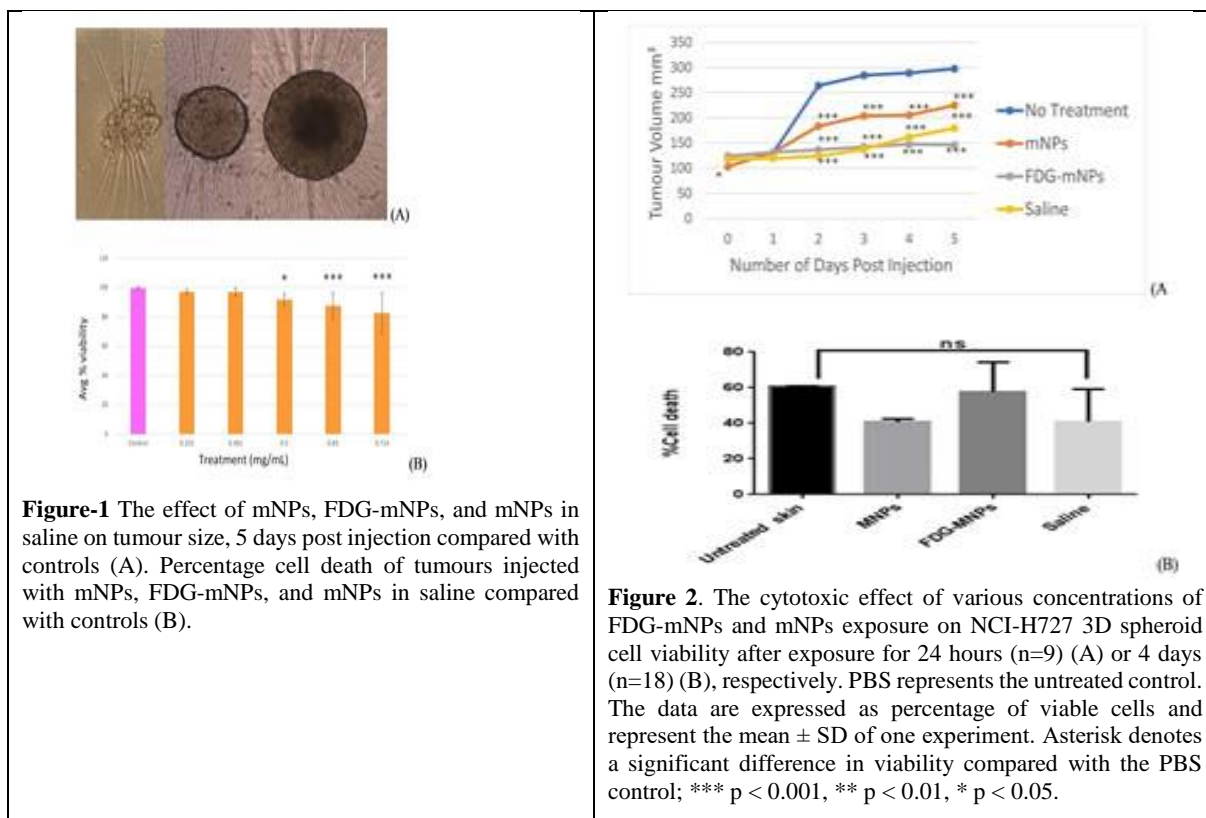
Figure 2 shows the cytotoxic effect of FDG-mNP exposure on NCI-H727 3D spheroidal culture cell viability. The results showed that FDG-mNP and mNP-treated cell viability were significantly reduced when spheroids were dosed above 500 µg/mL.

3D spheroid models mimic the natural tumour microenvironment as they exhibit increased intracellular signalling, extracellular matrix deposition and increased cell-cell junctions. These conditions mean oxygen/nutrient diffusion is limited and gradients start to form. This results in layers forming within the spheroid. This morphology accurately represents naturally forming tumours and is therefore potentially useful and advantageous when testing anti-cancer therapeutics.

One-way ANOVA found that only concentrations above 5 mg/mL have a significant effect on reducing cell viability by as much as 15%. At day 0 cells simply aggregate in the bottom of the well. Cell interaction leads to cell-cell adhesion and the formation of small spheres. By day 12, cells become compacted and distinct layers form.

Effect in MAC-16 Tumour Bearing Mice:

When comparing tumour volume measurements taken on day 0 compared to day 5, a significant increase in tumour volume was observed in mNP-treated mice (day 0: 103 vs day 5: 225 mm)



## Conclusion

We conclude that there appears to be no definitive correlation between mNP concentration and spheroid cell viability, in the experiments undertaken. Tumour volume, (an indicator of therapeutic response), over 5 days post-injection indicated that the rate of FDG-mNP and saline treated tumour growth remained low over the 5 day period. Furthermore, tumour volume decreased to a significant degree compared to untreated and mNP-treated tumours and those exposed to saline (the tumour receiving the FDG-mNP injection decreased in volume by about 50%, relative to the untreated mouse). This in vivo study suggests that in MAC-16 bearing mice, FDG-mNPs and, unexpectedly, saline can be administered intratumorally to slow down the rate of tumour progression.

We note that our pilot study involved the use of few mice, but nevertheless, we feel that our approach of using FDG-mNP to potentially treat tumours is worthy of further investigation.

**Acknowledgement:** This research was funded in part through the NIH/NCI Cancer Support Grant P30 CA008748

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## OP45- Eco-friendly Synthesis of Graphene Oxide for Removal of Pollutants from Aquatic Environments

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Increasing industrial activities with population causes a serious number of pollutants released into the environment day by day. These pollutants represent an important environmental and public health problem. Among these pollutants, lead is one of the most serious environmental poisons throughout the world. Currently, there is a strong interest in finding green alternative options to remediate lead pollution. Adsorption is one of the techniques which is fast, inexpensive, and effective methods used to remove pollutants from aquatic environments. Graphene is an attractive material which has excellent mechanical, electrical, thermal and optical properties. Graphene and graphene-based materials (graphene oxide, reduced graphene oxide) are widely used in extensive range of fields such as electronics, energy storage, catalysis and several environmental applications. Currently, graphene can be synthesized by mechanical exfoliation, chemical vapor deposition, epitaxial growth and chemical synthesis methods. Although the first three methods might be preferred for specific device assembly, the challenge is still being large-scale of manufacturing. Chemical synthesis might provide practical approach to large-scale graphene oxide. To chemical synthesis of graphene oxide from graphite powder, there are various methods such as Brodie, Staudenmaier, Hummers, Modified Hummers, Tour methods. Hummers method is the most common way to produce graphene oxide. In this method graphite which is starting material, oxidized by treatment with  $\text{KMnO}_4$  and  $\text{NaNO}_3$  in concentrated  $\text{H}_2\text{SO}_4$  and further being reduced using reducing agent to obtain graphene. Unfortunately, Hummers method produce toxic gases  $\text{NO}_2$ ,  $\text{N}_2\text{O}_4$  and heat. Besides, chemical reduction process usually involves toxic reduction agents such as hydrazine, hydroquinone. Those chemical agents have toxic effects and harm both human health and the environment. On the other hand, Tour's method serves a great advantage over others since it does not generate toxic gases and the reaction temperature is easily controlled during reaction.

In this study, synthesis of graphene oxide is demonstrated with an eco-friendly and facile approach (Tour's method). The structure and morphological properties of the prepared nano-materials were characterized by means of scanning electron microscopy (SEM), transmission, X-Ray diffraction (XRD), Fourier transform infrared spectrometry (FT-IR), and BET surface area analysis. The graphene oxide obtained by environmentally friendly ways is aimed to be used in the removal of lead pollution as adsorbent. The effects of parameters such as solution pH, contact time, initial lead ( $\text{Pb(II)}$ ) concentration and temperature on the adsorption efficiency were studied. In addition, theoretical aspects of such adsorption process such as adsorption isotherms and thermodynamics has been investigated.

**Keywords:** Graphene oxide, Tour's method, Green Synthesis, Water Pollution, Removal of lead



## OP46- Stability Constants of Actinide Metal Ions with Selected Polyphenols

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Polyphenols are a complex group of substances that have gained enormous attention in the last years, because of their biological properties, their ecological role and natural antioxidant capacity. The dissociation constants of polyphenolic acids are biologically important physicochemical parameters. They constitute important data for the thorough understanding of certain chemical phenomena such as activity and rates, biological uptake and receptor binding of substances at the molecular level<sup>1</sup>. Complexes of Th(IV), UO<sub>2</sub>(II) metal ions with gallic acid (GaA), sinamic acid (SinA), and Caffeic acid (CafA) was studied in aqueous medium using the potentiometric technique at 25 °C ( $I = 0.10 \text{ mol}^{-1}\text{dm}^{-3} \text{ KNO}_3$ ). Stability constants of polyphenol complexes were obtained from the pH-metric data using the program HYPERQUAD2013 for each metal ion<sup>2</sup>. The formation of monomeric and polymeric species was taken into consideration for each ion at 25°C. Their biological behavior was studied *in vitro* at MCF-7 and A-549 cell lines. According to the results it could be concluded that complexes of actinide group (UO<sub>2</sub>, Th) metals ligands with polyphenolic ligands (gallic acid, caffeic acid, cinnamic acid) which have antioxidant properties are effective on cancer cell lines.

**Keywords:** Polyphenols; Actinides, Stability Constants, Potentiometry

**Acknowledgement:** This Project was supported by Istanbul University, Scientific Research Project Numbered as ONAP 18875.

## **OP47- The Decline of Glutathione Reductase Activity by Copper (II) Oxide Nanoparticles and Copper (II) Chloride**

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Copper is an important trace element for many organisms, but overexposure of metals can induce a broad spectrum of toxic effects on animal and vegetable species <sup>1</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 ( $\beta$ -nicotinamide adenine dinucleotide 2'-phosphate reduced) by GR enzyme <sup>2</sup>.

In this study, GR from baker's yeast (*Saccharomyces cerevisiae*) exposed to 0, 25, 50, 100, 250 and 500 mg/L copper (II) oxide nanoparticles (CuO NPs) and copper (II) chloride (CuCl<sub>2</sub>). The results of present study showed that CuO NPs inhibit the GR enzyme competitively while CuCl<sub>2</sub> inhibits the GR enzyme noncompetitively.

**Key Words:** Glutathione Reductase, Copper (II) Oxide Nanoparticles, Copper (II) Chloride, Nanoparticles.

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## OP48- Targeted Lipidomic Investigation of Royal Jelly Samples as a Rich Source of Sterols and Fatty Acids

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**Abstract**-Scientific studies show that royal jelly (RJ) has health-promoting properties due to its bioactive lipids. The aim of the study is the lipidome enlightening of these lipid compositions based on targeted quantification of 38 different fatty acids and 9 sterols with novel analytical methods. Total lipid contents were measured via soxhlet extraction. Fatty acids and sterol analysis have been performed using developed methods at GC-MS. 10-HDA analysis was also optimized using HPLC-PDA. RJ contains an average of 2.9% lipid according to analysis results. 10-HDA analysis validated that RJ is the major natural source of this compound. Linolelaidic acid, stearic acid, palmitic acid, and cis-9-oleic acid were also quantified. Stigmasterol was the lowest phytosterol. Beta-sitosterol was at the highest abundance with mean values of 149.6 µg/100g. Osteosterol, campesterol, fucosterol, cholesterol, and desmosterol were also determined. Described values may be used as quality markers and expected averages to identify the adulterations.

### Introduction

Royal jelly (RJ) is a yellowish and milky bee product secreted from the hypopharyngeal and mandibular glands of young worker bees, used for feeding larvae for the first three days and for the entire life of the queen bee [1]. The chemical structure of RJ consists of lipids, proteins, and sugars, especially water. Scientific studies show that the health-promoting properties of RJ are due to bioactive lipids, proteins, and phenolic compounds [2]. This remarkable nutraceutical possesses numerous functional properties, such as anti-inflammatory, antibacterial, antioxidant, antiaging, hypotensive, immune-activating, and antitumoral activities [3]. The RJ lipids are effective in regulating immune response and decreasing the risk factor in the development of heart disease. The majority of lipids in royal jelly are fatty acids (FA's), mostly in free form and rarely in esters [4]. Besides fatty acids, RJ lipids also contain major amounts of phytosterols which could be claimed as "cholesterol-lowering" molecules. The aim of the study is the quantitative investigation of these valuable compounds in RJ samples as targeted lipidome profile enlightening based on targeted quantification of 38 different fatty acids and 9 sterols with novel analytical methods using gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC) instrumentation.

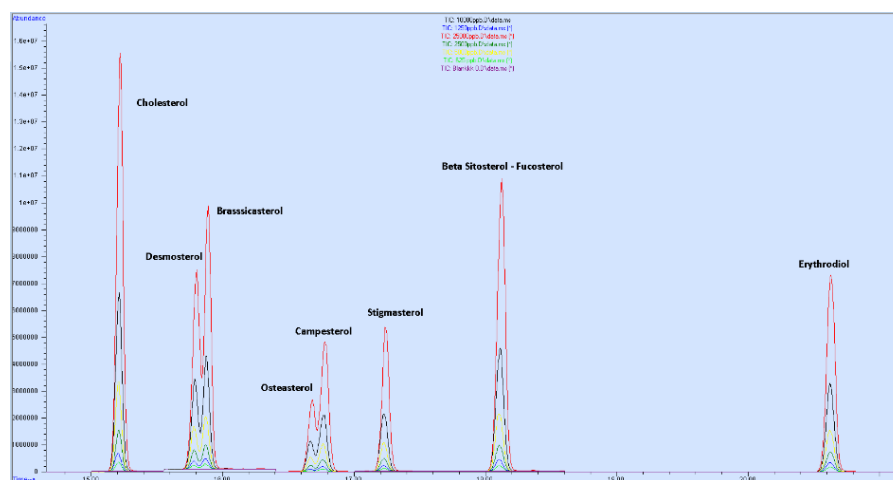
### Method & Materials

Royal jelly samples were produced under controlled conditions during the 2020 season. Total lipid contents were measured applying soxhlet extraction. For FA's analysis, FAME mix. contains 37 components used for external calibration. Agilent® 6890B GC system equipped with the 5977A MSD system was used. Separations were achieved by means of Agilent® DB-23 60 m x 0.25 mm, 0.25µm column applying gradient elution. Run time was 23 min. and the injection volume was 1 µl at split mode. 10-HDA analysis was conducted at Waters® Alliance HPLC-PDA system in 10 min. Isocratic elution accomplished using acidified 20% methanol as mobile phase. 280 nm wavelength was chosen and the column was Thermo® Hypersil Gold BDS 150 x 4.0 mm, 3 µm. For sterol analysis, the GC inlet was operated in splitless mode (280°C, 1.4 µl injection). Agilent® DB-5-MS capillary column (30m x 0.25 mm i.d., 0,25 µm) and gradient elution was used at same MS configuration. External calibration was established regarding 9 sterols ranging

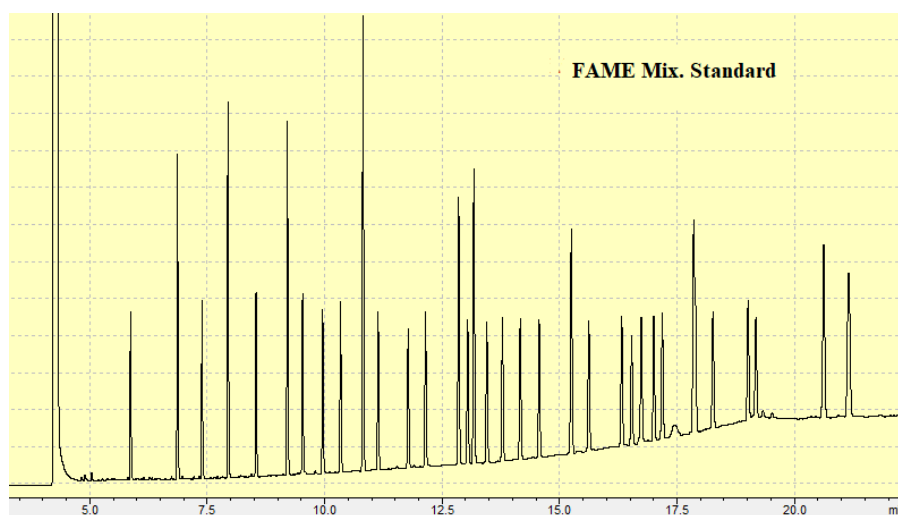
from 0.6 to 25 mg/L. Sample pretreatment for FA's involved solid-liquid extraction using isopropanol-diethyl ether mixture followed by dehydration with Na<sub>2</sub>SO<sub>4</sub>. Extracts were evaporated and reconstituted with acidified methanol for methylation and hexane was added for back-extraction. Conjugated sterols released by acid hydrolysis. Then, salting-out assisted liquid-liquid extraction was applied using hexane. Cold saponification under non-oxidative conditions was performed using methanolic NaOH. Unsaponifiable hexane layer was neutralized and evaporated before derivatization with BSTFA-TMCS and pyridine. For 10-HDA analysis, samples were weighed and extracted with ultrasound-assisted extraction using 20% methanol.

## Results & discussion

The RJ contains an average of 2.9% fat according to analysis results. We tried to determine the sterol and free fatty acid concentrations within this value. Sterol method was validated to cover a range of 0.2-500 µg/100g, achieving mean recoveries at 89-95% with a standard deviation range of 2-6%. The method had a detection limit of 0.2 µg/100g for all molecules. MS response was linear with correlation coefficients ( $R^2$ ) of 0.998. Representative chromatograms of calibration points were given in **figure 1**. No sample contained detectable level of brassicasterol and erythrodiol. Stigmasterol was the lower sterol substance among the 7 present sterols with the average of 0.86 µg/100g concentration. Beta-sitosterol was at the highest abundance with mean values of 149.6 µg/100g. Osteosterol, campesterol, fucosterol, cholesterol and desmosterol followed this result with the average values of 101.8 µg/100g, 38.1 µg/100g, 27.57 µg/100g, 4.83 µg/100g and 1.28 µg/100g respectively. These values were also in agreement with the literature. The total sterol contents quantified just after harvest varied from 55.02 µg/100g to 853.2 µg/100g. For fatty acid analysis, calibration was established using FAME mix. standard and methyl esters of 37 fatty acids were resolved with high efficiency as shown in **figure 2**. To the best of our knowledge 10-HDA is the highest abundant fatty acid in royal jelly. Besides of it, linolelaidic acid was observed to be predominant fatty acid among the analyzed 37 FA's. Its mean value was 590.2 µg/g. Stearic acid, palmitic acid and cis-9-oleic acid were also quantified at higher rates with the concentrations of 431.9 µg/g, 202.9 µg/g and 105.6 µg/g respectively. Octanoic, lauric, arachidic, gamma-linoleic, linolenic, arachidonic and eicosapentaenoic acids were also found at minor quantities at RJ samples. Mean concentration of 25 mg/g at 10-HDA analysis validated that RJ is the major natural source of this compound.



**Figure 1.** Illustration of the peak linearities for sterol calibration.



**Figure 2.** Separation of 37 FAME components.

### Conclusion

RJ can be seemed to be a rich source of nutritious compounds based on lipidome profiles. The analytical methods described in this study enlightened the major sterols and FA's in RJ. The values may be used as expected averages to identify the adulterations and can be considered as quality markers.

**Keywords;** Sterol, Fatty acid, Lipidome, Royal Jelly

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## OP49- Analysis of Isobaric Advanced Glycation End Products Instead of Furosine as an Early Stage Maillard Reaction Product for Quality Assessment of Food Samples

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**Abstract**-Furosine is the molecule that is formed during the acid hydrolysis of Amadori products and can be used for the evaluation of the glycation and food quality. As a novel approach, we screened two alternative isobaric advanced glycation end products (AGE's) simultaneously during furosine analysis. Royal jelly (RJ) and cookie samples were examined to consider the feasibility of pyralline and pyridosine molecules for quality assessment. We have acquired the mass spectrum at both oxidative and non-oxidative conditions along with acid hydrolysis. Pyralline and pyridosine were detected in both samples at high concentrations. Furosine compound was very labile at high temperatures and in the presence of oxygen. Furosine was also not detected in samples. Same m/z transitions of pyralline and pyridosine compounds let us track the stages of the Maillard reaction (MR). It was shown that AGE's are more amenable to be quantified rather than early MR products such as furosine.

### Introduction

The Maillard reaction (MR) is a common reaction in food substances and defined as “An array of non-enzymatic, parallel and consecutive, rearrangements and reactions”. MR products can limit the absorptions of nutrients [1]. Amadori compounds are the known first stable intermediate compounds resulting from the early MR. The subsequent oxidation of the Amadori product produces advanced glycation end products (AGEs). AGE's are a group of heterogeneous compounds which are known as brown and fluorescent cross-linking substances such as CML, pyridosine, and pyralline [2]. Cookies are highly prone to be a source of MR products due to both the roasting process and high sugar and protein contents. Royal jelly (RJ) is a secretion from the hypopharyngeal and mandibular glands of worker bees [3]. In products based on royal jelly (RJ) and cookie, MR takes place mainly between the amino group of lysine and reducing sugars. Furosine is recognized as a quality indicator in a number foods [4]. The furosine assay is widely accepted method for assessing the formation of Amadori compounds during the early MR. Royal jelly and cookie samples were examined to consider the feasibility of pyralline and pyridosine molecules as AGE's instead of furosine for quality assessment.

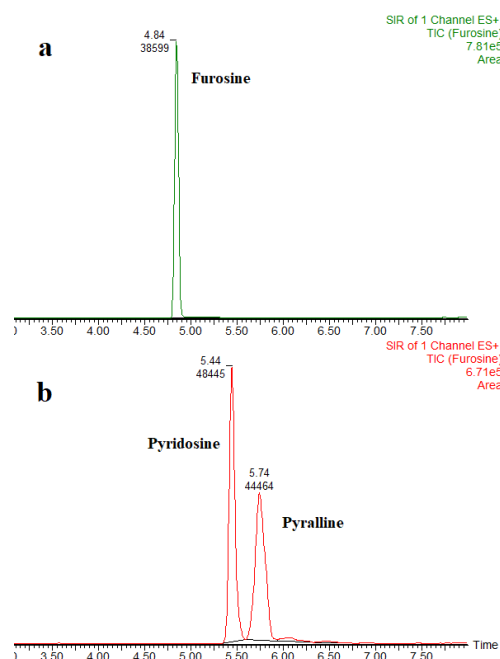
### Method & Materials

For the assessment of the alternative AGE products, we have acquired the mass spectrum at both oxidative and non-oxidative conditions along with acid hydrolysis. The RJ sample were divided into three parts and stored in the dark at -18°C, at +2/+5 °C, and room temperature. Cookie samples were prepared according to the same pretreatment and the results were compared based on furosine amount & stability and pyridosine & pyralline signal intensities. RJ or cookie, equivalent to 400 mg was weighted in a hermetically closed vessel, to which 32 mL of 8 mol/L HCl was added. The sample was blown out by a stream of N<sub>2</sub>, and heated for 23 h at 110°C. Hydrolysates were cooled and filtrated then diluted with LC mobile phase A and injected into UPLC-ESI-MS/MS system. Alternatively, the same samples were prepared without nitrogen purging. Total protein quantitation was accomplished using the Kjeldahl method and the protein results were given in mg/100g protein unit. The chromatographic analysis was performed on Waters® ACQUITY UPLC (Waters®, Milford, MA, USA) system equipped Waters® Xevo TQ

tandem quadrupole mass spectrometer. Separation was performed on the Thermo Synchronis HILIC column (150 x 2.1 mm, i.d., 1.7  $\mu$ m) by using gradient elution. Furosine contents of the samples were calculated by using the external standard calibration curve prepared from the standard solutions of furosine ranging between 0.025 and 0.20 mg/L. Due to the lack of analytical standard of pyralline and pyridosine, the resulting compounds were also calculated relatively by means of the same calibration plot.

## Results & discussion

We have quantified the amount of two different AGE's with the same mass transition (255.2 m/z) along with furosine. Thanks to our high-resolution HILIC separation, as shown in **Figure 1**, pyridosin, furosine and pyralline molecules were analyzed in high specificity and in short run time.



**Figure 1. a-** Furosine standard at 250  $\mu$ g/L concentration, **b-** Separation of pyridosine and pyralline as AGE's.

Pyralline and pyridosine were detected at both cookie and royal jelly with high concentrations. Storage of royal jelly at room temperature has been identified as improper due to increase at AGE's. Nitrogen purged furosine standard prepared in 8N HCL solution at 250  $\mu$ g/L concentration and incubated at 110°C. Furosine was not monitored after 1-hour incubation. Furosine was also not detected at the cookie and royal jelly samples independent from the conditions that of nitrogen purged or not. Reported values for total pyralline and pyridosine was ranged from 85 mg/100g protein to 135 mg/100g protein for cookie samples. Royal jelly was analyzed in order to compare the effects of different storage conditions. Total quantified pyralline and pyridosine concentrations were 205 mg/100g protein for 4°C storage, 190.7 mg/100g protein for -20°C storage, and 2012 mg/100g protein for room temperature storage. Significant ( $p < 0.05$ ) increases in the concentration of total AGE's were found in sample that was stored at room temperature which indicates the predominance of pyralline and pyridosine and inappropriateness of the room temperature storage. The decrease in furosine content was the sign of the development of the MR from early-stage to advanced-stage which could be observed as increases in the concentration of AGE's. Same m/z transitions let us to track the stages of MR. It was



shown that AGE compounds are more amenable to be quantified rather than early MR products such as furosine in the cookie and royal jelly samples.

### Conclusion

The novel analytical method showed that roasting and improper storage caused the MR to be shifted in the direction of early to an advanced stage. Our suggestion is to use pyralline and pyridosine molecules when monitoring glycation due to the inappropriateness of the furosine compound based on its labile properties.

**Keywords;** Maillard Reaction, Furosine, LC-MS/MS, Glycation

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## OP50- Aptamer-based Nanobiosensing Strategies Using Graphene Oxide Devoted for Clinical Analysis

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With the studies carried out in recent years, a better understanding of aptamers biology and their binding properties for target molecules has enabled their use in different areas, including bioimaging, drug delivery systems, targeted therapeutic and aptamer-based biosensors, aptasensors. Aptamers, also known as chemical antibodies, are synthetic ssDNA or RNA oligonucleotides which possess high affinity and specificity toward target molecules by folding into tertiary structures<sup>1</sup>.

In our present study, GO was deposited onto pencil graphite electrode (PGE) from its solution in DMSO/H<sub>2</sub>O. Graphene oxide (GO) in combination with aptamers have enhanced the performance of the electrochemical aptasensors by providing high surface area, thus rapid electron transfer<sup>2</sup>. After optimization studies of the electrode surface nanomodification, aptamers specific for target biomarkers were covalently attached to the sensor surface via coupling between the carboxylic groups of the GO and the amino groups of the 5' aminohexyl modified aptamer. The constructed aptasensor was used for detection and quantification of target molecules in solutions with different concentrations. Using GO nanoparticles allowed us to increase the sensitivity of the proposed biosensor. The relative electrochemical signal of the aptasensor increased linearly with target biomarker concentration. In addition, the sensor showed high precision and repeatability. Aptamer-based nanobiosensors designed in this study showed high sensitivity and specificity in detection of target biomarker molecules. In future we are planning to carry out stability studies and application of this nanobiosensor with real biological samples for further possible development of point-of-care diagnostic tools.

**Keywords:** graphene oxide, nanobiosensor, Electrochemical Impedance Spectroscopy (EIS), aptamers, clinical analysis

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## OP51- Elemental Composition, Antioxidant Activities and Identification of Volatile Compounds in Goji Berries

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Goji berry (GB), which is a very powerful antioxidant, is used in Chinese medicine to reduce cholesterol, lower blood pressure, and clean blood. GB is an authentic fruit with orange, red color and tangy flavor<sup>1,2</sup>. It is aimed to determine metal, antioxidant and volatile organic components of the dried GB sample of Turkish origin, which has been increasingly used all over the world in recent years.

The volatile organic compounds found in GB samples were extracted and determined by headspace-solid phase microextraction-gas chromatograph-mass spectrometer (HS-SPME-GC/MS) using commercial SPME fiber. The elemental composition of GB sample was analyzed by ICP-OES after acid digestion. Antioxidant capacity of GB sample was determined by DPPH radical-scavenging activity method. The volatile organics of GB sample were also determined by hydrodistillation method.

The elements determined in the GB samples were Na, K, Mg, Ca, Zn, Fe, Cu, Mn, P, Se and V and were found at different levels. The extraction efficiencies of polyacrylate and polydimethylsiloxane/carboxen/divinylbenzene SPME fibers were found higher. The optimum extraction temperature and extraction time for the SPME method were determined as 60 °C and 60 min, respectively. Some identified compounds are limonene, safranal, nonanal, geranyl acetone, benzophenone, fatty acids like ethyl palmitate, methyl oleate, methyl linoleate, etc.

The identified compounds in GB by SPME method were higher than the hydrodistillation method. The samples showed similar contents of elemental composition with the previous work<sup>2</sup>. The black GB sample has the highest antioxidant activity.

**Keywords:** Goji berry, HS-SPME-GC/MS, element composition, volatile compounds, antioxidant

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**OP52- Alterations in Superoxide Dismutase Activity and Malondialdehyde Level of Blood Tissue of *Cyprinus carpio* Caught from the Polluted and Unpolluted Regions in the Atatürk Dam Lake**

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Atatürk Dam Lake, Turkey's largest dam, is contaminated with anthropogenic pollutants. *Cyprinus carpio* is the most common fish in the Atatürk Dam Lake and is inevitably exposed to the pollutants in the reservoir. In the present study, the changes in superoxide dismutase (SOD) activity and malondialdehyde (MDA) level were determined in the blood tissue of *C. carpio* captured from the Sıtlıce region, the polluted area, and the Samsat region, the clean area, in August 2012. In fish from the Sıtlıce region relative to the Samsat region, it was observed a decline in the SOD activity and an elevation in the MDA level. The results of this research indicate that untreated wastewaters caused significant oxidative stress by dysregulation in the antioxidant system and *C. carpio* in Sıtlıce site was undergoing this stress. Also, the oxidative changes in the fish may suggest a potential health hazard of the wastewaters to aquatic organisms in the dam lake.

**Key Words:** Atatürk Dam Lake, *Cyprinus carpio*, Pollution, Superoxide Dismutase, Malondialdehyde.

**Acknowledgment:** This work was supported by the Research Found of Adiyaman University, Project Number FEFBAP2011/0008.

## Virtual Presentations

### VP1- Preparation of Murexide -Alginate Hydrogel Beads For Strontium Detection Using RGB Coordinates

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Wastewater from nuclear power plants contains radioisotopes, suspended, and dissolved solid materials. Radionuclides of strontium ( $\text{Sr}^{2+}$ ), because of its greater yield in the nuclear fission process and longer half-lives, is among the most hazardous and dangerous radionuclides in the nuclear waste effluents and it can be found in almost all radioactively contaminated areas, mainly in the aqueous phases <sup>1</sup>.

In this study, we prepared alginate beads that were modified with Murexide (Ammonium purpurate) probe molecule to detect the strontium ions. As a probe molecule, Murexide was immobilized to the alginate beads. The colour changes of the beads in the presence of strontium ions were determined quantitatively using RGB (red, green, blue) colour coordinate values <sup>2</sup>. The RGB values of the Murexide-alginate beads were evaluated using ImageJ (Image Java). This enables us to obtain the colour change by simply determining of the RGB coordinates corresponding to the colour of the sample. The optimization of suitable conditions for colorimetric detection of strontium ions was studied. The influence of solution pH, Murexide concentration, Strontium concentration in the solution, the reaction temperature and the reaction time when responding to strontium detection in aqueous solution were optimized.

Colorimetric methods have their own advantages such as simplicity, high sensitivity and selectivity, and a reasonable response time. The colorimetric coordinate method provides a convenient and simple technique for naked-eye metal ion detection. This method, which can be conveniently and easily monitored by the naked eye, are appropriate for monitoring of target metal ions and potential application in on-site detection owing to their simplicity and portability. This colorimetric detection of strontium ion is handily analysed to determine the red-green-blue (RGB) value using portable devices such as desktop digital scanner and mobile phone camera, quantitatively.

We believe that it is a simple, convenient and rapid detection method for can be a potential candidate for practical applications such as on-site test and monitoring of the elements.

**Keywords:** Strontium, Alginate bead, Murexide, RGB colour coordinate.

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**Acknowledgement:** This work was financially supported by Ege University, Scientific Research Foundation under the Grant No. FDK-2020-2210.

## **VP2- Permeable Reactive Barrier Design for The Immobilization of Strontium Radionuclide**

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Accidents in nuclear reactors and leaks in nuclear waste are the most important sources of highly toxic radionuclides and fission products spreading into the environment. Due to this spread, underground and surface water resources are exposed to radioactive contamination. Sr-90 is the most common product of nuclear fission and is a potential biohazard cause for living organisms. Studies are carried out to develop new treatment technologies to prevent the spread of this radionuclide from contaminated environments to clean environments. Recently, various materials have been developed and reported for the removal of radionuclides from the aqueous phase.

Permeable reactive barrier (PRB) technology has gained importance with the emergence of in-situ treatment technologies that have been studied extensively in recent years. Zeolite, diatomite, clay and sepiolite are natural, economical and readily available materials. Besides, it has been used as a barrier material due to its high sorption and ion exchange capacity, large surface area and reactivity advantages.

In this study; in the case of a nuclear accident, the effectiveness of natural zeolite, diatomite, clay and sepiolite barrier materials were tested separately to prevent the penetration of Sr radionuclide to the environments. BET, XRD, XRD and FTIR characterization tests of barrier materials have been completed. Some properties of these barrier materials such as swelling degree, hydraulic conductivity, particulate size and selectivity against target pollutant were investigated. Optimum operating conditions (solution pH, Sr concentration, contact time, barrier material thickness and the effect of other ions) of natural zeolite and diatomite barrier materials were determined. Optimization studies for the permeable reactive barrier system were carried out using the Full Factorial Experimental Design Method.

This study is first to demonstrate immobilization of Sr from contaminated groundwater by PRB system.

**Keywords:** *Strontium, PRB, natural barrier, remediation*

**Acknowledgement:** This work was financially supported by TÜBİTAK under the Grant No. 118F455

### VP3- Green Synthesis of Iron Nanoparticles Using Galium aparine Extract, Their Characterization and Antioxidant Activity

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Nanotechnology has found applications in industry, pharmaceuticals, medicine, electronics and tissue engineering. Iron nanoparticles (FeNPs) attract much attention in nanotechnology due to their high intrinsic reactivity, biocompatibility, surface modifiability and high surface / volume ratio of their surface areas. It is very important to produce iron oxide nanoparticles with green chemistry due to their non-toxic and environmentally friendly properties<sup>1</sup>.

Antioxidants are substances that inhibit formation of free radicals or react with free radicals. Free radicals are formed in the body as a result of sunlight, chemical reactions and natural cell metabolism. Increased free radicals disrupt pro-oxidant/anti-oxidant balance and oxidative stress occurs. Oxidative stress causes various pathological effects such as DNA damage, carcinogenesis, diabetes and cellular degeneration. Many plants in nature have different biological activities due to the presence of its active compounds such as phenols, terpenoids<sup>2</sup>.

*Galium aparine* has antioxidant, antidiabetic, antimicrobial, anticancer and hepatoprotective properties, since it includes alkaloids, carbohydrates, tannins and phenolic compounds. In this study, the extraction of the *Galium aparine* was carried out by maceration method using water as a solvent. The obtained extract was reacted with FeCl<sub>3</sub> and iron nanoparticles were synthesized. Iron nanoparticles were characterized with Ultraviolet–visible and Fourier-transform infrared spectroscopy. The antioxidant capacities of the original extract and its state after the synthesis of iron nanoparticles were investigated by DPPH (2,2-Diphenyl-1-picrylhydrazyl), ABTS (2,2'-Azino-bis( 3-ethylbenzothiazoline-6-sulfonic acid) method. According to the results obtained, iron nanoparticle synthesis increases the antioxidant capacity of the plant extract.

**Keywords:** *Galium aparine*, iron nanoparticles, antioxidant activity

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## **VP4- Nanomaterial-free and Carbon nanotube-based Rapid Electrochemical Genosensor for the detection of Escherichia coli**

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*Escherichia coli* (E. coli) is a gram-negative and non-spore rod-shaped bacterium and it cause to problems at gastrointestinal system such as renal and hepatic troubles or diarrhea.<sup>1,2</sup> The determination of this bacteria from many samples, such as drinking water, is important for the protection of human health. Traditional determinations such as live cell counts of food pathogenic bacteria, selective isolation of bacteria and immunoassays may be available, but they are time consuming, labor intensive, unsuitable for on-site detection and require highly trained persons.<sup>3,4</sup> For this reason, in our study, as an alternative to these existing methods, a reliable and easy-to-use electrochemical genosensor that provides DNA-based E.coli analysis in a short time was designed.

The single-use pencil graphite electrode (PGE) was modified with the corresponding capture probe specifically that recognizes E. coli DNA, and then hybridization with target sequences was performed sensitively. Electrochemical investigation of DNA hybridization was monitored using differential pulse voltammetry (DPV) based on the intrinsic guanine response observed at 1.0 V potential. The developed genosensor reached the nM level of detection limit with 5 min of detection time.

On the other hand, nanomaterial free and carbon nanotube modified PGE was prepared and the hybridization based E. coli analysis was carried out. The analyzes were repeated 1st, 7th, 14th and 30th days after the electrode surface preparation. The results showed that electrodes whose surface had been modified before could also perform E. coli analysis in about 5 minutes. Future work for this direction is to detect E. coli and different bacteria in a very short time using disposable screen printed electrodes (SPEs) developed to have diagnostic kit capability.

**Keywords:** *Escherichia coli*; Diagnosis kit, genosensor, carbon nanotube, pencil graphite electrode, differential pulse voltammetry.

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## **VP5- Investigation Of Biological Activities Of Water And Methanol Extracts Of Hazelnut Leaf-Blueberry (Ericaceae), Buckwheat -Sorrel (Polygonaceae) Species The Role Of These Species In Our Nutrition**

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In many studies, it has been stated that plants contain high levels of natural chemical components and eliminate the effects of free radicals in the fight against various diseases. In this context, determining the biological activities of plant species is important in terms of its use in the treatment of many diseases.

In this study, the antioxidant activities<sup>1</sup> and pancreatic lipase/mushrooms tyrosinase inhibition properties<sup>2</sup> of heather leaves and blueberry (*Ericaceae*) and, buckwheat and sorrel (*Polygonaceae*) species were investigated.

DPPH method was used to determine the antioxidant activities of plants<sup>1</sup>. According to the results, it was observed that the methanol extracts of the plants generally showed better DPPH scavenging effect than the aqueous extracts. Blueberry methanol extract (IC<sub>50</sub> = 0.029 ± 0.002 mg / ml) exhibited the highest antioxidant activity, while the aqueous extract of heather leaf (0.078 ± 0.002 mg / ml) showed the lowest antioxidant activity. While methanol extracts of plants used in the study did not show tyrosinase inhibition, it was observed that buckwheat aqueous extract inhibited tyrosinase the most among the extracts studied. Lipase inhibition activity was not observed for aqueous and methanol extracts of plants.

When our study is evaluated in the light of other studies, it is thought that the differences in the results are due to the differences in the climate and soil between the places where the plants are grown, and the storage conditions. It is thought that including these four plants with rich antioxidant activity in the diet will have an important effect on the treatment and prevention of many diseases in terms of health. It is thought that these herbs can be used to prevent pigmentation disorders in pharmaceutical and cosmetic products due to their inhibitory effect on tyrosinase enzyme. It is thought that these herbs can be used to prevent pigmentation disorders in pharmaceutical and cosmetic products due to their inhibitory effect on tyrosinase enzyme activity.

**Keywords:** DPPH, Tyrosinase, Inhibition

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